IMU-838, a Small Molecule DHODH Inhibitor in Phase 2 Clinical Trial for Multiple Sclerosis, Shows Potent Anti-EBV Activity in Cell-Culture-Based Systems: Potential Additional Benefits in Multiple Sclerosis Treatment

Immunic THERAPEUTICS

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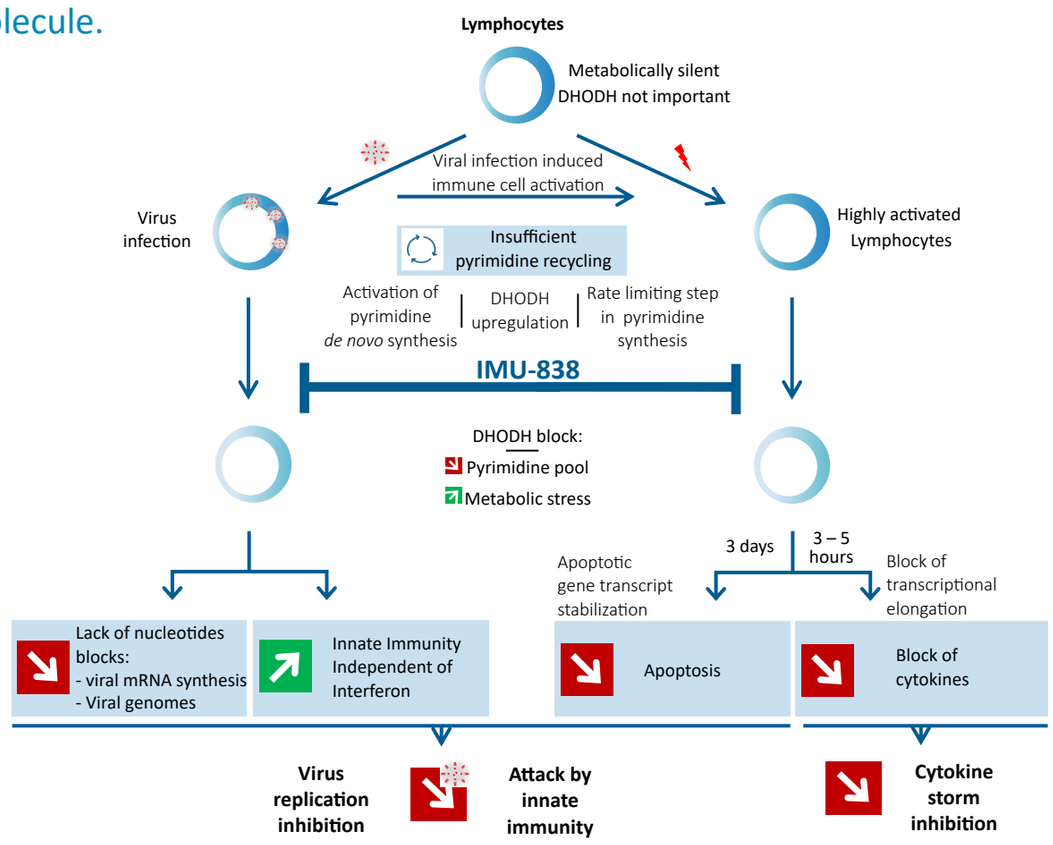
Background[1-4]

IMU-838, in development for multiple sclerosis (MS) treatment, is a safe and orally available dihydroorotate dehydrogenase (DHODH) inhibitory small molecule.

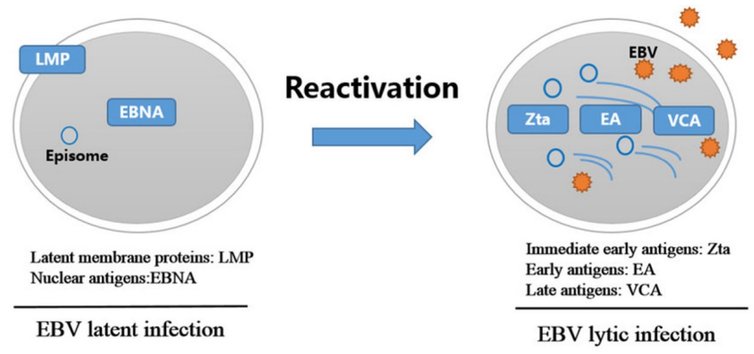


Viral infections have been linked to MS pathogenesis, and one of the most studied viral complications in MS disease is Epstein-Barr virus (EBV) infection. Here, we investigated IMU-838 as a potential EBV reactivation inhibitor.

Methods[5-9]

1. Lytic EBV reactivation of Raji or B95-8 cells by 12-O-tetradecanoylphorbol-13-acetate (TPA) was evaluated by fluorescent staining for Zta/BZLF1 and visual counting in triplicates (mean ± SD). Raji cells contain a defective EBV genome and can not produce late viral proteins. B95-8 are EBV producer cells.

2. Raji cells, superinfected with B95-8 viral stock, were microscopically evaluated for cell aggregate formation. Extracellular EBV genome copies were assessed by qPCR in triplicates (mean ± SD).



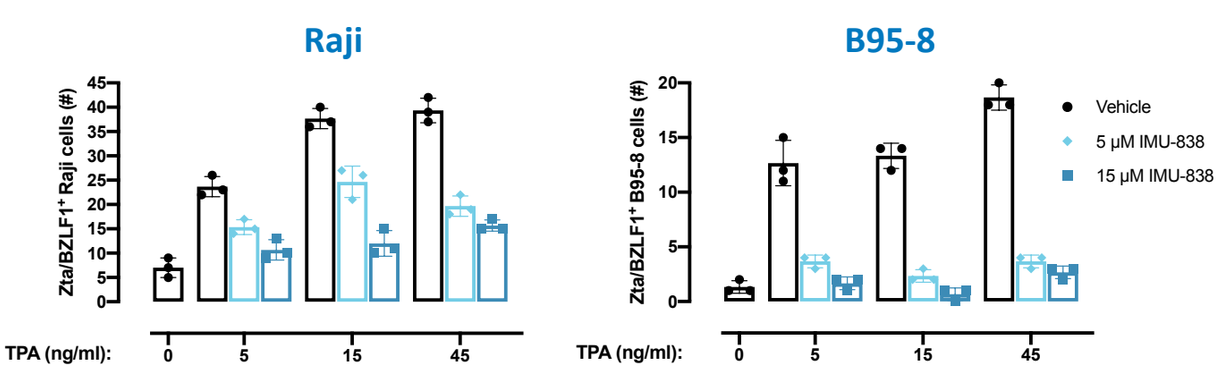
3. Anti-human IgG (anti-hIgG) stimulation induces EBV reactivation in low-level producer Akata-BX1 cells (carrying an EBV-GFP reporter). EBV is measured via GFP-based automated fluorometry on cell lysates in 3-4 replicates.

4. T81GFP (epithelial cell line) containing a OriLyt-GFP reporter, were infected with the B95-8 EBV strain. EBV reactivation is measured via GFP-based automated fluorometry on cell lysates in 6 replicates.

Neutral Red Assay was used for monitoring drug-induced cytotoxicity.

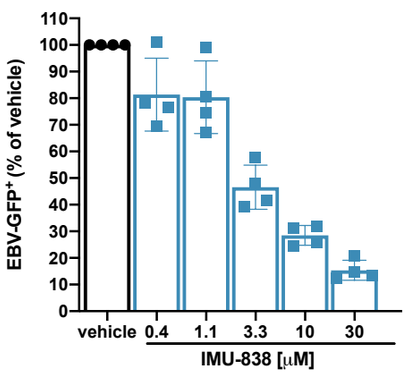
All assays were performed with 2% FCS medium unless stated otherwise.

IMU-838 showed concentration-dependent anti-EBV activity in Raji and B95-8 cells under TPA stimulation



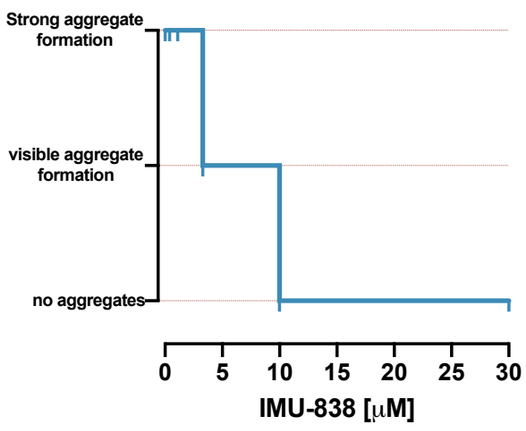
Using an indirect immunofluorescence (IF)-based microscopic readout, IMU-838 produced a concentration-dependent reduction of the immediate early antigen, Zta, at 4 days post infection (dpi). In parallel, the NRA staining indicated no cytotoxic effects of IMU-838 with concentrations up to 45 µM.

IMU-838 showed concentration-dependent anti-EBV activity to spontaneous lytic EBV production and upon an anti-hIgG stimulation



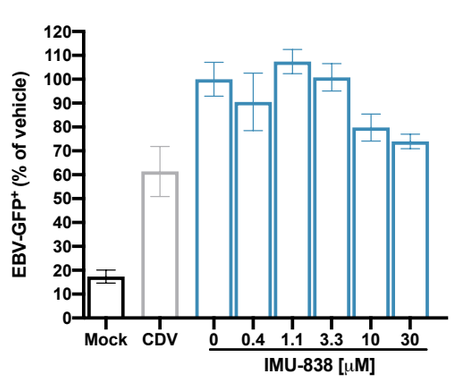
IMU-838 effect was assessed in Akata-BX1-EBV-GFP cells stimulated with anti-human IgG for 4-5 dpi. EC_{50} is 3.5 ± 1.2 µM (n = 4). CC_{50} values were >30 µM with 57% viability at 30 µM. FCS contains uridine, which can be taken up by the cell. Since these cells are low-level EBV producers, the pyrimidine demand is relatively low and can therefore be partially rescued by higher FCS levels in the medium. 10% FCS instead of 2%, partially reversed EBV inhibition as seen by an increase in EC_{50} values (16.8 ± 3.3 µM; n = 5), which was tested ± anti-hIgG stimulation. This indicates that pyrimidine depletion, as effected by IMU-838, may represent the main mode of action in the inhibition of EBV replication.

IMU-838 exerted anti-EBV activity at concentrations of 3.3–30 µM in a superinfection assay



IMU-838 activity was assessed in a superinfection assay. In the negative control condition, no aggregates were observed. In contrast, strong aggregate formation was observed in the vehicle condition. IMU-838 reduced aggregate formation in a concentration-dependent way, with the strongest effects seen with 10 and 30 µM.

IMU-838 also indicated a tendency of concentration-dependent inhibition of EBV in T81GFP cells



The epithelial derived cell line T81GFP contains a reporter transcribing GFP in response to lytic EBV infection. IMU-838 produced a partial inhibition of EBV activity at the highest concentrations. As a positive control, the anti-cytomegaloviral drug cidofovir (CDV) was included. 30 µM of IMU-838 effected a similar level of inhibition as 100 µM CDV. CDV and 10-30 µM of IMU-838 showed low cytotoxicity based on visual microscopic inspection.

Conclusions

- **IMU-838 comprises a promising EBV-inhibitory potential *in vitro* at concentrations reached in patients**
- **Phase 2 clinical trial data show activity for IMU-838 in MS**
- **Next to the anti-inflammatory capacity, IMU-838's antiviral activity might provide additional benefits for patients by preventing EBV reactivation**

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