**Methods**

This systematic review is reported in accordance with PRISMA guidelines. The protocol for this systematic review is registered with PROSPERO under the registration number CRD42022315324.

**Search strategy and selection criteria**

Initial database searches were conducted in PubMed, Embase, and Scopus and included studies from Jan 1, 1998, to Jan 13, 2022. Three follow-up studies and one new study published between Jan 14, 2022, and Dec 31, 2023, were identified and included after the initial search.

Eligible studies had to report outcomes among three or more people living with perinatal HIV. Studies of persons co-infected with other diseases or disorders were excluded. The intervention of interest was the initiation of ART following diagnosis of perinatally-acquired HIV-1. The primary outcomes of interest were total HIV-1 DNA concentrations in PBMCs and associated factors (e.g., age of ART initiation, duration of ART). The HIV-1 reservoir was defined as total HIV-1 DNA copies per million (cpm) PBMCs following ART initiation. Log10 HIV-1 DNA cpm PBMCs were compared across treatment initiation groups; studies that reported cpm were converted to log10 HIV-1 DNA cpm PBMCs. Studies reporting on HIV-1 DNA in CD4+ T cells were excluded. Studies that only report HIV-1 DNA in the context of HIV-1 diagnosis, baseline, or pre-ART HIV-1 DNA levels were excluded. However, cross-sectional studies reporting at least one post-ART HIV-1 DNA value were included. Studies reporting less than three participants were excluded. Only study designs in English were included.

**Data extraction and eligibility**

Data extraction was done in duplicate (MH and DP). Studies needed to report changes in HIV-1 DNA at a minimum to be included in this review. Results from the literature search were uploaded into Covidence, a systematic review management software, to streamline the article screening process. Inclusion and exclusion criteria were added to Covidence for reviewers to reference throughout screening.

Two reviewers (MH and DP), working independently in pairs, screened a total of 4753 records for eligibility (Supp. Fig. 1). From this pool, 1844 records were identified as duplicates and removed, and the title and abstract screening excluded an additional 2638 records. Reviewers (MH and DP) then assessed the full texts of the remaining 269 articles, of which 234 were excluded for the following reasons. One article focused on the wrong population (i.e., HIV-positive cohort infected by means other than perinatal transmission), and 70 records did not contain full texts (e.g., oral abstracts). An additional 34 articles were excluded due to study design ineligibility and lack of empirical data, 7 articles did not assess the intervention of interest, and 5 articles were written in a non-English language. The most frequently cited reason for exclusion was a wrong outcome; 117 articles were excluded because HIV-1 DNA values were not available. While many studies stated measuring cell-associated HIV-1 DNA, studies were excluded if these data were not explicitly reported in the text.

A total of 39 studies were identified from the initial search. 12 studies were excluded from further analyses. Five of these studies were case reports. Four reported total HIV-1 DNA per million CD4+ T cells, rather than cpm PBMC. Two reported total HIV-DNA decay over time. One study reported total HIV-DNA decay over time per million PBMC. Thus, a total of 26 studies met eligibility criteria for inclusion.

**Data Analysis**

All graphing and analyses were performed by R (version 4.2.2).

**Role of Funding Source**

The funders of the study had no role in study design, data collection, data interpretation, or writing of the report.

**Supplementary Figure 1:**

Flow diagram of PRISMA chart for the literature search.

Abbreviations: PRISMA; Preferred Reporting Items for Systematic Reviews and Meta-analysis

**Supplemental Table 1:**

Summary table of 26 studies included in the systematic review with first author, year of publication, doi, subgroups defined by author for whom HIV-1 DNA was reported, median age at ART initiation, drug regimen, median age at time of total HIV-1 DNA measurement, sample size for whom HIV-1 DNA was reported, median total HIV-1 DNA (log10 cpm PBMC), country of origin and cohort reported.

 \* Values were converted to months or log10.

ART=antiretroviral therapy. VS=viral suppression. NRTI=nucleoside reverse transcriptase inhibitor. NNRTI=non-nucleoside reverse transcriptase inhibitor. PI=protease inhibitor. NR=not reported.