Poster 314

TLR9 Agonist Triggers Potent Intestinal Antiviral Response in HIV+ Individuals on ART

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BACKGROUND

- HIV-1 persists in infected individuals despite ART.
- While persistent HIV-1 becomes transcriptionally active in the presence of latency reversing agents, these agents have little effect on the size of the viral reservoir in vivo.
- Improved killing of infected cells is essential to achieve viral eradication.[1]
- Towards this goal, we tested whether a TLR9 agonist, specifically MGN1703 [2] (Lefitolimod) could enhance antiviral immunity in vivo.

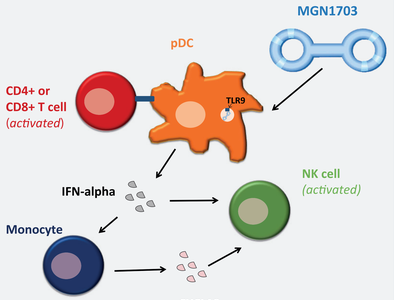
AIMS

Define MGN1703 treatment outcomes related to:

(i) TLR9 agonist-induced intestinal immune activity

(ii) TLR9 agonist-associated changes in HIV-1 persistence in the intestine.

PATHWAY OF TLR9 STIMULATION



METHODS

- Single-arm phase 1b/2a clinical trial
- HIV-1+ adults received MGN1703 (Lefitolimod; Mologen AG, Berlin, Germany) (60 mg s.c.) twice weekly for 4 weeks (Fig. 1A)
- Sigmoid biopsies were collected from 11 participants at baseline and 24 hours after the last dose in week 4 (Fig. 1A)
- Quantitative IHC for MX1, ISG15, IL-21, CXCL10 or MPO[3]
- RNA-sequencing of colonic mononuclear cells[4]
- Quantitative PCR for integrated viral DNA[5]

FIGURE 1

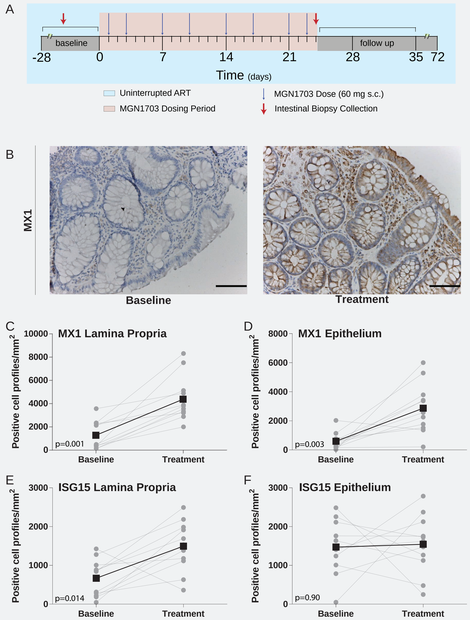


Figure 1. MGN1703 induced an increase in cell profiles producing the antiviral proteins MX1 or ISG15 in the sigmoid colon. (A) The timing of MGN1703 dosing and biopsy sample collection are illustrated. **(B)** Representative images for MX1 IHC (brown staining) from biopsies collected prior to dosing (baseline) and during the 4th dosing week (treatment) are presented. Scale bars=100μm. **(C-F)** Plots show the number of cell profiles expressing MX1 (C-D) or ISG15 (E-F) at baseline and during MGN1703 treatment (n=11 in all graphs). A cell profile was recorded as positive, when IHC signal was associated with a nucleus. Gray circles indicates results for each participant. Paired data connected with gray lines. Black squares represent the mean value for all participants at the indicated time point. Black lines connect longitudinal mean data points. (C and E) The numbers of positive cell profiles per mm^2 within the lamina propria regions are illustrated. (D and F) The numbers of positive cell profiles per mm^2 within the epithelial regions are illustrated. Statistics: Wilcoxon matched-pairs signed rank test.

FIGURE 2

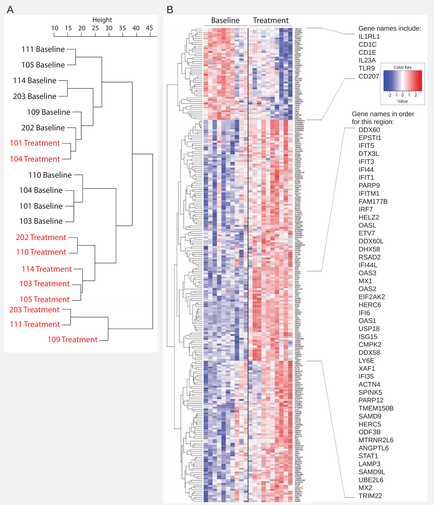


Figure 2. MGN1703 induced significant regulation of 248 genes in intestinal mononuclear cells, including many genes involved in type I interferon responses. (A) Euclidian complete link dendrogram reveals that groups cluster according to baseline and treatment (n=10). **(B)** RNASeq heat map was generated using standardized Z-scores of the genes that were significantly regulated (up or down) in intestinal mononuclear cells after adjusting for a false discovery rate <0.05. Individual participants (n=10) are depicted in the columns and one gene is depicted per row. The dendrogram reveals the relatedness of individual genes.

FIGURE 3

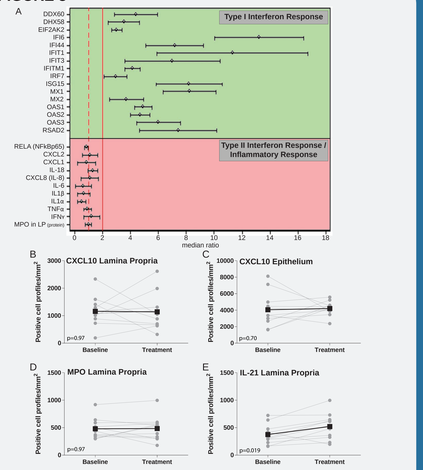


Figure 3. MGN1703 did not induce inflammation in the sigmoid colon. (A) Key type I interferon response and inflammatory parameters from the RNASeq and IHC analyses were assessed. In this forest plot representation of an equivalence evaluation, an upregulation of 2-fold from baseline was conservatively set as the threshold for concluding that upregulation had been induced. Confidence intervals in the forest plot are at a 10% significance level, such that the comparison to the upper equivalence limit is performed at an alpha of 0.05 (n=10 for gene expression parameters and 11 for the MPO in LP protein parameter). **(B-E)** Plots show the number of cell profiles expressing CXCL10 (B-C), MPO (D), or IL-21 (E) at baseline and during MGN1703 treatment (n=11 in all graphs). Gray circles indicates results for each participant. Paired data connected with gray lines. Black squares represent the mean value for all participants at the indicated time point. Black lines connect longitudinal mean data points. (B, D and E) The numbers of positive cell profiles per mm^2 within the lamina propria regions are illustrated. (C) The numbers positive cell profiles per mm^2 within the epithelial regions are illustrated. Statistics: Wilcoxon matched-pairs signed rank test.

FIGURE 4

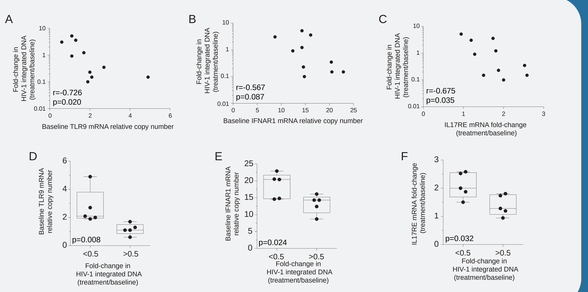


Figure 4. MGN1703-induced reductions in intestinal mononuclear cell HIV-1 DNA copies is associated with a higher baseline TLR9 and IFNAR1 expression. (A, B and C) Bi-variant plots show the fold change in the copies of integrated HIV-1 DNA (left axis) versus the baseline number of TLR9 transcripts (A), the baseline number of IFNAR1 (IFN-α receptor alpha chain) transcripts (B) or the MGN1703-induced fold-change in transcription of IL17RE (IL-17 receptor E) (C). Spearman correlation r and p values are shown. **(D, E and F)** Participant data for the baseline number of TLR9 transcripts (D), the baseline number of IFNAR1 transcripts (E) or the MGN1703-induced fold-change in transcription of the IL17RE (F) were stratified based on whether the fold-change in integrated HIV-1 DNA copies was greater than 0.5 or less than 0.5. Statistics: Wilcoxon matched-pairs signed rank test.

RESULTS

- MGN1703 induced a strong increase in the number of cell profiles producing the antiviral proteins MX1 or ISG15 (Fig. 1)
- The samples clustered hierarchially in a treatment-dependent manner (Fig. 2A)
- 248 genes were significantly regulated (up or down) (Fig. 2B)
- MGN1703 treatment led to significant increases in the regulation of multiple interferon-stimulated genes and type I interferon pathway signaling molecules (Fig. 2B)
- No significant regulation of IFN-γ and type II interferon response genes (Fig. 3A)
- No change in the number of cell profiles positive for myeloperoxidase (an indicator of general inflammation) or CXCL10 (interferon γ-induced protein 10) (Fig. 3B-E)
- Higher TLR9 and IFNAR1 gene expression prior to MGN1703 treatment is associated with fewer copies of integrated HIV-1 DNA following treatment (Fig. 4)

CONCLUSION

- MGN1703 treatment improved immune surveillance via a type I interferon response in the intestines of HIV-1+ individuals on ART.
- MGN1703 did not induce inflammation in the sigmoid colon.
- Baseline gene expression levels for TLR9 and IFNAR1 may significantly impact the downstream efficacy of a TLR9 agonist in the intestines.
- These findings strongly support the continued development of MGN1703 as an enhancer of innate immune surveillance in HIV-1 eradication approaches.

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