Concept Paper

Hydroxychloroquine as Prophylactic Adjunct to BCG Vaccination in High-Risk Neonates: A Hypothesis for Triple Efficacy in Tuberculosis Prevention, BCG-Complication Mitigation, and SCID Safety

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Hydroxychloroquine as Prophylactic Adjunct to BCG Vaccination in High-Risk Neonates: A Hypothesis for Triple Efficacy in Tuberculosis Prevention, BCG-Complication Mitigation, and SCID Safety

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Abstract

Background: BCG vaccination at birth remains the cornerstone of neonatal tuberculosis (TB) protection in high-burden countries. However, BCG induces severe, potentially fatal disseminated infection (BCGosis) in infants with Severe Combined Immunodeficiency (SCID). Saudi Arabia's 2019 policy delay of BCG to six months of age—intended to reduce BCGitis in SCID—leaves the vast immunocompetent neonatal population unprotected against TB during their most vulnerable window. **Hypothesis:** We propose that prophylactic hydroxychloroquine (HCQ), administered concurrently with BCG at 28 days of life in high-risk neonates, can achieve triple efficacy: (1) TB protection through maintenance of early BCG immunization, (2) BCG-complication mitigation through HCQ's containment mechanisms, and (3) extended safety window for SCID diagnosis prior to irreversible BCGosis. The biological rationale rests on three pillars: HCQ's direct intraphagosomal antimycobacterial activity, its anti-inflammatory attenuation of BCGitis, and its established safety profile via transplacental maternal administration. Critically, it is BCG itself—not HCQ—that acts as the trained immunity inducer, epigenetically reprogramming innate immune cells (monocytes, NK cells, macrophages) via H3K4me3 and H3K27ac histone modifications to mount enhanced responses to subsequent mycobacterial challenge. **Evidence Base:** Bernatowska et al. (2022) demonstrated that BCGosis in SCID occurs exclusively in the NK^- phenotype, implicating IFN-γ deficiency as the decisive pathogenic mechanism. HCQ is documented to alkalinize the phagolysosomal compartment, directly impairing mycobacterial replication, and to exert anti-inflammatory effects via TNF-α and IL-1β suppression. BCG, by contrast, is the established trained immunity inducer—epigenetically reprogramming monocytes and NK cells via mTOR-dependent pathways. These distinct and complementary roles create a biologically coherent basis for a BCG + HCQ prophylactic adjunct strategy. **Conclusion:** The HCQ + BCG approach, if validated, offers a pragmatic, cost-effective intervention for countries combining high consanguinity rates with significant TB burden. We call for a prospective, randomized controlled pilot trial in high-risk neonatal populations to evaluate this triple-efficacy hypothesis before broader implementation.

Keywords: BCG vaccine; hydroxychloroquine; SCID; tuberculosis; neonatal vaccination; IFN-γ; BCGosis; high-risk neonates; triple efficacy

1. Introduction

Bacillus Calmette-Guérin (BCG) vaccination at birth is one of the most widely administered vaccines in the world, providing critical protection against disseminated tuberculosis (TB), tuberculous meningitis, and miliary TB in early infancy [1,2]. In countries with significant TB burden,

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such as Saudi Arabia (estimated TB incidence 8.4 per 100,000 population in 2023, reflecting a sustained 63.5% decline since 2000) [25], timely BCG vaccination remains a public health imperative of the first order, particularly given continued TB transmission in high-density urban and migrant-worker populations. as Saudi Arabia (estimated TB incidence 8.4 per 100,000 population in 2023, there is challenge because there are millions of residents at suadia arabia from many nationalities the ministry of health do great efforts to catch tuberculosis all over the country with mobile teams under DOTs protocol under supervision of national tuberculosis program , timely BCG vaccination remains a public health imperative of the first order, particularly given continued TB transmission in high-density urban and migrant-worker populations (25)

Yet BCG carries a known and serious risk: in infants with primary immunodeficiencies—most critically Severe Combined Immunodeficiency (SCID)—the live attenuated mycobacterium can cause uncontrolled disseminated BCG disease (BCGosis), with reported mortality reaching 13% in international cohorts [3]. The SCID incidence in Saudi Arabia is estimated at approximately 1:2,906 live births, driven by high consanguinity rates, making this risk clinically significant at a national level [4].

In 2019, Saudi Arabia adopted a policy of deferring BCG vaccination to six months of age, ostensibly to allow SCID to manifest clinically before BCG is administered. While this policy reduces BCGitis in the small number of SCID infants, it creates an unacknowledged public health deficit: the overwhelming majority of immunocompetent neonates (estimated at greater than 99.97% of all births) are left completely unprotected against TB for 180 days—a period of maximal vulnerability [5].

Aldhaheri et al. (2025) documented reduced BCGitis complications under the delayed vaccination policy, but their study focused exclusively on SCID patients at a single tertiary centre and did not evaluate the population-level TB consequences of the delay [5]. The landmark international experience—TREC-based neonatal screening with BCG at 28 days, as practiced in the United Kingdom, Slovenia, and the Netherlands—demonstrates that early vaccination is safely achievable when SCID is identified proactively [6,7].

We propose a novel hypothesis: that prophylactic hydroxychloroquine (HCQ), administered concurrently with BCG at 28 days of life in a defined high-risk neonatal population, may achieve what no single strategy has achieved to date— a ***triple efficacy*** comprising (1) TB protection through early BCG vaccination, (2) reduction of BCG-related complications through HCQ's containment mechanisms, and (3) provision of a diagnostic safety window for SCID identification. In this framework, BCG provides the trained immunity stimulus while HCQ provides the containment safety net.

2. Scientific Rationale: The IFN-γ Axis and the NK Cell Determinant

2.1. The Decisive Role of NK Cells in BCGosis Prevention

The most instructive dataset regarding BCGosis pathogenesis comes from Bernatowska et al. (2022), whose 40-year retrospective analysis at the Children's Memorial Health Institute (CMHI) in Warsaw provides critical mechanistic insights [8]. Among 72 SCID patients vaccinated with the BCG Moreau substrain at birth, BCGosis occurred in **11 out of 72 patients (15%)—and critically, exclusively in those with the NK⁻ SCID phenotype**. Not a single case of BCGosis was recorded among the 36 patients with NK⁺ SCID phenotype ($p < 0.000001$).

This finding is not merely epidemiological: it identifies NK cell–derived IFN-γ as the decisive innate immune determinant protecting against BCGosis. NK cells constitute the primary source of IFN-γ in neonates, where adaptive T-cell responses are not yet mature. IFN-γ activates macrophage mycobactericidal pathways—including reactive oxygen species generation, phagolysosomal acidification, and autophagy—creating the bactericidal environment necessary to contain BCG replication [8,9].

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When NK cells are absent (NK⁻ phenotype), this protective circuit collapses, and the live BCG organism proliferates unchecked. The logical therapeutic consequence of this insight is: if we can augment IFN-γ availability and/or directly suppress mycobacterial replication by pharmacological means, we may be able to shift the risk-benefit equation sufficiently to permit earlier, safer BCG vaccination in at-risk neonates.

2.2. Hydroxychloroquine: A Pharmacological IFN-γ Amplifier and Antimycobacterial Agent

Hydroxychloroquine (HCQ) is a lysosomotropic 4-aminoquinoline with a well-established safety profile in neonates and infants, including those exposed in utero and postnatally in the context of neonatal lupus [10]. Its immunological and antimicrobial mechanisms are uniquely suited to our hypothesis:

- **Mechanism 1 – Phagolysosomal Alkalinization and Direct Antimycobacterial Activity:** HCQ accumulates within lysosomes and phagolysosomes, raising intravacuolar pH. Crowle and May demonstrated that chloroquine at therapeutic concentrations kills intracellular Mycobacterium tuberculosis in cultured human macrophages by impairing the acidic intraphagosomal environment required for mycobacterial survival and replication [11]. Rolain et al. further documented that phagolysosomal alkalinisation is the shared antimicrobial mechanism of chloroquine and its hydroxyl analogue across bacterial, fungal, and viral pathogens, including mycobacterial species [12]. This provides a direct antimycobacterial effect independent of host immune status.
- **Mechanism 2 – Anti-inflammatory Attenuation of BCGitis:** HCQ inhibits TLR7/TLR9 signalling and downstream production of pro-inflammatory cytokines including TNF-α, IL-1β, and IL-6 [13,14]. This anti-inflammatory effect attenuates the local inflammatory response at the BCG inoculation site (BCGitis) and may reduce systemic inflammatory complications without abolishing the BCG-induced adaptive immune response. Importantly, HCQ does NOT augment NK cell IFN-γ production in this context; rather, it is BCG itself that induces NK cell activation and trained immunity reprogramming via mTOR-dependent epigenetic pathways (see Section 2.3).
- **Mechanism 3 – Autophagy Enhancement and Mycobacterial Clearance:** HCQ's effects on the autophagic machinery, particularly LC3-associated phagocytosis (LAP), enhance the delivery of intracellular mycobacteria to the degradative lysosomal compartment. This autophagy-mediated killing pathway is a central mechanism of macrophage-mediated mycobacterial clearance and operates in a manner complementary to IFN-γ–mediated activation [15,16].
- **Mechanism 4 – Anti-inflammatory Modulation Reducing BCGitis Severity:** HCQ's well-documented anti-inflammatory properties—including inhibition of pro-inflammatory cytokine cascades (TNF-α, IL-1β, IL-6)—may attenuate the local inflammatory response at the BCG inoculation site, potentially reducing BCGitis (localized lymphadenitis) without abolishing the protective immune response to the vaccine [17,18].

2.3. BCG as the Trained Immunity Inducer: The Epigenetic Engine of Protection

A fundamental distinction must be drawn between the roles of BCG and HCQ in the proposed protocol. It is ***BCG—not HCQ—that constitutes the trained immunity inducer*** in this strategy. This distinction is not merely semantic; it is mechanistically essential and was confirmed when HCQ was shown to suppress, rather than induce, trained immunity.

BCG is the paradigmatic trained immunity vaccine. Following BCG vaccination, monocytes, macrophages, and NK cells undergo epigenetic reprogramming via mTOR-dependent signalling: histone modifications including H3K4me3 and H3K27ac are deposited at promoters of pro-inflammatory genes, creating a durable "immune memory" in innate cells that enhances their functional capacity upon subsequent challenge with unrelated pathogens [9,22]. This non-specific protective effect—documented across diverse infections including respiratory viruses, Candida, and

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Leishmania—is the mechanism underlying BCG's well-established heterologous protective benefits beyond TB.

Critically, Rother et al. (Cell Reports Medicine, 2020) demonstrated that hydroxychloroquine **inhibits trained immunity** at both functional and epigenetic levels, via suppression of mTOR signalling in the lysosomal compartment [22]. This means HCQ blocks the very pathway through which BCG induces trained immunity. This finding has a critical implication for the proposed protocol: HCQ should be administered at the **minimum effective dose and for the shortest necessary duration**, and its timing relative to BCG vaccination must be optimised to avoid impairing BCG-induced trained immunity reprogramming. We propose that HCQ be discontinued by Day 28 post-vaccination (unless SCID is confirmed), by which time the critical BCG-induced epigenetic reprogramming window is expected to be complete.

> ⚠ **Mechanistic Correction: HCQ Role in This Protocol**
>
> HCQ is NOT a trained immunity inducer and must not be described as one. Its role in this protocol is strictly as a CONTAINMENT ADJUNCT: (1) limiting BCG replication in macrophages via phagolysosomal alkalinisation, and (2) attenuating excessive local inflammation (BCGitis). BCG is the trained immunity inducer. HCQ's mTOR-suppressing activity may partially antagonise BCG-induced epigenetic reprogramming if co-administered at high doses over extended periods—this is a critical limitation requiring formal evaluation in Phase 3 of the proposed research programme. The minimum effective HCQ dose and shortest duration must be used.

3. The Triple Efficacy Hypothesis

We hypothesize that HCQ administered prophylactically alongside BCG vaccination at 28 days of life in a high-risk neonatal population achieves three simultaneous objectives—constituting what we term "Triple Efficacy":

Efficacy	Target Outcome	Proposed Mechanism
Efficacy 1	**TB Protection for Immunocompetent Neonates**	BCG at 28 days restores the protective window eliminated by delayed vaccination policies. The first month of life is the period of highest TB vulnerability, particularly for tuberculous meningitis. Early BCG confers 73–83% protection against disseminated TB and near-complete protection against TB meningitis in this age group.
Efficacy 2	**BCG Complication Mitigation in High-Risk Infants**	HCQ's direct intraphagosomal antimycobacterial activity contains BCG replication in macrophages. HCQ anti-inflammatory properties (TNF-α, IL-1β suppression) attenuate BCGitis at the inoculation site. BCG itself provides the trained immunity stimulus—HCQ's role is containment, not immune priming. Duration-limited HCQ use minimises antagonism of BCG-induced epigenetic reprogramming.

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Efficacy 3	**Extended Safe Diagnostic Window for SCID**	HCQ prophylaxis during the first weeks post-vaccination creates a pharmacological safety net while TREC-based SCID screening results are processed. If SCID is confirmed, HCQ therapy is escalated to full anti-BCGosis protocol. If screening is negative, HCQ is discontinued and BCG immunity develops unimpeded.

4. Defining the High-Risk Neonatal Population

Not all neonates require the proposed adjunct strategy. We propose a tiered risk stratification framework identifying candidates for the BCG + HCQ prophylaxis protocol:

- **Tier 1 – Highest Priority: Neonates born to consanguineous parents in countries with SCID incidence ≥1:10,000 (including Saudi Arabia, UAE, Kuwait, Bahrain, Iran, Pakistan, and North African countries), awaiting TREC screening results at the time of BCG administration.**
- **Tier 2 – High Priority: Siblings or first-degree relatives of previously diagnosed SCID patients who decline to defer vaccination and where TREC screening is not yet available.**
- **Tier 3 – Intermediate Priority: Premature neonates in high-TB-burden settings where NICU discharge and subsequent primary care follow-up creates a high risk of missed vaccination appointments, resulting in unvaccinated status at six months under the delayed policy.**
- **Tier 4 – Population Level (if policy adopted): All neonates in countries with TB incidence >10/100,000 and consanguinity rates >20%, where the population-level risk of TB in unvaccinated infants outweighs the risk of BCG complications even without individual risk stratification.**

5. Proposed Clinical Protocol (For Pilot Trial Evaluation)

5.1. BCG Vaccination Component

BCG vaccination administered intradermally at 28 days of life per WHO and IUATLD standard protocol [1]. BCG substrain selection should prioritize the Moreau substrain where available, given Bernatowska et al.'s documented superior safety profile in SCID patients—including a 15% BCGosis rate compared to 34% with other substrains (p=0.0012)—attributable to PDIM/PGL lipid virulence factor deficiency and IS6110 insertion in the PhoP regulatory region [8].

5.2. HCQ Prophylaxis Component: Safety Considerations and Research Prerequisites

⚠ IMPORTANT SAFETY CAVEAT

HCQ is not approved for direct administration to neonates and infants under 6 years of age for long-term use by any major regulatory authority. The 4-aminoquinoline class has documented heightened toxicity in this age group. Furthermore, no randomised controlled trial has evaluated direct HCQ administration in neonates. All existing neonatal safety data derive from indirect exposure (in utero or via breast milk) and cannot be extrapolated directly to scheduled oral dosing. The dosing parameters below are therefore strictly provisional and theoretical, intended solely to frame the design of a prospective pharmacokinetic and safety study. They must NOT be implemented in clinical practice prior to formal trial evaluation and regulatory approval.

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Phase 1 Prerequisite – Pharmacokinetic and Safety Study (Required Before Any Clinical Use): Before any clinical application of this protocol, a dedicated pharmacokinetic (PK) and safety study in healthy term neonates must be conducted. This study should characterise: (i) plasma and whole-blood HCQ concentrations at steady state; (ii) time to therapeutic phagolysosomal concentration; (iii) elimination half-life in neonates; and (iv) safety biomarkers including ECG (QTc interval), complete blood count, liver enzymes, and ophthalmological assessment. Only after PK data confirm a favourable safety profile should a Phase 2 efficacy trial proceed.

Provisional Theoretical Dosing (For Trial Design Purposes Only): Based on extrapolation from indirect neonatal exposure data and paediatric rheumatology literature, a theoretical starting dose of 3–5 mg/kg/day orally, weight-adjusted, is proposed for evaluation in a PK study. This range is informed by: (i) estimates of neonatal exposure via breast milk (0.1–0.2 mg/kg/day), which have not produced adverse events in available case series [21]; and (ii) the lowest effective antimycobacterial concentrations documented in macrophage models [11,12]. Commencing 48 hours prior to BCG is proposed to allow steady-state tissue concentration before mycobacterial challenge.

Provisional Duration: 28 days post-vaccination if TREC screening is negative. If TREC screening is positive or indeterminate, continue under close immunological supervision until diagnostic workup is complete.

Mandatory Pre-treatment Screening – G6PD Deficiency: G6PD deficiency is a contraindication to HCQ and is significantly more prevalent in populations with high consanguinity and African, Middle Eastern, or South Asian ancestry—precisely the populations targeted by this hypothesis. Universal G6PD screening must therefore be a prerequisite for enrolment in any trial and for any future clinical application. Neonates with confirmed G6PD deficiency are excluded from the proposed protocol.

Monitoring Parameters (Provisional, For Trial Design): Weekly ECG monitoring for QTc prolongation; complete blood count and reticulocyte count at Day 7 and Day 14; liver function tests at Day 14; ophthalmological assessment at baseline and Day 28. Given the short intended duration, the risk of chloroquine retinopathy is considered very low, but must be formally evaluated.

5.3. SCID Screening Integration

The protocol is designed to function in parallel with TREC-based neonatal screening. Countries where TREC screening is not yet implemented should consider this protocol as a bridge strategy, combined with active promotion of universal SCID screening as the primary long-term solution. The 28-day screening model implemented in the United Kingdom, Slovenia, and the Netherlands provides the operational template [6,7].

5.4. Decision Algorithm Post-Screening

- TREC screening NEGATIVE (normal): HCQ discontinued at Day 28. BCG vaccination completed. Standard follow-up resumed.
- TREC screening POSITIVE (low TRECs): HCQ continued as anti-BCGosis prophylaxis. Full immunological workup initiated. HSCT team notified. HCQ escalated or anti-TB therapy initiated based on clinical trajectory.
- TREC screening INDETERMINATE: HCQ continued pending repeat TREC and flow cytometry. BCG site monitored weekly.

6. Contextualization Within International Evidence

The scientific foundation for this hypothesis draws on converging evidence streams:

First, Bernatowska et al.'s 40-year CMHI dataset [8] provides the clearest mechanistic evidence that BCGosis is an NK cell–IFN-γ–dependent phenomenon. The BCG Moreau substrain's superior safety—attributed to its genetic deficiency in PDIM and PGL lipid virulence factors—suggests that

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both the host immune axis (IFN-γ) and the pathogen's virulence determinants are amenable to modification.

Second, Marciano et al.'s international SCID cohort [3] (349 patients, 17 countries) establishes the baseline BCGosis rate of 34% in SCID and a 13% mortality rate, quantifying the magnitude of harm that any mitigation strategy must address.

Third, HCQ's antimycobacterial activity has been demonstrated at concentrations achievable in macrophage compartments with standard dosing, including against clinical isolates of Mycobacterium tuberculosis [11,12,15]. Pharmacokinetic modelling indicates that HCQ achieves relevant intraphagolysosomal concentrations at doses comparable to those used in rheumatological practice [19].

Fourth, The precedent of HCQ use in neonates exposed to maternal anti-Ro/SSA antibodies—where HCQ is administered from birth to prevent neonatal lupus cardiac complications—provides an established safety and dosing framework applicable to our proposed protocol [10,20].

7. Limitations and Critical Appraisal

We acknowledge the following limitations and uncertainties inherent in a hypothesis paper of this nature, which must be addressed in prospective research:

- **Absence of direct clinical evidence: No randomised controlled trial has yet evaluated HCQ as prophylaxis against BCGosis. All evidence cited is mechanistic, observational, or derived from analogous clinical contexts. This hypothesis requires prospective validation before any clinical implementation.**
- **Regulatory and safety barrier – direct neonatal administration: HCQ is not approved for direct use in neonates and is formally contraindicated in children under 6 years for long-term use. Existing neonatal safety data derive exclusively from indirect exposure via maternal administration. A formal pharmacokinetic and toxicology study in neonates is a mandatory prerequisite before any clinical application.**
- **G6PD deficiency – contraindication in target population: The populations with highest SCID risk (Middle East, North Africa, South Asia) also carry the highest prevalence of G6PD deficiency, which is a contraindication to HCQ due to risk of haemolytic anaemia. Universal G6PD screening must precede enrolment in any trial, and will necessarily exclude a proportion of the highest-risk neonates from the proposed protocol.**
- **Potential immunosuppression of BCG protective response: HCQ's immunomodulatory properties raise the theoretical concern that it may attenuate the very T-cell and antibody responses that BCG is intended to generate. This possibility must be evaluated by measuring BCG-specific immune responses (tuberculin skin test conversion, IFN-γ ELISPOT, anti-mycobacterial antibody titres) in HCQ-exposed vs. control groups in a pilot trial.**
- **Interaction with BCG strain-specific immunogenicity: Bernatowska et al. documented genetically distinct BCG substrains with different virulence and immunogenicity profiles. Whether HCQ's effects differ across substrains (Moreau, Danish, Pasteur) remains unstudied and is an important variable for trial design.**
- **Neonatal pharmacokinetics of HCQ: Although HCQ is used in neonates in the context of maternal connective tissue disease, formal pharmacokinetic studies in healthy neonates are limited. A pharmacokinetic sub-study should be integrated into any pilot trial.**
- **Ethical considerations: Randomizing high-risk infants to BCG alone (without HCQ) in a trial setting requires careful ethical justification, given the known risk of BCGosis. A phased approach—beginning with observational pharmacokinetic and safety data—is strongly recommended.**
- **Clinical field observations of vaccine access: In the Saudi context, operational failures of the delayed BCG policy (missed 6-month appointments, vaccine unavailability at primary health care centers, infants exceeding 12 months without BCG) suggest that any alternative strategy must account for real-world implementation realities beyond laboratory efficacy.**

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8. Future Research Agenda

We propose the following stepwise research programme to evaluate this hypothesis:

- **Phase 1 – In vitro mechanistic validation:** BCG replication assay in human neonatal macrophages and NK cells with and without HCQ, measuring IFN-γ secretion, intracellular mycobacterial counts, and LC3-associated phagocytosis activation.
- **Phase 2 – Neonatal pharmacokinetics:** Single-centre pharmacokinetic study of HCQ at 5 mg/kg/day in healthy term neonates aged 28 days, measuring plasma concentrations, whole-blood concentrations, and safety biomarkers.
- **Phase 3 – Pilot safety and immunogenicity RCT:** Multicentre randomised controlled trial in high-consanguinity, high-TB-burden settings (Saudi Arabia, Kuwait, Iran) comparing BCG + HCQ vs. BCG alone in Tier 1 neonates (consanguineous parents, awaiting TREC results). Primary outcome: BCGitis/BCGosis rate at 3 months. Secondary outcomes: BCG-specific immune response (TST conversion at 3 months, IFN-γ ELISPOT), HCQ plasma concentrations, adverse events.
- **Phase 4 – Integration with TREC screening scale-up:** Parallel advocacy and implementation research to promote universal TREC-based neonatal SCID screening as the definitive long-term solution, with the BCG + HCQ protocol serving as a bridge strategy during the implementation gap.
- **Phase 5 – Antenatal maternal HCQ priming trial (see Section 9):** Dedicated RCT evaluating antenatal maternal HCQ in high-risk consanguineous pregnancies, measuring cord blood HCQ concentrations, neonatal NK cell counts and IFN-γ secretion, and BCGosis rates at 3 months post-vaccination.

9. Extended Hypothesis: Antenatal Maternal HCQ as a Transplacental Neonatal Priming Strategy

The safety barriers to direct neonatal HCQ administration detailed in Section 5.2 prompt consideration of an alternative delivery route that eliminates these barriers entirely: maternal administration of HCQ during the third trimester in high-risk consanguineous pregnancies, exploiting documented transplacental transfer to achieve therapeutic neonatal concentrations from the moment of birth—without any direct neonatal dosing.

9.1. Transplacental Transfer: The Pharmacological Foundation

HCQ crosses the placenta efficiently. Costedoat-Chalumeau et al. demonstrated that cord blood HCQ concentrations are comparable to maternal concentrations at delivery, confirming near-complete transplacental equilibration [21]. HCQ has an exceptionally long elimination half-life of 40–50 days; in neonates with immature hepatic metabolism, this half-life is expected to be longer still, meaning therapeutically relevant HCQ concentrations would persist for weeks post-birth—precisely the window during which BCG is administered and BCGosis risk is highest.

This pharmacokinetic profile creates a unique opportunity: a mother receiving HCQ for the final 8–12 weeks of pregnancy delivers a neonate who is, in pharmacological terms, already pre-loaded with HCQ at therapeutic concentrations, with no direct neonatal dosing whatsoever.

9.2. Regulatory and Safety Advantage over Direct Neonatal Administration

Parameter	Direct Neonatal HCQ	Antenatal Maternal HCQ
Safety data	❌ No RCT in neonates; contraindicated <6 years	✅ Thousands of pregnancies documented; well-characterised profile

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Regulatory status	❌ Not approved for direct neonatal use	✅ Accepted in pregnancy for SLE, RA, anti-Ro/SSA prophylaxis
Dosing complexity	❌ Weight-adjusted neonatal dosing; palatability problems	✅ Standard adult oral dose (200–400 mg/day)
G6PD risk	⚠ Must screen neonate; excludes some target cases	✅ G6PD screened in mother; neonatal G6PD checked at birth before BCG
Concentration at birth	⚠ Zero; requires post-natal loading period	✅ Therapeutic levels from Day 0 via transplacental transfer
Duration of neonatal cover	⚠ Depends on neonatal adherence and PK	✅ Extended by prolonged neonatal elimination half-life (>40 days expected)

9.3. Proposed Biological Mechanisms: Transplacental NK Cell Priming

The rationale extends beyond passive drug transfer. We propose three distinct mechanisms by which antenatal HCQ exposure may actively prime fetal innate immunity:

- **NK Cell Maturation Priming:** Fetal NK cells undergo active maturation during the third trimester. HCQ modulates innate immune signalling via TLR7/TLR9 inhibition, attenuating pro-inflammatory cytokine cascades (TNF-α, IL-1β, IL-6) that could otherwise cause excessive fetal inflammatory activation [13,14]. This immunomodulatory environment may support more regulated NK cell maturation in utero. This mechanism is conceptually analogous to the neonatal lupus prevention pathway, where maternal HCQ modulates fetal cardiac conduction system development via transplacental immunomodulation.
- **BCG-Induced Trained Immunity: The Primary Mechanism:** BCG is the established trained immunity inducer, epigenetically reprogramming monocytes, macrophages, and NK cells via H3K4me3 and H3K27ac histone modifications at pro-inflammatory gene promoters [9,22]. This mTOR-dependent pathway is activated by BCG's mycobacterial cell wall components, particularly muramyl dipeptide and β-glucan analogues. Fetal NK cells begin to mature and acquire this reprogramming capacity in the third trimester. HCQ transplacentally present at birth provides containment support while BCG, administered at 28 days, delivers the trained immunity stimulus. Critically, HCQ must be limited in duration to avoid antagonising BCG's mTOR-dependent epigenetic reprogramming (Rother et al., 2020 [22]).
- **Macrophage Phagolysosomal Pre-conditioning:** Fetal tissue macrophages exposed to HCQ in utero will have elevated intraphagolysosomal pH at birth. This pre-conditioned state may provide an immediate antimycobacterial advantage when BCG is administered postnatally, before neonatal HCQ tissue levels decline.

9.4. Critical Caveat: The NK⁻ SCID Limitation

> ⚠ **Fundamental Biological Limitation of the Antenatal Strategy**
>
> This strategy cannot protect the NK⁻ SCID phenotype from BCGosis. As established by Bernatowska et al. [8], BCGosis in SCID occurs exclusively in infants with genetically absent NK cells. No pharmacological priming strategy—antenatal or postnatal—can activate NK cells that do not exist. Therefore, antenatal maternal HCQ does not replace TREC-based neonatal

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SCID screening, which remains the definitive solution for NK^- SCID. Rather, it offers a complementary protective layer for: (i) immunocompetent neonates at population level; (ii) NK^+ SCID phenotypes whose NK cells are present but functionally suboptimal; and (iii) the diagnostic safety window pending SCID screening results.

9.5. Proposed Eligibility Criteria for Antenatal HCQ Priming Pilot Trial

- **Inclusion:** Consanguineous pregnancy (first- or second-degree); gestational age 26–28 weeks at enrolment; singleton; no maternal contraindication to HCQ.
- **Exclusion:** Maternal G6PD deficiency; QTc >460 ms; known retinal disease; concurrent QTc-prolonging medications; multiple gestation; known fetal structural anomaly.
- **Intervention:** HCQ 200 mg twice daily from 28 weeks gestation until delivery. Cord blood sampling at birth for HCQ concentration, NK cell enumeration (CD56/CD16), and BCG-stimulated whole-blood IFN-γ assay.
- **Primary outcomes:** Cord blood HCQ concentration; neonatal NK cell functional activity at Day 0 and Day 28 (pre-BCG). Secondary outcome: BCGitis/BCGosis rate at 3 months post-BCG.

10. Conclusions

The current binary choice—delay BCG and leave immunocompetent neonates unprotected from TB, or vaccinate at birth and risk BCGosis in undetected SCID patients—is a false dichotomy. The scientific evidence reviewed here identifies two biologically coherent alternative paths that may be pursued in parallel.

The first path—postnatal BCG + HCQ at 28 days—achieves triple efficacy: TB protection, BCG complication mitigation, and extended SCID diagnostic safety window. The second path—antenatal maternal HCQ—offers a superior safety profile by converting the mother into the pharmacological vehicle, delivering therapeutic neonatal HCQ concentrations from birth via transplacental transfer, while leveraging the well-established safety record of HCQ in pregnancy. Neither path replaces TREC-based neonatal SCID screening, which remains the definitive solution for NK^- SCID.

Both hypotheses are grounded in the pathogenic mechanism elucidated by Bernatowska et al.: BCGosis is an IFN-γ–dependent, NK cell–mediated phenomenon. The complementary roles of BCG and HCQ are clearly delineated: BCG is the trained immunity inducer—epigenetically reprogramming innate immune cells via mTOR-dependent histone modifications to confer durable heterologous protection—while HCQ is the containment adjunct, limiting BCG replication via phagolysosomal alkalinisation and attenuating inflammatory complications. HCQ does not replace BCG's immune-priming function; it creates the pharmacological safety net within which BCG can be administered earlier and more safely.

This paper is not a clinical recommendation. It is a call to rigorous in vitro, pharmacokinetic, and clinical trial research. The answer to the questions posed here has implications for every country combining high consanguinity rates with significant TB burden across the Middle East, North Africa, Central Asia, and South Asia.

The infants who cannot wait for the perfect policy are being born today. They deserve the most creative, evidence-driven thinking the scientific community can offer.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. The following three supplementary tables accompany this manuscript and are provided as Supplementary File 1 (BCG_Strains_HCQ_Tables.docx). They constitute Tables S1, S2, and S3 referenced in the text. Global BCG Vaccine Strains: Genetic Classification (Early/Late group), PDIM/PGL Virulence Factors, BCGosis Rates in SCID Patients, TB Efficacy, and SCID Safety Ranking for 12 strains including BCG Moreau (Polish), BCG Denmark (1331), BCG Pasteur, BCG Russia (Moscow), BCG Japan (Tokyo), BCG

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Glaxo, BCG Tice (USA), BCG Connaught (Canada), BCG Prague (Czech), BCG Rio de Janeiro (Brazilian daughter of Moreau), BCG Sweden, and BCG Sofia; Hydroxychloroquine (HCQ) Mechanisms Relevant to BCG Adjunct Strategy: Evidence Assessment for 6 mechanisms including phagolysosomal alkalinisation (CONFIRMED), anti-inflammatory BCGitis attenuation (CONFIRMED), LC3-associated phagocytosis (PLAUSIBLE), NK cell modulation (INDIRECT), transplacental priming (NEEDS TRIAL), and trained immunity induction (REFUTED — HCQ inhibits trained immunity per Rother et al. 2020); BCG Strain Safety Ranking for SCID-Risk Neonates: Policy Recommendations including current Saudi Arabian BCG substrain (BCG Russia) and evidence-based recommendation for transition to BCG Moreau (Polish substrain).

Author Contributions: AKA: Concept, hypothesis formulation, literature synthesis, manuscript preparation, and final responsibility for all content.

Data Availability Statement: This is a hypothesis paper. No primary data were generated. All evidence cited is from published, peer-reviewed literature referenced below.

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Appendix A. Comprehensive Comparison of Global BCG Vaccine Strains — Virulence, Safety in SCID, and Implications for HCQ Adjunct Strategy

Understanding BCG strain-specific virulence is central to the HCQ adjunct hypothesis: the magnitude of HCQ benefit will differ by strain. The following table synthesises data from WHO safety documentation [6], Zhang et al.'s landmark 13-strain SCID mouse virulence study [22], Bernatowska et al.'s 40-year clinical SCID cohort [8], and Marciano et al.'s international SCID registry [3] to provide the most comprehensive comparative framework available for BCG strain selection in high-risk neonates.

GROUP 1: HIGHEST VIRULENCE — Most BCGosis Risk in SCID

BCG Strain	Country of Use	DU2 Group	SCID Mouse Survival (days)	PDIM/PGL Lipids	Reactogenicity (WHO)	BCGosis in Human SCID	HCQ Priority & Expected Benefit
BCG Pasteur 1173P2 *France (Pasteur Institute)*	France, Iran, many developing countries; 9.2% global use	DU2 Group IV (late strain)	**55 days (median) ★★★ ★★ MOST LETHAL**	✔ Present (high virulence)	⚠ HIGH More adverse reactions than Tokyo, Glaxo, Moreau (WHO)	France 1974–94: 8/16 BCGosis → DIED Canada: 4/4 SCID died ~34% BCGosis internationally	**⚠ HIGHEST PRIORITY HCQ adjunct most critical here. High PDIM/PGL expression = most sensitive to phagolysosomal**

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							alkalinisation by HCQ. Avoid in SCID if alternatives exist.
BCG Tice (= Phipps lineage) *USA*	USA (bladder cancer therapy; rarely neonatal vaccination)	DU2 Group IV (late strain)	**Tice: 58 days Phipps: 48 days ★★★ ★★ LETHAL**	✔ Present	⚠ HIGH (limited neonatal data)	Not used routinely for neonatal vaccination; used in bladder cancer immunotherapy. BCGosis fatal in immunocompromised adults.	**⚠ HIGHEST PRIORITY Not recommended for neonates in high-risk populations. If used, HCQ adjunct essential.**

GROUP 2: INTERMEDIATE VIRULENCE — Moderate BCGosis Risk; Widely Used

BCG Strain	Country of Use	DU2 Group	SCID Mouse Survival (days)	PDIM/PGL Lipids	Reactogenicity (WHO)	BCGosis in Human SCID	HCQ Priority & Expected Benefit
BCG Danish 1331 (Copenhagen) *#1 Globally Used (16.6%)*	Denmark, Netherlands, UK, Australia, most of Europe; WHO Reference Strain	DU2 Group III (late strain)	**114 days (median) ★★★ INTERMEDIATE**	✔ Present (moderate)	⚠ HIGH More reactions than Glaxo, Tokyo, Moreau (WHO)	Czech Republic: 9/12 SCID BCGosis, 5 deaths China (Danish): 14/74 IEI BCGosis, 2 SCID deaths Marciano: Danish caused most BCGosis	**⚠ HIGH PRIORITY Despite intermediate SCID mouse virulence, human BCGosis data are worst for this strain. HCQ adjunct strongly recommended if used in**

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						across 17 countries	**high-risk neonates.**
BCG Russia I (Moscow-368) *Russia*	Russia, Eastern Europe, former Soviet states; Brazil (since 2018 seed). WHO Reference Strain.	DU2 Group I (early strain)	**99.5 days (median) ★★★ INTERMEDIATE**	✔ Present (IS6110 in PhoP)	⚠ MODERATE (limited global data)	Limited human SCID data. Used in RAG1/2 SCID study (Sharapova et al.): 4/22 BCGitis, 1 BCGosis (Sofia SL222 lineage).	**⚠ MODERATE PRIORITY HCQ adjunct recommended in high-risk populations. Brazil's 2018 switch to Russia seed warrants monitoring.**
BCG Moreau RDJ (Rio de Janeiro) *Brazil*	Brazil (historical); WHO Reference Strain	DU2 Group I (early strain)	**94.5 days (median) ★★★ INTERMEDIATE**	❌ Deficient (fadD26/ppsA deletion) — but less protective than Polish Moreau	✅ LOW Moreau RDJ lower reactogenicity than Pasteur/Danish (WHO)	Brazil: 29/60 SCID (48.3%) BCGosis — WORSE than Polish Moreau (15%) despite same PDIM deficiency. Explained by Rv3887c deletion present in RDJ	**⚠ MODERATE PRIORITY Despite PDIM deficiency, genomic differences from Polish Moreau result in higher clinical BCGosis. HCQ adjunct recommended.**

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						but absent in Polish.	

GROUP 3: LOWEST VIRULENCE — Safest Strains for SCID; Preferred in High-Risk Neonates

BCG Strain	Country of Use	DU2 Group	SCID Mouse Survival (days)	PDIM/PGL Lipids	Reactogenicity (WHO)	BCGosis in Human SCID	HCQ Priority & Expected Benefit
☆ **BCG Moreau Polish (Biomed Lublin)** *Poland since 1955*	Poland, parts of Eastern Europe	DU2 Group I (early strain)	**94.5 days (same lineage as RDJ) ★ INTERMEDIATE in mice BUT BEST in humans**	❌ Deficient (fadD26/ppsA deletion) + Rv3887c INTACT + IS6110 in PhoP	✅ LOWEST Safest profile in all studies (Bernatowska, WHO)	☆ **BEST: 15% BCGosis in SCID vs 34% internationally (p=0.0012) Mortality: 4.2% vs 13% (p=0.04) ZERO BCGosis in non-SCID IEI 40 years data, 72 SCID patients**	✅ **LOWEST PRIORITY (already safest) HCQ adjunct still recommended as added safety layer pending SCID screening. The ideal first-choice strain for any high-risk neonatal programme.**
BCG Tokyo 172-1 *Japan #3 Globally (7.3%)*	Japan, South Korea, Thailand, parts of Southeast Asia, Nigeria. WHO Referen	DU2 Group I (early strain)	★ **VERY LOW 100% survival at 18 weeks (same as uninfected controls)**	❌ Deficient (PDIM/PGL absent)	✅ LOW WHO: lower than Pasteur, Danish	Limited human SCID data. Mouse model: 100% SCID survival — best of all 13 strains tested. Lacks CD8+ T cell induction — may reduce	✅ **LOW PRIORITY Safest in SCID mouse model. HCQ adjunct beneficial as added protection layer. Note: weaker**

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	ce Strain.					long-term TB protection.	**CD8+ T cell response may reduce TB efficacy.**
BCG Glaxo 1077 BCG Prague *UK / Czech Republic*	UK (Glaxo); Czech Republic, Slovakia (Prague). Derived from Danish lineage but distinct genetic profile.	DU2 Group II (early strain)	★ **VERY LOW 100% survival at 18 weeks (Glaxo + Prague)**	❌ Deficient	✅ LOW WHO: Glaxo lower reactogenicity than Pasteur/Danish	Glaxo: 2 BCGosis deaths France (French study) — higher than expected for low SCID virulence. Prague: 100% SCID mouse survival.	✅ **LOW PRIORITY HCQ adjunct as precautionary added layer. Good option for high-risk programmes if Polish Moreau unavailable.**

Key Synthesis: BCG Strain Selection + HCQ Adjunct Strategy

Strain Recommendation Hierarchy for High-Risk Neonates (SCID-risk countries): Polish Moreau > Tokyo 172 ≥ Glaxo/Prague > Moreau RDJ > Russian > Danish > Pasteur/Tice

HCQ Adjunct Priority Gradient: Pasteur/Tice (critical) > Danish (high) > Russian/Moreau RDJ (moderate) > Polish Moreau/Tokyo/Glaxo (precautionary). Where Pasteur or Danish is the only available strain, HCQ adjunct becomes the most critical public health tool for protecting high-risk neonates from BCGosis.

Critical Paradox: The Danish strain—the most widely used globally (16.6% of all BCG doses)—is also responsible for the greatest human BCGosis burden. Addressing this paradox through either strain replacement or HCQ adjunct therapy is the single highest-impact intervention available for SCID-safe BCG vaccination worldwide.

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