Abstract
The US is in the midst of a major drug epidemic fueled in large part by the widespread recreational use of synthetic opioids such as fentanyl. Unfortunately, medications approved for the treatment of opioid use disorders (OUD) are underutilized and/or not offered in many settings. Thus, persons with OUD are at significant risk for transmission of the human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV). Moreover, commonly abused substances can antagonize immune responses and promote viral replication. However, the impact of synthetic opioids on virus replication has never been explored.

We evaluated the impact of fentanyl using in vitro systems that replicate infectious viruses. Fentanyl was available as a highly purified analytical reference standard and used at concentrations of 1 ng, 100 ng, and 10 ug. Viral protein synthesis was quantified by ELISA, while apoptosis and cell death were measured by M30 or MTT assays, respectively. HCV replicative fitness was evaluated in a luciferase-based system. RNAseq was conducted to evaluate cellular gene regulation in the presence of fentanyl.

Low dose fentanyl had no impact on HCV replication in HuH7.5JFH1 hepatocytes; however, higher doses significantly enhanced HCV replication. In the HepG2.2.15 hepatocyte cell line, fentanyl caused a dose-dependent increase in HBV replication, although the same low dose that caused increased HBV replication had a minimal effect on HCV. A dose-dependent increase in HCV replicative fitness was observed in the presence of fentanyl. Similarly, fentanyl increased HIV replication in two lymphocyte cell lines. Addition of fentanyl resulted in significant accumulation of soluble caspase-cleaved keratin 18—a product of apoptosis—in two hepatocyte cell lines. Cell death was minimal at low drug concentrations. RNAseq identified a number of hepatocyte genes that were up or down regulated after fentanyl exposure including those related to apoptosis, viral gene expression, hepatocarcinogenesis, and NFkB.

Discussion
These preliminary data suggest that synthetic opioids promote viral replication in vitro but may have distinct effects depending on the drug dose and the viral target. Synthetic opioids also increased hepatic apoptosis, although their impact on cell death was minimal across a wide range of doses. RNAseq identified a number of hepatocyte genes that were up or down regulated after fentanyl exposure including those related to apoptosis, viral gene expression, hepatocarcinogenesis, and NFkB. However, these results were cell line-dependent and require validation by real-time PCR and quantitative protein expression.

As higher viral loads are associated with pathogenesis and virus transmission, in vivo studies are essential to an enhanced understanding of opioid-virus pathogenesis and for the development of new and optimized treatment strategies. A number of unanswered questions remain include:

- Do synthetic opioids influence viral pathogenesis as documented for other commonly abused substances?
- Do individual synthetic opioids differ in their proinflammatory effect and antiviral activities?
- Does opioid receptor antagonism counteract the effects of synthetic opioids on viral replication, thereby providing added benefit in individuals with opioid use disorders?
- What role does polysubstance use play in viral pathogenesis?
- Are there drug-drug interactions between synthetic opioids and currently prescribed antiviral medications for HIV, HBV, or HCV that must be considered in high-risk patient populations?
- What cellular pathways and microRNAs are regulated by synthetic opioids that could be exploited to control viral replication in virus-infected individuals with substance use disorders?

This work was funded in part by the National Institute of General Medical Sciences (award GM105414 to JTB).