Early antiretroviral therapy in children perinatally infected with HIV: a unique opportunity to implement immunotherapeutic approaches to prolong viral remission

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From the use of antiretroviral therapy to prevent mother-to-child transmission to the possibility of HIV cure hinted at by the Mississippi baby experience, paediatric HIV infection has been pivotal to our understanding of HIV pathogenesis and management. Daily medication and indefinite antiretroviral therapy is recommended for children infected with HIV. Maintenance of life-long adherence is difficult and the incidence of triple-class virological failure after initiation of antiretroviral therapy increases with time. This challenge shows the urgent need to define novel strategies to provide long-term viral suppression that will allow safe interruption of antiretroviral therapy without viral rebound and any associated complications. HIV-infected babies treated within a few days of birth have a unique combination of a very small pool of integrated viruses, a very high proportion of relatively HIV resistant naïve T cells, and an unparalleled capacity to regenerate an immune repertoire. These features make this group the optimum model population to investigate the potential efficacy of immune-based therapies. If successful, these investigations could change the way we manage HIV infection.

Introduction

Study of the pathogenesis and management of HIV in paediatric populations has contributed pivotaly to the collective understanding of the pathogen, from use of antiretroviral therapy to prevent mother-to-child transmission to the possibility of cure suggested by the circumstances surrounding the Mississippi baby. Babies infected vertically with HIV and treated within a few days after birth represent a unique opportunity to study novel approaches to HIV management and particularly therapeutic vaccines. These babies have a very small viral reservoir, rarely exhibit HIV-specific immunity, but still seem to maintain normal immune development. The unique combination of a very small pool of integrated viruses, a very high proportion of relatively HIV resistant naïve T cells, and an unparalleled capacity to regenerate an immune repertoire makes this group the optimum model population to investigate the potential efficacy of immune-based therapies.

Infants born with HIV infection have access to potent combinations of antiretroviral therapy, so that increasing numbers of children are surviving to adolescence and older. Despite this optimistic outlook, several questions still need to be addressed (panel 1). An estimated 3–4 million children are living with HIV, more than 90% of whom are in sub-Saharan Africa, and almost all of these infections were acquired through mother-to-child transmission. As a result of widespread use of preventive interventions such as the administration of antiretroviral drugs to mothers and their babies, elective caesarean section, and bottle feeding, vertical HIV transmission has diminished to less than 2% from mother to baby in resource-rich countries. Similar results have been achieved in resource-poor settings, in which these strategies have also been implemented. Although new HIV infections in children declined by 53% from 2001 to 2012 because of the effective implementation of techniques to prevent mother-to-child transmission, about 250 000 HIV-infected infants are still newly infected every year. Antiretroviral therapy has very effectively prevented mortality when initiated in infancy and international guidelines now recommend initiation of antiretroviral therapy in all infants younger than 12 months infected with HIV, irrespective of clinical and immunological variables. Thus, research can now focus on the effect of viral reservoirs in different antiretroviral therapy regimens started in early life. In terms of long-term viral control, evidence is growing to suggest that regimens containing lopinavir, if tolerated, started within the first year of life might be better than nevirapine regimens. Moreover, a potential role for the use of integrase inhibitors during infancy has been suggested. How the use of different or novel combinations of antiretroviral drugs will affect viral reservoirs is still unclear.

What did we learn from the Mississippi baby?

The Mississippi baby led scientists to think that very early and aggressive antiretroviral therapy in vertically infected infants could be sufficient to ensure HIV remission, defined as a prolonged period of undetectable plasma viraemia without antiretroviral therapy. The attempt to replicate this case with very early antiretroviral therapy (started within 48 h after birth) represents the objective of the IMPAACT1195 trial announced by the National Institute of Health. The Mississippi baby initiated antiretroviral therapy at 30 h after birth during the acute phase of the infection. The child had a spontaneous antiretroviral therapy interruption at age 18 months followed by a period of 27 months with undetectable
Furthermore, the instruments used to detect low viral load are recorded in less than 2–4 weeks after antiretroviral therapy was resumed.1,15 Therefore, at age 4 years, the child had a rebound of HIV RNA to nearly 20,000 copies per mL and antiretroviral therapy, could be achieved with very early treatment—namely, within a few hours after birth. However, at age 4 years, the child had a rebound of HIV RNA to nearly 20,000 copies per mL and antiretroviral therapy was resumed.1,15

This case shows that acute HIV infection targets could lead to a significant reduction in HIV reservoirs.1,16 However, the reasons for the long duration of viral suppression without antiretroviral therapy for 27 months in this child still need to be elucidated (panel 1) and thus far, these factors are difficult to reproduce with antiretroviral therapy alone. In most early treated cases, the virus could persist in different anatomical compartments as shown in several cohort studies.28–30

One implication of the use of early antiretroviral therapy worldwide is the increased number of HIV-infected children in therapy for many years who remain seronegative, leading to a growing demand by parents and patients to interrupt therapy. Whether a safe way can be achieved to interrupt treatment has become a pertinent and urgent question in the paediatric community. Paradoxically, although children who are seronegative are most likely to achieve a period of drug-free viral rebound after treatment interruption,24 The ethics of tissue biopsy collection and of lumbar punctures for research in children who are not able to provide consent are challenging, and thus far, have restricted the ability of researchers to examine these reservoir compartments (panel 1).23 However, use of these medical procedures in a subset of patients, such as in early treated, long-term virally suppressed adolescents,23 could guide therapeutic strategies for cure.

Virological and immunological benefits of early antiretroviral therapy in children

Emerging evidence suggests that the use of early antiretroviral therapy not only reduces HIV-1 related mortality but also preserves immune function and long-term control of viral production. Early antiretroviral therapy restricts the number of long-lived CD4 T cells that harbour HIV-1 DNA and viruses that are competent of replication.4,26 Importantly, early treatment also preserves the predominant naive CD4 cell populations and restricts the generation of memory cells. Data in early treated children suggest that within the small population of memory cells that do exist, the contribution of the proviral reservoir is greater in the short lived transitional memory than the long lived central memory CD4 T cells or naive CD4 cells pool,26 a profile reported in the post-treatment controllers from the VISCONTI cohort.27 Furthermore, early antiretroviral therapy is advantageous to restrict viral diversity and reduce escape mutations, both secondary to the absence of viral evolution over time. Immunologically, early control of viral replication through antiretroviral therapy preserves the normal development of the memory B-cell and T-cell compartments as shown in several cohort studies.28–30

Additionally, Schuetz and colleagues reported that early antiretroviral therapy initiation prevents the functional and quantitative loss of mucosal Th17 cells in addition to the induction of a normalisation of local and systemic T-cell activation.

Well established evidence suggests that most children who achieve sustained viral suppression since the first days of life show undetectable HIV antibodies and little, if any, cellular HIV-specific responses attributed to the absence of antigen stimulation.1,5,13,15,24–26,30 The few studies that investigated the relationship between serostatus and viral reservoir show a direct association between the concentration of HIV-specific antibodies and the size of the viral reservoir.1,16

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remission, as seen with the Mississippi baby, they might also be more susceptible to uncontrolled viral replication in the absence of HIV-specific immune responses. 28,32,33

**Therapeutic HIV vaccine research in early treated children**

Reasons to implement therapeutic HIV vaccine research in children are summarised in panel 2. Life-long drug treatment for antiretroviral therapy is recommended for the paediatric population. 36 Maintenance of life-long adherence to the therapy is difficult, the risk of viral resistance, and as yet unknown long-term toxic effects, represent major concerns. Children infected vertically with HIV have a progressively increased risk of developing triple-class virological failure after 5 years of antiretroviral therapy. 37 Because of the absence of reliable biomarkers that can predict safe antiretroviral therapy interruption in these children, such a strategy will need to be closely monitored and should ideally be investigated in clinical trials. The PENTA 11 trial 40 reported that although short periods of treatment interruption might be well tolerated and seem safe long term, they are unlikely to provide any long-term immunological advantages. 38 Therefore, the need to define strategies to provide long-term viral suppression and durable host immune control of HIV is urgent for safe interruption of antiretroviral therapy without viral rebound and the associated complications.

**Immunotherapeutic strategies tested in HIV-infected adults**

Several immunological strategies have been proposed to achieve viral remission without antiretroviral therapy. These include passive immunisation with broadly neutralising antibodies, which have been tested in primates, 41 in infected human beings, 42 and with a selected potent human monoclonal anti-CD4 binding site. 43 Another passive immunisation strategy targets the infected cell with a combination of antiretroviral drugs and monoclonal antibodies. 44 These approaches could produce in a baculovirus expression system. HIV-1-gag vaccine was DNA vaccines are lasting specific cellular immune responses and functional antibodies in healthy individuals. 52–55 DNA vaccines are easily stored, and can be manufactured on a large scale. 56–58

Panel 2: Reasons to implement therapeutic HIV vaccine research in children

**Reasons to do therapeutic HIV vaccine studies in children infected with HIV perinatally**

- Knowledge of timing of HIV exposure in infants allows for prompt diagnosis and treatment 25
- All international guidelines recommend early antiretroviral therapy in all newly infected infants 15
- The antiretroviral therapy recommended for the paediatric population needs lifelong medication with high risk of long-term toxic effects 26
- A large number of children living with HIV will continue to need care and treatment after 2020 23
- Vertically HIV-infected children have a progressively increased risk of developing triple-class virological failure after 5 years of antiretroviral therapy 27

**Reasons why early antiretroviral therapy treated children represent a unique model population to investigate immunotherapeutic strategies**

- Children have a much more active thymus than adults and a greater capacity for immune regeneration which permits evaluation of vaccines specific immune responses 18–20
- Children treated early with antiretroviral therapy can become seronegative and not have HIV-specific cellular immune responses leading to uncontrolled viral replication 21,22
- Young infants have high immune tolerance, low immune activation, high naive CD4+ T cells, and low central memory cells, all features that are, conducive for a reduced and less diverse HIV reservoir and a great potential for effective response to vaccinations 23,24

Immunotherapeutic approaches to enhance host immunity to control viral replication have so far shown small clinical effects or reduction in viral load in the context of treatment interruption. One pioneering study 25 in adults with HIV on single drug therapy (zidovudine) showed an improved 2-year survival with frequently repeated immunisations of the gp160 glycoprotein produced in a baculovirus expression system. HIV-1-specific immune responses elicited by dendritic cell vaccines significantly changed HIV RNA concentrations after antiretroviral therapy interruption in patients treated in the early stages of the disease. 26 Patients treated with antiretroviral therapy were given dendritic cells loaded ex vivo with HIV lipopeptides with good control of viral replication related to the efficacy of the vaccine. 26,27

The efficacy of peptide-based HIV-1 vaccines have also been disappointing. The Vacc-4x vaccine was shown to significantly lower the viral setpoint after treatment interruption in adults, but without clinical benefit. 59 Li and colleagues 60 reported one therapeutic trial with a recombinant adenovirus-5-based HIV-1 gag vaccine was associated with only a slight transient effect on residual viraemia. 61 These studies with single vaccines have not shown convincing long-term efficacy in controlling HIV in the absence of antiretroviral therapy. Therefore, our view is that a prime DNA-boost (vector-based) schedule could be more promising for early treated children. Such approaches have been shown to induce broad and long lasting specific cellular immune responses and functional antibodies in healthy individuals. 62–65 DNA vaccines are safe, immunogenic in prime-boost strategies, stable, easily stored, and can be manufactured on a large scale. The HlVIS vaccine, consisting of a multigene, multisubtype A, B, C HIV-DNA vaccine, has been tested in
HIV-infected adults and elicits novel HIV-specific cellular immune responses, especially to gag antigens. Mosaic genes, including strong immunogens of several HIV strains, are of particular interest. A mixture of two mosaic HIV env genes, which included the single strains HXB2 and MN, the M group consensus sequence, and the two-valent and three-valent mosaic sequences, increased both cellular and antibody responses in macaques whereas mixtures of plasmids encoding three selected HIV-C gp140 envs elicited high concentrations of neutralising antibodies in guinea pigs.

Another genetic vaccine approach links together the optimum 18-mer T-cell epitopes of gag, pol, vif, and nef. This construct has shown good induction of CD8 cells in animals but has yet to be tested in human beings. In healthy adults, broad and strong cellular immunity was shown with a heterogeneous vaccination strategy that primes with DNA and then boosts with recombinant virus or adenovirus-based HIV genes, with or without adjuvant.

Among several vectors that could carry HIV gene inserts, vaccinia virus, BCG, and measles virus have been included in non-HIV vaccination programmes for children and could therefore be advantageous as boosting strategies in future paediatric HIV vaccine studies. Modified vaccinia virus Ankara (MVA) is a multiclade multigene HIV DNA vaccine. RT is reverse transcriptase.

### Table: HIV vaccine studies in paediatric settings

<table>
<thead>
<tr>
<th>Vaccine type</th>
<th>Infant (0–6 months)</th>
<th>Child (6–16 years)</th>
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<tbody>
<tr>
<td></td>
<td>PACG 230</td>
<td>PACG 236</td>
</tr>
<tr>
<td></td>
<td>Prophylactic</td>
<td>Prophylactic</td>
</tr>
<tr>
<td>rgp120 (MN) + adjuvant (alum/MF-59)</td>
<td>vCP1452 ± rgp120</td>
<td>MVA/HIVA (HIV-1 clade A gag p24/p37 + CD8 T-cell epitope)</td>
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<thead>
<tr>
<th>Number of patients</th>
<th>188</th>
<th>20</th>
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<tbody>
<tr>
<td>Population under study</td>
<td>Exposed infants</td>
<td>Vertically HIV infected children</td>
</tr>
<tr>
<td>Study design</td>
<td>Multicentre, phase 1/2, randomised, placebo-controlled study</td>
<td>Single centre, phase 1, open-label, randomised, no treatment controlled study</td>
</tr>
<tr>
<td>Schedules</td>
<td>Birth and ages 2 or 4, 8, and 12 weeks</td>
<td>Aged 20 weeks</td>
</tr>
<tr>
<td>Immunogenicity</td>
<td>Induce robust, durable env-specific IgG responses, including anti-V1V2 IgG responses in infant recipients of rgp120 (MN) + MF-59</td>
<td>Did not alone induce sufficient HIV-1-specific responses</td>
</tr>
</tbody>
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rgp120= recombinant gp120; vCP1452+HIV-1 canarypox vaccine 1452. MVA= modified vaccinia virus Ankara. HIVIS DNA= multiclade multigene HIV DNA vaccine. RT= reverse transcriptase.

Table: HIV vaccine studies in paediatric settings
immune responses that facilitate a comprehensive investigation into vaccine specific responses. For these reasons, we think that studies on new immunotherapeutic strategies in paediatric populations might be more informative than those in adults, even in the context of prophylactic vaccination as advocated by Fouda and colleagues.71

Present scenario and future perspectives of therapeutic HIV vaccines in childhood

Only five randomised clinical trials of HIV vaccine have been done in paediatric settings in the past 20 years and only one in the specialty of therapeutic vaccination (table).72-76 Two prophylactic HIV-1 vaccines in exposed infants were safe and immunogenic in phase 1 trials.72,73 These vaccines can induce robust and durable env-specific IgG responses, including antibodies (anti-V1V2 IgG) associated with a reduced risk of HIV-1 acquisition in the RV144 adult vaccine trial.77 Of note, the few data available that compare immunogenicity of similar HIV vaccines between adults and children show significantly better immune responses in the paediatric population.77,78

An initial attempt to increase HIV-specific responses during the infection, with the aim to control viral replication, has been tested in children by planned repeated controlled exposure to the autologous virus. This approach resulted in increased CD8 T-lymphocyte responses to HIV antigens and a reduction of viremia; however in these individuals large amounts of viral diversification was noted. This heterogeneity was attributed to either increased viral replication or immunological pressure.79

To date, the only therapeutic HIV vaccine trial in children was a phase 2a open-label study with the DNA-based HIVIS vaccine (table).78 In this population, a better cellular response was transiently recorded in HIV-specific antigens compared with an age-matched group of children infected with HIV and treated with antiretroviral therapy. The vaccine was also shown to be safe.80 Although, to obtain broader and durable responses in every child, the priming DNA should be followed by a boosting vector-based vaccine, as previously described in every child, the priming DNA should be followed by a boosting vector-based vaccine, as previously described in every child.

The model of early treated children with perinatal HIV infections will contribute to the understanding of the mechanisms behind HIV remission, which will be translated into novel and more effective immunotherapeutic strategies than at present. An important first step will be to extensively characterise existing cohorts of early treated children as a potential trial population, including timing of treatment, size of viral reservoir, and presence of HIV-specific immune responses. These data might drive the choice of a personalised or a generalised immune intervention approach. In these future trials, the immunological and virological endpoints must be defined and closely monitored; these should include several relevant immune responses related to viral control, immune activation markers, and estimates of the viral reservoir. The latter should assess intracellular HIV DNA and RNA and detect HIV RNA in plasma with ultrasensitive methods.80 Moreover, inclusion of the assessment of replication-competent viral reservoirs and analysis of viral evolution is crucial. A systems biology approach in combination with a mechanistic mathematical model could be used to help to predict vaccine induced immunity in the context of early treatment to improve trial design.81,82

Together these approaches could provide unique insights into the effect of immunotherapy on the viral reservoir and define the basis for achieving an effective immunotherapeutic strategy to prolong viral remission in perinatally infected individuals.

Contributors
All authors helped with the conception of this Personal View. NK and PP wrote the first draft, and managed all subsequent revisions. KL, SP, EN, DMG, PRoj, WB, SB, PZ, VC, AC, BW, CF, MAM-F, ADR, JA, DP, CG, and PRks provided comments on the draft. All authors reviewed the article and approved the final submission.

Declaration of interests
We declare no competing interests. The views expressed by JA are those of the author and should not be construed to represent the positions of the US Army or the Department of Defense.

Acknowledgments
The work of the authors is supported by PENTA Foundation and by the Children Hospital Bambino Gesù.

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