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Detection of Nascent Mitochondrial DNA Using Click Chemistry

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Since the introduction of the nucleoside reverse transcriptase inhibitor (NRTI) azidothymidine (AZT) in 1987, the use of highly active antiretroviral therapy (HAART) has been the predominant approach for the treatment of HIV infection. Chronic use of NRTIs can lead to serious side effects stemming from mitochondrial toxicity due to inhibition of the mitochondrial DNA (mtDNA) polymerase gamma. As mtDNA replication decreases and is eventually depleted from NRTI exposure, the expression of mtDNA-encoded proteins necessary for mitochondrial respiration is severely decreased and leads to mitochondrial dysfunction, compromising the cell's ability to produce energy and ultimately to survive. While methods have been previously established to assess the effects of drugs on mtDNA content, effective approaches to acquire upstream temporal information about the status of mtDNA replication has been elusive.

By use of the exogenous addition of 5-ethynyl-2′-deoxyuridine (EdU), a thymidine analog which contains an alkyne functional group detectable with click chemistry, measurements of newly synthesized mtDNA can be made. Click-based chemistry provides an elegant mechanism for attaching a small molecule reporter to an incorporated thymidine analog. The practice of its use for labeling nuclear DNA has been well established over the last three years; however, applications for its use in labeling mtDNA are only now being developed.

When EdU treatment is combined with addition of aphidicolin, a polymerase alpha inhibitor, nuclear DNA replication is effectively reduced, enabling quantitation of nascent mtDNA synthesis. This method, combined with other fluorescence-based detection approaches, including the HCS Mitochondrial Health assay (Invitrogen) and various antibodies, creates an effective new multi-parametric strategy for determining the effect of drug treatments on mtDNA replication rates and mitotoxicity in general. Given the sensitivity with which mitochondrial status can indicate pre-lethal cytotoxicity and the amenability of this method to combination with other fixed endpoint measurements by high content imaging and analysis, this approach has particular applicability in the development of less toxic NRTIs.