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1263W94 inhibits the phosphorylation of EBV DNA polymerase processivity factor BMRF1 by EBV protein kinase BGLF4.

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1263W94 (5,6-dichloro-2-(isopropylamino)-1- β-L-ribofuranosyl-1H-benzimidazole) is a potent inhibitor of human cytomegalovirus (HCMV) replication that is currently in Phase I/II clinical trials. 1263W94 had no activity against herpes simplex virus (types 1 and 2), but was active against Epstein-Barr virus (EBV). Previously, we have shown that the compound inhibits EBV DNA replication in a nucleotide-independent manner and affects the phosphorylation of the BMRF1 protein (EBV DNA polymerase processivity factor). The mutations that cause 1263W94 resistance in HCMV have been mapped to the UL97 gene (viral protein kinase). EBV possesses a UL97 homolog, the BGLF4 gene, that we are proposing as the target for 1263W94. We have now cloned BGLF4 ORF and tested the ability of its protein product to phosphorylate BMRF1 protein as well as the nucleoside analog, ganciclovir (GCV). The results show, first, that BMRF1 protein may be phosphorylated to some extent by cellular kinases, but the hyperphosphorylated form which is affected by 1263W94 appears only when co-expressed with BGLF4. Second, GCV was phosphorylated by the BGLF4 product, but to a lesser extent than by the HCMV kinase, UL97. Thus, the EBV BGLF4 product, which is homologous to HCMV UL97, is presumably a kinase and phosphorylates not only the EBV DNA polymerase processivity factor, BMRF1, but also GCV. Phosphorylation of BMRF1 is inhibited by 1263W94. We infer that 1263W94 impairs the ability of the EBV protein kinase to hyperphosphorylate BMRF1 and this phosphorylation may be important for BMRF1 function and viral replication. Work is in progress to characterize further BGLF4 protein kinase and to understand the importance of BMRF1 phosphorylation for its functions.