Summary of the results

The main study finding: We saw no significant difference in measures of HIV viral reservoir; total HIV DNA or viral outgrowth at weeks 16 & 18 after randomisation between study arms. Whilst this finding is disappointing, it is valuable that we have shown for the first time definitive, unambiguous results.

The results of this study do not mean that HIV cure is not possible. They also do not mean that the kick and kill approach does not work. They mean that taken together, the way and the time at which we measured the HIV reservoir, the vaccination we used in this trial with the dose and length of treatment with vorinostat, had no additional impact on measures of the HIV reservoir over Antiretroviral therapy (ART) alone.

This study had **outstanding commitment from the participants** with 100% attendance to primary endpoint study visits and no loss to follow-up and 97% adherence to intervention.

THANK YOU!! because without your support, commitment and enthusiasm this study would not have been successful.

There is **not enough evidence from this trial to recommend all study participants from the control arm now receive vaccination and vorinostat in addition to their ART**.

ART with integrase inhibitor agents (in most cases with 4-drug combination) started close to the time of HIV acquisition was acceptable, well-tolerated and **very efficient at controlling viral replication,** allowing the body's immune system to rapidly recover. Whilst we have shown dramatic reductions in HIV viral load and reservoir measures for all people in the trial who started ART very close to the time of HIV infection, based on the results so far, we cannot recommend interrupting ART.

The tests we have available to measure the HIV reservoir are constantly improving but are still not perfect. It could be that we measured the outcome (at 16-18 weeks) too soon after ART was started or too soon after the intervention. **We would very much like to continue to follow up all study participants to see if there is a delayed impact on viral reservoir over time**.

The vaccine and vorinostat as used in this study design were safe.

Both the vaccine and vorinostat worked as expected. The measured T-cell responses to HIV were significantly higher in the participants who had the vaccinations compared to the control. The vorinostat worked to alter gene expression, to a level similar to that previously reported 2 hours after dosing.

As ART is so effective, this study highlights the **importance of having a control arm when measuring the impact of any novel intervention on measures of HIV reservoir**. All future trials in HIV cure should compare the outcomes to a control ART only arm. The risks vs benefits of taking new medication must be balanced against the effectiveness and safety of current ART.

This document describes the results of the study. If you have any questions about it, please speak to your doctor or research nurse.

The following pages explain the trial process and results in more detail.

What the trial set out to do:

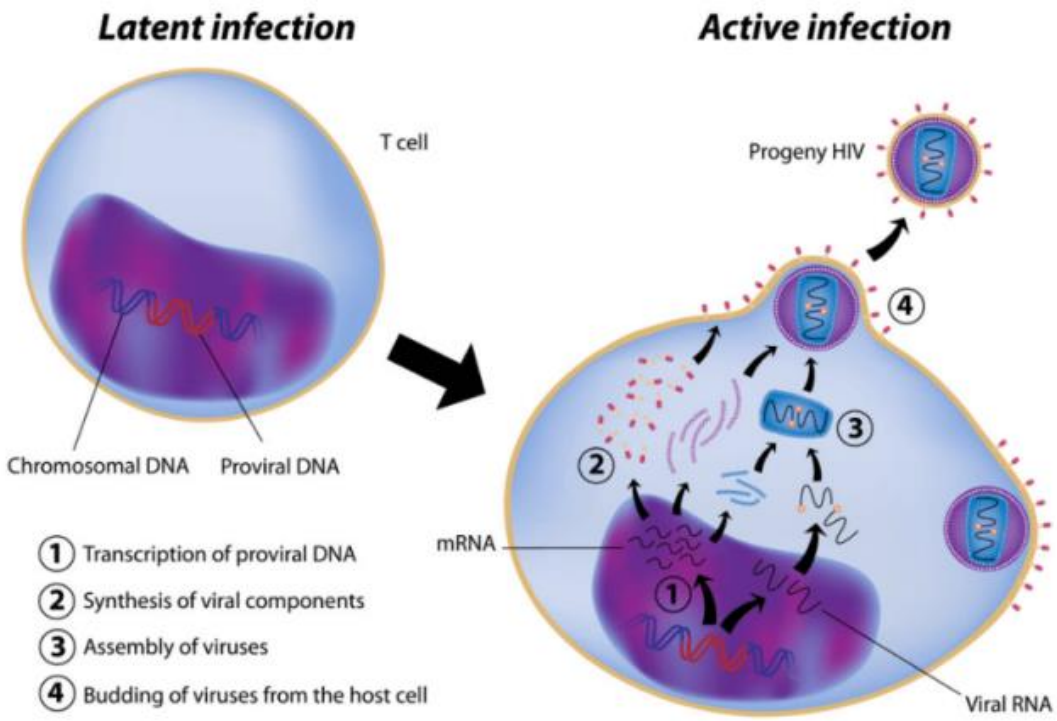
The RIVER trial was designed to test whether the idea of “kick and kill” might work as a way of reducing the size of the HIV reservoir. Specifically, RIVER is the first randomised study designed to compare the effect of immediate ART started at the time of very recent HIV infection, with ART + vaccine and vorinostat on measures of the HIV reservoir.

The kick and kill approach used in this study

- the **“kick”** was ten doses of 400mg tablets of Vorinostat taken every three days
- the “**kill”** was two vaccinations; a “prime” with ChAdV63.consv. and a “boost” with MVA.HIVconsv.

What is the HIV reservoir and how did we measure it’s behaviour?

The figure shows a cartoon of what we think a latently infected cell and an active cell might look like. Both are normal CD4+ T-cells that have a section of HIV genetic material (red) inside their own DNA (blue).



Latently infected cells seem to function normally and do not look any different from cells that do not have HIV DNA inside them, so they are “invisible” to the immune system.

New virus particles are not released from these latently infected cells unless they become activated. If ART medicines are in the blood then even if the latently infected cell gets activated the medicines will block any new virus being released from the cell and so there is no rise in HIV viral load in the blood.

Vorinostat, the drug used in this trial, activates the latently infected cells to start producing virus proteins. This represents the “kick” of the cure strategy we test in the RIVER trial.

Any latently infected cell that is forced to express virus proteins is then no longer ignored by the immune system. The vaccination used in the RIVER trial encourages the immune system to recognise any activated reservoir cells and destroy them.

The latently infected cells, for people on ART with undetectable HIV viral loads, are very rare in the blood (probably less than 1 in every million CD4+ T-cells) and so the body can replace those cells that might be killed by this approach with new healthy cells very rapidly.

In RIVER these are the HIV reservoir measurements we made:

1. To measure the viral reservoir in this study we counted the number of CD4+ T-cells that look like a latently infected cell: this is the number of CD4+ T-cells that have detectable HIV DNA inside them for every million CD4+ T-cells in the blood sample (Total HIV DNA/million CD4+ T-cells). This is the main measure of HIV reservoir for the trial.
2. We also measured if we could stimulate the blood cells in a test tube, to see if virus particles would grow out from the blood cells (like the cell on the right-hand side above) (viral outgrowth assay, VOA).
3. We did very sensitive tests in research laboratories to see if we can find very low levels of virus particles (Ultra-sensitive viral load < 1 copy HIV RNA/ml).
4. And the same as (3) for virus proteins (p24 assay).
5. To check if the vaccine was working to increase the immune responses to HIV proteins we measured in the test tube how the CD4+ T-cells and CD8+ T-cells reacted to stimulation with virus proteins (HIV-specific immunity, ICS and VIA).
6. We checked if vorinostat worked how we expected it to by measuring cell gene activation (HDAC inhibition).
7. Finally, the other very important outcome that we looked at in RIVER is safety and how well tolerated the medicines and vaccine were. This is detailed in the Safety section below.

Study design:

We enrolled 60 study participants across [6] centres in London and Brighton. All study participants had confirmed recent HIV infection and all started on ART within a maximum of four weeks from their date of HIV diagnosis. Once their viral load was suppressed to < 50 copies HIV RNA/ml, participants were randomly allocated to one of two study groups, on a 1:1 basis; 30 in each arm.

the control group *ART alone*

the intervention group *ART + vaccine + vorinostat.*

The main measure of the trial - measurement (1) above – the total HIV DNA/million CD4+ T-cells was counted at weeks 16 and 18 after the random allocation into one of the two study groups.

Results in more detail:

83 participants were screened and in total 60 study participants were enrolled into the trial. All participants were men, the average age was 32 years, and average CD4+ T-cell count at randomisation was 708 cells/mm^3.

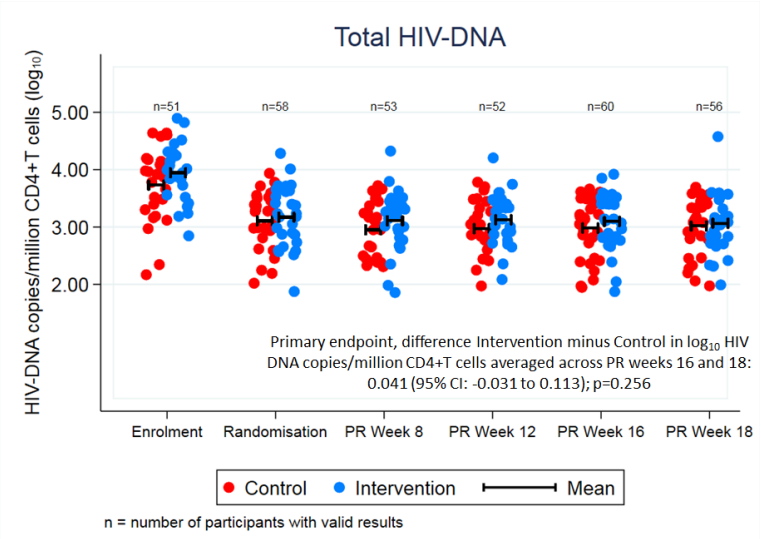
No participant withdrew nor was lost from the study after randomisation. In the experience of the clinicians and investigators, this is outstanding.

All study participants stayed on ART throughout the trial and only three changed ART regimen after randomisation, for toxicity reasons. Self-reported non-adherence (missing any dose in the past seven days) was recorded at 6% of all visits across all participants, with no difference between the arms.

All 30 participants in the intervention arm received two vaccines as scheduled; 27 participants received all 10 doses of vorinostat as scheduled.

Main measure: Total HIV DNA/million CD4+ T-cells at weeks 16 and 18 after randomisation

At enrolment the average total HIV-DNA of all participants was 3.84 $\log_{10}$ copies/million CD4+ T-cells. This decreased to 3.14 at randomisation. Total HIV-DNA then decreased further by weeks 16/18. There was no significant difference between the two arms in terms of the total HIV DNA measures at weeks 16 & 18 (red compared with blue in the graph).



The figure shows the changes in measures of total HIV DNA where a dot represents the results of each individuals' blood sample. The red dots are measures of total HIV DNA for people who were in the control arm of the trial, and blue dots are for those in the intervention arm. We compare the average level of HIV DNA between the red and the blue. We show that whilst there is a very large drop (I log is a very big drop in the measure of HIV reservoir), from around 4.00 at the beginning to 3.00 after almost a year), this drop was similar for both arms of the trial (the average for the red and blue dots are around the same level). There is a wide range of the levels of HIV DNA for each time point measured. We would like to try to explain this with more research laboratory tests from the stored blood samples, for example some people have very low levels of HIV DNA right from the start (nearly 2.00) and others start with very high levels (nearly 5.00). We would like to better understand why this is and what this might mean.

Did cell stimulation lead to virus production in a test-tube? The viral outgrowth assays (VOA)

There was no significant change in VOA test results between the study arms from randomisation to the final study visit at week 16: for both the control and intervention arms, virus could be detected on stimulation of CD4+ T-cells for 60% of individuals at randomisation and a similar 64% at week 16.

However, we saw a very high proportion of people (around 40%) where we could not grow virus from their cells in this assay, higher than we would anticipate for people on ART who started at later stage disease. This might show how effective ART is at limiting virus activity.

Did the two vaccinations improve the immune responses to HIV? HIV-specific T-cell responses:

Participants in the intervention arm showed significantly higher HIV-specific CD4+ T-cell responses than those participants who did not receive the vaccinations in the control arm.

Did the HIV-specific T-cell responses kill HIV test cells in a test-tube? CD8+ T-cell antiviral activity - viral inhibition:

To test if the vaccine had stimulated CD8+ T-cells to recognise and kill HIV-infected cells, CD8+ killer T-cells from the study participants were added into a test-tube that contained CD4+ T-cells expressing HIV. This test is called the *viral inhibition assay (VIA).* The measure of participants from the control arm inhibiting virus in this test decreased over time, suggesting that on ART the number and function of the HIV-specific killer CD8+ T-cells in the blood declined, whereas in the intervention arm there remained strong HIV inhibition in this test over time.

Did the vorinostat work how we expected it to – did it affect cell gene expression; HDAC inhibition?

For participants in the intervention arm of the trial, blood samples were taken before a dose of vorinostat and two hours after. Twenty-two patients in the intervention arm provided a total of 41 pre and post dose results. Averaged across all time-points, acetylation two hours post vorinostat had increased by a factor of 3.2 compared to pre vorinostat, with no difference between visits. This means that the vorinostat worked on histone deacetylation as we would have expected and to a level similar to previous reports using this dose of the drug.

Note: Results for the ultra-sensitive viral load assay (point 3 above) and the p24 assay (point 4 above) are not yet completed.

Safety

Safety reporting for studies of new agents is very strict and requires full reporting of any symptoms whether they are thought to be related to the study or the study intervention or not. The events are graded for severity where grade 4 is a very serious event and grades 1 and 2 are minor symptoms such as discomfort, rash, and headache.

There were no deaths, pregnancies in a partner, cancer, or pre-specified vaccine related adverse events. Only one serious adverse event was reported post-randomisation, which was not considered to be related to the study or drug intervention.

Clinical adverse events were reported for 97% of participants in the intervention and 73% in the control arm. The number of adverse events per individual was higher in the intervention arm (median 3 vs. 1 event per person). However, the maximum grade was higher in the control arm, with six participants presenting with a grade 3 event (1 event for each of the following: hepatitis; influenza; *Herpes* proctitis; shingles; wrist injury; back pain) vs. 1 individual in intervention (back pain).

A clinical adverse event possibly, probably or definitely related to the vaccines was reported for 53% of intervention participants. However, most events were mild, only three patients had a grade 2 event (tiredness, pain at injection site, rash), and none a grade 3 or 4 clinical event. 93% of vaccinated participants reported any local or systemic solicited vaccine event, with maximum grade 1 in 37%, grade 2 in 43%, and grade 3 in 13% (1 pain/chills post MVA, 1 pain/headache post MVA vaccine, 1 itching post ChAd vaccine, 1 chills/tiredness post ChAd vaccine & tiredness post MVA vaccine; all reported on the patient diary).

A clinical adverse event related to vorinostat was reported for 76% participants who started vorinostat. Again, most events were mild, six patients had a grade 2 event (1 diarrhoea, 2 dry mouth, 3 tiredness, 1 night sweats, 1 impaired glucose tolerance), and none a grade 3 or 4 clinical event.

Laboratory events (those based on flags raised by blood analysis in the lab) were reported in 75% of all participants, with no difference between the arms (p=0.58). 3% of participants reported a grade 4 event (1 intervention: raised liver function test; 1 control: raised creatine kinase (CK), and another 3% a grade 3 event (1 intervention: raised CK; 1 control: low glucose).

Further information

If you have any questions about the RIVER trial, please speak to your doctor or research nurse. RIVER trial is registered with the clinical trials.gov (#NCT02336074) and ISRCTN (#ISRCTN 83717528).

The RIVER trial was sponsored by Imperial College London. It was funded by MRC, MSD and GSK. For more information visit www.cherub.uk.net