Lower Inflammatory Biomarkers in Children Randomized to Prolonged Cotrimoxazole Prophylaxis

Poster 914

Andrew J. Prendergast[1], Mutsa Bwakura Dangarembizi[2], Victor Musiime[3], Joseph Lutaakome[4], Adeodata Kekitiinwa[5], Godfrey Pimundu[3], Annie Shonhai[2], Moira Spyer[6], Alex Szubert[6], A. Sarah Walker[6], Nigel Klein[7] and the ARROW Trial Team

[1] Queen Mary University of London, London, UK; [2]University of Zimbabwe, Harare, Zimbabwe; [3]Joint Clinical Research Centre, Kampala, Uganda; [4]UVRI/MRC Uganda Research Unit on AIDS, Entebbe, Uganda; [5]Baylor-Uganda, Paediatric Infectious Diseases Clinic, Mulago Hospital, Kampala, Uganda; [6]MRC Clinical Trials Unit at UCL, London, UK; [7]Institute of Child Health, London, UK.

Contact details
MRC Clinical Trials Unit at UCL
email: rmjlasw@ucl.ac.uk

www.arrowtrial.org

ABSTRACT (updated)

Background: Cotrimoxazole (CTX) prophylaxis reduces morbidity and mortality in HIV-infected children on long-term ART, despite high-level pathogen resistance. Children taking CTX also have improved growth and reduced anemia, for reasons that are unclear. We hypothesized that benefits of CTX may be partly driven by reductions in inflammation.

Methodology: 758 children/adolescents (median (IQR) age 7 (4,11) years) in the ARROW trial in Uganda/Zimbabwe were randomized to stop (n=382) vs continue (n=376) daily CTX after median (IQR) 2.1 (1.8,2.2) years on ART. Inflammatory biomarkers (CRP, TNF-α, IL-6, sCD14) were measured by ELISA on cryopreserved plasma in a subset of 304 children at randomization (155 stop, 149 continue), then at 12, 24, and 96 weeks. Those co-enrolled in a cross-sectional metabolic substudy also had albumin, total protein and lipid profile measured 3 years after ART initiation. Change in $\log_{10}$ cytokine levels after randomization were modelled using normal interval regression, adjusting for site performing the assay.

Results: CRP was significantly higher in those randomized to stop CTX at 12, 24 and 96 weeks after ART initiation (2.19-fold [95% CI 1.15-4.17], p=0.02; 1.97-fold [1.22-3.20], p=0.006; and2.16-fold [1.18-3.95], p=0.01, higher, respectively; N=193, 264, 202 to date). There was a trend towards higher TNF-α in the stop CTX group at week 12 (1.14-fold higher vs continue [0.99-1.30], p=0.06), but this had disappeared by 24 weeks (p=0.33) and 96 weeks (p=0.42). For sCD14 and IL-6, there was no evidence of a difference between stop vs continue CTX groups at week 12 (p=0.16 and p=0.20 respectively); by 24 weeks differences had widened (difference stop vs continue: sCD14 1.10-fold higher [0.99-1.21], p=0.06; IL6 1.18-fold higher [1.01-1.38], p=0.04), but were absent at week 96 (sCD14 p=0.89; IL-6 p=0.78). At a single measurement taken at median 48 (IQR 36-60) weeks after randomization in 323 children, albumin was non-significantly lower in those randomized to stop CTX (difference -0.99 g/L vs continue [-2.01,0.04] p=0.06), with further non-significant trends towards higher total protein (+1.56 g/L vs continue [-0.03,+3.15] p=0.054) and LDL cholesterol (+0.11mmol/L vs continue [-0.04,+0.26] p=0.15).

Conclusions: Children on long-term ART randomized to stop CTX showed a rise in inflammatory biomarkers compared to those continuing CTX, in particular with increases in CRP persisting for two years after stopping prophylaxis. Previously reported benefits of CTX, particularly for anemia and growth, may therefore be partly driven by reductions in chronic inflammation, either directly through the well-described immunomodulatory properties of CTX, or indirectly through modulation of microbial translocation.

BACKGROUND

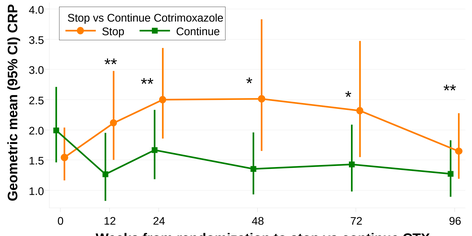
- Cotrimoxazole (CTX) prophylaxis reduces morbidity and mortality in HIV-infected children on long-term ART, despite high-level pathogen resistance (NEJM 2014)
- Children taking CTX also have improved growth and reduced anemia, for reasons that are unclear
- We hypothesized that benefits of CTX may be partly driven by reductions in inflammation

METHODOLOGY

- ARROW (ISCRTN24791884) was a randomized trial in 1206 HIV-infected children in Uganda and Zimbabwe
- **758 children/adolescents (median (IQR) age 7 (4,11) years) were randomized to stop (n=382) vs continue (n=376) daily CTX after median (IQR) 2.1 (1.8,2.2) years on ART (NEJM 2014)**
  - Those continuing CTX had significantly lower rates of hospitalizations (for both malaria and non-malaria infections) and lower rates of anaemia
- Inflammatory biomarkers (CRP, TNF-α, IL-6, sCD14) were measured by ELISA on cryopreserved plasma at randomization, then at 12 (Zimbabwe only), 24 and 96 weeks, in all children randomized from Zimbabwe and Ugandan children co-enrolled in an immunology substudy (poster #1181)
  - Some children enrolled in the immunology substudy in Uganda and Zimbabwe also had results at weeks 48 and 72
  - Analysis included only children who had a baseline measurement available (within the 13 weeks preceding randomization to stop vs continue CTX; 96% within the prior 4 days); N=293
  - $\log_{10}$ absolute cytokine levels after ART initiation were modelled using interval regression adjusting for assay site
- Those co-enrolled in a cross-sectional metabolic substudy 3 years after ART initiation in two ARROW sites (Joint Clinical Research Centre, Uganda; University of Zimbabwe, Harare) also had albumin, total protein and lipid profile measurements available

RESULTS

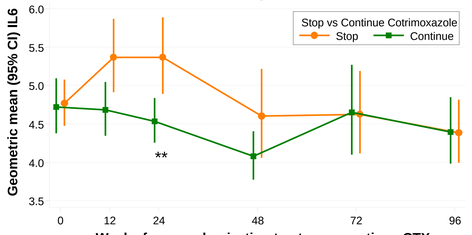
Figure 1: CRP (mg/L) after randomization to stop vs continue CTX



	Week 12	Week 24	Week 48	Week 72	Week 96
N stop/continue	100 / 93	135 / 129	65 / 73	82 / 84	100 / 102
FC from w0: stop	2.27	1.74	0.86	1.06	1.12
FC from w0: cte	1.04	0.88	0.47	0.64	0.52
FC(stop:continue) [95% CI] p-value	2.19 [1.15,4.17] P=0.02	1.97 [1.22,3.20] P=0.006	1.85 [0.94,3.65] P=0.08	1.67 [0.91,3.07] P=0.10	2.16 [1.18,3.95] P=0.01

Note: FC=fold change. 293 (149 stop, 144 continue) children with baseline and ≥1 later values
** 0.05<p<0.10 ** p<0.05 (full results in table)*

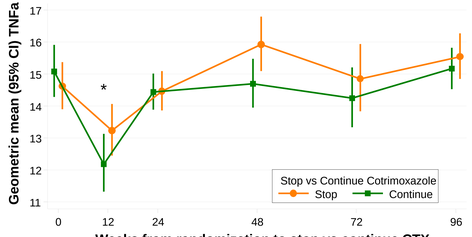
Figure 2: IL6 (pg/mL) after randomization to stop vs continue CTX



	Week 12	Week 24	Week 48	Week 72	Week 96
N stop/continue	98 / 94	131 / 129	63 / 69	79 / 81	99 / 101
FC from w0: stop	1.13	1.13	0.87	0.92	0.90
FC from w0: cte	1.02	0.95	0.83	1.00	0.92
FC(stop:continue) [95% CI] p-value	1.11 [0.95,1.29] P=0.20	1.18 [1.01,1.38] P=0.04	1.05 [0.86,1.28] P=0.60	0.92 [0.75,1.11] P=0.38	0.98 [0.83,1.15] P=0.78

Note: FC=fold change. 286 (144 stop, 142 continue) children with baseline and ≥1 later values

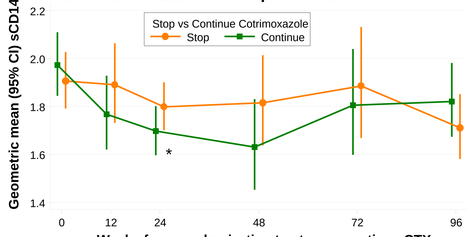
Figure 3: TNFα (pg/mL) after randomization to stop vs continue CTX



	Week 12	Week 24	Week 48	Week 72	Week 96
N stop/continue	99 / 93	135 / 129	63 / 72	81 / 84	100 / 102
FC from w0: stop	0.95	0.99	1.03	1.00	1.04
FC from w0: cte	0.84	0.94	0.95	0.95	1.00
FC(stop:continue) [95% CI] p-value	1.14 [0.99,1.30] P=0.06	1.04 [0.96,1.44] P=0.33	1.08 [0.98,1.20] P=0.13	1.04 [0.92,1.18] P=0.49	1.04 [0.94,1.15] P=0.42

Note: FC=fold change. 289 (145 stop, 144 continue) children with baseline and ≥1 later values
** 0.05<p<0.10 ** p<0.05 (full results in table)*

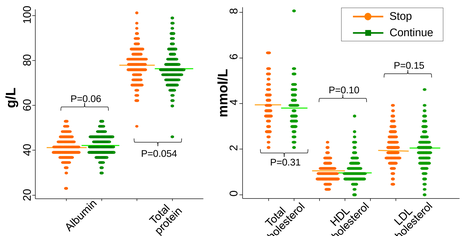
Figure 4: sCD14 (x10⁶ pg/mL) after randomization to stop vs continue CTX



	Week 12	Week 24	Week 48	Week 72	Week 96
N stop/continue	100 / 93	135 / 129	65 / 73	82 / 84	100 / 103
FC from w0: stop	1.01	0.94	0.94	1.00	0.89
FC from w0: cte	0.91	0.86	0.84	0.98	0.88
FC(stop:continue) [95% CI] p-value	1.11 [0.96,1.30] P=0.16	1.10 [0.99,1.21] P=0.06	1.11 [0.94,1.31] P=0.20	1.02 [0.86,1.22] P=0.82	1.01 [0.88,1.16] P=0.89

Note: FC=fold change. 290 (146 stop, 144 continue) children with baseline and ≥1 later values

Figure 5: Albumin, total protein and lipids 3 years after ART initiation



Note: light lines show means

3 years after ART initiation	Albumin (g/L)	Total protein (g/L)	Total cholesterol (mmol/L)*	HDL cholesterol (mmol/L)	LDL cholesterol (mmol/L)
N stop/continue	159/164	159/164	82/87	159/163	159/164
Mean difference (stop-continue) [95% CI] p-value	-0.99 [-2.01,0.04] P=0.06	+1.56 [-0.03,+3.15] P=0.054	+0.15 [-0.13,+0.43] P=0.31	-0.08 [-0.18,+0.02] P=0.10	+0.11 [-0.04,+0.26] P=0.15

*Only measured in the Zimbabwean site

SUMMARY and CONCLUSIONS

- **Children on long-term ART randomized to stop CTX showed a rise in inflammatory biomarkers compared to those continuing CTX**
  - **Differences in IL6, sCD14 and TNFα were transient (Figures 2-4)**
  - **But increases in CRP persisted for two years after stopping prophylaxis (Figure 1)**
  - **Lower albumin, higher total protein and LDL cholesterol may be driven by higher levels of inflammation in children stopping cotrimoxazole**
- **Previously reported benefits of CTX, particularly for anemia and growth, may therefore be partly driven by reductions in chronic inflammation, either directly through the well-described immunomodulatory properties of CTX, or indirectly through modulation of microbial translocation**

We thank all the patients and staff from all the centres participating in the ARROW trial. Joint Clinical Research Centre, Kampala, Uganda: P Mugyenyi, V Musiime, R Keishanyu, VD Afayo, J Bwomezi, J Byaruhanga, P Erimu, C Karungi, H Kizito, WS Namala, J Namusanje, R Nandugwa, TK Najjuko, E Natukunda, M Ndigendawani, SO Nsiyona, R Kibenge, B Bainomuhwezi, D Sseremba, J Tezikyabbiri, CS Tumusiime, A Balaba, A Mugumya, F Nghania, D Mwebesa, M Mutumba, E Bagurukira, F Odongo, S Mubokyi, M Ssenyonga, M Kasango, E Lutalo, P Oronon; MRC/UVRI Uganda Research Unit on AIDS, Entebbe, Uganda: P Munderi, P Nahirya-Ntege, R Katuramu, J Lutaakome, F Nankya, G Nabulime, I Sekamatte, J Kyarimpa, A Ruberantwari, R Sebukyu, G Tushabe, D Wangi, M Musinguzi, M Aber, L Matama, D Nakitto-Kesi; Baylor-Uganda, Paediatric Infectious Disease Centre, Mulago Hospital, Uganda: A Kekitiinwa, P Musoke, S Bakeera-Kitaka, R Namuddu, P Kasirye, A Babirye, J Asello, S Nakalanzi, NC Ssemambo, J Nakafeero, J Tikabibamu, G Musoba, J Ssanyu, M Kisekka; University of Zimbabwe, Harare, Zimbabwe: KJ Nathoo, MF Bwakura-Dangarembizi, F Mapinge, E Chidziva, T Mhute, T Vhembo, R Mandidewa, M Chipiti, R Dzapasi, C Katanda D Nyoni, GC Tinago, J Bhiri, S Mudzingwa, D Muchabaiwa, M Phiri, V Masore, CC Marozva, SJ Maturure, S Tsikirayi, L Munetsi, KM Rashirai, J Steamer, R Nhema, W Bikwa, B Tambawoga, E Mufuka; Medical Research Council Clinical Trials Unit, London, UK:DM Gibb, MJ Thomason, AS Walker, AD Cook, B Naidoo-James, MJ Spyer, C Male, AJ Glabay, LK Kendall, J Crawley, AJ Prendergast. Independent ARROW Trial Monitors: I Machingura, S Ssenyonjo. Trial Steering Committee:I Weller (Chair), E Luyirika, H Lyall, E Malianga, C Mwansambo, M Nyathi, F Miiro, DM Gibb, A Kekitiinwa, P Mugyenyi, P Munderi, KJ Nathoo, AS Walker; Observers S Kinn, M McNeil, M Roberts, W Snowden. Data and Safety Monitoring Committee: A Breckenridge (Chair), C Hill, J Matenga, A Pozniak, J Tumwine. Endpoint Review Committee: G Tudor-Williams (Chair), H Barigye, HA Mujuru, G Ndeezi; Observers: S Bakeera-Kitaka, MF Bwakura-Dangarembizi, J Crawley, V Musiime, P Nahirya-Ntege, A Prendergast, M Spyer.

Funding: ARROW was funded by the UK Medical Research Council and the UK Department for International Development (DFID). Viiv Healthcare/GlaxoSmithKline donated first-line drugs for ARROW and provided funding for VL assays.