The Impact of Immunoglobulin in Acute HIV Infection on the HIV Reservoir: A Randomized Controlled Trial

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Background:

Despite the huge success of antiretroviral therapy (ART), a cure is needed to reduce ongoing morbidity and unsustainable costs of maintaining those living with HIV on lifelong ART. Current antiretroviral therapy potently suppresses human immunodeficiency virus type 1 (HIV-1) replication, however proviral infection still persists in a small pool of latently infected cells (the 'reservoir'). Targeting the reservoir is critical for a HIV cure and evidence suggests that a combination of interventions will be required [1].

Immediate ART following infection can minimize the size and complexity of the latent reservoir [2,3] and in a minority of individuals can lead to post-treatment control [4,5]. Additional interventions are required; proposed strategies include the passive infusion of neutralizing antibodies [6] and kick and kill approaches to induce HIV reactivation to reveal hidden virus [7]. Intravenous immunoglobulin is used in a variety of autoimmune conditions and has been shown in chronic HIV infection to temporarily reduce viral reservoir in the presence of ART [7,8].

We propose that giving intravenous immunoglobulin at acute infection will induce a change in reservoir before the set point has been reached, thus conferring a long term benefit on viral reservoir. We investigated whether the addition of immunoglobulin could cause reactivation of virus which would be re-suppressed by resident ART thereby reducing long term immune activation and HIV viral reservoir.

Methods

This was a prospective, proof of concept 48- week randomized study. Acutely HIV-infected adults, defined as HIV antibody negative with p24/PCR DNA positive or HIV antibody positive with a previous HIV negative test in the preceding 3 months or HIV incident assay (estimating virus acquired within 3 months) were enrolled at a single site over four months. At enrolment all subjects initiated 4-drug antiretroviral therapy (tenofovir, entricitabine, ritonavir boosted darunavir and raltegravir). Blood samples were collected at baseline, week 4, 12, 19, 24, 36 and 48. Individuals who had an undetectable HIV viral load (< 50 copies HIV RNA/ml) at week 19 were randomised to receive a five day infusion of intravenous immunoglobulin (Octagam® 10%: 30g per day) or to remain in the ART alone arm. All individuals had optional rectal biopsies obtained by flexible sigmoidoscopy at w19, w24 and

w48. The study was approved by the National Research Ethics Service (12/LO/0977) and registered at the European Clinical Trials Database (2011-001982-42).

Ten subjects were recruited within four months; one individual was subsequently withdrawn and replaced as virological suppression had not been achieved by w19.

Reservoir and Residual viraemia

Low copy viral load was measured with an internally controlled ultrasensitive quantitative real-time RT-PCR able to detect 3 copies/ml. Purified peripheral blood CD4 T cells were analyzed by qPCR for HIV-1 DNA (Total and Integrated) as reported elsewhere [3].

For preparation of the gut biopsies for HIV reservoir quantitation, a commercial kit was used (Qiagen, Cat. No. 56404), and the manufacturer's protocol modified as follows to ensure maximum yield of DNA. Biopsy sections were removed from paraffin blocks using a scalpel and placed into 1.5ml micro centrifuge tubes. Samples were washed repeatedly with xylene and ethanol until the precipitate had disappeared, then dried at room temperature. Biopsy sections were incubated at 37°C and DNA extracted using a commercial kit (QIAAMP DNA FFPE Tissue Extraction Kit, ID 56404, Qiagen (Hilden, Germany)). The resulting DNA was eluted into 200µl and analysed by qPCR using the same assays for peripheral blood CD4 cells detailed above.

Bacterial translocation

Plasma bacterial 16s DNA was quantified by real time PCR [9]. All samples were run in duplicate.

Immune activation and lymphocyte subsets

PBMC were stained with the anchor markers (CD3- VioBlue, CD4(VIT4)-VioGreen, CD8-APC) (Miltenyi Biotec) and a Live/Dead marker Near IR- APC-Cy7 (ThermoFisher Scientific) plus either an activation panel (CD25(3G10)-PE, CD38-PE-Vio770, CD69-FITC, Anti-HLA-DR-PerCP)(Miltenyi Biotec) or an exhaustion panel (TIGIT-PE, TIM-3-FITC, LAG-3-PerCPeF710, PD1-PE-Cy7) (eBioscience). Cells were run on a MACSQuant and analysed with FlowJo software v10 (Miltenyi Biotec).

Immunohistochemistry

Rectal biopsy sections were stained for CD4 (goat polyclonal, Novus Biologicals) and CD8 antibodies (rabbit polyclonal, Abcam). Images were analysed in ImageJ and the distribution of cells determined as described elsewhere [9].

Statistical Analysis

All outcome measures were compared using the paired T-test after confirming normal distribution of variables. The primary outcome was week 0 to week 48 and secondary outcomes from week 19 (randomisation) to week 48.

Results

Patient characteristics

All 10 individuals were male. The mean age was 31 years, mean (SD) baseline CD4 was 593.3 cells/ml (177.68), HIV viral load 6.0 $\log_{10}$ copies/ml (6.35) and CD4:CD8 ratio = 0.61 (0.3) (Table 1). All individuals had wild type virus and maintained virological suppression from w19 to w48. Immunoglobulin therapy was well tolerated and no viral blips (above < 50 copies/ml) occurred during IVIG therapy. Spearman's rank correlation showed a non-statistically significant negative relationship between baseline viral load and baseline total HIV DNA (correlation coefficient = -0.21: p= 0.56).

Primary outcome:

- **HIV-1 reservoir**

Total HIV DNA in PBMCs decreased in both arms from baseline to w48 (immunoglobulin arm -3.70 $\log_{10}$ copies/million CD4 cells ; control arm -3.87 $\log_{10}$ copies/million CD4 cells) with no significant difference in change between the arms (CI -6548.5,11482.97;p=0.49). Furthermore no significant change between the arms was observed from w19 to w48 for total HIV DNA in PBMCs [immunoglobulin arm +2.09 $\log_{10}$, control arm -3.10 $\log_{10}$ copies/million CD4 cells **(**CI -2553,5363; p=0.38)] or low copy viral load (immunoglobulin arm +5.2 $\log_{10}$ copies/million CD4 cells; control arm +12.25 $\log_{10}$ copies/million CD4 cells; (CI -40.87,26.77; p=0.57).

In the gut, total HIV DNA declined in both groups, with no significant differences between arms between w19 to w48 (immunoglobulin arm 4.72 log10, control arm -4.84 log10 copies/million CD4 cells; p=0.55). Furthermore there was no difference in change in the number of CD4 T cells for w19 to w48 (immunoglobulin arm +2.87, control arm -6.07 cells;

p= 0.17] or in CD8 T cells for w19 to w48 [(immunoglobulin arm -4.53, control arm -1.53; p= 0.60] in the gut over time (Table 2).

- **Bacterial translocation**

Bacterial translocation increased from w19 to w48 in the immunoglobulin arm (24.1 copies 16SDNA/ml) and control arm (14.8 copies 16S DNA/ml) with no difference between the groups (CI -83.09, 101.87: p: 0.8)].

- **T-cell activation and exhaustion**

There were no significant changes from w19 to w48 in the level of activation of CD4 + T cells and CD8+ T cells (% CD4CD25, % CD4CD38, % CD4CD69, % CD4 HLA DR, % CD8 HLA DR, CD8CD69, CD8CD38 and CD8CD25) or level of immune exhaustion of markers (Pd1 , Tim3 Lag3) on CD4 + T cells and CD8+ T cells .

Discussion

The latent infection of resting CD4+ T-lymphocytes constitutes a major obstacle for the eradication of HIV. We sought to explore whether the temporary reduction in viral reservoir associated with immunoglobulin use in chronic infection [7,8] could induce a longer term effect in those treated at acute HIV infection before viral set point has been reached.

Immunoglobulin therapy was well tolerated but did not induce any viral blips during therapy. We found no impact of immunoglobulin on viral reservoir in the blood or gut or any surrogate markers of HIV (including immune activation, immune exhaustion or bacterial translocation) from baseline to w48. The finding that such non-specific antibodies play no role in HIV cure is highly topical given that several HIV-specific monoclonal antibodies—in particular, PGT121, VRC01, and VRC03—do exhibit anti-latent viral reservoir properties [10, 11] and clinical trials are underway.

The rapid recruitment to this study and fact that all participants volunteered for the optional gut biopsies highlights the willingness of individuals with acute HIV infection to take part in HIV cure research.

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Table 1: Baseline characteristics of participants

	Treatment (n=5)	Control (n=6)	P-value
Gender: Male	5(100%)	6(100%)	NA
Ethnicity: - **White** - **Black**	5(100%) 0(0)	5(83.33%) 1(16.67%)	1.00
HIV Genotype: - **Wild type virus**	5	6	1.00
Age	31.2(3.3)	31.3(5.4)	0.96
HIV Plasma Viral Load (log_{10} copies/million CD4 cells)	4.82(4.82)	6.28(6.48)	0.20
CD4:CD8	0.71(0.35)	0.5(0.22)	0.3
CD4 T cell count	678(218.75)	508.6(72.45)	0.16
Total HIV DNA (log_{10} copies/million CD4 cells)	3.80(3.43)	4.04(3.74)	0.14

Summary statistics are mean (SD) or count (%) as appropriate.

Table 2 : Results: comparison between treatment and control group

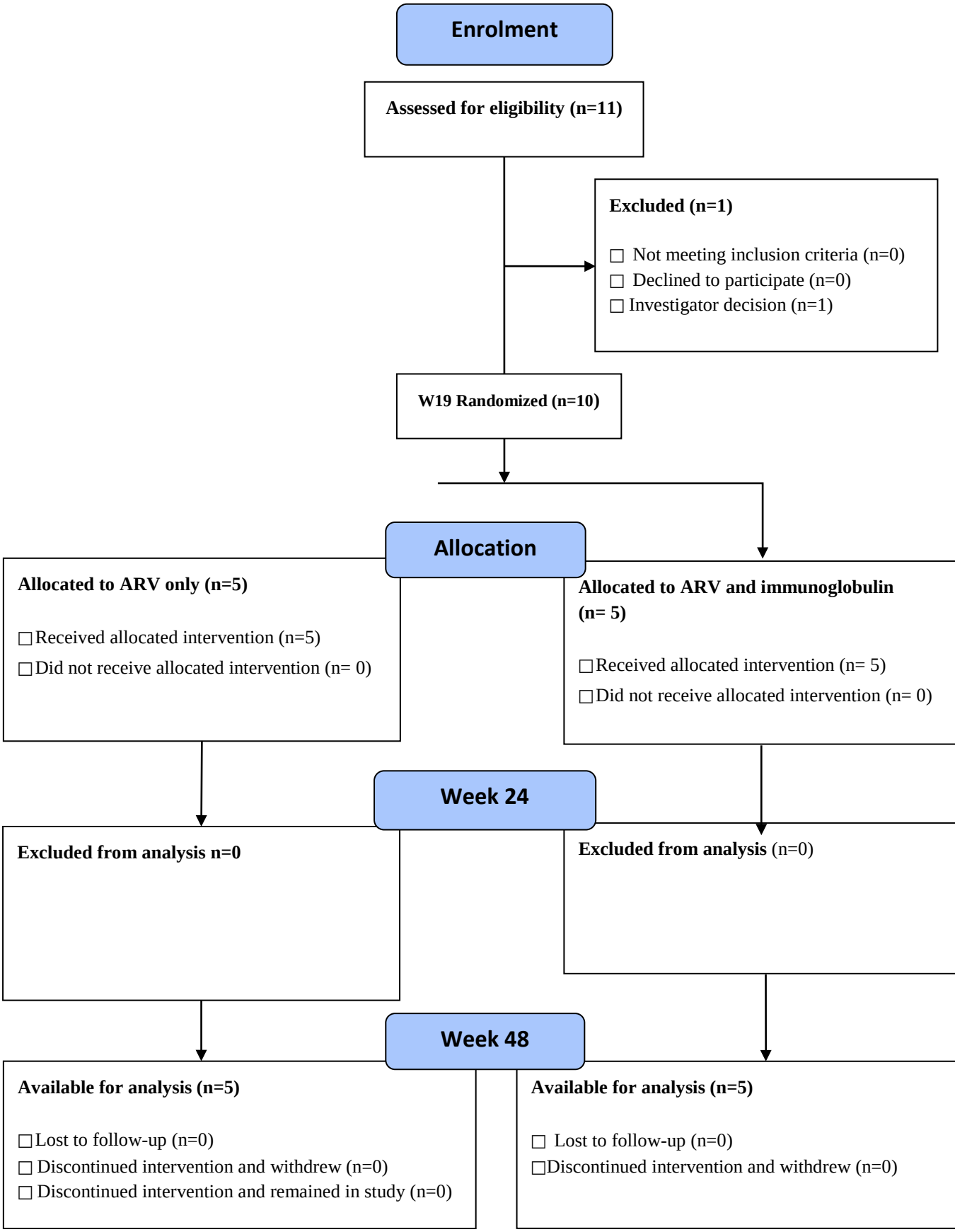
	Changes from week 19 to 48			
	Mean change			
	treatment group n=5	**control group n=5**	**difference between groups (95% CI)**	**P-value**
Reservoir				
Total HIV DNA log_{10} copies/million CD4 cells in PBMCs	2.09	-3.10	3.14 (-3.40, 3.72)	0.38
Total HIV DNA log_{10} copies/million CD4 cells in gut	4.72	-4.84	5.09 (-5.54,5.77)	0.55
Low copy RNA copies/ml	5.2	12.25	-7.05(-40.87,26.77)	0.57
Gut immunology				
CD4 cell number	2.87	-6.07	8.93(-5.09,22.96)	0.17
CD8 cell number	-4.53	-1.53	-3(-15.95,9.95)	0.6
Microbial translocation				
16S RNA copy/nl	24.16	14.77	9.39(-83.09,101.87)	0.8
Immune activation				
CD8+CD25+	4.09	-0.88	4.97(-1.07,11)	0.08
CD8+CD38+	18.25	5	13.25(-47.63,74.12)	0.5
CD8+CD69+	4.63	0.58	4.04(-14.32,22.41)	0.46
CD8+HLA.DR+	2.14	-0.1	2.25(-7.54,12.03)	0.43
CD4+CD25+	13.18	5.95	7.23(-15.43,29.9)	0.19
CD4+CD38+	7.85	-4.6	12.45(-22.22,47.11)	0.3
CD4+CD69+	2.64	0.79	1.85(-7.82,11.52)	0.55
CD4+HLA.DR+	2.32	1.73	0.59(-4.06,5.24)	0.71
Immune exhaustion				
CD8+ Lag3	0.55	-3.15	3.69(-19.28,26.67)	0.43
CD8+ Pd1	5.81	3.96	1.85(-18.29,21.99)	0.79
CD8+ Tim3	13.27	0.94	12.33(-26.82,51.48)	0.28
CD4+ Lag3	0.76	-1.52	2.28(-7.79,12.36)	0.32
CD4+ Pd.1	5.91	6.95	-1.04(-59.92,57.84)	0.89

CD4+ Tim3	7.13	1.93	5.2(-21.09,31.49)	0.37

Table 3: **Adverse events**

	Treatment group	**Control group**
Serious event	None	None
Number of AE (any)	2	0
Event name	"Cold"; "Conjunctivitis"	NA
Intensity:	Mild	NA
AE status	New AE	NA
Study drug action	None	NA
AE outcome	Resolved	NA
Relationship to study drug	Unlikely	NA
Concomitant Medication given	No	NA

Figure 1: Consort diagram



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