 doi:10.20944/preprints201705.0209.v1

Review

Influenza virus infection, interferon response, viral counter-response and apoptosis

Jung Min Shim [1], Jinhee Kim [2], Ji-Young Min [3] and Denis E. Kainov [4,5] *

[1] Institut Pasteur Korea, Gyeonggi-do 13488, South Korea; jungmin.shim@ip-korea.org
[2] Institut Pasteur Korea, Gyeonggi-do 13488, South Korea; jinhee.kim@ip-korea.org
[3] Institut Pasteur Korea, Gyeonggi-do 13488, South Korea; jiyoung.min@ip-korea.org
[4] Institut Pasteur Korea, Gyeonggi-do 13488, South Korea; denikaino@gmail.com
[5] Institute of Technology, University of Tartu, Tartu 50090, Estonia; denikaino@gmail.com
* Correspondence: denikaino@gmail.com; Tel.: +358-50-415-5460

Academic Editors: Eric O. Freed and Marc Kvansakul
Received: 30 May 2017; Accepted: date; Published: date

Abstract: Human influenza A viruses (IAVs) cause global pandemics and epidemics, which remain serious threats to public health because of the shortage of effective means of control. To combat the surge of viral outbreaks, new treatments are urgently needed. Developing new virus control modalities requires better understanding of virus-host interactions. Here we describe how IAV infection triggers cellular apoptosis, and how this process can be exploited towards development of new therapeutics, which might be more effective than the currently available anti-influenza drugs.

Keywords: influenza virus; apoptosis; antiviral agent; innate immunity; host response

1. Introduction

Influenza A and B viruses cause seasonal epidemics and are culprits of 3-5 million annual cases of hospitalization and 250 000 – 500 000 deaths [1, 2]. Influenza infections could show mild symptoms like cough, sore throat, runny nose, fever, headache, and muscle pain [3].

Influenza A viruses also cause global pandemics. The pandemics occur when a novel subtype of IAV emerges, typically from an animal origin [4]. In the 20th century alone, four pandemics were recorded. The most severe pandemic "Spanish Flu" swept the continents in 1918-1919, affected estimated 500 million people and caused over 40 million deaths [5]. The most recent pandemic in 2009 emerged when the swine-origin virus started to infect humans [6]. So called "Swine Flu", it is an ongoing threat that would result in devastating consequences if not controlled.

Oseltamivir, zanamivir and peramivir are amongst commonly used drugs to treat influenza [7]. However, certain amino acid changes in the viral neuraminidase (NA) protein have been reported to give a rise of drug-resistant IAV strains to reduce the efficacy of current treatment [8, 9]. Therefore the critical question remains: what will be the next generation of anti-influenza drugs that is less likely to result in resistant-related evolution of IAV?

In the light of discoveries on virus-host interactions, we attempt to summarize our knowledge on IAV replication and cellular antiviral responses with a particular focus on apoptosis. We demonstrate how this information could be used to identify potential antiviral agents not only against influenza virus but also other viral diseases.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 May 2017 doi:10.20944/preprints201705.0209.v1

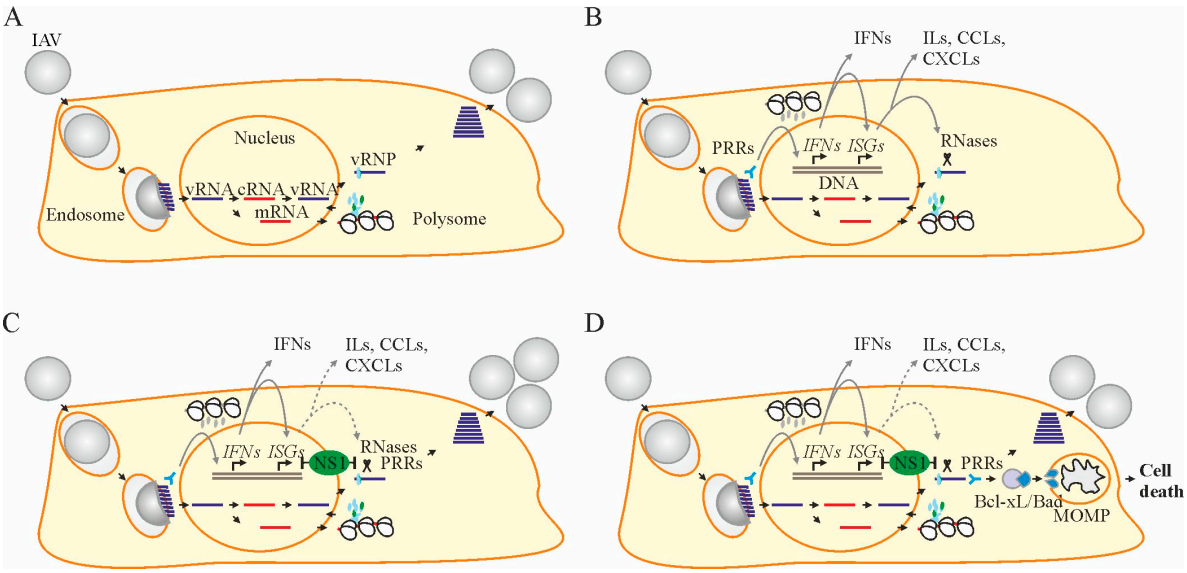

Figure 1. IAV replication cycle and cellular antiviral responses. (A) The viral replication cycle starts when the HA proteins recognize surface receptors of the host. IAV particles are taken up by endocytosis. In the late endosomes viral and endosomal membranes fuse together to expose viral vRNPs, which then become uncoated from the lipid envelope and matrix protein M1 shell. The vRNPs are imported to the nucleus, where the viral genes direct the production of new viral components. Newly synthesized viral genomes and proteins assemble into new virus particles on the plasma membrane before budding and being released from the cell to infect other cells. (B) Anti-IAV interferon response. When IAV enters the cells, PRRs sense vRNA and initiate the transcription of IFN genes. Once transcribed, IFNs mediate the expression of interferon stimulated genes (ISGs) in self and neighboring non-infected cells. ISGs encode different antiviral proteins including RNases, which degrade vRNA in infected cells. ISGs also encode interleukins (ILs), chemokines (CXCLs and CCLs) and other cytokines to recruit immune cells to the site of infection. (C) IAV NS1 protein can hinder the cellular IFN-ISG response by binding with viral RNA, cellular DNA or other cellular factors. The viral replication cycle resumes. (D) Apoptosis in IAV-infected cell. Cell initiate apoptosis in response to a large amount of vRNA. PRRs recognize vRNA and transduce signals to anti-apoptotic Bcl-2 proteins. Bcl2 proteins release pro-apoptotic proteins to initiate MOMP, ATP degradation and caspase 3 activation. This results in cell death.

2. IAV structure and replication cycle

IAV is an enveloped virus which belongs to the Orthomyxoviridae family [10]. The genome is comprised of eight single-stranded RNA segments to encode total 10-13 proteins depending on the virus strain. Among these, nucleoprotein (NP) and three viral polymerase subunits (PA, PB1 and PB2) bind viral RNA (vRNA) to make viral ribonucleoproteins (vRNPs). The vRNPs are surrounded by lipid membrane embedded with hemagglutinin (HA), NA, the ion channel M2 and the matrix protein M1. Some proteins are only expressed in the infected cells and not present in virion, for example nonstructural protein 1 (NS1), PB1-F2, PA-X and N40. Two gene segments are alternatively spliced to produce NS1/NEP and M1/M2proteins [10].

IAVs are divided into subtypes based on the structure of virus surface glycoproteins HA and NA. Currently, there are 18 known subtypes of HA (H1-18) and 11 of NA (N1-11) [11]. Only a limited number of IAV subtypes including H1N1 and H3N2 are capable of infecting humans.

IAV replication cycle begins when the HA proteins bind to sialic acid on the surface of epithelial cells of respiratory tract, dendritic cells, type II pneumocytes, alveolar macrophages or retinal epithelial cells (Fig. 1A) [12].

After binding, virus particles are internalized by endocytosis [13]. Following the internalization, IAVs are transported to late endosomes where the acidic environment facilitates HA-mediated fusion of the viral and endosomal membrane, followed by the dissociation and degradation of M1 from

 doi:10.20944/preprints201705.0209.v1

vRNPs [14, 15]. The vRNPs enter the nucleus through nuclear pore complexes [16]. In the nucleus viral genomic RNA is transcribed into positive-sense mRNA using viral polymerase [17, 18]. The polymerase snatches 5′ Caps from cellular RNA, performs RNA-dependent RNA polymerization and 3′ RNA polyadenylation to make viral pre-mRNA. Two of the eight viral pre-mRNAs are then spliced by cellular machinery. The viral proteins are translated from mRNA in the cytoplasm by host cell ribosomes, some of which imported to the nucleus to initiate replication of vRNA. The replication of vRNA occurs in two steps: the synthesis of cRNA (the positive-sense RNA strand) that is complementary to the full-length vRNA; and the copying of cRNA into new negative-sense vRNAs. Newly assembled vRNPs and viral proteins are transported to the lipid domains on the apical side of the cell plasma membrane, where virions are assembled and budded by NA [19].

Approximately 0.18%–0.21% of the amino acids in IAVs proteins mutate every year due to the error-prone nature of viral RNA-polymerase [20]. Some of these mutations cause antigenic drift, which allows emerging viruses to evade host immunity developed from previous IAV infections/vaccinations or drug treatment. The viruses can also undergo reassortment of genetic segments to generate even greater variations and sometimes antigenic shift. These genetic variations are potential causes of epidemic and pandemic [10].

3. Early anti-viral response: cellular interferon and viral counteract

When IAV enters the cells, stimulus-specific signals are transduced along the interferon signaling pathway in order to activate antiviral mechanisms (Fig. 1B) [21]. Pattern recognition receptors (PRRs) play a pivotal role in detecting invading virus and subsequent activation of innate response [22]. In particular, Toll-like receptors 3 and 7 (TLR-3 and -7) are specialized in recognizing vRNA to activate transcription of interferon genes *IFNB1, IL28A, IL29, IL28B, IFNG, IFNA1, IFNA2* and *IFNW1*. Interferons (IFNs), thereafter, mediate the expression of interferon stimulated genes (ISGs). When secreted extracellularly, IFNs can also stimulate transcription of ISGs in neighboring non-infected cells to protect these cells from potential viral invasion [23-25]. ISGs encode various proteins with antiviral properties: ribonucleases (OASL, OAS1, ISG20) to degrade viral RNA, E3-ligases and ubiquitin-like molecules (HERC5, Trim25, and ISG15) to destroy viral proteins, metabolic enzymes (COX2, IDO and 25HC) to catalyze the production of immuno- and neuro-modulators (prostaglandin H2, kynurenine and oxysterol 25-hydroxycholesterol), as well as cytokine-processing factors (GBP1, GBP4, GBP5, MX1 and MX2) and cytokines (IL1B, IL8, IL6, CXCL10, CCL5) to activate and recruit immune cells to the site of infection [26-31]. As a result, ISG products can inhibit viral replication in infected cells, alert non-infected cells for potential infections, attract immune cells and alarm central nervous system (CNS) about ongoing infection.

In counter-response to cellular IFNs, however, IAVs have evolved to utilize non-structural protein NS1 (Fig. 1C) [32]. Synthesized from the ribosomes within few hours of infection, multifunctional NS1 can circumvent antiviral responses [33]. For example, NS1 can interact with vRNA and its replication intermediates to prevent recognition by cellular PRRs and RNAses [34-37]. By directly binding with cellular DNA, NS1 can block the transcription of innate antiviral genes [38]. It can also bind TRIM25, ISG15, GBP1 and other ISG products to inhibit their functions at transcriptional, post-transcriptional, translational and post-translational [39].

The manifestation of IAV infection depends on cellular IFN responses versus viral counter-responses. Efficient activation of IFN responses and timely activation of viral counter-responses might influence the severity of infection; hence, it is critical to consider timing of both aspects when developing antiviral drugs.

4. **Apoptosis in IAV-infected cells**

When the IFN responses fail to control IAV replication, cells may activate a secondary antiviral response via programmed death called apoptosis (Fig. 1D). During this process, PRRs including RIG-I, MDA5, PKR (encoded by ISGs: *IFIH1, DDX58* and *EIF2AK2*) recognize accumulating vRNA and activate pro-apoptotic apoptotic machinery that direct the fate of IAV-infected cells [40]. The anti-

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 May 2017 doi:10.20944/preprints201705.0209.v1

apoptotic (Bcl-2, Bcl-xL, and Bcl-w) and pro-apoptotic (Bax, Bak, Bad, Bim, Bid, Puma, and Noxa) Bcl-2 proteins dissociate/associate to start a cascade of reactions leading to mitochondria membrane permeabilization (MOMP), followed by cytochrome C release, apoptosome activation, ATP degradation and eventually cell death [41-44]. As the initial trigger of this process, concentration of vRNA is therefore a critical rate-limiting factor in apoptosis. Alternatively, if the viral load is high, apoptosis can be initiated during virus entry.

All Bcl-2 proteins contain Bcl2-homology 3 (BH3) domains, which are essential for protein-protein interactions and functions [45]. Other cellular proteins including UACA, PAWR, FLII, Trim21, IMMT, 14-3-3, EFHD2, DHX9, DDX3, NLRP3 and LRRFIP2 as well as viral factors M2, PB1-F2, NS1, HA and NP may play important roles in Bcl-2-dependent apoptosis by stabilizing or altering the interactions of BH3-domain proteins in infected cells [44, 46-48]. However, further studies are required to verify their specific functions in apoptosis.

5. Bcl-2 inhibitors as antiviral compounds

Bcl-2 dependent apoptosis represents an excellent target for antiviral drug development. First Bcl-2 inhibitor (Bcl2i) ABT-737 was engineered for cancer treatment based on the structure of Bad bound to Bcl-xL in order to mimic Bad BH3-peptide [49, 50]. Several derivatives have been developed to have enhanced pro-apoptotic effect as well as pharmacokinetic properties, and the resulting products ABT-263 and ABT-199 are orally bioavailable [51, 52]. Another group of Bcl2i was discovered using high-throughput screening [53]. This includes WEHI-539 and its derivatives, A-1331852 and A-1155463. ABT-263 is currently in clinical trials as an anticancer drug candidate, ABT-199 is approved to treat multiple lymphoid malignancies, while others are in preclinical development [45, 54].

These compounds (ABT-737, ABT-263, ABT-199, WEHI-539, A-1331852, A-1155463) have different affinities for Bcl-2 proteins [40], but all can universally induce premature death of IAV-infected cells at concentrations not toxic for non-infected cells [44] (Bulanova et al., unpublished). However, only ABT-263, A-1331852 and A-1155463 could effectively limit viral replication and spread. The most likely explanation for the effects is the high affinity of these compounds to Bcl-xL and their ability to alter its structure and interactions with other Bcl-2 proteins. ABT-263, unlike A-1155463, causes irreversible thrombocytopenia [55, 56], which makes A-1155463 a better candidate for antiviral testing in animals. Moreover, A-1155463 has half-maximum efficacy concentration (EC_{50}) less than 10 nM, whereas the value of ABT-263 is 80 nM. In addition, half-maximum cytotoxic concentration (CC_{50}) value of A-1155463 is higher than that of A-1331852, whereas EC50 of both are below 10 nM. Thus, A-1155463 could represent an antiviral lead candidate, which would reinforce the necessary therapeutic arsenal for the treatment of influenza and perhaps other viral diseases.

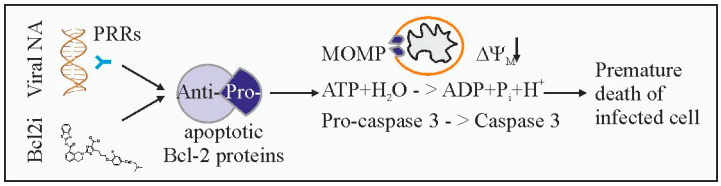

Figure 2. Schematic diagram showing how chemical inhibitors of Bcl2 proteins induce premature death of cells containing viral nucleic acids. PRRs recognize intracellular viral NAs and send signals to anti-apoptotic Bcl-2 proteins. Bcl2 proteins release pro-apoptotic proteins to initiate mitochondria membrane permeabilization (MOMP), ATP degradation and caspase 3 activation. This results in cell death. Bcl2i act synergistically with viral NA and thereby facilitate the cell death.

6. Conclusions

Cellular antiviral responses including IFN response and apoptosis are employed in order to inhibit virus replication and spread. IAVs have evolved to gain mechanisms to disconcert these responses to ensure viral replication and spread. The knowledge of host-virus interaction can be exploited in development of pharmacological interventions to control and treat IAV infections. In particular, novel antiviral drugs inducing premature apoptosis of infected cells without affecting non-infected cells may be developed.

A-1155463 could serve as a lead compound in this process. Treatment of IAV infections with A-1155463 or its analogues may reduce the use of NA-directed drugs, and thus slow down evolutional selection of drug-resistant viral strains. Timely treatment with A-1155463 may also reduce the use of antibiotics, which are utilized for treatment of secondary bacterial infections. This will limit the development of emerging antibiotic-resistant bacteria. Thus, further development of A-1155463 may warrant emergence of better drugs for treatment/prevention of influenza and perhaps other viral diseases.

Acknowledgments: This work was funded by Mobilitas pluss top researcher grant (contract No MOBTT39, to DK), IP-Korea and Bio&Medical Technology Development Program of the NRF and the Ministry of Science, ICT and Future Planning, Republic of Korea (grant No. 2014K1A4A7A01074646, to JYM).

Author Contributions: All authors analyzed the literature and wrote the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Global Burden of Disease Study, C., Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2015, 386 (9995), 743-800.
2. Lafond, K. E.; Nair, H.; Rasooly, M. H.; Valente, F.; Booy, R.; Rahman, M.; Kitsutani, P.; Yu, H.; Guzman, G.; Coulibaly, D.; Armero, J.; Jima, D.; Howie, S. R.; Ampofo, W.; Mena, R.; Chadha, M.; Sampurno, O. D.; Emukule, G. O.; Nurmatov, Z.; Corwin, A.; Heraud, J. M.; Noyola, D. E.; Cojocaru, R.; Nymadawa, P.; Barakat, A.; Adedeji, A.; von Horoch, M.; Olveda, R.; Nyatanyi, T.; Venter, M.; Mmbaga, V.; Chittaganpitch, M.; Nguyen, T. H.; Theo, A.; Whaley, M.; Azziz-Baumgartner, E.; Bresee, J.; Campbell, H.; Widdowson, M. A.; Global Respiratory Hospitalizations-Influenza Proportion Positive Working, G., Global Role and Burden of Influenza in Pediatric Respiratory Hospitalizations, 1982-2012: A Systematic Analysis. PLoS Med 2016, 13 (3), e1001977.
3. WHO, Influenza (Seasonal). http://www.who.int/mediacentre/factsheets/fs211/en/.
4. Horimoto, T.; Kawaoka, Y., Influenza: lessons from past pandemics, warnings from current incidents. Nat Rev Microbiol 2005, 3 (8), 591-600.
5. Taubenberger, J. K.; Morens, D. M., 1918 Influenza: the mother of all pandemics. Emerg Infect Dis 2006, 12 (1), 15-22.
6. Fineberg, H. V., Pandemic preparedness and response--lessons from the H1N1 influenza of 2009. N Engl J Med 2014, 370 (14), 1335-42.
7. Belanov, S. S.; Bychkov, D.; Benner, C.; Ripatti, S.; Ojala, T.; Kankainen, M.; Kai Lee, H.; Wei-Tze Tang, J.; Kainov, D. E., Genome-Wide Analysis of Evolutionary Markers of Human Influenza A(H1N1)pdm09 and A(H3N2) Viruses May Guide Selection of Vaccine Strain Candidates. Genome Biol Evol 2015, 7 (12), 3472-83.
8. Bouvier, N. M.; Palese, P., The biology of influenza viruses. Vaccine 2008, 26 Suppl 4, D49-53.

9. CDC, Influenza Antiviral Medications: Summary for Clinicians. https://www.cdc.gov/flu/professionals/antivirals/summary-clinicians.htm.
10. Spanakis, N.; Pitiriga, V.; Gennimata, V.; Tsakris, A., A review of neuraminidase inhibitor susceptibility in influenza strains. Expert Rev Anti Infect Ther 2014, 12 (11), 1325-36.
11. Hussain, M.; Galvin, H. D.; Haw, T. Y.; Nutsford, A. N.; Husain, M., Drug resistance in influenza A virus: the epidemiology and management. Infect Drug Resist 2017, 10, 121-134.
12. Min, J. Y.; Subbarao, K., Cellular targets for influenza drugs. Nat Biotechnol 2010, 28 (3), 239-40.
13. van de Wakker, S. I.; Fischer, M. J. E.; Oosting, R. S., New drug-strategies to tackle viral-host interactions for the treatment of influenza virus infections. Eur J Pharmacol 2017.
14. Watanabe, T.; Kawaoka, Y., Influenza virus-host interactomes as a basis for antiviral drug development. Curr Opin Virol 2015, 14, 71-8.
15. Muller, K. H.; Kakkola, L.; Nagaraj, A. S.; Cheltsov, A. V.; Anastasina, M.; Kainov, D. E., Emerging cellular targets for influenza antiviral agents. Trends Pharmacol Sci 2012, 33 (2), 89-99.
16. Keener, A. B., Host with the most: Targeting host cells instead of pathogens to fight infectious disease. Nat Med 2017, 23 (5), 528-531.
17. Soderholm, S.; Fu, Y.; Gaelings, L.; Belanov, S.; Yetukuri, L.; Berlinkov, M.; Cheltsov, A. V.; Anders, S.; Aittokallio, T.; Nyman, T. A.; Matikainen, S.; Kainov, D. E., Multi-Omics Studies towards Novel Modulators of Influenza A Virus-Host Interaction. Viruses 2016, 8 (10).
18. Pinsent, A.; Fraser, C.; Ferguson, N. M.; Riley, S., A systematic review of reported reassortant viral lineages of influenza A. BMC Infect Dis 2016, 16, 3.
19. Werner, J. L.; Steele, C., Innate receptors and cellular defense against pulmonary infections. J Immunol 2014, 193 (8), 3842-50.
20. Edinger, T. O.; Pohl, M. O.; Stertz, S., Entry of influenza A virus: host factors and antiviral targets. J Gen Virol 2014, 95 (Pt 2), 263-77.
21. White, J. M.; Whittaker, G. R., Fusion of Enveloped Viruses in Endosomes. Traffic 2016, 17 (6), 593-614.
22. Banerjee, I.; Miyake, Y.; Nobs, S. P.; Schneider, C.; Horvath, P.; Kopf, M.; Matthias, P.; Helenius, A.; Yamauchi, Y., Influenza A virus uses the aggresome processing machinery for host cell entry. Science 2014, 346 (6208), 473-7.
23. Pumroy, R. A.; Ke, S.; Hart, D. J.; Zachariae, U.; Cingolani, G., Molecular determinants for nuclear import of influenza A PB2 by importin alpha isoforms 3 and 7. Structure 2015, 23 (2), 374-84.
24. Te Velthuis, A. J.; Fodor, E., Influenza virus RNA polymerase: insights into the mechanisms of viral RNA synthesis. Nat Rev Microbiol 2016, 14 (8), 479-93.
25. Reguera, J.; Gerlach, P.; Cusack, S., Towards a structural understanding of RNA synthesis by negative strand RNA viral polymerases. Curr Opin Struct Biol 2016, 36, 75-84.
26. Lakdawala, S. S.; Fodor, E.; Subbarao, K., Moving On Out: Transport and Packaging of Influenza Viral RNA into Virions. Annu Rev Virol 2016, 3 (1), 411-427.
27. Killip, M. J.; Fodor, E.; Randall, R. E., Influenza virus activation of the interferon system. Virus Res 2015, 209, 11-22.
28. Bowie, A. G.; Unterholzner, L., Viral evasion and subversion of pattern-recognition receptor signalling. Nat Rev Immunol 2008, 8 (12), 911-22.
29. Liu, S. Y.; Sanchez, D. J.; Aliyari, R.; Lu, S.; Cheng, G., Systematic identification of type I and type II interferon-induced antiviral factors. Proc Natl Acad Sci U S A 2012, 109 (11), 4239-44.

30. Schoggins, J. W.; Wilson, S. J.; Panis, M.; Murphy, M. Y.; Jones, C. T.; Bieniasz, P.; Rice, C. M., A diverse range of gene products are effectors of the type I interferon antiviral response. Nature 2011, 472 (7344), 481-5.
31. Soderholm, S.; Anastasina, M.; Islam, M. M.; Tynell, J.; Poranen, M. M.; Bamford, D. H.; Stenman, J.; Julkunen, I.; Sauliene, I.; De Brabander, J. K.; Matikainen, S.; Nyman, T. A.; Saelens, X.; Kainov, D., Immuno-modulating properties of saliphenylhalamide, SNS-032, obatoclax, and gemcitabine. Antiviral Res 2016, 126, 69-80.
32. Melchjorsen, J.; Kristiansen, H.; Christiansen, R.; Rintahaka, J.; Matikainen, S.; Paludan, S. R.; Hartmann, R., Differential regulation of the OASL and OAS1 genes in response to viral infections. J Interferon Cytokine Res 2009, 29 (4), 199-207.
33. Ludwig, S.; Wolff, T., Influenza A virus TRIMs the type I interferon response. Cell host & microbe 2009, 5 (5), 420-1.
34. Gaelings, L.; Soderholm, S.; Bugai, A.; Fu, Y.; Nandania, J.; Schepens, B.; Lorey, M. B.; Tynell, J.; Vande Ginste, L.; Le Goffic, R.; Miller, M. S.; Kuisma, M.; Marjomaki, V.; De Brabander, J.; Matikainen, S.; Nyman, T. A.; Bamford, D. H.; Saelens, X.; Julkunen, I.; Paavilainen, H.; Hukkanen, V.; Velagapudi, V.; Kainov, D. E., Regulation of kynurenine biosynthesis during influenza virus infection. FEBS J 2017, 284 (2), 222-236.
35. Dudek, S. E.; Nitzsche, K.; Ludwig, S.; Ehrhardt, C., Influenza A viruses suppress cyclooxygenase-2 expression by affecting its mRNA stability. Sci Rep 2016, 6, 27275.
36. Meunier, E.; Broz, P., Interferon-inducible GTPases in cell autonomous and innate immunity. Cell Microbiol 2016, 18 (2), 168-80.
37. Gold, E. S.; Diercks, A. H.; Podolsky, I.; Podyminogin, R. L.; Askovich, P. S.; Treuting, P. M.; Aderem, A., 25-Hydroxycholesterol acts as an amplifier of inflammatory signaling. Proc Natl Acad Sci U S A 2014, 111 (29), 10666-71.
38. Ayllon, J.; Garcia-Sastre, A., The NS1 protein: a multitasking virulence factor. Curr Top Microbiol Immunol 2015, 386, 73-107.
39. Anastasina, M.; Schepens, B.; Soderholm, S.; Nyman, T. A.; Matikainen, S.; Saksela, K.; Saelens, X.; Kainov, D. E., The C terminus of NS1 protein of influenza A/WSN/1933(H1N1) virus modulates antiviral responses in infected human macrophages and mice. J Gen Virol 2015, 96 (8), 2086-91.
40. Bornholdt, Z. A.; Prasad, B. V., X-ray structure of NS1 from a highly pathogenic H5N1 influenza virus. Nature 2008, 456 (7224), 985-8.
41. Min, J. Y.; Li, S.; Sen, G. C.; Krug, R. M., A site on the influenza A virus NS1 protein mediates both inhibition of PKR activation and temporal regulation of viral RNA synthesis. Virology 2007, 363 (1), 236-43.
42. Min, J. Y.; Krug, R. M., The primary function of RNA binding by the influenza A virus NS1 protein in infected cells: Inhibiting the 2'-5' oligo (A) synthetase/RNase L pathway. Proc Natl Acad Sci U S A 2006, 103 (18), 7100-5.
43. Li, S.; Min, J. Y.; Krug, R. M.; Sen, G. C., Binding of the influenza A virus NS1 protein to PKR mediates the inhibition of its activation by either PACT or double-stranded RNA. Virology 2006, 349 (1), 13-21.
44. Anastasina, M.; Le May, N.; Bugai, A.; Fu, Y.; Soderholm, S.; Gaelings, L.; Ohman, T.; Tynell, J.; Kyttanen, S.; Barboric, M.; Nyman, T. A.; Matikainen, S.; Julkunen, I.; Butcher, S. J.; Egly, J. M.; Kainov, D. E., Influenza virus NS1 protein binds cellular DNA to block transcription of antiviral genes. Biochim Biophys Acta 2016, 1859 (11), 1440-1448.
45. Hale, B. G.; Randall, R. E.; Ortin, J.; Jackson, D., The multifunctional NS1 protein of influenza A viruses. J Gen Virol 2008, 89 (Pt 10), 2359-76.

46. Tran, A. T.; Cortens, J. P.; Du, Q.; Wilkins, J. A.; Coombs, K. M., Influenza virus induces apoptosis via BAD-mediated mitochondrial dysregulation. J Virol 2013, 87 (2), 1049-60.
47. McLean, J. E.; Datan, E.; Matassov, D.; Zakeri, Z. F., Lack of Bax prevents influenza A virus-induced apoptosis and causes diminished viral replication. J Virol 2009, 83 (16), 8233-46.
48. Hinshaw, V. S.; Olsen, C. W.; Dybdahl-Sissoko, N.; Evans, D., Apoptosis: a mechanism of cell killing by influenza A and B viruses. J Virol 1994, 68 (6), 3667-73.
49. Kakkola, L.; Denisova, O. V.; Tynell, J.; Viiliainen, J.; Ysenbaert, T.; Matos, R. C.; Nagaraj, A.; Ohman, T.; Kuivanen, S.; Paavilainen, H.; Feng, L.; Yadav, B.; Julkunen, I.; Vapalahti, O.; Hukkanen, V.; Stenman, J.; Aittokallio, T.; Verschuren, E. W.; Ojala, P. M.; Nyman, T.; Saelens, X.; Dzeyk, K.; Kainov, D. E., Anticancer compound ABT-263 accelerates apoptosis in virus-infected cells and imbalances cytokine production and lowers survival rates of infected mice. Cell Death Dis 2013, 4, e742.
50. Ashkenazi, A.; Fairbrother, W. J.; Leverson, J. D.; Souers, A. J., From basic apoptosis discoveries to advanced selective BCL-2 family inhibitors. Nat Rev Drug Discov 2017, 16 (4), 273-284.
51. Delbridge, A. R.; Grabow, S.; Strasser, A.; Vaux, D. L., Thirty years of BCL-2: translating cell death discoveries into novel cancer therapies. Nat Rev Cancer 2016, 16 (2), 99-109.
52. Ong, J. D.; Mansell, A.; Tate, M. D., Hero turned villain: NLRP3 inflammasome-induced inflammation during influenza A virus infection. J Leukoc Biol 2017, 101 (4), 863-874.
53. Herold, S.; Ludwig, S.; Pleschka, S.; Wolff, T., Apoptosis signaling in influenza virus propagation, innate host defense, and lung injury. J Leukoc Biol 2012, 92 (1), 75-82.
54. Subramanian, T.; Vijayalingam, S.; Kuppuswamy, M.; Chinnadurai, G., Interaction of cellular proteins with BCL-xL targeted to cytoplasmic inclusion bodies in adenovirus infected cells. Virology 2015, 483, 21-31.
55. Lee, E. F.; Czabotar, P. E.; Smith, B. J.; Deshayes, K.; Zobel, K.; Colman, P. M.; Fairlie, W. D., Crystal structure of ABT-737 complexed with Bcl-xL: implications for selectivity of antagonists of the Bcl-2 family. Cell Death Differ 2007, 14 (9), 1711-3.
56. Kvansakul, M.; Hinds, M. G., The Bcl-2 family: structures, interactions and targets for drug discovery. Apoptosis 2015, 20 (2), 136-50.
57. Souers, A. J.; Leverson, J. D.; Boghaert, E. R.; Ackler, S. L.; Catron, N. D.; Chen, J.; Dayton, B. D.; Ding, H.; Enschede, S. H.; Fairbrother, W. J.; Huang, D. C.; Hymowitz, S. G.; Jin, S.; Khaw, S. L.; Kovar, P. J.; Lam, L. T.; Lee, J.; Maecker, H. L.; Marsh, K. C.; Mason, K. D.; Mitten, M. J.; Nimmer, P. M.; Oleksijew, A.; Park, C. H.; Park, C. M.; Phillips, D. C.; Roberts, A. W.; Sampath, D.; Seymour, J. F.; Smith, M. L.; Sullivan, G. M.; Tahir, S. K.; Tse, C.; Wendt, M. D.; Xiao, Y.; Xue, J. C.; Zhang, H.; Humerickhouse, R. A.; Rosenberg, S. H.; Elmore, S. W., ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. Nat Med 2013, 19 (2), 202-8.
58. Vandenberg, C. J.; Cory, S., ABT-199, a new Bcl-2-specific BH3 mimetic, has in vivo efficacy against aggressive Myc-driven mouse lymphomas without provoking thrombocytopenia. Blood 2013, 121 (12), 2285-8.
59. Lessene, G.; Czabotar, P. E.; Sleebs, B. E.; Zobel, K.; Lowes, K. N.; Adams, J. M.; Baell, J. B.; Colman, P. M.; Deshayes, K.; Fairbrother, W. J.; Flygare, J. A.; Gibbons, P.; Kersten, W. J.; Kulasegaram, S.; Moss, R. M.; Parisot, J. P.; Smith, B. J.; Street, I. P.; Yang, H.; Huang, D. C.; Watson, K. G., Structure-guided design of a selective BCL-X(L) inhibitor. Nat Chem Biol 2013, 9 (6), 390-7.
60. Roberts, A. W.; Huang, D., Targeting BCL2 With BH3 Mimetics: Basic Science and Clinical Application of Venetoclax in Chronic Lymphocytic Leukemia and Related B Cell Malignancies. Clin Pharmacol Ther 2017, 101 (1), 89-98.

 doi:10.20944/preprints201705.0209.v1

61. Leverson, J. D.; Phillips, D. C.; Mitten, M. J.; Boghaert, E. R.; Diaz, D.; Tahir, S. K.; Belmont, L. D.; Nimmer, P.; Xiao, Y.; Ma, X. M.; Lowes, K. N.; Kovar, P.; Chen, J.; Jin, S.; Smith, M.; Xue, J.; Zhang, H.; Oleksijew, A.; Magoc, T. J.; Vaidya, K. S.; Albert, D. H.; Tarrant, J. M.; La, N.; Wang, L.; Tao, Z. F.; Wendt, M. D.; Sampath, D.; Rosenberg, S. H.; Tse, C.; Huang, D. C.; Fairbrother, W. J.; Elmore, S. W.; Souers, A. J., Exploiting selective BCL-2 family inhibitors to dissect cell survival dependencies and define improved strategies for cancer therapy. Sci Transl Med 2015, 7 (279), 279ra40.

62. Tao, Z. F.; Hasvold, L.; Wang, L.; Wang, X.; Petros, A. M.; Park, C. H.; Boghaert, E. R.; Catron, N. D.; Chen, J.; Colman, P. M.; Czabotar, P. E.; Deshayes, K.; Fairbrother, W. J.; Flygare, J. A.; Hymowitz, S. G.; Jin, S.; Judge, R. A.; Koehler, M. F.; Kovar, P. J.; Lessene, G.; Mitten, M. J.; Ndubaku, C. O.; Nimmer, P.; Purkey, H. E.; Oleksijew, A.; Phillips, D. C.; Sleebs, B. E.; Smith, B. J.; Smith, M. L.; Tahir, S. K.; Watson, K. G.; Xiao, Y.; Xue, J.; Zhang, H.; Zobel, K.; Rosenberg, S. H.; Tse, C.; Leverson, J. D.; Elmore, S. W.; Souers, A. J., Discovery of a Potent and Selective BCL-XL Inhibitor with in Vivo Activity. ACS Med Chem Lett 2014, 5 (10), 1088-93.