Discovery of Smallpox Inhibitors that Block Processive DNA synthesis

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Smallpox virus (*variola*) was responsible for the deaths of hundreds of millions prior to its global eradication in 1980. Currently, there is concern that certain organizations may possess or obtain their own *variola* stocks. In response to such a threat, the United States must have at its disposal supplies of both vaccine as well as new antiviral compounds that can be used to respond rapidly to an outbreak of smallpox. The viral processivity factors are ideal drug targets since they function specifically with their cognate DNA polymerases and have no homology to cellular proteins. Processivity factors enable their DNA polymerases to incorporate nucleotides continuously without dissociating from the DNA template. The vaccinia virus (VV) DNA polymerase E9 protein becomes processive in the presence of the VV processivity proteins A20 and D4R. Predictably, since each of these VV proteins is at least 97% homologous to its corresponding protein of *variola* virus, therapeutics of VV processive DNA synthesis will likely target the same function in *variola* and prevent viral replication. We describe a mechanistic Rapid Plate Assay that we have developed to screen small chemical libraries of more than 52,000 compounds for their abilities to block processive DNA synthesis. From this primary screen we have obtained a small number of interesting antiviral compounds that are able to block viral plaque formation with low cytotoxicity. Structural analogs, identified computationally, are currently being evaluated for greater efficacy as vaccinia virus inhibitors. Strategies for continued evaluation are presented.