IAP ANTAGONISM PROMOTES PD-1 BLOCKADE-MEDIATED ELIMINATION OF HIV IN HUMANIZED MICE

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Background

The immune checkpoint programmed cell death protein 1 (PD-1) plays a major role in T cell exhaustion in cancer and chronic HIV infection. Inhibitor of apoptosis protein antagonists (IAPa) reverse HIV latency and costimulate T-cells through modulation of NF-kB signaling in vitro.

Method

We asked in this study whether a new IAPa would stimulate the potency of an anti-human PD-1 monoclonal antibody (mAb) to reduce HIV loads in humanized mice.

Results

Four weeks of Anti-PD-1 mAb treatment decreased the PD-1+ CD8+ cell population among CD45+ in blood by 22% compared to vehicle, while IAPa co-treatment reduced it by 50%. Anti-PD-1 mAb administration reduced HIV load in blood by 94% with detectable levels in 8 of 8 mice, and addition of the IAPa further enhanced this reduction from 94 to 97% with undetectable levels in 5 of 8 mice. 2 weeks after drug treatment interruption, Anti-PD-1 mAb administration had reduced HIV loads in CD4+ cells also in all tissues analyzed compared to vehicle, including spleen (5.6 to 2 log in viral RNA copies), lymph nodes (5.6 to 1.1 log in viral RNA copies), liver (5.4 to 1.6 log in viral RNA copies), lung (5.6 to 2 log in viral RNA copies) and thymic organoid (5.5 to 1.2 log in viral RNA copies). IAPa further enhanced the anti-PD-1-mediated reduction of HIV tissue loads achieving a >5 log reduction in all tissues analyzed, notably with undetectable levels in some individual organs; spleen (5.6 to 0.2 log in viral RNA copies), lymph nodes (5.6 to 0.2 log in viral RNA copies), liver (5.4 to 0.3 log in viral RNA copies), lung (5.6 to 0.2 log in viral RNA copies) and thymic organoid (5.5 to 0.1 log in viral RNA copies). Following the 4 weeks of in vivo treatments, ex vivo anti-CD3/CD28 stimulation increased the ability to activate CD8+ T cells in infected mice having received in vivo anti-PD-1 treatment by 7.9-fold (5 to 39.6%), and an additional increase by 1.7-fold in mice having received IAPa co-treatment (39.6 to 67.3%).

Conclusion

These findings demonstrate for the first time that an IAPa greatly enhances the effects of an immune checkpoint inhibitor on antiviral immunity resulting in undetectable HIV titers in blood and organs of humanized mice. This suggests that the combination of two distinct classes of immunomodulatory agents constitutes a promising immunotherapeutic approach to cure HIV.

Results

Fig 1. Experimental design for D1143 and anti-PD-1 mAb treatments and blood and tissue analyses in HIV-infected BLT mice

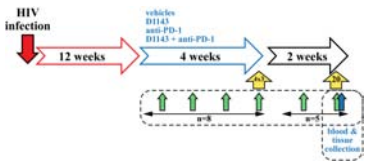
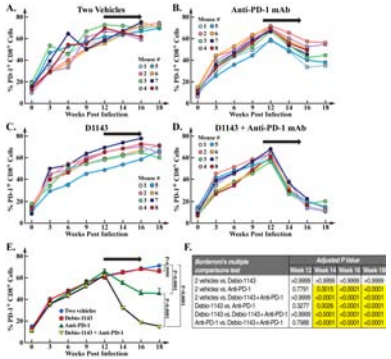
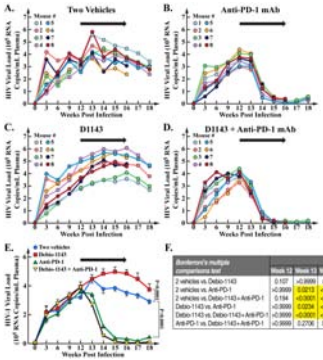


Fig 2. PD-1 expression on human CD8+ T cells from HIV-infected BLT mice



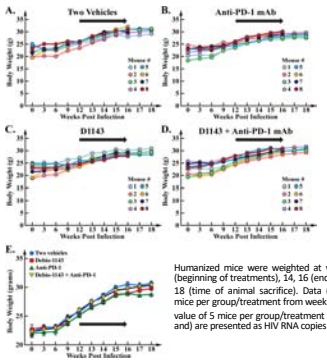
Blood was collected at week 0, 3, 6, 9, 12 (beginning of treatments), 14, 16 (end of treatments) and 18 (time of animal sacrifice) and isolated PBMCs stained with directly conjugated anti-human CD8 and anti-human PD-1 antibodies and analyzed by FACS. Data (median value of 8 mice per group/treatment and median value of 5 mice per group/treatment from week 16 to 18) are presented as percentage of human PD-1+ CD8+ T cells.

Fig 3. HIV loads in blood of HIV-infected BLT mice



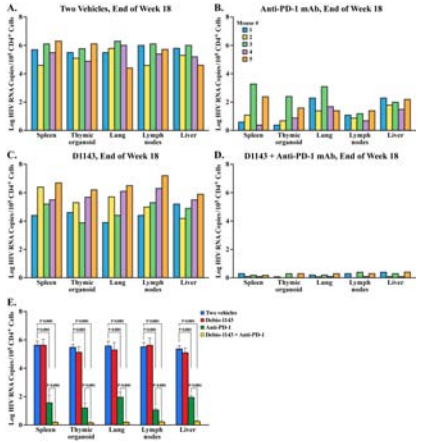
Blood was collected at week 0, 3, 6, 9, 12 (beginning of treatments), 14, 16 (end of treatments) and 18 (time of animal sacrifice). HIV RNA levels in plasma were quantified using one-step reverse transcriptase quantitative real-time PCR as described previously [51–52]. Data (median value of 8 mice per group/treatment from week 0 to 16 and median value of 5 mice per group/treatment from week 16 to 18) are presented as HIV RNA copies per mL of plasma.

Fig 4. Body weight of HIV-infected BLT mice



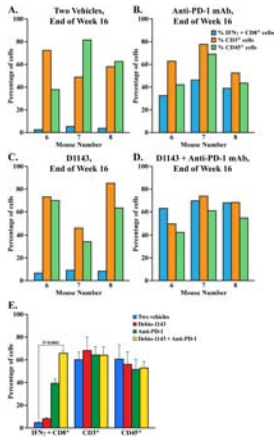
Humanized mice were weighted at week 0, 3, 6, 9, 12 (beginning of treatments), 14, 16 (end of treatments) and 18 (time of animal sacrifice). Data (median value of 8 mice per group/treatment from week 0 to 16 and median value of 5 mice per group/treatment from week 16 to 18 and) are presented as HIV RNA copies per mL of plasma.

Fig 5. HIV loads in tissues of HIV-infected BLT mice



Selected tissues were collected at week 18 (time of animal sacrifice). HIV RNA levels in isolated human CD4+ cells were quantified using one-step reverse transcriptase quantitative real-time PCR as described previously [51–52]. Data (median value of 5 mice per group/treatment) are presented as HIV RNA copies per 100,000 isolated CD4+ cells.

Fig 6. *Ex vivo* IFNγ production by human CD8+ effector T cells isolated from HIV-infected BLT mice



At the end of week 16 –end of the four treatments–percentages of human CD3+ and CD45+ cells in blood of HIV-infected BLT mice were quantified by FACS. Isolated human CD45+ cells were stimulated *ex vivo for 24 h with anti-CD3 and anti-* CD28 antibodies and the percentage of IFNγ+ CD8+ cells was quantified by FACS. Data (median value of 5 mice per group/treatment) are presented as percentages of CD3+, CD45+ and IFNγ+ CD8+ cells.

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