Antiviral Research 217 (2023) 105698

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Inhibition of SIRT2 promotes death of human cytomegalovirus-infected peripheral blood monocytes via apoptosis and necroptosis

Jennifer Cheung [a], Stacy Remiszewski [b], Lillian W. Chiang [b], Ejaz Ahmad [c], Mohan Pal [c], SM Ashikur Rahman [c], Zaneta Nikolovska-Coleska [c,d], Gary C. Chan [a,*]

[a] Department of Microbiology & Immunology, SUNY Upstate Medical University, Syracuse, NY, 13210, USA
[b] Evrys Bio, LLC, Pennsylvania Biotechnology Center, Doylestown, PA, 18902, USA
[c] Department of Pathology, University of Michigan Medical School, Ann Arbor, MI, 48109, USA
[d] Rogel Cancer Center, University of Michigan Medical School, Ann Arbor, MI, USA

ARTICLE INFO

Keywords:
Cytomegalovirus
Apoptosis
Necroptosis
Small-molecule inhibitor
Sirtuin 2
Monocyte

ABSTRACT

Peripheral blood monocytes are the cells predominantly responsible for systemic dissemination of human cytomegalovirus (HCMV) and a significant cause of morbidity and mortality in immunocompromised patients. HCMV establishes a silent/quiescent infection in monocytes, which is defined by the lack of viral replication and lytic gene expression. The absence of replication shields the virus within infected monocytes from the current available antiviral drugs that are designed to suppress active replication. Our previous work has shown that HCMV stimulates a noncanonical phosphorylation of Akt and the subsequent upregulation of a distinct subset of prosurvival proteins in normally short-lived monocytes. In this study, we found that SIRT2 activity is required for the unique activation profile of Akt induced within HCMV-infected monocytes. Importantly, both therapeutic and prophylactic treatment with a novel SIRT2 inhibitor, FLS-379, promoted death of infected monocytes via both the apoptotic and necroptotic cell death pathways. Mechanistically, SIRT2 inhibition reduced expression of Mcl-1, an Akt-dependent antiapoptotic Bcl-2 family member, and enhanced activation of MLKL, the executioner kinase of necroptosis. We have previously reported HCMV to block necroptosis by stimulating cellular autophagy. Here, we additionally demonstrate that inhibition of SIRT2 suppressed Akt-dependent HCMV-induced autophagy leading to necroptosis of infected monocytes. Overall, our data show that SIRT2 inhibition can simultaneously promote death of quiescently infected monocytes by two distinct death pathways, apoptosis and necroptosis, which may be vital for limiting viral dissemination to peripheral organs in immunosuppressed patients.

1. Introduction

Human cytomegalovirus (HCMV) is a member of the *Herpesviridae* family of viruses with seropositivity rates ranging from 40 to 100% in adults worldwide (Cannon et al., 2010; Zuhair et al., 2019). HCMV infection is typically asymptomatic in immunocompetent individuals but can cause mononucleosis-like symptoms (Fiala et al., 1977). HCMV is also linked to various inflammatory conditions, such as atherosclerosis, and certain cancers, including breast cancer and glioblastomas (Cobbs et al., 2002; Geisler et al., 2019; Nikitskaya et al., 2016; Rahman et al., 2019). However, HCMV is a significant cause of morbidity and mortality in immunocompromised patients (Emery, 2001). Due to the immunosuppression necessary to prevent organ rejection, patients receiving bone marrow or solid organ transplants are among the most vulnerable to severe HCMV disease. HCMV is the most common viral opportunistic infection following hematopoietic stem cell and solid organ transplants, with disease incidence reaching 50–75% of lung transplant and 50% of kidney transplant recipients (Azevedo et al., 2015; Patel and Paya, 1997). In these vulnerable patient populations, HCMV can spread to and trigger inflammation in a multitude of organ systems, including the lungs, brain, esophagus, intestines, and eyes, potentially leading to multi-organ failure, organ rejection, or even death

Abbreviations: MOI, multiplicity of infection; SSC, side scatter; FSC, forward scatter; SRB, Sulforhodamine B; p, phospho; HPI, hours post-infection; DPI, days post-infection; h, hour; RT, room temperature; SEM, standard error of the mean; G, gravitational force; AV, Annexin-V; PI, propidium iodide.

* Corresponding author. Department of Microbiology and Immunology, SUNY Upstate Medical University, 750 East Adams Street, Syracuse, NY, 13210, USA.
E-mail address: chang@upstate.edu (G.C. Chan).

https://doi.org/10.1016/j.antiviral.2023.105698
Received 24 May 2023; Received in revised form 2 August 2023; Accepted 4 August 2023
Available online 9 August 2023

(Azevedo et al., 2015). Preventing systemic dissemination of the virus is an integral component to relieving the disease burden caused by HCMV.

During acute HCMV infection, monocytes are key cells that mediate systemic dissemination (Chan et al., 2012a; Manez et al., 1996; Smith et al., 2004a; Taylor-Wiedeman et al., 1994). HCMV establishes a quiescent infection in monocytes, during which no viral lytic genes are expressed and productive viral replication is stalled (Hargett and Shenk, 2010; Krishna et al., 2017; Rossetto et al., 2013; Sinclair and Sissons, 1996; Smith et al., 2021; Wagenknecht et al., 2015). Subsequently, the virus uses monocytes as "Trojan horses" to disseminate throughout the body while evading the immune system (Elder et al., 2019). Upon extravasation into tissues, infected monocytes differentiate into long-lived, replication-permissive macrophages allowing for infection of organs (Chan et al., 2008a, 2012a, 2012b; Cojohari et al., 2020; Min et al., 2020; Smith et al., 2004a; Soderberg-Naucler et al., 2001). This viral dissemination strategy limits the effectiveness of current HCMV antivirals. At present, the limited repertoire of antiviral therapeutics approved for HCMV—ganciclovir, valganciclovir, maribavir, cidofovir, foscarnet, and letermovir—all exclusively block the lytic phase of HCMV's lifecycle and are unable to target quiescently infected monocytes to prevent spread (Crumpacker, 1992; Kendle and Fan-Havard, 1998; Maertens et al., 2019; Matthews and Boehme, 1988; Patil et al., 2010; Verghese and Schleiss, 2013). Consequently, while prophylactic and pre-emptive use of HCMV antivirals has dramatically reduced the incidence of early HCMV disease, rebound infection and late-onset disease remains a significant issue once antiviral therapy has been discontinued in organ transplant recipients (Boeckh et al., 2003; Singh, 2005). In addition, severe drug-associated toxicities from long-term use of HCMV antivirals and the rise of drug-resistant HCMV strains have further increased the need for novel antiviral therapies to reduce the disease burden of HCMV (Jacobson, 1992; Kendle and Fan-Havard, 1998; Perrottet et al., 2009). One promising avenue of drug development is the targeting of host proteins modulated by HCMV infection and vital to the survival of infected cells.

A key biological hurdle that HCMV must overcome is extending the normally short 48–72 h lifespan of monocytes without the support of viral antiapoptotic proteins (Patel et al., 2017; Whitelaw, 1966). Apoptosis is the major pathway through which surveilling monocytes are programmed to die after entering circulation from the bone marrow in the absence of differentiation stimuli (Mangan et al., 1993). HCMV rapidly blocks the intrinsic biological programming of monocytes to undergo apoptosis (Chan et al., 2010; Cojohari et al., 2016; Collins-McMillen et al., 2018; Collins-McMillen et al., 2015; Peppenelli et al., 2016; Peppenelli et al., 2018; Reeves et al., 2012). HCMV-induced antiapoptotic mechanisms triggered within infected monocytes occur through binding of viral glycoproteins to host cellular receptors (Chan et al., 2010; Mahmud et al., 2020; Peppenelli et al., 2016; Yurochko et al., 1992, 1997). The distinct receptor combination and subsequent kinetics of activation following engagement of the virion to the cell surface initiates a noncanonical PI3K/Akt signaling cascade, which leads to the preferential phosphorylation of Akt at residue S473 (Cojohari et al., 2016; Mahmud et al., 2020; Peppenelli et al., 2018). This unique Akt phosphorylation signature mediates an HCMV-specific transcriptional and translational profile, leading to the upregulation of a distinct subset of antiapoptotic proteins necessary for the survival of HCMV-infected monocytes (Cojohari et al., 2016; Mahmud et al., 2020; Peppenelli et al., 2018). However, once apoptosis is inhibited, infected monocytes initiate necroptosis, a "trapdoor" cell death pathway as an antiviral countermeasure (Mocarski et al., 2015), which can be rapidly suppressed by HCMV in monocytes (Altman et al., 2020), and in other cell types (Fletcher-Etherington et al., 2020; Upton and Chan, 2014; Upton et al., 2008, 2010, 2012). Akt is a known regulator of necroptosis and in certain cases is required for its suppression (Liu et al., 2014; McNamara et al., 2013). Together, these studies suggest that targeting the Akt pathway could prevent HCMV from impeding both apoptosis and necroptosis, thus destining infected monocytes for cell death.

Sirtuins (SIRTs) are a family of seven evolutionarily conserved NAD^+-dependent deacylases and have been implicated in both apoptosis and necroptosis (Ding and Hao, 2021; Kozako et al., 2018; Luo et al., 2019; Song et al., 2021; Xu et al., 2019). Sirtuins are able to modulate Akt activity by directly deacetylating the pleckstrin homology (PH) domain of Akt, enabling its localization to the cell membrane and subsequent activation (Sundaresan et al., 2011). SIRT2 is the predominantly cytoplasmic member of the sirtuin family, which directly binds to and is required for the full activation of Akt (Dan et al., 2012; Ramakrishnan et al., 2014). FLS-359 is a newly described allosteric SIRT2 modulator that inhibits the growth and spread of HCMV in cultured fibroblasts and mouse models of infection (Roche et al., 2023). In biochemical assays, the drug binds to SIRT2 and alters its thermal denaturation profile; it is selective for inhibition of SIRT2 deacetylation versus Sirt1 and Sirt3, the Sirts most closely related to SIRT2; and it is substrate selective, inhibiting SIRT2 deacetylation but not demyristoylation. The engagement of FLS-359 with SIRT2 was confirmed in an X-ray structure at 1.8 Å resolution; and its biological activity was confirmed by demonstrating that the drug induces hyperacetylation of the SIRT2 target, α-tubulin, and causes degradation of c-Myc, well-known consequences of SIRT2 inhibition (Jing et al., 2016; Liu et al., 2013; North et al., 2003).

We show here that inhibition of SIRT2 using a novel small-molecule inhibitor, FLS-379 (Roche et al., 2023), leads to the death of HCMV-infected monocytes via both the apoptotic and necroptotic pathways. The novel SIRT2 inhibitor exerted its proapoptotic effect through the Akt pathway by blocking HCMV-induced S473 Akt phosphorylation and preventing the downstream upregulation of Mcl-1. Concurrently, SIRT2 inhibition led to increased activation of MLKL, the executioner kinase of necroptosis, following HCMV infection. Taken together, these data support SIRT2 inhibition as a new strategy through which HCMV-infected monocytes can be eliminated by simultaneously counteracting virus-induced antiapoptotic and anti-necroptotic mechanisms.

2. Materials and methods

2.1. Human peripheral blood monocyte isolation and culture

Isolation of human peripheral blood monocytes was performed as previously described (Chan et al., 2010; Smith et al., 2004a; Yurochko and Huang, 1999). Blood was drawn from voluntary random donors by venipuncture, diluted in RPMI-1640 media (ATCC, Manassas, VA), and centrifuged through Ficoll-Paque cell separation medium (Cytiva, Uppsala, Sweden) to remove red blood cells and neutrophils. Mononuclear cells were collected and washed with saline to remove the platelets and then separated by centrifugation through a Percoll (GE Healthcare, Wilkes-Barre, PA) gradient (40% and 48.6%). More than 95% of isolated peripheral blood mononuclear cells were monocytes, as determined by $CD14^+$ or $CD16^+$ staining. The cells were washed with saline, resuspended in RPMI-1640 medium, and counted. All experiments were performed in 0%–0.5% human serum at 37 °C in a 5% CO_2 incubator, unless otherwise stated. SUNY Upstate Medical University Institutional Review Board and Health Insurance Portability and Accountability Act guidelines for the use of human subjects were followed for all experimental protocols in our study. For the inhibitor studies, the following reagents were used: FLS-359 and FLSX-008 (SIRT2 inhibitors from Evrys Bio) (Roche et al., 2023) and UM-116 (an Mcl-1 inhibitor from the Nikolovska-Coleska lab).

2.2. Virus preparation and infection

Human embryonic lung (HEL) 299 fibroblasts (CCL-137; American Type Culture Collection, Manassas, VA) were cultured in Dulbecco modified Eagle medium (DMEM) (Corning, Manassas, VA) with 100 U/mL Penicillin and 100 μg/mL Streptomycin (Life Technologies, Carlsbad, CA), 2.5 μg/mL Plasmocin (Invivogen, San Diego, CA), and 10%

fetal bovine serum (FBS) (Sigma). Upon reaching confluence, the fibroblasts were infected with HCMV (strain TB40/E) in DMEM supplemented with 4% FBS. Virus was purified from the supernatant on a 20% sorbitol cushion to remove cellular contaminants and resuspended in RPMI-1640 medium. A multiplicity of infection (MOI) of 5 was used for each experiment, as >99% of monocytes were infected with TB40E particles (Chan et al., 2009b). Mock infection was performed by adding an equivalent volume of RPMI-1640 medium to monocytes.

2.3. Flow cytometry

Monocytes were washed in phosphate-buffered saline (PBS) and stained with fluorescein isothiocyanate (FITC)-annexin V (Life Technologies) and propidium iodide (PI) stain (Life Technologies) to detect dead and dying cells. After staining, the cells were analyzed by flow cytometry using an LSRFortessa cell analyzer and BD FACSDiva software (BD Biosciences, Franklin Lakes, NJ). Our gating strategy on forward scatter (FSC)/side scatter (SSC) was set to include both cells in the early stages of apoptosis (decreased FSC and increased SSC compared to those for viable cells) and cells in the late stages of apoptosis (decreased FSC and decreased SSC compared to those of viable cells).

2.4. Sulforhodamine B (SRB) cytotoxicity assay

Monocytes were plated in 96-well plates and cultured in RPMI-1640 media supplemented with 0.1% human serum. Cytotoxicity was measured using an SRB assay (Abcam, Cambridge, UK). Briefly, the monocytes were fixed for 1 h (h) at 4 °C, the wells were gently washed 3 times with dH_2O, and the adherent cells were stained with SRB staining solution for 15 min protected from light. The stain was removed, and the wells were washed 4 times with washing solution. The protein-bound dye was solubilized with solubilization solution, and the absorbance was measured at 565 nm.

2.5. Western blot analysis

Monocytes were harvested in a modified radioimmunoprecipitation assay (RIPA) buffer (50 mM Tris-HCl [pH 7.5], 5 mM EDTA, 100 mM NaCl, 1% Triton X-100, 0.1% SDS, 10% glycerol) supplemented with protease inhibitor cocktail (Sigma) and phosphatase inhibitor cocktails 2 and 3 (Sigma) for 30 min (min) on ice. The lysates were cleared from the cell debris by centrifugation at 4 °C (5 min, 21130×*g*) and stored at −20 °C until further analysis. Protein samples were solubilized in Laemmli SDS-sample nonreducing (6×) buffer (Boston Bioproducts, Boston, MA) supplemented with β-mercaptoethanol (Amresco, Solon, OH) by incubating at 95 °C for 10 min. Equal amounts of total protein from each sample were loaded in each well, separated by SDS-polyacrylamide gel electrophoresis, and transferred to polyvinylidene difluoride membranes (Bio-Rad, Hercules, CA). Blots were blocked in 5% bovine serum albumin (BSA) (Fisher Scientific, Waltham, MA) for 1 h at room temperature (RT) and then incubated with primary antibodies overnight at 4 °C. The following antibodies were used: anti-Akt, anti-phospho (p)-Akt (S473), anti-p-Akt (T308), anti-Mcl-1, anti-RIP3, anti-p-RIP3, anti-MLKL, and anti-p-MLKL (Cell Signaling, Danvers, MA). The blots were then incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (Cell Signaling), and chemiluminescence was detected using the Amersham ECL Prime Western Blotting Detection reagent (GE Healthcare).

2.6. Immunoprecipitation

Monocytes were pretreated with a Mcl-1 inhibitor or DMSO vehicle for 1 h, and then infected with HCMV for 24 h. Monocyte lysates were harvested in NP-40 lysis buffer (1 mM DTT, 50 mM Tris-HCl [pH 7.4], 1 mM EDTA, 150 mM NaCl, 0.5% (v/v) NP-40) supplemented with protease inhibitor cocktail (Sigma-Aldrich) and phosphatase inhibitor cocktails 2 and 3 (Sigma-Aldrich) for 30 min on ice. The lysates were cleared from the cell debris by centrifugation at 4 °C (5 min, 21130×*g*). Input controls were collected and stored at −20 °C until further analysis. Lysates equivalent to 160 μg of total protein were incubated with 2 μg of antibody recognizing Mcl-1 (Invitrogen, Waltham, MA) or IgG isotype controls (Santa Cruz, Dallas, TX) overnight at 4 °C. Dynabeads protein G (Invitrogen) were added to the lysates and incubated at 4 °C for 4 h. The protein G beads with bound protein complexes were magnetically separated and washed with lysis buffer, followed by elution of the protein complexes. The samples were then prepared for SDS-polyacrylamide gel electrophoresis and analyzed by Western blot analysis.

2.7. Synthesis and characterization of UM-116

The UM-116 Mcl-1 inhibitor was synthesized through a presented route in Supplemental Fig. S1. Detailed chemistry and characterization are provided in the supplemental material and Supplemental Fig. S2.

2.8. Fluorescence polarization (FP) binding assay

Sensitive and quantitative FP-based binding assay was developed and optimized to determine the binding affinity of small-molecule inhibitors to the recombinant Mcl-1 protein. Fluorescent labeled Bid BH3-peptide, FAM-ϵ-Ahx-Bid peptide (QEDIIRNIARHLAQVGDSMDR), was used as a competitive probe. The assay buffer was 20 mM phosphate (pH 7.4), 50 mM NaCl, 1 mM EDTA, and 0.05% Pluronic F68 with a final DMSO concentration of 4%. All experiments were performed in a final volume of 125 μL using black 96-well plates (Corning no. 3792) and analyzed with Synergy H1 Hybrid BioTek plate reader at excitation and emission wavelengths of 485 nm and 530 nm, respectively, after incubation for 3 h at room temperature. Dissociation constant (K_d) of the FAM-BID was determined to be 5.99 ± 1.12 nM, demonstrating stable signal up to 24h (Fig. S3). Based on the K_d value, the concentration of the Mcl-1 protein used in the competitive binding experiments was 20 nM and the fluorescent probe, FAM-BID, was fixed at 2 nM. IC_{50} values were determined by nonlinear regression fitting of the competition curves (GraphPad Prism 7.0 Software) and converted into K_i values as previously described (Nikolovska-Coleska et al., 2004).

2.9. Bio-layer interferometry (BLI)

To confirm and determine the direct binding of UM-116 to Mcl-1 protein, the BLI experiments were performed as per our previous report (Kump et al., 2020) at room temperature on OctetRED96 (PALL/ForteBio) in black 96-well plates (Greiner bio-one, # 655209). The biotinylated Mcl-1 (10 μg/ml) was immobilized and saturated on Superstreptavidin (SSA) Biosensors (Sartorius, #18–5057) for 10 min. Association and dissociation cycles were fixed for 10 min. The same FP binding assay buffer (20 mM phosphate pH 7.4, 50 mM NaCl, 1 mM EDTA, and 0.05% Pluronic F68) was used for custom, baseline, and dissociation steps, while buffer containing serially diluted compound, UM-116 with final DMSO concentration of 4% DMSO was used for association. Association and dissociation cycles were fixed at 10 min each. Kinetic data were collected and processed with the Data Analysis software provided by ForteBio. All experiments were analyzed with referencing by subtraction of the buffer sensorgrams. Plotting the response nm values of the binding sensorgrams with the respective compound concentration was used for steady state analysis and calculation of the K_d value.

2.10. Immunofluorescence

Cell monolayers plated in glass-bottom plates were fixed for 15 min in 4% paraformaldehyde (PFA) (Sigma) in PBS, and then washed three times with PBS. Cell permeabilization and blocking of nonspecific

binding were performed by incubating the cells with 0.1% Triton X-100, 5% BSA, and 1:10 FcR blocking reagent (Miltenyi, Bergisch Gladbach, Germany) in PBS for 30 min at room temperature. The cells were then incubated overnight at 4 °C in a humidified chamber with an anti-LC3 antibody (Cell Signaling). Cells were washed in PBS and then incubated with an anti-rabbit secondary antibody conjugated to Alexa Fluor 488 (Cell Signaling) for 1 h, protected from light. Cells were washed in PBS and then incubated with Hoechst 33342 nuclear stain (Thermo Fisher) for 10 min before being analyzed on a Nikon Eclipse Ti80 epifluorescence microscope (Nikon, Melville, NY). Digitized images were resized, organized, thresholded, and labeled using ImageJ software, an open Java-based image processing program developed at the National Institutes of Health. Puncta per cell were counted from at least 30 unique cells per donor per treatment. The mean puncta per cell was then calculated.

2.11. Statistical analysis

All experiments were performed independently a minimum of 3 times using primary monocytes isolated from different blood donors. Survival data sets obtained from primary monocytes inherently have substantial variation due to donor variability. Consequently, data are displayed as matched experimental data points from individual donors in a side-by-side comparison. Data were analyzed using a two-way Student's *t*-test comparison or two-way ANOVA with GraphPad Prism software (GraphPad) and expressed as the mean ± the standard error of the mean (SEM). P values of less than 0.05 were considered statistically significant.

3. Results

3.1. Inhibition of SIRT2 triggers death of HCMV-infected monocytes

HCMV induces the survival of infected monocytes in the absence of viral lytic protein expression via viral glycoprotein binding to cellular surface receptors (Chan et al., 2010; Cojohari et al., 2016; Collins-McMillen et al., 2018; Collins-McMillen et al., 2015; Mahmud et al., 2020; Peppenelli et al., 2016; Peppenelli et al., 2018; Stevenson et al., 2014). This interaction triggers a noncanonical activation of the Akt signaling pathway, stimulating a preferential phosphorylation of Akt at S473 that is distinct from the T308/S473 phosphorylation ratio induced by normal myeloid growth factors (Cojohari et al., 2016; Mahmud et al., 2020; Peppenelli et al., 2018). HCMV-activated Akt leads to the upregulation of a unique subset of cellular antiapoptotic proteins (Cojohari et al., 2016; Mahmud et al., 2020; Peppenelli et al., 2018). SIRT2 directly binds and deacetylates Akt allowing for the phosphorylation and

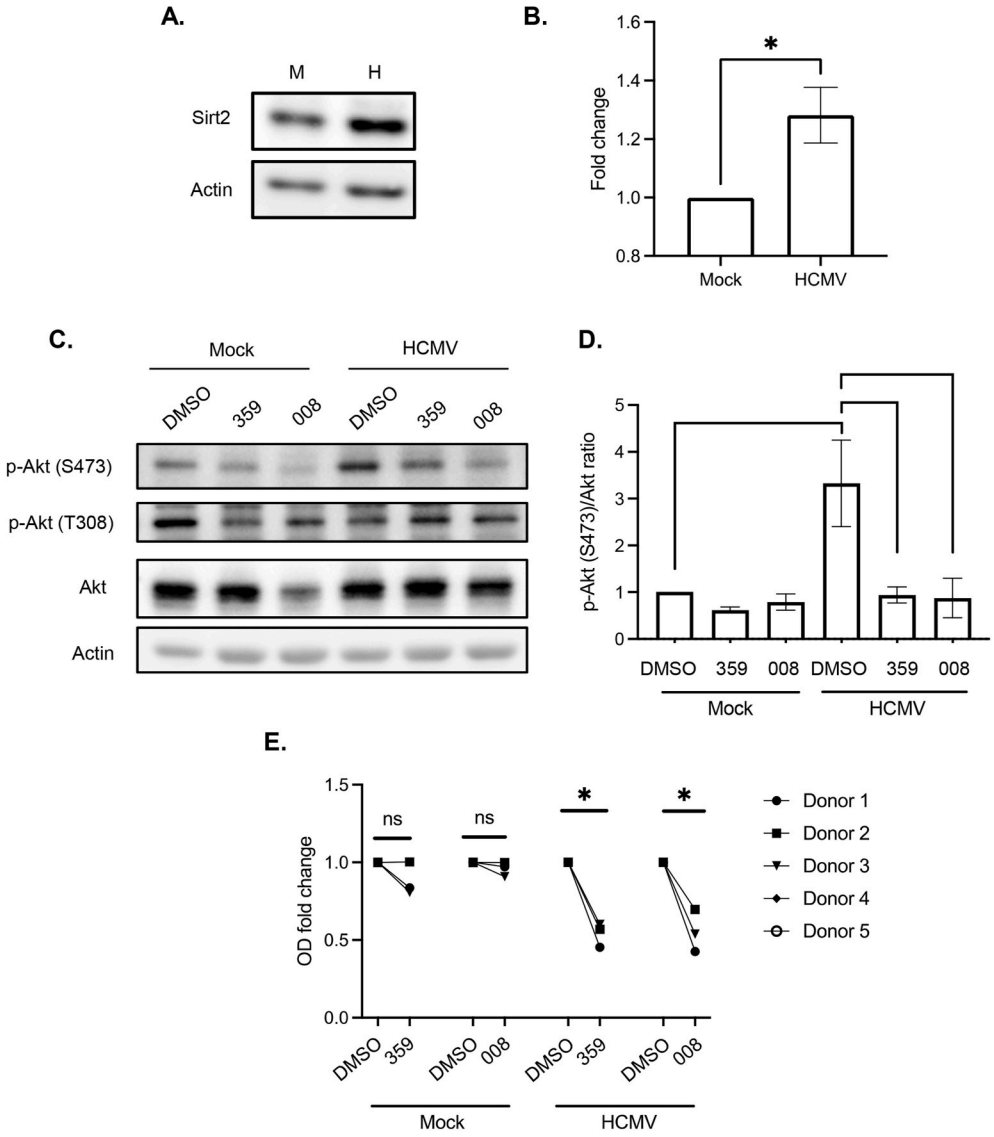

Fig. 1. Inhibition of SIRT2 promotes death of HCMV-infected monocytes. (A, B) Primary monocytes were harvested from peripheral blood and mock or HCMV infected for 24 h (h). Expression levels of SIRT2 were determined by immunoblotting. Membranes were probed for β-actin as a loading control. (C, D) Monocytes were treated with DMSO (vehicle control), 5 μM FLS-359 (359), or 5 μM FLSX-008 (008) (SIRT2 inhibitors) starting 1 h prior to infection, then mock or HCMV infected for 24 h. Expression levels of Akt and p-Akt were determined by immunoblotting. (E) Primary monocytes were mock infected or HCMV infected for 72 h and then treated with 5 μM FLS-359 or FLSX-008 at 1 dpi. Cells were subjected to an SRB cytotoxicity assay and the OD measured at 565 nm. The fold change in OD was normalized to the mock-infected DMSO-treated control. Data are representative of 3–5 independent blood donors. *, $p \leq 0.05$.

subsequent activation of Akt in several cell types, including cells of the myeloid lineage (Dan et al., 2012; Ramakrishnan et al., 2014). We found HCMV infection increased the expression of SIRT2 protein relative to mock infection at 24 h post infection (hpi), suggesting SIRT2 involvement in the activation of Akt within HCMV-infected monocytes (Fig. 1A and B). To investigate the role of SIRT2 in promoting the unique Akt activity induced by HCMV following infection of peripheral blood monocytes, we utilized a highly selective small-molecule inhibitor of SIRT2, FLS-359 (Roche et al., 2023). FLSX-008, another SIRT2 inhibitor, was also utilized in these studies to reduce the possibility that the effects of FLS-359 are due to off-target effects. We found that prophylactic treatment with SIRT2 inhibitors attenuated the HCMV-mediated Akt phosphorylation at S473 (Fig. 1C and D). HCMV infection did not stimulate T308 Akt phosphorylation, consistent with our previous findings (Cojohari et al., 2020), and the SIRT2 inhibitors had no effect on basal levels. Given the importance of Akt activity in the enhanced viability of HCMV-infected monocytes, we examined whether SIRT2-mediated Akt activation was necessary for the survival of infected cells. Monocytes were infected with HCMV immediately post-isolation from peripheral blood, followed by treatment with FLS-359, FLSX-008, or vehicle control at 1 dpi (day post-infection), when infected monocytes exhibit maximum viability (Chan et al., 2010; Chan et al., 2012a; Chan et al., 2012b; Collins-McMillen et al., 2015). At 3 dpi, we performed SRB viability assays and observed a significant increase in the death of HCMV-infected cells treated with the SIRT2 inhibitors and minimal death of uninfected cells (Fig. 1E). Taken together, these data indicate SIRT2 as a potential antiviral cellular target to selective eliminate quiescently HCMV-infected monocytes.

3.2. SIRT2 inhibition triggers apoptosis and necroptosis of HCMV-infected monocytes

In the absence of differentiation stimuli, peripheral blood monocytes rapidly undergo apoptosis after 48–72 h in the circulation (Patel et al., 2017; Whitelaw, 1966). When apoptosis is blocked, monocytes can initiate necroptosis as a "trapdoor" death pathway (Altman et al., 2020; Galluzzi et al., 2017; Ke et al., 2016; Lee et al., 2019). However, HCMV can block both pathways to ensure the survival of infected cells, and subsequently the systemic dissemination of the virus (Altman et al., 2020; Chan et al., 2010; Chan et al., 2012b; Cojohari et al., 2016; Collins-McMillen et al., 2018; Collins-McMillen et al., 2015; Peppenelli et al., 2016; Peppenelli et al., 2018; Reeves et al., 2012). Thus, we asked which death pathway were the SIRT2 inhibitors acting through to promote death of infected monocytes. Using annexin-V (AV) as a marker of apoptosis and propidium iodide (PI) as a marker for cell death, flow cytometry was performed to determine monocyte viability, as well as to differentiate between apoptotic and necroptotic cells. In agreement with our previous studies (Altman et al., 2020; Chan et al., 2010, 2012b;

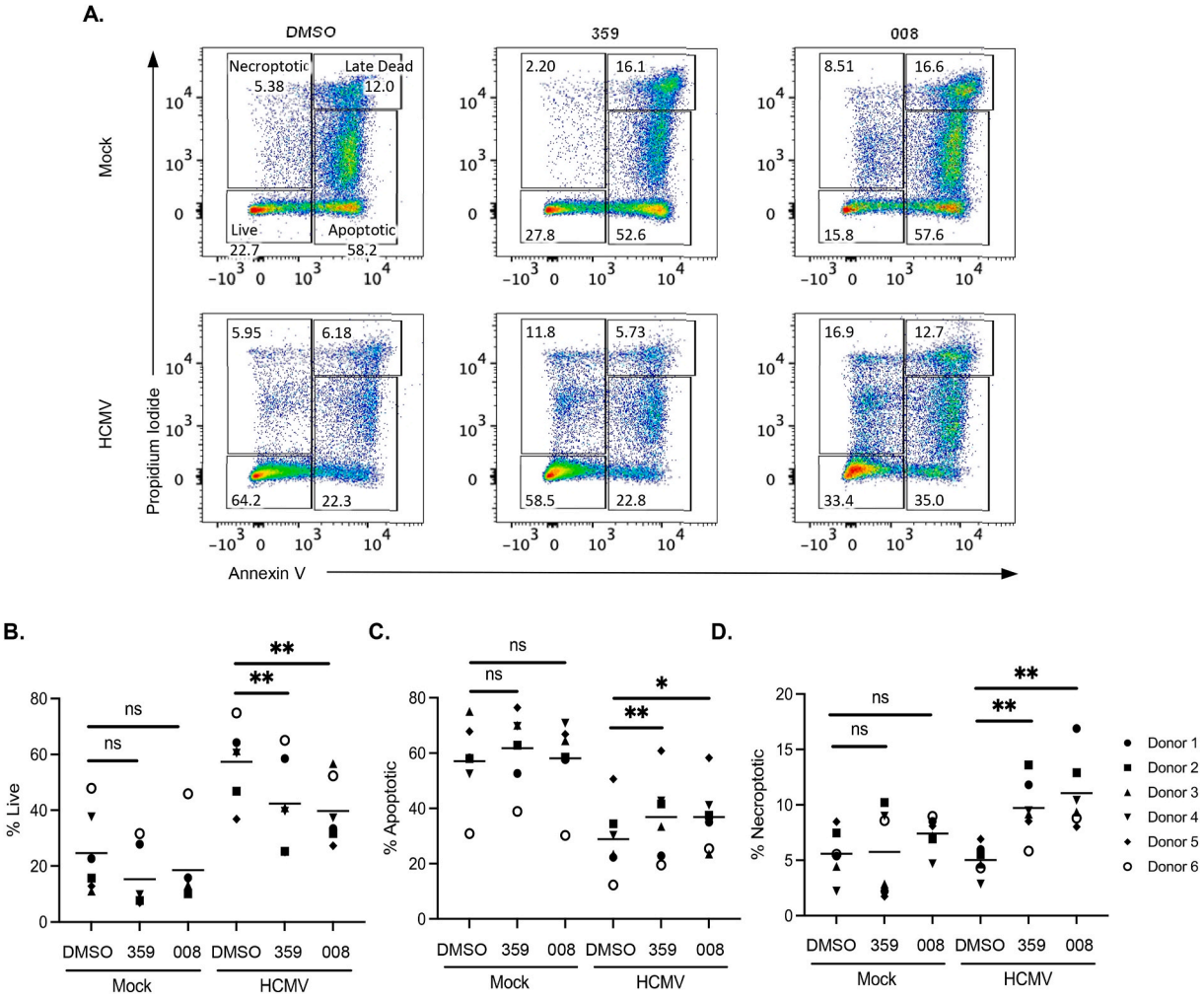

Fig. 2. Therapeutic treatment with SIRT2 inhibitors of HCMV-infected monocytes increases apoptosis and necroptosis. (A–D) Monocytes were mock infected or HCMV infected for 48 h and subsequently treated with DMSO, 5 μM FLS-359, or 5 μM FLSX-008 at 24 hpi. Monocytes were stained with annexin (AV)-FITC and propidium iodide (PI). Viability was analyzed by flow cytometry. Gates represent live (B), apoptotic (C), necroptotic (D), and late dead cell populations. (B–D) Horizontal lines indicate the mean result of the independent experiments. Data are representative of 6 independent blood donors. **, $p \leq 0.01$; *, $p \leq 0.05$.

Cojohari et al., 2016; Peppenelli et al., 2016, 2018), HCMV stimulated an increase in the percentage of live cells (AV negative, PI negative), thus also confirming infection of monocytes (Fig. 2). The inhibitors were then tested as a therapeutic treatment administered to infected monocytes at 24 hpi. Cells were treated with 5 μM of either inhibitor since this concentration abrogated SIRT2-mediated activation of Akt in HCMV-infected monocytes (Fig. 1C and D). Inhibition of SIRT2 shifted cells from the live cell population to both the "apoptotic" (AV positive, PI negative) and "necroptotic" (AV negative, PI positive) gates (Fig. 2A–D). With treatment post-infection, there was a reduction of ~30% of live infected cells in the presence of FLS-359 and FLSX-008 relative to the vehicle-treated control, indicating SIRT2 is necessary for the survival of HCMV-infected monocytes (Fig. 2B). Although uninfected cells appear to have some sensitivity to death induced by SIRT2 inhibition, the decrease in viability was highly variable and not statistically significant, which is likely due to uninfected monocytes already undergoing apoptosis in the absence of infection or activation stimuli (Fig. 2A). Consistent with the decrease in viability of infected monocytes, FLS-359 and FLSX-008 induced a ~1.4-fold increase in apoptosis and a ~2.3-fold increase in necroptosis of HCMV-infected cells (Fig. 2D). There was no significant effect on the levels of apoptosis or necroptosis of the mock-infected cells with SIRT2 inhibition (Fig. 2B–D). Next, we examined the effects of prophylactic treatment of the SIRT2 inhibitors on the viability of infected monocytes. Prophylactic SIRT2 inhibitor treatment reduced the frequency of live infected cells by ~40% (Fig. 3A and B). Concomitantly, FLS-359 and FLSX-008 increased the levels of apoptotic infected cells by ~1.5-fold and necroptotic infected cells by ~2.2-fold (Fig. 3C and D). Prophylactic treatment of mock-infected cells with both SIRT2 inhibitors did not increase apoptosis. However, we observed a significant (or trending towards significant) effect of SIRT2 inhibitors on the viability and necroptosis of mock-infected cells. In contrast to therapeutic treatments, where cells were treated with drugs at 2 dpi, prophylactically treated uninfected cells were given SIRT2 inhibitors immediately upon isolation from peripheral blood, suggesting that SIRT2 may accelerate death of young monocytes exiting the bone marrow but not aged monocytes already circulating in the blood. Regardless, our data clearly demonstrate that SIRT2 inhibitors have enhanced ability to induce death, via apoptosis and necroptosis, of HCMV-infected monocytes relative to uninfected cells under both therapeutic and prophylactic conditions.

To validate that both apoptotic and necroptotic pathways are being activated in HCMV-infected monocytes treated with SIRT2 inhibitors, we treated infected monocytes with UM-116 (an inhibitor of Mcl-1) as a control for stimulation of only the apoptotic pathway. Mcl-1 binds and sequesters proapoptotic members of the Bcl-2 family of proteins, preventing the proapoptotic effector proteins from oligomerizing and forming pores in the mitochondrial outer membrane (Giam et al., 2008; Willis et al., 2007). UM-116 is a novel small-molecule inhibitor that directly binds to Mcl-1 with a K_d value of 1.7 ± 0.2 μM determined by BLI and a IC_{50} value of 0.54 ± 0.11 μM in competitive FP based assay

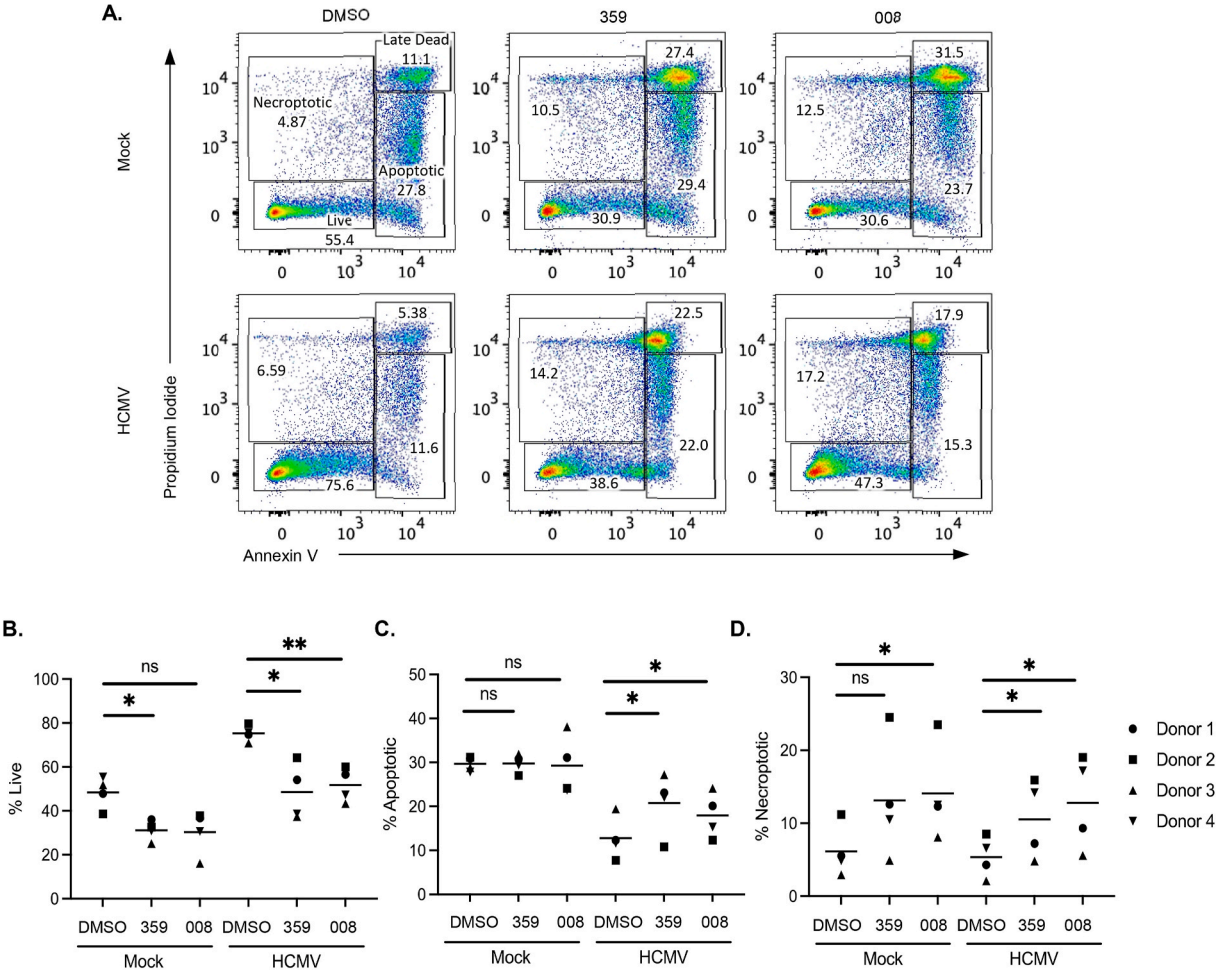

Fig. 3. Prophylactic treatment with SIRT2 inhibitors of HCMV-infected monocytes increases apoptosis and necroptosis. (A–D) Monocytes were pretreated with DMSO, 5 μM FLS-359, or 5 μM FLSX-008 starting 1 h prior to infection. Cells were then mock or HCMV infected for 48 h. Following infection, monocytes were stained with AV-FITC and PI. Viability was analyzed by flow cytometry. Gates represent live (B), apoptotic (C), necroptotic (D), and late dead cell populations. (B–D) Horizontal lines indicate the mean result of the independent experiments. Data are representative of 4 independent blood donors. **, $p \leq 0.01$; *, $p \leq 0.05$.

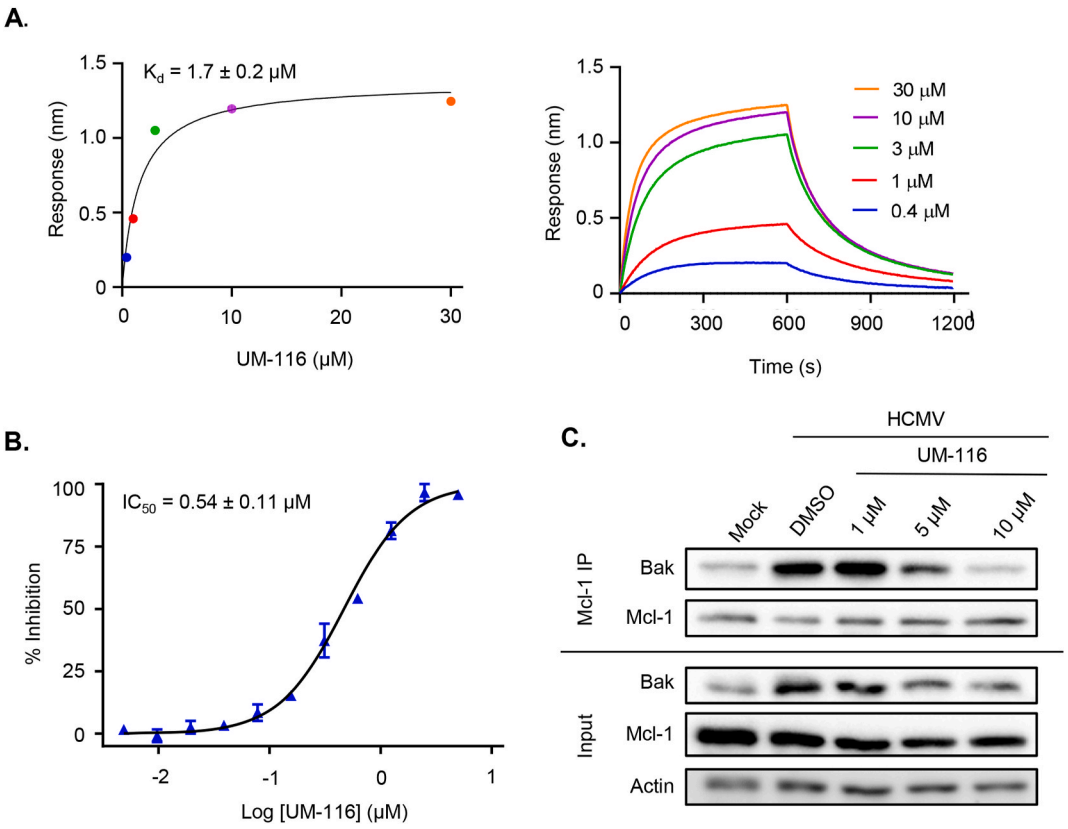

Fig. 4. UM-116 binds to Mcl-1 and disrupts protein-protein interactions with Bak in infected monocytes. (A) The binding affinity of UM-116 was determined by biolayer interferometry (BLI) with the presented sensorgrams of tested concentrations and (B) by competitive fluorescence polarization (FP) assay using fluorescent labeled Bid BH3 peptide. (C) Monocytes were treated with increasing concentrations of UM-116 (a Mcl-1 inhibitor) or DMSO for 1 h, followed by mock or HCMV infection for 24 h. Mcl-1 was immunoprecipitated using an anti-Mcl-1 antibody and the level of Bak bound to Mcl-1 was determined by immunoblotting. Membranes were probed for β-actin as a loading control. Data are representative of at least 3 independent blood donors.

(Fig. 4A and B). We confirmed that UM-116 inhibits Mcl-1 by competitively binding and blocking protein-protein interactions with its binding partner Bak in a dose dependent manner (Fig. 4C). Treatment with UM-116 confirmed that inhibiting Mcl-1 stimulated cell death strictly though apoptosis without a significant increase in necroptosis (Fig. 5). Taken together, our data indicate that SIRT2 inhibition may be a bona fide target to stimulate death of quiescently HCMV-infected monocytes via simultaneous activation of both the apoptotic and necroptotic pathways.

3.3. SIRT2 regulates apoptotic and necroptotic factors within HCMV-infected monocytes

Engagement of HCMV glycoproteins to cellular receptors modulate the phosphorylation ratio between Akt residues S473 and T308, which is known to regulate Akt substrate specificity (Jacinto et al., 2006; Yung et al., 2011), to increase the expression of select prosurvival proteins and the subsequent survival of infected monocytes (Altman et al., 2020; Chan et al., 2010, 2012b; Cojohari et al., 2016; Peppenelli et al., 2016, 2018). Mcl-1 is a critical Akt-dependent antiapoptotic protein that is upregulated following HCMV infection to a much greater extent than following treatment with normal myeloid growth factors (Chan et al., 2010; Peppenelli et al., 2016, 2018). Consequently, we examined whether SIRT2 inhibitors might be exerting their proapoptotic effect on infected monocytes by preventing the Akt-dependent upregulation of Mcl-1. As expected, HCMV infection increased Mcl-1 expression. However, treatment with FLS-359 and FLSX-008 dramatically attenuated the expression of Mcl-1 in HCMV-infected monocytes to levels observed in mock-infected cells (Fig. 6A and B). This abrogation of Mcl-1 expression indicates that SIRT2 inhibition may relieve the block on apoptosis in infected monocytes by preventing HCMV's Akt-dependent upregulation of Mcl-1.

HCMV's circumvention of apoptosis results in the inactivation of caspase 8, which then prompts infected monocytes to initiate necroptosis (Altman et al., 2020). Necroptosis is dependent on the sequential activation of RIPK1, RIPK3, and ultimately MLKL. MLKL acts as the final executioner kinase of the necroptosis pathway by undergoing phosphorylation, oligomerizing, and translocating to form pores in the plasma membrane (Cho et al., 2009). We have previously shown HCMV to stimulate cellular autophagy to block necroptosis between RIPK3 activation and the final MLKL activation steps, ensuring survival of infected monocytes (Altman et al., 2020). Since our data suggests SIRT2 inhibition leads to necroptosis, we examined if SIRT2 inhibition circumvents the blockage between RIPK3 and MLKL within infected monocytes. We confirmed that HCMV infection alone induced activation of RIPK3 relative to mock infection, indicating that infected monocytes have initiated the early steps of necroptosis (Fig. 6C and D). However, SIRT2 inhibition has no effect on RIPK3 activation in the context of infection, suggesting that the pro-necroptotic effect observed with the SIRT2 inhibitors occurs downstream of RIPK3. Indeed, treatment with the SIRT2 inhibitors FLS-359 and FLSX-008 enhanced the phosphorylation of MLKL in HCMV-infected monocytes without significantly affecting phosphorylation levels in mock-infected cells (Fig. 6E and F). Our data here supports a role for SIRT2 during HCMV infection in the regulation of apoptosis, via Akt-dependent expression of Mcl-1, and of necroptosis, via regulation of MLKL activation.

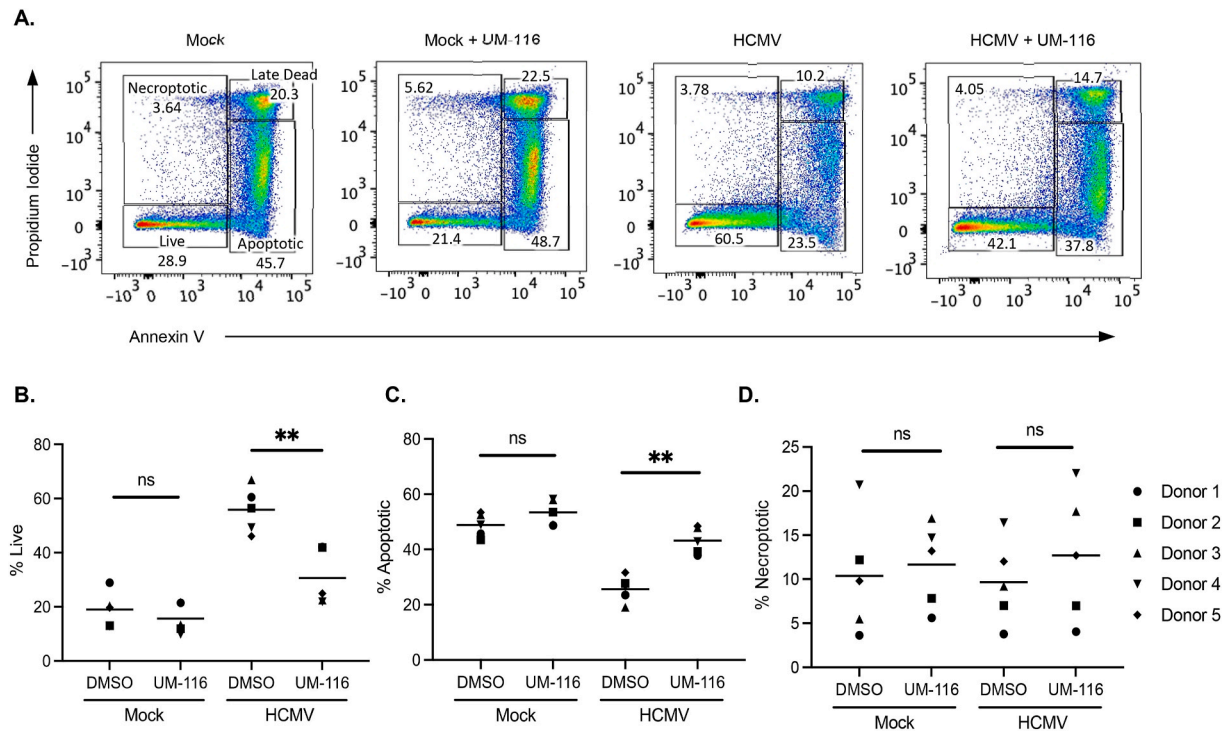

Fig. 5. Mcl-1 inhibition stimulates apoptosis, but not necroptosis, of HCMV-infected monocytes. (A–D) Monocytes were mock infected or HCMV infected for 48 h, followed by treatment with DMSO or 5 μM UM-116 for 24 h. Monocytes were stained with AV-FITC and PI. Cell viability was analyzed by flow cytometry. Gates represent live (B), apoptotic (C), necroptotic (D), and late dead cell populations. (B–D) Horizontal lines indicate the mean result of the independent experiments. Data are representative of 5 independent blood donors. **, $p \leq 0.01$; *, $p \leq 0.05$.

3.4. Formation of autophagosomes during HCMV infection is mediated by Akt

Our group has previously reported that HCMV infection stimulates the necroptotic pathway through RIPK3 phosphorylation, but concomitant induction of cellular autophagy, as determined by measuring autophagic flux and autophagosome accumulation, prevents activation of MLKL and the subsequent execution of necroptosis (Altman et al., 2020). We observed that SIRT2 inhibition promotes MLKL activation without affecting RIPK3 activity. Thus, we sought to determine whether reducing autophagosome accumulation is the mechanism through which the SIRT2 inhibitors relieve the block on necroptosis. After treating monocytes with SIRT2 inhibitors for 1 h and then infecting with HCMV for 2 h, we performed immunofluorescence analysis of LC3-II, a cytoplasmic protein that is lipidated and localized to autophagosomes during their maturation (Tanida et al., 2008). HCMV infection increased the mean number of puncta per cell by 85% relative to mock infection. Treatment with FLS-359 and FLSX-008 resulted in a 37.5% and 40.5% reduction of LC3-II puncta per cell, respectively, compared to the infected vehicle control, indicating a decrease in autophagosome accumulation following SIRT2 inhibition (Fig. 7A and B). Due to the central role that HCMV's unique activation of Akt plays in survival and many other cellular functions, we examined whether SIRT2's regulatory role in autophagosome formation could be mediated by Akt. Treatment with MK2206, an inhibitor of Akt, reduced the average number of LC3-II puncta per cell by 41.1%, mirroring treatment with the SIRT2 inhibitors (Fig. 7A and B). Our data here indicate that the unique activation of Akt by HCMV enhances cellular autophagosome accumulation and that preventing Akt activation, via SIRT2 inhibition, could lead to diminished autophagy resulting in necroptosis of infected monocytes.

4. Discussion

Monocytes are a key cell population responsible for hematogenous dissemination of HCMV, despite not being permissive to viral replication and having a short 48 h lifespan (Chan et al., 2012a; Manez et al., 1996; Patel et al., 2017; Taylor-Wiedeman et al., 1994). HCMV has evolved several mechanisms to prolong the survival of infected monocytes allowing for the differentiation of infected cells into replication permissive macrophages (Altman et al., 2020; Chan et al., 2010; Chan et al., 2012b; Cojohari et al., 2020; Cojohari et al., 2016; Collins-McMillen et al., 2015; Peppenelli et al., 2016; Peppenelli et al., 2018; Smith et al., 2004a). The primary route through which monocytes are programmed to die is apoptosis, which can be accelerated as part of an intrinsic antiviral defense response against the virus (Barber, 2001; Mangan et al., 1993). Monocytes also possess the molecular machinery necessary to shift course to an alternative antiviral cell death response pathway called necroptosis if apoptosis is impeded during a virus infection (Altman et al., 2020; Lee et al., 2019). Through long co-evolution with humans, HCMV appears to have developed mechanisms to counteract cell death pathways during infection of monocytes independent of *de novo* synthesized viral proteins. In this study, we demonstrate that HCMV utilizes the cellular deacetylase SIRT2 to simultaneously block both the apoptotic and necroptotic death pathways during quiescent infection of monocytes.

In our proposed model, SIRT2 acts as a central regulator of both apoptosis and necroptosis through modulation of Akt activity following HCMV infection of monocytes (Fig. 8). HCMV infection aberrantly modifies cellular signaling pathways in a manner reminiscent of cancer, with the PI3K/Akt pathway being a central pathway altered by the virus (Chan et al., 2008b, 2009a, 2010; Cojohari et al., 2016; Hoxhaj and Manning, 2020; Luo et al., 2003; Mahmud et al., 2020; Peppenelli et al., 2018; Smith et al., 2004b, 2007). Under canonical stimulation by normal myeloid growth factors, Akt is dually phosphorylated at residues S473 and T308 (Alessi et al., 1996; Cojohari et al., 2020; Mahmud et al., 2020; Peppenelli et al., 2016, 2018). In contrast, binding of HCMV glycoproteins to cellular receptors initiates a signaling cascade in monocytes that leads to a noncanonical Akt phosphorylation profile

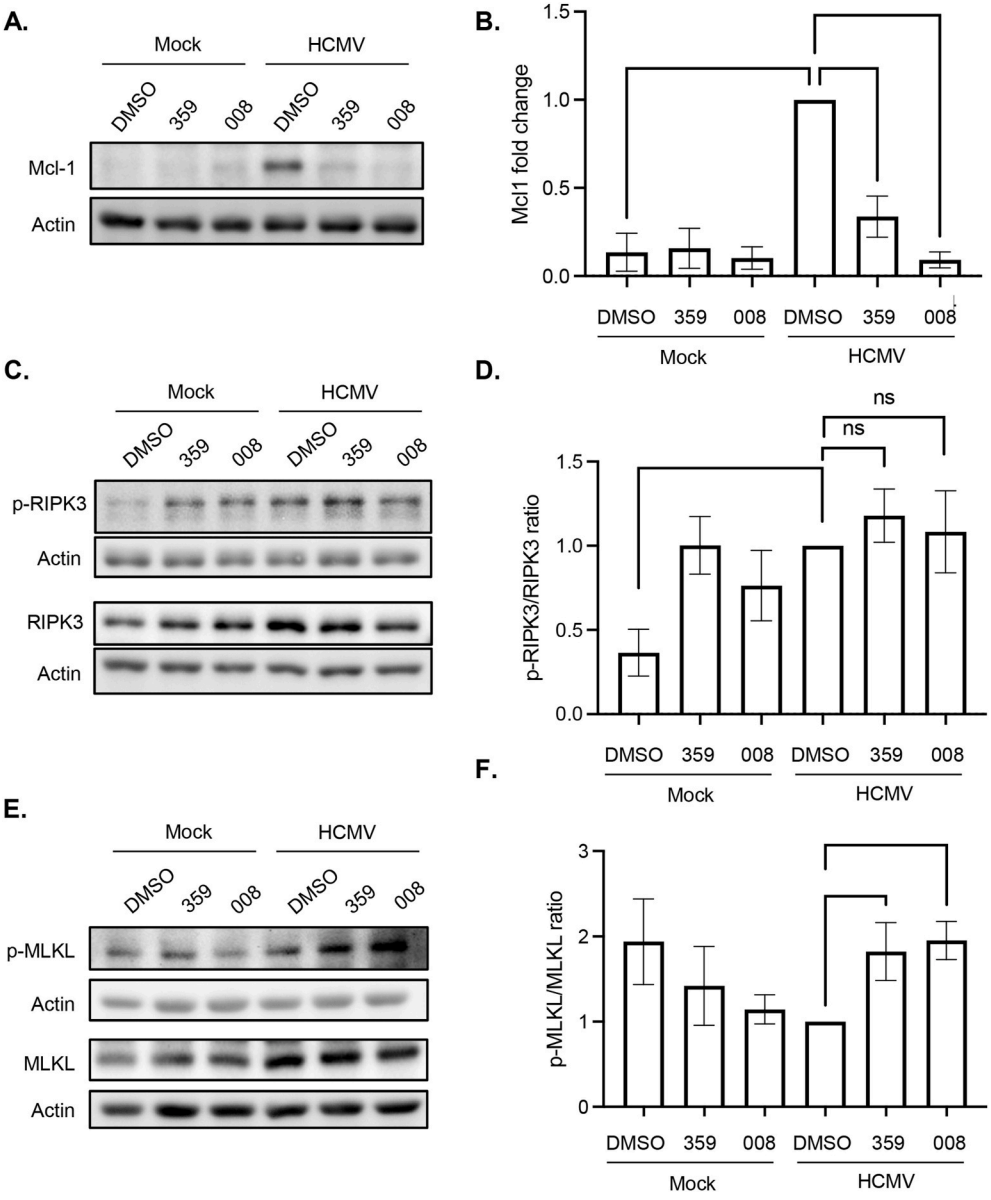

Fig. 6. SIRT2 regulates the expression and activity of factors involved in apoptosis and necroptosis. (A–F) Monocytes were treated with DMSO, 5 μM FLS-359, or 5 μM FLSX-008 1 h prior to infection. Cells were then mock infected or HCMV infected for 24 h. Expression levels of Mcl-1, RIPK3, p-RIPK3, MLKL, and p-MLKL were determined by immunoblotting. Membranes were probed for β-actin as a loading control. Data are representative of at least 4 independent blood donors. ***, $p \leq 0.001$; **, $p \leq 0.01$; *, $p \leq 0.05$.

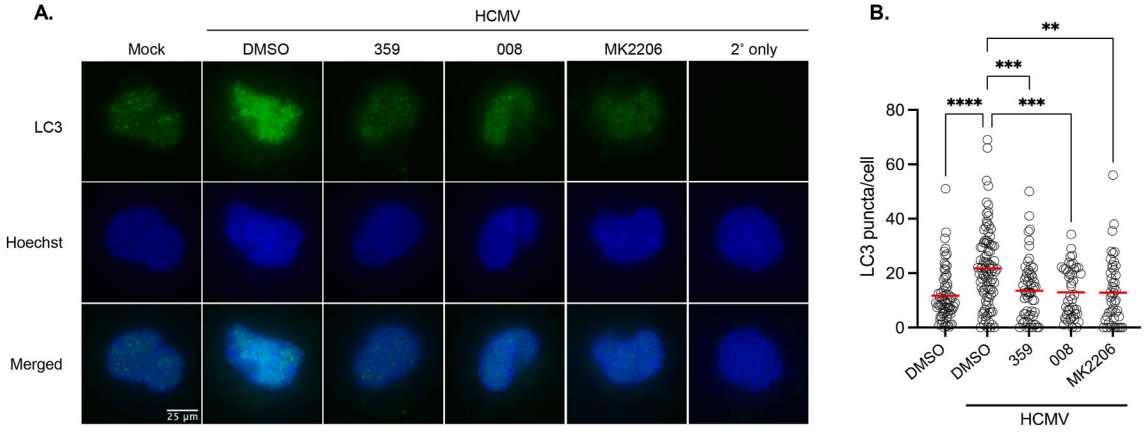

Fig. 7. SIRT2 and Akt are required for HCMV-induced autophagosome accumulation in infected monocytes. (A, B) Monocytes were treated with DMSO, 5 μM FLS-359, 5 μM FLSX-008, or 10 μM MK2206 (an Akt inhibitor) 1 h prior to infection. Cells were mock infected or HCMV infected for 2 h, followed by immunofluorescent analysis with an anti-LC3 antibody (green) and Hoechst staining (blue). (B) LC3 puncta per cell were counted using FIJI and plotted with the mean (red line). Data are representative of at least 3 independent blood donors. ****, $p \leq 0.0001$; ***, $p \leq 0.001$; **, $p \leq 0.01$; *, $p \leq 0.05$.

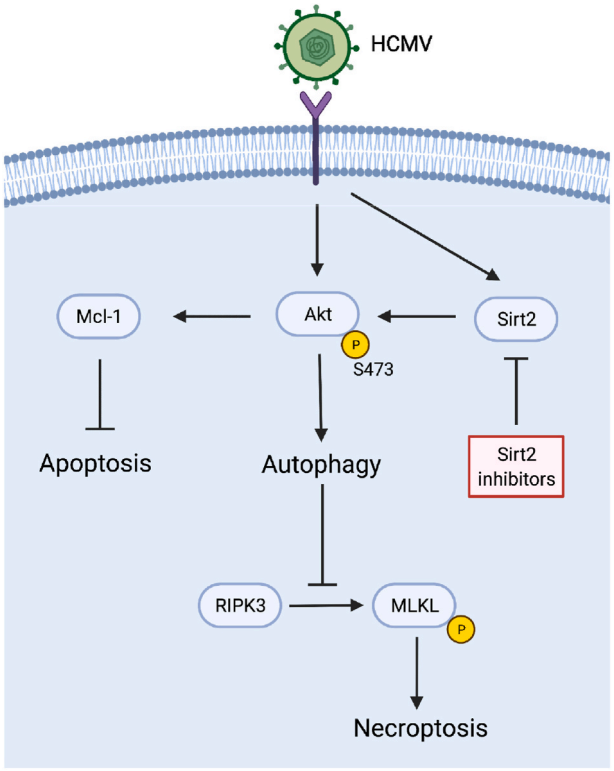

Fig. 8. Proposed model for SIRT2 regulation of apoptosis and necroptosis in HCMV-infected monocytes. In the absence of myeloid differentiation factors, monocytes undergo programmed cell death within 48 h after entering the peripheral circulation. Upon HCMV binding to cellular receptors, Akt is preferentially phosphorylated at S473 to trigger pro-survival pathways that circumvent apoptosis and necroptosis. The unique phosphorylation of Akt during HCMV infection leads to the increased expression of antiapoptotic proteins such as Mcl-1. HCMV-induced activation of Akt also enhances cellular autophagy, which blocks phosphorylation of MLKL during necroptosis. SIRT2-mediated deacetylation of Akt is required for HCMV's unique activation of Akt in monocytes. Inhibition of SIRT2 relieves the viral-induced blocks on both the apoptotic and necroptotic death pathways by reducing expression of Mcl-1 and by reducing autophagy to allow the activation of MLKL, thus promoting death of HCMV-infected monocytes through dual concurrent mechanisms.

wherein S473 is preferentially phosphorylated (Cojohari et al., 2020; Mahmud et al., 2020; Peppenelli et al., 2016, 2018), which also occurs in some cancers (Fernandes et al., 2013; Kerr, 2011; Opel et al., 2007; Tsurutani et al., 2006). SHIP1 normally acts as a negative regulator of Akt by opposing the action of PI3K, but in some transformed cells, SHIP1 amplifies Akt activity through the specific phosphorylation of S473 (Fernandes et al., 2013; Kerr, 2011). We have previously shown a similar dysregulation of SHIP1 during HCMV infection of monocytes where HCMV upregulates SHIP1, which then acts as a positive regulator of Akt by mediating the S473 site-specific phosphorylation (Cojohari et al., 2016). The distinct activation profile of Akt leads to a HCMV-specific transcriptomic landscape that includes the increased expression of a distinct subset of antiapoptotic proteins not upregulated during growth factor-induced signaling (Chan et al., 2008b, 2009a, 2010; Cojohari et al., 2016; Mahmud et al., 2020; Peppenelli et al., 2016, 2018). Here, we show HCMV-induced SIRT2 likely mediates deacetylation of Akt that permits HCMV's unique glycoprotein-initiated signaling to impart a distinctive Akt activation profile.

A primary function of HCMV-activated Akt is to prevent the intrinsic biological programming of monocytes to undergo apoptosis and the acceleration of apoptosis due to the initiation of antiviral defenses. The phosphorylation profile of Akt dictates its downstream substate specificity (Jacinto et al., 2006; Yung et al., 2011) and we have found that the preferential phosphorylation of S473 leads to the upregulation of a unique milieu of antiapoptotic proteins, including Mcl-1, HSP27, and XIAP (Chan et al., 2010, 2012b; Cojohari et al., 2016; Peppenelli et al., 2016, 2018). However, upon inhibition of apoptosis via blockage of procaspase 8 cleavage, the trapdoor cellular defensive death pathway necroptosis is opened (Altman et al., 2020; Galluzzi et al., 2017; Ke et al., 2016; Lee et al., 2019; Mocarski et al., 2011, 2015; Upton et al., 2008, 2010, 2012). We have shown infected monocytes initiate the early steps of necroptosis involving the phosphorylation of RIPK3 (Altman et al., 2020). However, HCMV rapidly blocks the progression of necroptosis preventing the activation of the final executioner protein MLKL (Altman et al., 2020). Inhibition of MLKL, and therefore necroptosis, is dependent on the HCMV-induced stimulation of cellular autophagy (Altman et al., 2020). Our current study now demonstrates that the HCMV-specific activation of Akt promotes autophagosome accumulation and that preventing the activation of Akt likely reduces HCMV-induced autophagy, leading to necroptosis. Based on SIRT2 acting as a gateway allowing HCMV glycoproteins to uniquely regulate Akt activity, our study supports the targeting of SIRT2 as a means of eliminating quiescently HCMV-infected monocytes via apoptosis and/or necroptosis.

Our study also identifies an unusual relationship between HCMV-activated Akt and autophagy. Traditionally, Akt has an inhibitory role in the regulation of cellular autophagy. Akt is directly implicated as a negative regulator of autophagy via phosphorylation of Beclin 1, a component of the Beclin 1-Vsp34-Vsp15 autophagy nucleation core complex (Kang et al., 2011; Wang et al., 2012). Additionally, Akt is thought to negatively regulate autophagy through activation of mTOR (Egan et al., 2011; Heras-Sandoval et al., 2014; Kim et al., 2011; Kim and Guan, 2015; Song et al., 2018), which we have shown to be rapidly activated in HCMV-infected monocytes following viral entry (Altman et al., 2019; Peppenelli et al., 2018). Yet, we found a positive relationship between Akt and autophagy, as abrogating Akt activity via direct or SIRT2-mediated inhibition attenuated autophagy. Why HCMV-activated Akt has the opposite effect on autophagy remains unknown. Aberrant microenvironments, such as those during cancer or viral infections, can have profound effect on the cellular signaling network leading to atypical biological outcomes. For example, AMPK traditionally negatively regulates mTOR activity, however HCMV infection uncouples the AMPK-mTOR signaling axis allowing both AMPK and mTOR to be activated simultaneously during both lytic and silent infections (Altman et al., 2019, 2020; Kudchodkar et al., 2007; Moorman et al., 2008). Another instance is the conversion of SHIP1 from a negative to positive regulator of AKT during HCMV infection (Cojohari et al., 2016). How HCMV modulates signaling and protein activities to allow Akt to promote autophagy during viral infection of monocytes is an important avenue of future studies.

Overall, our study demonstrates the potential utility of targeting SIRT2 as an antiviral strategy. Targeting host proteins as an antiviral strategy offers several advantages over targeting viral proteins, including limiting drug resistance and the potential for broad-spectrum antiviral activity (Kumar et al., 2020; Tripathi et al., 2021). The SIRT2 inhibitor FLS-359 has been shown to possess antiviral activity against a wide range of both DNA and RNA viruses, including members of the herpesvirus, flavivirus, orthomyxovirus, hepadnavirus, and coronavirus families (Roche et al., 2023). FLS-359 has been demonstrated to be well-tolerated in animal studies with no weight loss or adverse clinical signs (Roche et al., 2023). Unlike knockout mouse models of the other members of the Sirtuin family, SIRT2 knockout mice are viable without significant phenotypic abnormalities (Ciarlo et al., 2017; Finkel et al., 2009). SIRT2 inhibition has previously been shown to inhibit growth of HCMV during lytic infection in fibroblast cells (Mao et al., 2016; Roche et al., 2023). This study demonstrates that SIRT2 inhibition does not only inhibit HCMV lytic replication, but it also promotes death of quiescently infected monocytes. The lack of lytic viral protein expression in quiescently infected monocytes is an obstacle that has prevented

all the current direct-targeted antivirals from being fully effective against HCMV. In the case of transplant recipients, although the current HCMV antivirals have been highly efficacious at reducing HCMV disease in the most vulnerable first few months immediately post-transplantation, antiviral therapies must eventually be discontinued due to drug toxicities, leading to later rebound HCMV reactivation of virus from quiescently infected monocytes that have extravasated into peripheral organ tissue (Singh, 2005). An ideal antiviral regimen would target the virus in both cells with active viral replication and cells that are silently harboring the virus during dissemination. Thus, the ability of a single SIRT2 inhibitor to target both lytic and quiescent stages of the HCMV lifecycle could have major implications to prognosis of high-risk transplant recipients.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

We thank Christine Burrer in the Department of Microbiology and Immunology at SUNY Upstate Medical University for technical support, maintenance of lab operations, and assistance with virus growth and isolation. This work was supported by grants National Institute of Allergy and Infectious Disease (R01AI141460) to G.C. Chan, National Heart, Lung, and Blood Institute (R01HL139824) to G.C. Chan, National Cancer Institute (R01CA217141) to Z. Nikolovska-Coleska and AACR Bayer Innovation and Discovery Award to Z. Nikolovska-Coleska.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.antiviral.2023.105698.

References

Alessi, D.R., Andjelkovic, M., Caudwell, B., Cron, P., Morrice, N., Cohen, P., Hemmings, B.A., 1996. Mechanism of activation of protein kinase B by insulin and IGF-1. EMBO J. 15, 6541–6551.

Altman, A.M., Mahmud, J., Nikolovska-Coleska, Z., Chan, G., 2019. HCMV modulation of cellular PI3K/AKT/mTOR signaling: new opportunities for therapeutic intervention? Antivir. Res. 163, 82–90. https://doi.org/10.1016/j.antiviral.2019.01.009.

Altman, A.M., Miller, M.J., Mahmud, J., Smith, N.A., Chan, G.C., 2020. Human cytomegalovirus-induced autophagy prevents necroptosis of infected monocytes. J. Virol. 94 https://doi.org/10.1128/jvi.01022-20.

Azevedo, L.S., Pierrotti, L.C., Abdala, E., Costa, S.F., Strabelli, T.M., Campos, S.V., Ramos, J.F., Latif, A.Z., Litvinov, N., Maluf, N.Z., Caiaffa Filho, H.H., Pannuti, C.S., Lopes, M.H., Santos, V.A., Linardi Cda, C., Yasuda, M.A., Marques, H.H., 2015. Cytomegalovirus infection in transplant recipients. Clinics 70, 515–523. https://doi.org/10.6061/clinics/2015(07)09.

Barber, G.N., 2001. Host defense, viruses and apoptosis. Cell Death Differ. 8, 113–126. https://doi.org/10.1038/sj.cdd.4400823.

Boeckh, M., Nichols, W.G., Papanicolaou, G., Rubin, R., Wingard, J.R., Zaia, J., 2003. Cytomegalovirus in hematopoietic stem cell transplant recipients: current status, known challenges, and future strategies. Biol. Blood Marrow Transplant. 9, 543–558. https://doi.org/10.1016/s1083-8791(03)00287-8.

Cannon, M.J., Schmid, D.S., Hyde, T.B., 2010. Review of cytomegalovirus seroprevalence and demographic characteristics associated with infection. Rev. Med. Virol. 20, 202–213. https://doi.org/10.1002/rmv.655.

Chan, G., Bivins-Smith, E.R., Smith, M.S., Smith, P.M., Yurochko, A.D., 2008a. Transcriptome analysis reveals human cytomegalovirus reprograms monocyte differentiation toward an M1 macrophage. J. Immunol. 181, 698–711.

Chan, G., Bivins-Smith, E.R., Smith, M.S., Yurochko, A.D., 2008b. Transcriptome analysis of NF-kappaB- and phosphatidylinositol 3-kinase-regulated genes in human cytomegalovirus-infected monocytes. J. Virol. 82, 1040–1046. https://doi.org/10.1128/JVI.00864-07.

Chan, G., Bivins-Smith, E.R., Smith, M.S., Yurochko, A.D., 2009a. NF-kappaB and phosphatidylinositol 3-kinase activity mediates the HCMV-induced atypical M1/M2 polarization of monocytes. Virus Res. 144, 329–333. https://doi.org/10.1016/j.virusres.2009.04.026.

Chan, G., Nogalski, M.T., Bentz, G.L., Smith, M.S., Parmater, A., Yurochko, A.D., 2010. PI3K-dependent upregulation of Mcl-1 by human cytomegalovirus is mediated by epidermal growth factor receptor and inhibits apoptosis in short-lived monocytes. J. Immunol. 184, 3213–3222. https://doi.org/10.4049/jimmunol.0903025.

Chan, G., Nogalski, M.T., Stevenson, E.V., Yurochko, A.D., 2012a. Human cytomegalovirus induction of a unique signalsome during viral entry into monocytes mediates distinct functional changes: a strategy for viral dissemination. J. Leukoc. Biol. 92, 743–752. https://doi.org/10.1189/jlb.0112040.

Chan, G., Nogalski, M.T., Yurochko, A.D., 2009b. Activation of EGFR on monocytes is required for human cytomegalovirus entry and mediates cellular motility. Proc. Natl. Acad. Sci. U. S. A. 106, 22369–22374. https://doi.org/10.1073/pnas.0908787106.

Chan, G., Nogalski, M.T., Yurochko, A.D., 2012b. Human cytomegalovirus stimulates monocyte-to-macrophage differentiation via the temporal regulation of caspase 3. J. Virol. 86, 10714–10723. https://doi.org/10.1128/JVI.07129-11.

Cho, Y.S., Challa, S., Moquin, D., Genga, R., Ray, T.D., Guildford, M., Chan, F.K., 2009. Phosphorylation-driven assembly of the RIP1-RIP3 complex regulates programmed necrosis and virus-induced inflammation. Cell 137, 1112–1123. https://doi.org/10.1016/j.cell.2009.05.037.

Ciarlo, E., Heinonen, T., Theroude, C., Herderschee, J., Mombelli, M., Lugrin, J., Pfefferle, M., Tyrrell, B., Lensch, S., Acha-Orbea, H., Le Roy, D., Auwerx, J., Roger, T., 2017. Sirtuin 2 deficiency increases bacterial phagocytosis by macrophages and protects from chronic staphylococcal infection. Front. Immunol. 8, 1037. https://doi.org/10.3389/fimmu.2017.01037.

Cobbs, C.S., Harkins, L., Samanta, M., Gillespie, G.Y., Bharara, S., King, P.H., Nabors, L. B., Cobbs, C.G., Britt, W.J., 2002. Human cytomegalovirus infection and expression in human malignant glioma. Cancer Res. 62, 3347–3350.

Cojohari, O., Mahmud, J., Altman, A.M., Peppenelli, M.A., Miller, M.J., Chan, G.C., 2020. Human cytomegalovirus mediates unique monocyte-to-macrophage differentiation through the PI3K/SHIP1/Akt signaling network. Viruses 12. https://doi.org/10.3390/v12060652.

Cojohari, O., Peppenelli, M.A., Chan, G.C., 2016. Human cytomegalovirus induces an atypical activation of Akt to stimulate the survival of short-lived monocytes. J. Virol. 90, 6443–6452. https://doi.org/10.1128/JVI.00214-16.

Collins-McMillen, D., Chesnokova, L., Lee, B.J., Fulkerson, H.L., Brooks, R., Mosher, B.S., Yurochko, A.D., 2018. HCMV Infection and Apoptosis: How Do Monocytes Survive HCMV Infection? https://doi.org/10.3390/v10100533 Viruses 10.

Collins-McMillen, D., Kim, J.H., Nogalski, M.T., Stevenson, E.V., Chan, G.C., Caskey, J.R., Cieply, S.J., Yurochko, A.D., 2015. Human cytomegalovirus promotes survival of infected monocytes via a distinct temporal regulation of cellular Bcl-2 family proteins. J. Virol. 90, 2356–2371. https://doi.org/10.1128/jvi.01994-15.

Crumpacker, C.S., 1992. Mechanism of action of foscarnet against viral polymerases. Am. J. Med. 92, 3s–7s. https://doi.org/10.1016/0002-9343(92)90329-a.

Dan, L., Klimenkova, O., Klimiankou, M., Klusman, J.H., van den Heuvel-Eibrink, M.M., Reinhardt, D., Welte, K., Skokowa, J., 2012. The role of sirtuin 2 activation by nicotinamide phosphoribosyltransferase in the aberrant proliferation and survival of myeloid leukemia cells. Haematologica 97, 551–559. https://doi.org/10.3324/haematol.2011.055236.

Ding, T., Hao, J., 2021. Sirtuin 2 knockdown inhibits cell proliferation and RAS/ERK signaling, and promotes cell apoptosis and cell cycle arrest in multiple myeloma. Mol. Med. Rep. 24 https://doi.org/10.3892/mmr.2021.12400.

Egan, D., Kim, J., Shaw, R.J., Guan, K.L., 2011. The autophagy initiating kinase ULK1 is regulated via opposing phosphorylation by AMPK and mTOR. Autophagy 7, 643–644. https://doi.org/10.4161/auto.7.6.15123.

Elder, E., Krishna, B., Williamson, J., Aslam, Y., Farahi, N., Wood, A., Romashova, V., Roche, K., Murphy, E., Chilvers, E., Lehner, P.J., Sinclair, J., Poole, E., 2019. Monocytes latently infected with human cytomegalovirus evade neutrophil killing. iScience 12, 13–26. https://doi.org/10.1016/j.isci.2019.01.007.

Emery, V.C., 2001. Investigation of CMV disease in immunocompromised patients. J. Clin. Pathol. 54, 84–88. https://doi.org/10.1136/jcp.54.2.84.

Fernandes, S., Iyer, S., Kerr, W.G., 2013. Role of SHIP1 in cancer and mucosal inflammation. Ann. N. Y. Acad. Sci. 1280, 6–10. https://doi.org/10.1111/nyas.12038.

Fiala, M., Heiner, D.C., Turner, J.A., Rosenbloom, B., Guze, L.B., 1977. Infectious mononucleosis and mononucleosis syndromes. West. J. Med. 126, 445–459.

Finkel, T., Deng, C.X., Mostoslavsky, R., 2009. Recent progress in the biology and physiology of sirtuins. Nature 460, 587–591. https://doi.org/10.1038/nature08197.

Fletcher-Etherington, A., Nobre, L., Nightingale, K., Antrobus, R., Nichols, J., Davison, A. J., Stanton, R.J., Weekes, M.P., 2020. Human cytomegalovirus protein pUL36: a dual cell death pathway inhibitor. Proc. Natl. Acad. Sci. U. S. A. 117, 18771–18779. https://doi.org/10.1073/pnas.2001887117.

Galluzzi, L., Kepp, O., Chan, F.K., Kroemer, G., 2017. Necroptosis: mechanisms and relevance to disease. Annu. Rev. Pathol. 12, 103–130. https://doi.org/10.1146/annurev-pathol-052016-100247.

Geisler, J., Touma, J., Rahbar, A., Soderberg-Naucler, C., Vetvik, K., 2019. A review of the potential role of human cytomegalovirus (HCMV) infections in breast cancer carcinogenesis and abnormal immunity. Cancers 11. https://doi.org/10.3390/cancers11121842.

Giam, M., Huang, D.C., Bouillet, P., 2008. BH3-only proteins and their roles in programmed cell death. Oncogene 27 (Suppl 1), S128–S136. https://doi.org/10.1038/onc.2009.50.

Hargett, D., Shenk, T.E., 2010. Experimental human cytomegalovirus latency in CD14+ monocytes. Proc. Natl. Acad. Sci. U. S. A. 107, 20039–20044. https://doi.org/10.1073/pnas.1014509107.

Heras-Sandoval, D., Perez-Rojas, J.M., Hernandez-Damian, J., Pedraza-Chaverri, J., 2014. The role of PI3K/AKT/mTOR pathway in the modulation of autophagy and the clearance of protein aggregates in neurodegeneration. Cell. Signal. 26, 2694–2701. https://doi.org/10.1016/j.cellsig.2014.08.019.

Hoxhaj, G., Manning, B.D., 2020. The PI3K-AKT network at the interface of oncogenic signalling and cancer metabolism. Nat. Rev. Cancer 20, 74–88. https://doi.org/10.1038/s41568-019-0216-7.

Jacinto, E., Facchinetti, V., Liu, D., Soto, N., Wei, S., Jung, S.Y., Huang, Q., Qin, J., Su, B., 2006. SIN1/MIP1 maintains rictor-mTOR complex integrity and regulates Akt phosphorylation and substrate specificity. Cell 127, 125–137. https://doi.org/10.1016/j.cell.2006.08.033.

Jacobson, M.A., 1992. Review of the toxicities of foscarnet. J. Acquir. Immune Defic. Syndr. 5 (Suppl 1), S11–S17.

Jing, H., Hu, J., He, B., Negron Abril, Y.L., Stupinski, J., Weiser, K., Carbonaro, M., Chiang, Y.L., Southard, T., Giannakakou, P., Weiss, R.S., Lin, H., 2016. A SIRT2-selective inhibitor promotes c-Myc oncoprotein degradation and exhibits broad anticancer activity. Cancer Cell 29, 297–310. https://doi.org/10.1016/j.ccell.2016.02.007.

Kang, R., Zeh, H.J., Lotze, M.T., Tang, D., 2011. The Beclin 1 network regulates autophagy and apoptosis. Cell Death Differ. 18, 571–580. https://doi.org/10.1038/cdd.2010.191.

Ke, X., Lei, L., Li, H., Li, H., Yan, F., 2016. Manipulation of necroptosis by Porphyromonas gingivalis in periodontitis development. Mol. Immunol. 77, 8–13. https://doi.org/10.1016/j.molimm.2016.07.010.

Kendle, J.B., Fan-Havard, P., 1998. Cidofovir in the treatment of cytomegaloviral disease. Ann. Pharmacother. 32, 1181–1192. https://doi.org/10.1345/aph.17312.

Kerr, W.G., 2011. Inhibitor and activator: dual functions for SHIP in immunity and cancer. Ann. N. Y. Acad. Sci. 1217, 1–17. https://doi.org/10.1111/j.1749-6632.2010.05869.x.

Kim, J., Kundu, M., Viollet, B., Guan, K.L., 2011. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nat. Cell Biol. 13, 132–141. https://doi.org/10.1038/ncb2152.

Kim, Y.C., Guan, K.L., 2015. mTOR: a pharmacologic target for autophagy regulation. J. Clin. Invest. 125, 25–32. https://doi.org/10.1172/JCI73939.

Kozako, T., Mellini, P., Ohsugi, T., Aikawa, A., Uchida, Y.I., Honda, S.I., Suzuki, T., 2018. Novel small molecule SIRT2 inhibitors induce cell death in leukemic cell lines. BMC Cancer 18, 791. https://doi.org/10.1186/s12885-018-4710-1.

Krishna, B.A., Poole, E.L., Jackson, S.E., Smit, M.J., Wills, M.R., Sinclair, J.H., 2017. Latency-associated expression of human cytomegalovirus US28 attenuates cell signaling pathways to maintain latent infection. mBio 8. https://doi.org/10.1128/mBio.01754-17.

Kudchodkar, S.B., Del Prete, G.Q., Maguire, T.G., Alwine, J.C., 2007. AMPK-mediated inhibition of mTOR kinase is circumvented during immediate-early times of human cytomegalovirus infection. J. Virol. 81, 3649–3651. https://doi.org/10.1128/JVI.02079-06.

Kumar, N., Sharma, S., Kumar, R., Tripathi, B.N., Barua, S., Ly, H., Rouse, B.T., 2020. Host-Directed antiviral therapy. Clin. Microbiol. Rev. 33 https://doi.org/10.1128/CMR.00168-19.

Kump, K.J., Miao, L., Mady, A.S.A., Ansari, N.H., Shrestha, U.K., Yang, Y., Pal, M., Liao, C., Perdih, A., Abulwerdi, F.A., Chinnaswamy, K., Meagher, J.L., Carlson, J.M., Khanna, M., Stuckey, J.A., Nikolovska-Coleska, Z., 2020. Discovery and characterization of 2,5-substituted benzoic acid dual inhibitors of the anti-apoptotic Mcl-1 and Bfl-1 proteins. J. Med. Chem. 63, 2489–2510. https://doi.org/10.1021/acs.jmedchem.9b01442.

Lee, A.C.Y., Zhang, A.J.X., Chu, H., Li, C., Zhu, H., Mak, W.W.N., Chen, Y., Kok, K.H., To, K.K.W., Yuen, K.Y., 2019. H7N9 influenza A virus activation of necroptosis in human monocytes links innate and adaptive immune responses. Cell Death Dis. 10, 442. https://doi.org/10.1038/s41419-019-1684-0.

Liu, P.Y., Xu, N., Malyukova, A., Scarlett, C.J., Sun, Y.T., Zhang, X.D., Ling, D., Su, S.P., Nelson, C., Chang, D.K., Koach, J., Tee, A.E., Haber, M., Norris, M.D., Toon, C., Rooman, I., Xue, C., Cheung, B.B., Kumar, S., Marshall, G.M., Biankin, A.V., Liu, T., 2013. The histone deacetylase SIRT2 stabilizes Myc oncoproteins. Cell Death Differ. 20, 503–514. https://doi.org/10.1038/cdd.2012.147.

Liu, Q., Qiu, J., Liang, M., Golinski, J., van Leyen, K., Jung, J.E., You, Z., Lo, E.H., Degterev, A., Whalen, M.J., 2014. Akt and mTOR mediate programmed necrosis in neurons. Cell Death Dis. 5, e1084 https://doi.org/10.1038/cddis.2014.69.

Luo, G., Jian, Z., Zhu, Y., Zhu, Y., Chen, B., Ma, R., Tang, F., Xiao, Y., 2019. Sirt1 promotes autophagy and inhibits apoptosis to protect cardiomyocytes from hypoxic stress. Int. J. Mol. Med. 43, 2033–2043. https://doi.org/10.3892/ijmm.2019.4125.

Luo, J., Manning, B.D., Cantley, L.C., 2003. Targeting the PI3K-Akt pathway in human cancer: rationale and promise. Cancer Cell 4, 257–262. https://doi.org/10.1016/s1535-6108(03)00248-4.

Maertens, J., Cordonnier, C., Jaksch, P., Poiré, X., Uknis, M., Wu, J., Wijatyk, A., Saliba, F., Witzke, O., Villano, S., 2019. Maribavir for preemptive treatment of cytomegalovirus reactivation. N. Engl. J. Med. 381, 1136–1147. https://doi.org/10.1056/NEJMoa1714656.

Mahmud, J., Miller, M.J., Altman, A.M., Chan, G.C., 2020. Human cytomegalovirus glycoprotein-initiated signaling mediates the aberrant activation of Akt. J. Virol. 94 https://doi.org/10.1128/JVI.00167-20.

Manez, R., Kusne, S., Rinaldo, C., Aguado, J.M., St George, K., Grossi, P., Frye, B., Fung, J.J., Ehrlich, G.D., 1996. Time to detection of cytomegalovirus (CMV) DNA in blood leukocytes is a predictor for the development of CMV disease in CMV-seronegative recipients of allografts from CMV-seropositive donors following liver transplantation. J. Infect. Dis. 173, 1072–1076. https://doi.org/10.1093/infdis/173.5.1072.

Mangan, D.F., Mergenhagen, S.E., Wahl, S.M., 1993. Apoptosis in human monocytes: possible role in chronic inflammatory diseases. J. Periodontol. 64, 461–466.

Mao, G., Li, H., Ding, X., Meng, X., Wang, G., Leng, S.X., 2016. Suppressive effects of sirtinol on human cytomegalovirus (hCMV) infection and hCMV-induced activation of molecular mechanisms of senescence and production of reactive oxygen species. Mech. Ageing Dev. 158, 62–69. https://doi.org/10.1016/j.mad.2015.12.005.

Matthews, T., Boehme, R., 1988. Antiviral activity and mechanism of action of ganciclovir. Rev. Infect. Dis. 10 (Suppl 3), S490–S494. https://doi.org/10.1093/clinids/10.supplement_3.s490.

McNamara, C.R., Ahuja, R., Osafo-Addo, A.D., Barrows, D., Kettenbach, A., Skidan, I., Teng, X., Cuny, G.D., Gerber, S., Degterev, A., 2013. Akt Regulates TNFalpha synthesis downstream of RIP1 kinase activation during necroptosis. PLoS One 8, e56576. https://doi.org/10.1371/journal.pone.0056576.

Min, C.K., Shakya, A.K., Lee, B.J., Streblow, D.N., Caposio, P., Yurochko, A.D., 2020. The differentiation of human cytomegalovirus infected-monocytes is required for viral replication. Front. Cell. Infect. Microbiol. 10, 368. https://doi.org/10.3389/fcimb.2020.00368.

Mocarski, E.S., Guo, H., Kaiser, W.J., 2015. Necroptosis: the Trojan horse in cell autonomous antiviral host defense. Virology 479–480, 160–166. https://doi.org/10.1016/j.virol.2015.03.016.

Mocarski, E.S., Upton, J.W., Kaiser, W.J., 2011. Viral infection and the evolution of caspase 8-regulated apoptotic and necrotic death pathways. Nat. Rev. Immunol. 12, 79–88. https://doi.org/10.1038/nri3131.

Moorman, N.J., Cristea, I.M., Terhune, S.S., Rout, M.P., Chait, B.T., Shenk, T., 2008. Human cytomegalovirus protein UL38 inhibits host cell stress responses by antagonizing the tuberous sclerosis protein complex. Cell Host Microbe 3, 253–262. https://doi.org/10.1016/j.chom.2008.03.002.

Nikitskaya, E., Lebedeva, A., Ivanova, O., Maryukhnich, E., Shpektor, A., Grivel, J.C., Margolis, L., Vasilieva, E., 2016. Cytomegalovirus-productive infection is associated with acute coronary syndrome. J. Am. Heart Assoc. 5 https://doi.org/10.1161/JAHA.116.003759.

Nikolovska-Coleska, Z., Wang, R., Fang, X., Pan, H., Tomita, Y., Li, P., Roller, P.P., Krajewski, K., Saito, N.G., Stuckey, J.A., Wang, S., 2004. Development and optimization of a binding assay for the XIAP BIR3 domain using fluorescence polarization. Anal. Biochem. 332, 261–273. https://doi.org/10.1016/j.ab.2004.05.055.

North, B.J., Marshall, B.L., Borra, M.T., Denu, J.M., Verdin, E., 2003. The human Sir2 ortholog, SIRT2, is an NAD+-dependent tubulin deacetylase. Mol. Cell 11, 437–444. https://doi.org/10.1016/s1097-2765(03)00038-8.

Opel, D., Poremba, C., Simon, T., Debatin, K.M., Fulda, S., 2007. Activation of Akt predicts poor outcome in neuroblastoma. Cancer Res. 67, 735–745. https://doi.org/10.1158/0008-5472.CAN-06-2201.

Patel, A.A., Zhang, Y., Fullerton, J.N., Boelen, L., Rongvaux, A., Maini, A.A., Bigley, V., Flavell, R.A., Gilroy, D.W., Asquith, B., Macallan, D., Yona, S., 2017. The fate and lifespan of human monocyte subsets in steady state and systemic inflammation. J. Exp. Med. 214, 1913–1923. https://doi.org/10.1084/jem.20170355.

Patel, R., Paya, C.V., 1997. Infections in solid-organ transplant recipients. Clin. Microbiol. Rev. 10, 86–124. https://doi.org/10.1128/CMR.10.1.86.

Patil, A.J., Sharma, A., Kenney, M.C., Kuppermann, B.D., 2010. Valganciclovir in the treatment of cytomegalovirus retinitis in HIV-infected patients. Clin. Ophthalmol. 4, 111–119. https://doi.org/10.2147/opth.s3248.

Peppenelli, M.A., Arend, K.C., Cojohari, O., Moorman, N.J., Chan, G.C., 2016. Human cytomegalovirus stimulates the synthesis of select Akt-dependent antiapoptotic proteins during viral entry to promote survival of infected monocytes. J. Virol. 90, 3138–3147. https://doi.org/10.1128/JVI.02879-15.

Peppenelli, M.A., Miller, M.J., Altman, A.M., Cojohari, O., Chan, G.C., 2018. Aberrant regulation of the Akt signaling network by human cytomegalovirus allows for targeting of infected monocytes. Antivir. Res. 158, 13–24. https://doi.org/10.1016/j.antiviral.2018.07.015.

Perrottet, N., Decosterd, L.A., Meylan, P., Pascual, M., Biollaz, J., Buclin, T., 2009. Valganciclovir in adult solid organ transplant recipients: pharmacokinetic and pharmacodynamic characteristics and clinical interpretation of plasma concentration measurements. Clin. Pharmacokinet. 48, 399–418. https://doi.org/10.2165/00003088-200948060-00006.

Rahman, M., Dastmalchi, F., Karachi, A., Mitchell, D., 2019. The role of CMV in glioblastoma and implications for immunotherapeutic strategies. OncoImmunology 8, e1514921. https://doi.org/10.1080/2162402X.2018.1514921.

Ramakrishnan, G., Davaakhuu, G., Kaplun, L., Chung, W.C., Rana, A., Atfi, A., Miele, L., Tzivion, G., 2014. SIRT2 deacetylase is a novel AKT binding partner critical for AKT activation by insulin. J. Biol. Chem. 289, 6054–6066. https://doi.org/10.1074/jbc.M113.537266.

Reeves, M.B., Breidenstein, A., Compton, T., 2012. Human cytomegalovirus activation of ERK and myeloid cell leukemia-1 protein correlates with survival of latently infected cells. Proc. Natl. Acad. Sci. U. S. A. 109, 588–593. https://doi.org/10.1073/pnas.1114966108.

Roche, K.L., Remiszewski, S., Todd, M.J., Kulp 3rd, J.L., Tang, L., Welsh, A.V., Barry, A. P., De, C., Reiley, W.W., Wahl, A., Garcia, J.V., Luftig, M.A., Shenk, T., Tonra, J.R., Murphy, E.A., Chiang, L.W., 2023. An allosteric inhibitor of sirtuin 2 deacetylase activity exhibits broad-spectrum antiviral activity. J. Clin. Invest. 133 https://doi.org/10.1172/JCI158978.

Rossetto, C.C., Tarrant-Elorza, M., Pari, G.S., 2013. Cis and trans acting factors involved in human cytomegalovirus experimental and natural latent infection of CD14 (+) monocytes and CD34 (+) cells. PLoS Pathog. 9, e1003366 https://doi.org/10.1371/journal.ppat.1003366.

Sinclair, J., Sissons, P., 1996. Latent and persistent infections of monocytes and macrophages. Intervirology 39, 293–301.
Singh, N., 2005. Late-onset cytomegalovirus disease as a significant complication in solid organ transplant recipients receiving antiviral prophylaxis: a call to heed the mounting evidence. Clin. Infect. Dis. 40, 704–708. https://doi.org/10.1086/427506.
Smith, M.S., Bentz, G.L., Alexander, J.S., Yurochko, A.D., 2004a. Human cytomegalovirus induces monocyte differentiation and migration as a strategy for dissemination and persistence. J. Virol. 78, 4444–4453. https://doi.org/10.1128/jvi.78.9.4444-4453.2004.
Smith, M.S., Bentz, G.L., Smith, P.M., Bivins, E.R., Yurochko, A.D., 2004b. HCMV activates PI(3)K in monocytes and promotes monocyte motility and transendothelial migration in a PI(3)K-dependent manner. J. Leukoc. Biol. 76, 65–76. https://doi.org/10.1189/jlb.1203621.
Smith, M.S., Bivins-Smith, E.R., Tilley, A.M., Bentz, G.L., Chan, G., Minard, J., Yurochko, A.D., 2007. Roles of phosphatidylinositol 3-kinase and NF-kappaB in human cytomegalovirus-mediated monocyte diapedesis and adhesion: strategy for viral persistence. J. Virol. 81, 7683–7694. https://doi.org/10.1128/JVI.02839-06.
Smith, N.A., Chan, G.C., O'Connor, C.M., 2021. Modulation of host cell signaling during cytomegalovirus latency and reactivation. Virol. J. 18, 207. https://doi.org/10.1186/s12985-021-01674-1.
Soderberg-Naucler, C., Streblow, D.N., Fish, K.N., Allan-Yorke, J., Smith, P.P., Nelson, J. A., 2001. Reactivation of latent human cytomegalovirus in CD14(+) monocytes is differentiation dependent. J. Virol. 75, 7543–7554. https://doi.org/10.1128/JVI.75.16.7543-7554.2001.
Song, G., Lu, H., Chen, F., Wang, Y., Fan, W., Shao, W., Lu, H., Lin, B., 2018. Tetrahydrocurcumininduced autophagy via suppression of PI3K/Akt/mTOR in nonsmall cell lung carcinoma cells. Mol. Med. Rep. 17, 5964–5969. https://doi.org/10.3892/mmr.2018.8600.
Song, S., Ding, Y., Dai, G.L., Zhang, Y., Xu, M.T., Shen, J.R., Chen, T.T., Chen, Y., Meng, G.L., 2021. Sirtuin 3 deficiency exacerbates diabetic cardiomyopathy via necroptosis enhancement and NLRP3 activation. Acta Pharmacol. Sin. 42, 230–241. https://doi.org/10.1038/s41401-020-0490-7.
Stevenson, E.V., Collins-McMillen, D., Kim, J.H., Cieply, S.J., Bentz, G.L., Yurochko, A.D., 2014. HCMV reprogramming of infected monocyte survival and differentiation: a Goldilocks phenomenon. Viruses 6, 782–807. https://doi.org/10.3390/v6020782.
Sundaresan, N.R., Pillai, V.B., Wolfgeher, D., Samant, S., Vasudevan, P., Parekh, V., Raghuraman, H., Cunningham, J.M., Gupta, M., Gupta, M.P., 2011. The deacetylase SIRT1 promotes membrane localization and activation of Akt and PDK1 during tumorigenesis and cardiac hypertrophy. Sci. Signal. 4, ra46. https://doi.org/10.1126/scisignal.2001465.
Tanida, I., Ueno, T., Kominami, E., 2008. LC3 and autophagy. Methods Mol. Biol. 445, 77–88. https://doi.org/10.1007/978-1-59745-157-4_4.
Taylor-Wiedeman, J., Sissons, P., Sinclair, J., 1994. Induction of endogenous human cytomegalovirus gene expression after differentiation of monocytes from healthy carriers. J. Virol. 68, 1597–1604. https://doi.org/10.1128/JVI.68.3.1597-1604.1994.
Tripathi, D., Sodani, M., Gupta, P.K., Kulkarni, S., 2021. Host directed therapies: COVID-19 and beyond. Curr Res Pharmacol Drug Discov 2, 100058. https://doi.org/10.1016/j.crphar.2021.100058.
Tsurutani, J., Fukuoka, J., Tsurutani, H., Shih, J.H., Hewitt, S.M., Travis, W.D., Jen, J., Dennis, P.A., 2006. Evaluation of two phosphorylation sites improves the prognostic significance of Akt activation in non-small-cell lung cancer tumors. J. Clin. Oncol. 24, 306–314. https://doi.org/10.1200/jco.2005.02.4133.
Upton, J.W., Chan, F.K., 2014. Staying alive: cell death in antiviral immunity. Mol. Cell 54, 273–280. https://doi.org/10.1016/j.molcel.2014.01.027.
Upton, J.W., Kaiser, W.J., Mocarski, E.S., 2008. Cytomegalovirus M45 cell death suppression requires receptor-interacting protein (RIP) homotypic interaction motif (RHIM)-dependent interaction with RIP1. J. Biol. Chem. 283, 16966–16970. https://doi.org/10.1074/jbc.C800051200.
Upton, J.W., Kaiser, W.J., Mocarski, E.S., 2010. Virus inhibition of RIP3-dependent necrosis. Cell Host Microbe 7, 302–313. https://doi.org/10.1016/j.chom.2010.03.006.
Upton, J.W., Kaiser, W.J., Mocarski, E.S., 2012. DAI/ZBP1/DLM-1 complexes with RIP3 to mediate virus-induced programmed necrosis that is targeted by murine cytomegalovirus vIRA. Cell Host Microbe 11, 290–297. https://doi.org/10.1016/j.chom.2012.01.016.
Verghese, P.S., Schleiss, M.R., 2013. Letermovir treatment of human cytomegalovirus infection antiinfective agent. Drugs Future 38, 291–298. https://doi.org/10.1358/dof.2013.038.05.1946425.
Wagenknecht, N., Reuter, N., Scherer, M., Reichel, A., Muller, R., Stamminger, T., 2015. Contribution of the major ND10 proteins PML, hDaxx and Sp100 to the regulation of human cytomegalovirus latency and lytic replication in the monocytic cell line THP-1. Viruses 7, 2884–2907. https://doi.org/10.3390/v7062751.
Wang, R.C., Wei, Y., An, Z., Zou, Z., Xiao, G., Bhagat, G., White, M., Reichelt, J., Levine, B., 2012. Akt-mediated regulation of autophagy and tumorigenesis through Beclin 1 phosphorylation. Science 338, 956–959. https://doi.org/10.1126/science.1225967.
Whitelaw, D.M., 1966. The intravascular lifespan of monocytes. Blood 28, 455–464.
Willis, S.N., Fletcher, J.I., Kaufmann, T., van Delft, M.F., Chen, L., Czabotar, P.E., Ierino, H., Lee, E.F., Fairlie, W.D., Bouillet, P., Strasser, A., Kluck, R.M., Adams, J.M., Huang, D.C., 2007. Apoptosis initiated when BH3 ligands engage multiple Bcl-2 homologs, not Bax or Bak. Science 315, 856–859. https://doi.org/10.1126/science.1133289.
Xu, D., Jiang, X., He, H., Liu, D., Yang, L., Chen, H., Wu, L., Geng, G., Li, Q., 2019. SIRT2 functions in aging, autophagy, and apoptosis in post-maturation bovine oocytes. Life Sci. 232, 116639. https://doi.org/10.1016/j.lfs.2019.116639.
Yung, H.W., Charnock-Jones, D.S., Burton, G.J., 2011. Regulation of AKT phosphorylation at Ser473 and Thr308 by endoplasmic reticulum stress modulates substrate specificity in a severity dependent manner. PLoS One 6, e17894. https://doi.org/10.1371/journal.pone.0017894.
Yurochko, A.D., Huang, E.S., 1999. Human cytomegalovirus binding to human monocytes induces immunoregulatory gene expression. J. Immunol. 162, 4806–4816.
Yurochko, A.D., Hwang, E.S., Rasmussen, L., Keay, S., Pereira, L., Huang, E.S., 1997. The human cytomegalovirus UL55 (gB) and UL75 (gH) glycoprotein ligands initiate the rapid activation of Sp1 and NF-kappaB during infection. J. Virol. 71, 5051–5059. https://doi.org/10.1128/JVI.71.7.5051-5059.1997.
Yurochko, A.D., Liu, D.Y., Eierman, D., Haskill, S., 1992. Integrins as a primary signal transduction molecule regulating monocyte immediate-early gene induction. Proc. Natl. Acad. Sci. U. S. A. 89, 9034–9038.
Zuhair, M., Smit, G.S.A., Wallis, G., Jabbar, F., Smith, C., Devleesschauwer, B., Griffiths, P., 2019. Estimation of the worldwide seroprevalence of cytomegalovirus: a systematic review and meta-analysis. Rev. Med. Virol. 29, e2034 https://doi.org/10.1002/rmv.2034.