Title: The nucleoside analogues zidovudine, tenofovir alafenamide, and cladribine induce EBV lytic gene expression without subsequent viral replication

Short title: Nucleoside analogues induce EBV from latency

Introduction:

Converging evidence implicates a role for EBV in MS. However, the mechanism driving this association remains elusive and the limited list of anti-EBV drugs is a barrier to determining whether EBV could be a therapeutic target in MS.

Acyclovir and related anti-herpesvirals are nucleoside analogues (NAs) with efficacy against EBV replication. However, the anti-EBV effects of NAs licensed for alternative indications (e.g. cancer, MS, or infectious diseases) have not been thoroughly investigated. Intriguingly, antiretroviral NAs have been linked to MS remission in case reports. At least one such drug, zidovudine, is known to induce lytic gene expression in EBV+ cancers. Therefore, we assessed the efficacy of other NAs to induce EBV from latency.

Methods:

We tested cladribine, 3 anti-herpesviral NAs, and 8 anti-retroviral NAs using the EBV+ B-cell line HH514-16. We assessed induction of EBV by western blot, gene expression by RNA-seq, and lytic DNA replication by qPCR.

Results:

Two antiretroviral NAs (zidovudine and tenofovir alafenamide), as well as cladribine, were able to induce lytic EBV gene expression, and protein expression of the early lytic gene BMRF1.

Unlike the chemical agent butyrate, which causes lytic gene expression and viral DNA replication, zidovudine, tenofovir alafenamide, and cladribine induced EBV gene expression without EBV DNA replication.

Additionally, induction of EBV by zidovudine and cladribine was accompanied by increased expression of immune regulatory genes (CD80, CD86, CD40, ICAM1); inhibitory Fc receptor-like proteins (FcRLs) which may modulate BCR signaling; and CD1, with implications for viral antigen presentation.

Conclusions:

The nucleoside analogues zidovudine, tenofovir alafenamide, and cladribine induce EBV lytic gene expression without viral replication. Further clinical investigation of these NAs would be informative, specifically their effects on EBV viral load in MS, as these results suggest that they could aid clearance of latently infected EBV+ cells by direct apoptosis or immune recognition.