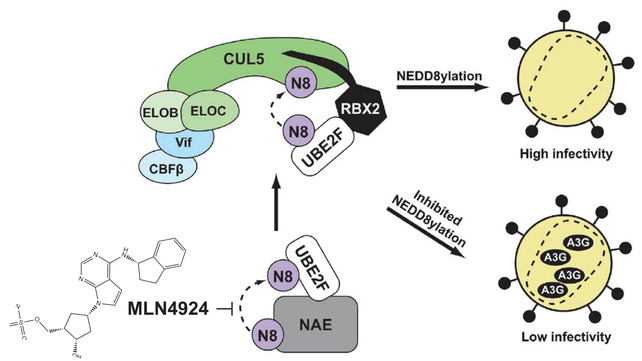
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MLN4924 Reduces HIV Transcription, Cell Survival, and HIV Infectivity After Latency Reversal In Vitro

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BACKGROUND & HYPOTHESIS



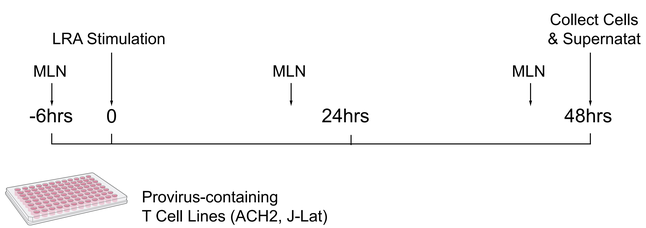
Adapted from Stanley et al. (2012)

Neddylation is a post- translational modification that is essential for regulation of many cellular processes, including transcription, by controlling protein degradation.

Neddylation activates Cullin-RING Ligases (CRLs) which polyubiquitinate proteins prior to proteasomal degradation. We studied the NEDD8-activating enzyme inhibitor MLN4924 (MLN), also known as pevonedistat, which can block degradation of HIV restriction factor APOBEC3G in newly infected cells by preventing the neddylation of CRL-Vif (1, 2). We hypothesized that inhibiting neddylation would also blunt infectivity of HIV-1 reactivated from latency.

We found that inhibiting neddylation reduced provirus transcription and virion production after latency reversing agents (LRAs) in provirus-containing T cell lines, as well as reducing virion infectivity. MLN also induced apoptosis of proliferating CD4+ T cells.

EXPERIMENTAL DESIGN



FUNDING

- NIH P01 5P01AI131346-04
- Immunology & Molecular Pathogenesis Training Program T32 (AI007476)
- Third Coast Center for AIDS Research, an NIH funded center (P30 AI117943)
- The Driskill Graduate Program in the Life Sciences, Northwestern University

RESULTS

FIGURE 1: MLN Reduces Intracellular p24

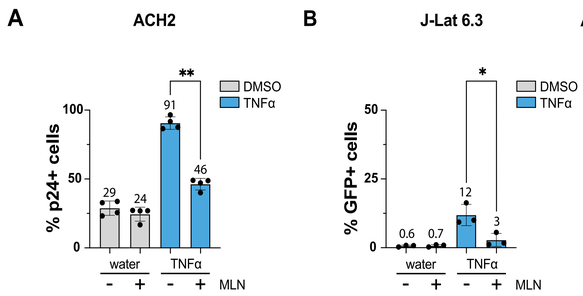


Fig. 1: Intracellular HIV p24 (or LTR-driven GFP) expression of ACH2 and J-Lat 6.3 cells measured by flow cytometry. Cells were treated for 48hrs with 10 ng/mL TNFα (blue) or water (grey) after 6 hr pretreatment with DMSO or 200 nM MLN. **A)** ACH2 cells **B)** J-Lat 6.3 **A-B)** n=4 biological replicates. Error bars show SD of biological replicates. * p<0.05, ** p<0.01.

FIGURE 2: MLN Reduces HIV RNA & LTR Initiation

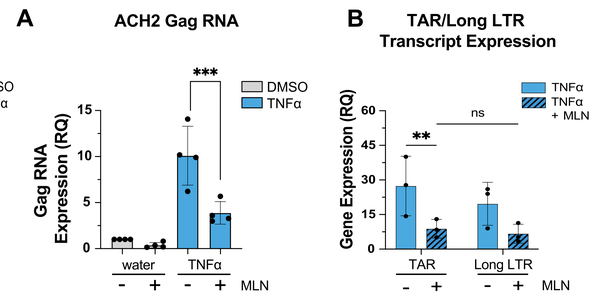


Fig. 2: A) HIV Gag RNA is reduced in ACH2 cells treated as in Figure 1 with 200 nM MLN. n=4 *** p<0.0005. **B)** TAR and Long LTR transcripts in ACH2 cells treated as in A. Decreased TAR with 200 nM MLN treatment signifies reduced transcriptional initiation at the HIV LTR. Comparable levels of TAR and Long LTR suggest no stalled elongation. n=3, ** p<0.0001.

FIGURE 3: MLN Reduces HIV Virion Infectivity

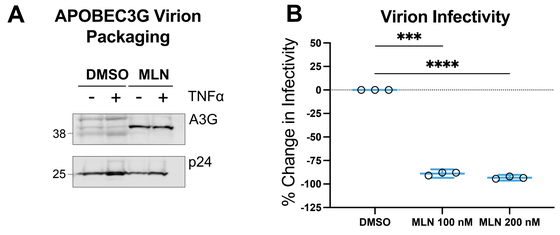


Fig. 3: A) A3G is increased in virions pelleted from ACH2 cells pretreated with 200 nM MLN **B)** Percent change in infectivity of virions from MLN (or DMSO) treated ACH2 cells treated as in A. Error bars show the 95% confidence interval of 3 biological replicates completed in technical triplicate. *** p<0.0002, **** p<0.0001.

FIGURE 4: MLN Induces Apoptosis in TCR-Activated, Uninfected CD4+ T Cells

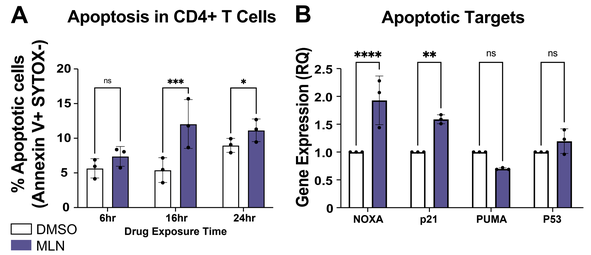


Fig. 4: A) CD4 T cells from 3 uninfected donors were activated for 48 hrs with anti-CD3/CD28 antibodies. Cells were then treated with 200 nM MLN or DMSO. Apoptosis was measured by flow cytometry after 6, 16, and 24hrs. **B)** Gene targets quantified via RT-qPCR. RNA was extracted 48 hrs after anti-CD3/28 stimulation. n=3. **A-D)** * p<0.05, ** p<0.005, *** p<0.0005.

FIGURE 5: MLN Reduces tat/rev msRNA in CD4+ T cells from ART-Suppressed PLWH

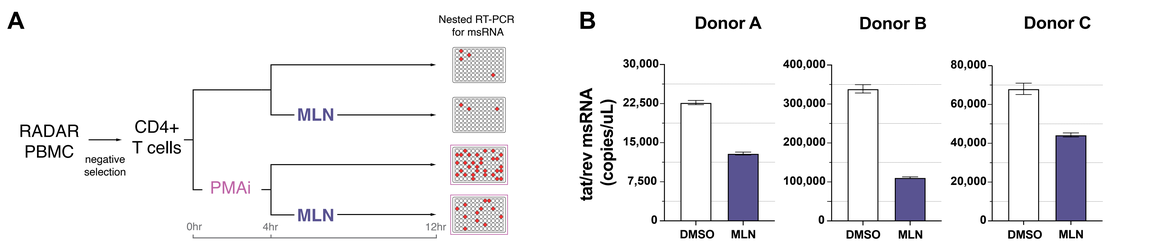


Fig. 5: MLN decreased reactivation of provirus by PMA and ionomycin (PMAi) from CD4+ T cells from ART-suppressed PLWH. **A)** Experimental set up of the TILDA assay (3). CD4+ T cells were treated with PMAi or DMSO for 12 hrs total. After 4 hrs, either 200 nM MLN or DMSO was added to cells for the remaining 8 hrs. A nested digital PCR platform was used to amplify tat/rev msRNA from each well and the Applied Biosystems QuantStudio Absolute Q Digital PCR System was used to quantify RNA copies/uL. **B)** msRNA copies/µL from CD4+T cells after PMAi stimulation (pink in panel A) followed by either DMSO (white bars) or MLN (purple bars). Error bars show 95% confidence interval.

CITATIONS

1) Stanley, D. J. et al. Inhibition of a NEDD8 Cascade Restores Restriction of HIV by APOBEC3G. PLoS Pathog 8, e1003085, doi:10.1371/journal.ppat.1003085 (2012).

2) Nekorchuk, M. D., Sharifi, H. J., Furuya, A. K., Jellinger, R. & de Noronha, C. M. HIV relies on neddylation for ubiquitin ligase-mediated functions. Retrovirology 10, 138, doi:10.1186/1742-4690-10-138 (2013).

3) Lungu C, Procopio FA, Overmars RJ, Beerkens RJJ, Voermans JJC, Rao S, Prins HAB, Rokx C, Pantaleo G, Vijver D, Mahmoudi T, Boucher CAB, Gruters RA, Kampen J. 2020. Inter-Laboratory Reproducibility of Inducible HIV-1 Reservoir Quantification by TILDA. Viruses 12:973.

CONCLUSIONS

Inhibiting neddylation:

- **decreases LRA-driven HIV transcription and virus production, & virion infectivity, in provirus containing T cell lines**
- **reduces latency reversal after PMA & ionomycin in CD4+ T cells from ART-suppressed PLWH**
- **induces apoptosis in TCR-activated, uninfected CD4+ T cells, but not in resting CD4+ T cells**

FUTURE DIRECTIONS

This work has been repeated using other LRAs, PMA and ionomycin, and BET-bromoinhibitor JQ1, with comparable results. Testing of additional LRAs, such as HDAC inhibitors, would clarify whether the impact of inhibiting neddylation will dampen HIV reactivation after other LRA classes. Our work highlights neddylation as a targetable mechanism to modulate proviral reactivation by LRAs, and MLN as a potential method to diminish reservoir replenishment after stopping ART. Investigation of the specific CRLs and their substrate proteins which are contributing to this phenotype could reveal novel pathways that could be interrogated in future HIV cure studies.

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