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Review

Development of cellular signaling pathway inhibitors as new antivirals against influenza

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ABSTRACT

Influenza virus exploits a number of cellular signaling pathways during the course of its replication, rendering them potential targets for new therapeutic interventions. Several preclinical approaches are now focusing on cellular factors or pathways as a means of treating influenza. By targeting host factors, rather than viral mechanisms, these novel therapies may be effective against multiple virus strains and subtypes, and are less likely to elicit viral drug resistance. The most promising candidates are inhibitors of intracellular signaling cascades that are essential for virus replication. This article reviews novel approaches and compounds that target the Raf/MEK/ERK signaling pathway, NF-κB signaling, the PI3K/Akt pathway and the PKC signaling cascade. Although these new antiviral strategies are still in an early phase of preclinical development, results to date suggest they offer a new approach to the treatment of influenza, supplementing direct-acting antiviral drugs.

Contents

1. Introduction

The number of intracellular signaling pathways that have been found to be essential for influenza virus replication has steadily increased over the past decade (Ludwig and Planz, 2008; Ludwig et al., 2003; Pleschka, 2008). In addition to influenza virus, other RNA and DNA viruses must interact with intracellular signaling mechanisms to ensure productive infection (Ludwig and Planz, 2008; Ludwig et al., 2006; Planz et al., 2001; Pleschka, 2008; Seth et al., 2006). Intracellular signaling pathways are therefore increasingly being studied as targets for novel antiviral therapies. Pathways that are required for the virus to cross intracellular barriers, such as the nuclear membrane, are most suitable for antiviral intervention. Influenza viruses must pass these barriers during the initial phase of replication, when the viral ribonucleoproteins

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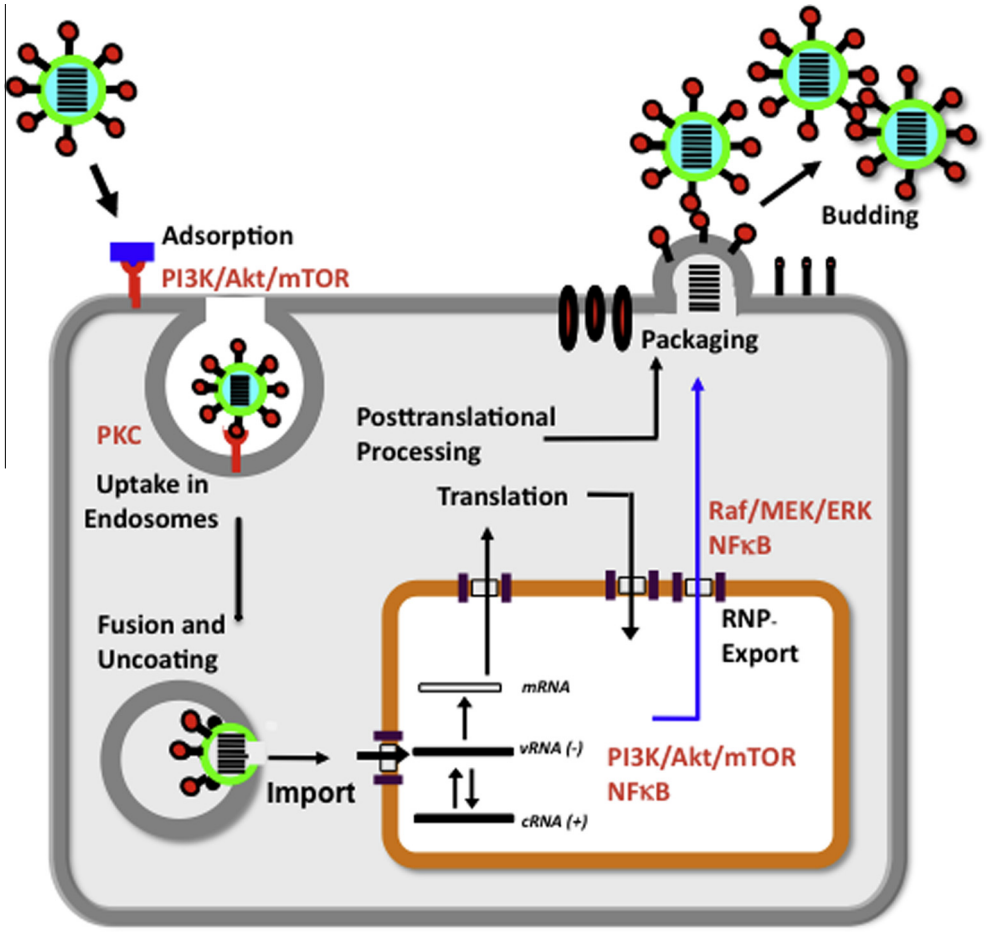


Fig. 1. Influenza virus replication cycle. Involvement of cellular Raf/MEK/ERK, NFκB, PI3K/Akt/mTOR and PKC pathways during the replication of influenza virus. Detailed information is given also in Table 1. Figure modified from (Ludwig et al., 2003).

(RNPs) are transported from the cytoplasm to the nucleus, and later in the replication cycle, when virus particles are released from the infected cell.

A potential advantage of antiviral strategies that target intracellular signaling pathways is that they are less likely to induce viral resistance than those that directly target viral replication, as has already been shown for several compounds (Ludwig et al., 2004; Mazur et al., 2007). However, development of resistance is dependent on multiple factors, including the specific pathway inhibited, its role in influenza virus replication and the level of pathway inhibition (i.e., at the global regulatory level vs. the specific effector level). On the other hand, potential adverse effects of inhibitors of intracellular signaling pathways must also be taken into consideration, since they interfere with the host cell machinery and with substantial cellular functions.

Intracellular signaling pathways are currently being evaluated as targets for many different medical indications. The most advanced development has occurred in the area of antitumor therapy, with an increasing number of compounds now in clinical studies or licensed for the treatment of human malignancies. As a consequence, there is an enormous amount of information about these compounds, as regards their pharmacokinetic and pharmacodynamic properties and adverse effects in humans. It would therefore be of great interest to investigate the antiviral potential of those compounds that have successfully passed Phase I clinical trials for other medical indications, and are suitable for oral administration. Because the target of influenza therapy is the respiratory epithelium, agents that could be delivered by aerosol are also of interest.

This article describes the potential of intracellular signaling pathways as targets for novel influenza therapies, focusing on the Raf/MEK/ERK signaling pathway, NF-κB signaling, the PI3K/Akt pathway and the PKC signaling cascade. In each case, a summary of the basic physiological features of the pathway is followed by a brief review of compounds that inhibit the pathway and have been shown to reduce influenza virus replication, including their *in vitro* and *in vivo* antiviral activity, safety and tolerability in patients, current developmental status and prospects for introduction into clinical use.

2. Development of Raf/MEK/ERK inhibitors against influenza virus infection

2.1. The Raf/MEK/ERK signaling pathway

The Ras-dependent Raf/MEK/ERK signaling pathway belongs to the family of so-called mitogen-activated protein kinase (MAPK) cascades and is one of the best studied signal transduction pathways. Since the discovery of MAP kinases more than 30 years ago a huge number of articles have been published on this topic. Almost all growth factors and cytokines that act through receptor tyrosine kinases, cytokine receptors or G-protein-coupled receptors initiate signaling via the Raf/MEK/ERK pathway (Fig. 1). Typically, ligand binding to receptor tyrosine kinases induces dimerization of the receptor and auto-phosphorylation of specific tyrosine residues in the C-terminal region. This generates binding sites for adaptor proteins, such as growth factor receptor-bound protein 2 (GRB2), which recruit the guanine nucleotide exchange

Table 1
Overview of cellular signaling pathways that play a supporting role in various stages of influenza virus replication.

Signaling pathway	Role of the pathway in support of viral replication	
Raf/MEK/ERK	- Nuclear release of viral ribonucleoprotein (RNP) complexes in the late stage of the replication cycle	Pleschka et al. (2001), Ludwig et al. (2006), Pleschka (2008) and Ludwig (2009)
NF-κB	- TRAIL- or FasL-mediated activation of caspases, resulting in enhanced nuclear export of viral RNPs by enhanced diffusion through the pores - Counteraction of type I IFN-induced gene expression - Differential regulation of viral RNA synthesis	Wurzer et al. (2003, 2004), Nimmerjahn et al. (2004), Ludwig et al. (2006), Wei et al. (2006), Ludwig and Planz (2008), Kramer et al. (2008), Pauli et al. (2008) and Kumar et al. (2008)
PI3K/Akt/mTOR	- Early entry uptake - Prevention of premature apoptosis - Viral RNA expression and RNP localization	Ehrhardt et al. (2006, 2007), Shin et al. (2007a,b), Zhirnov and Klenk (2007) and Ehrhardt and Ludwig (2009)
PKC	- Activation of Raf/MEK/ERK - Entry via late endosomes	Sieczkarski et al. (2003), Marjuki et al. (2006) and Ludwig (2009)

factor Sos at the plasma membrane. Sos activates the membrane-bound Ras by catalyzing the replacement of GDP with GTP. In its GTP-bound form, Ras leads to the stepwise phosphorylation and activation of the serine threonine kinase Raf (ARAF, BRAF and CRAF) to the plasma membrane, where they become activated by a complex interplay of phosphorylation events and protein–protein interactions. Raf acts as a MAP kinase kinase kinase (MAPKKK) and activates the dual-specificity kinase MEK1 and MEK2 (MAPK kinase/ERK kinase), which in turn catalyze the activation of the effector MAP kinases ERK1 and ERK2 (extracellular signal-regulated kinase).

Once activated, ERK1/ERK2 phosphorylate nuclear and cytoplasmic substrates involved in diverse cellular responses, such as cell proliferation, survival, differentiation, motility and angiogenesis (Widmann et al., 1999). The Raf/MEK/ERK pathway also regulates cytokine production, such as tumor necrosis factor-alpha (TNF-α) and interleukin-8. Inhibition of the pathway might therefore not only interfere with virus replication, but may also prevent the overabundant production of pro-inflammatory cytokines and chemokines known as "cytokine storm." This unbalanced cytokine expression is often correlated to severe pneumonia caused by several influenza virus strains, including the highly pathogenic avian H5N1 virus (Beigel et al., 2005; Chan et al., 2005; Cheng et al., 2011; de Jong et al., 2006). Influenza virus-mediated ERK activation contributes to cytokine production and airway inflammation (Mizumura et al., 2003). A study by Pinto and colleagues also demonstrated that, besides reducing virus titers, inhibition of MEK modulated pro-inflammatory cytokine expression (Pinto et al., 2011), another advantage of targeting this signaling pathway.

It is controversially debated whether treatment with inhibitors that interfere with cell proliferation would have a negative effect on the antiviral immune response. It is known that activation of the Raf/MEK/ERK signaling pathway is required for T_H2 cell differentiation, and that inhibiting this pathway supports the generation of T_H1 $CD4^+$ T-cells, which are required by the immune system for an efficient control of pathogens (Nakayama and Yamashita, 2010). Thus, besides the antiviral activity and regulation of pro-inflammatory cytokine production by MEK inhibitors, a third feature is their modulation of the T_H2 response supporting antigen presentation, activation and clonal expansion to T_H1 $CD4^+$ T-cells.

2.2. Raf/MEK/ERK pathway and influenza virus

More than a decade ago it was shown that activation of the Raf/MEK/ERK signaling pathway is a prerequisite for efficient influenza virus replication (Pleschka et al., 2001), and that virus titers are enhanced in cells with an activated Raf/MEK/ERK pathway. This has been demonstrated in MDCK cells in which the pathway was pre-activated by expression of constitutively active mutants of Raf or MEK (Ludwig et al., 2004; Marjuki et al., 2007; Olschlager et al., 2004). Similarly, in mice expressing constitutively active Raf kinase in type II alveolar epithelial cells, infection led to enhanced virus replication in the cells expressing the transgene (Olschlager et al., 2004). Strikingly, blockade of the Raf/MEK/ERK pathway with specific inhibitors strongly impaired the growth of all influenza A and B viruses tested (Ludwig et al., 2004; Olschlager et al., 2004; Pleschka et al., 2001).

Activation of the Raf/MEK/ERK signaling pathway is required by influenza virus for the efficient export of RNPs from the nucleus into the cytoplasm (Ludwig et al., 2004; Marjuki et al., 2007; Pleschka et al., 2001). Inhibition of the cascade leads to nuclear retention of the viral RNP complexes in late stages of the replication cycle (Fig. 1; Table 1). This suggests that the pathway controls RNP export, most probably by interfering with the activity of the viral nuclear export protein NEP (Pleschka et al., 2001), but the detailed mechanism by which the Raf/MEK/ERK pathway regulates RNP export is unknown. The role of phosphorylation of the viral NP and involvement of cellular factors are discussed in (Pleschka, 2008). Activation of the Raf/MEK/ERK pathway therefore might orchestrate the complex export of RNPs: on one hand, they must reside in the nucleus for sufficient replication and transcription of the viral genome in early stages of infection, while on the other, they have to be exported from the nucleus late in the replication cycle for budding of progeny virus at the cell membrane.

These early and late requirements for a supporting signal correlate well with the bi-phasic activation of ERK during the viral life cycle (Pleschka et al., 2001). Membrane accumulation of the viral HA protein and its tight association with lipid-raft domains (Fig. 2) triggers protein kinase C (PKC)-dependent activation of the Raf/MEK/ERK cascade via H-Ras late in the infection cycle, inducing RNP export (Eisenberg et al., 2006; Marjuki et al., 2006). Electron-dense patches at sites of virus membrane budding formed by viral HA, NA, M2 and M1 might be targets for signaling components, leading to activation of the Raf/MEK/ERK pathway. This late activation by membrane-accumulated HA might represent an auto-regulative mechanism, which coordinates RNP export to the stage when it is required for viral budding (Ludwig, 2009; Pleschka, 2008).

2.3. Compounds inhibiting the Raf/MEK/ERK pathway and influenza virus production

The requirement of Raf/MEK/ERK activation for efficient influenza virus replication suggests that this pathway could be a promising target for novel anti-influenza approaches. Mutations in the *ras* and *raf* genes, leading to hyperactivation of the Raf/MEK/ERK signaling pathway and uncontrolled cell proliferation, are the cause of nearly half of human malignancies, and aberrant receptor activation is frequently observed in certain tumors (Hynes and Lane, 2005). The Raf/MEK/ERK signaling pathway is therefore a perfect target for cancer therapy (Fremin and Meloche, 2010). The compounds U0126 and PD98059 were among the first

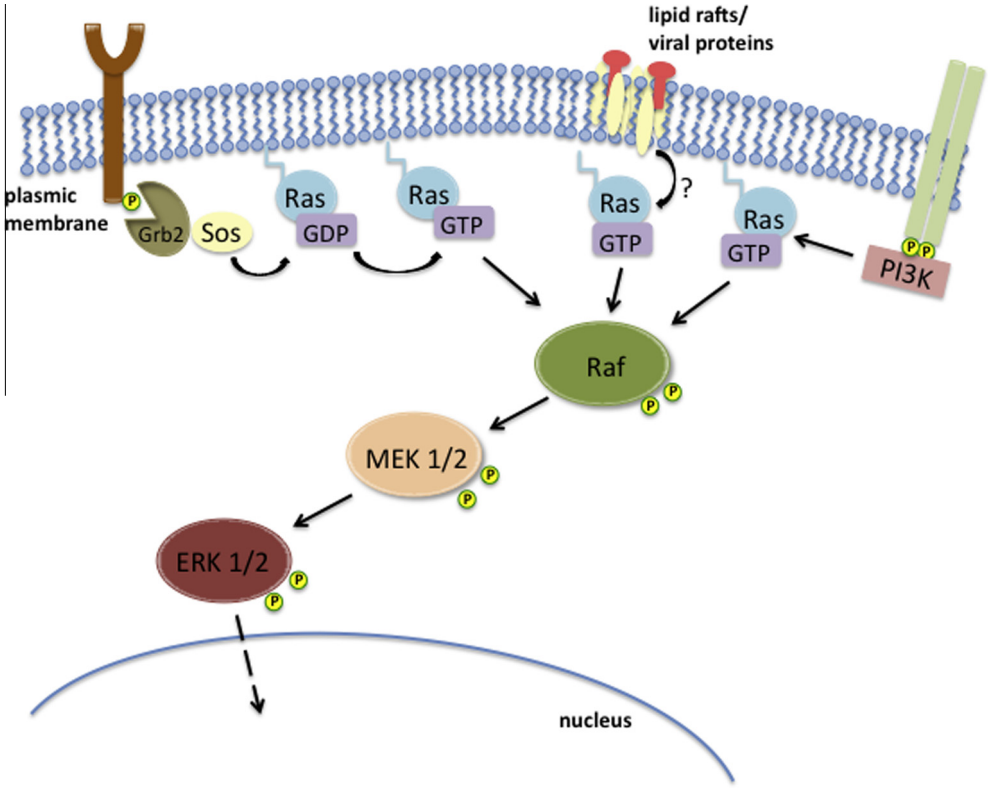


Fig. 2. Raf/MEK/ERK signaling in influenza virus infected cells. Influenza virus infection leads to activation of Raf via a GTP-bound form of Ras. This can be mediated either through receptor tyrosine kinases; by membrane accumulation of the viral HA protein and lipid-raft domains; or through PI3K signaling. Ras leads to the stepwise phosphorylation and activation of Raf. Once activated, Raf leads to phosphorylation and activation of MEK 1/2, which phosphorylates and consequently activates ERK 1/2. See text for details and abbreviations.

inhibitors available, but because of poor bioavailability, they never passed pre-clinical development for cancer treatment. Nevertheless, these inhibitors have been valuable tools for basic research in a number of fields.

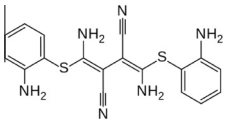
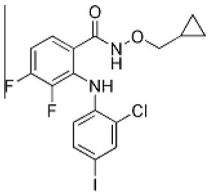
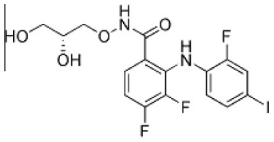
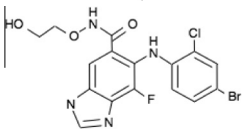
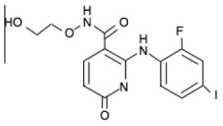
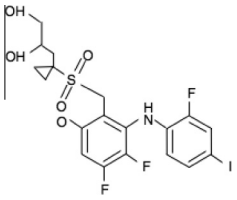
U0126 was the first MEK-inhibitor used to demonstrate that inhibiting the Raf/MEK/ERK pathway leads to reduction in influenza virus production in MDCK and A549 cells (Pleschka et al., 2001). Because of its limited pharmacological properties, U0126 is not suitable for oral treatment, and Pinto and colleagues demonstrated that delivery via the intraperitoneal route has only a slight antiviral effect (Pinto et al., 2011). We therefore decided to deliver U0126 as an aerosol. The limited bioavailability of U0126 was one of the reasons why it took ten more years to demonstrate – by aerosol-treatment – that the concept of inhibiting MEK to fight influenza is effective in mice (Droebner et al., 2011). Today, a large variety of MEK-inhibitors or dual inhibitors of the Raf/MEK/ERK signaling pathway are available for oral treatment, and are either in Phase I evaluation or have successfully passed clinical trials, and a few are licensed for cancer therapy (Fremin and Meloche, 2010). A summary of compounds that were tested for antiviral activity against influenza virus is found in Table 2.

CI-1040 (PD-184352), a benzhydroxamate derivative (Pfizer), is a small-molecule inhibitor of MEK1 and MEK2. It is considered as the second class of MEK-inhibitors and was tested in a Phase I trial against cancer, in which it was administered repeatedly for 21 days, and target suppression and antitumor activity were demonstrated (Lorusso et al., 2005). In another Phase II trial to assess the antitumor activity and safety of CI-1040 in breast cancer, colon cancer, non-small-cell lung cancer and pancreatic cancer, its activity was not sufficient (Rinehart et al., 2004), so that development as an antitumor compound was stopped. However, a first report demonstrated that CI-1040 efficiently inhibits influenza virus replication in MDCK cells (Droebner et al., 2011). It could therefore be worth continuing the preclinical development of the anti-influenza virus activity by CI-1040, because its pharmacological properties are already well characterized. Moreover, treatment times will be much shorter than for antitumor therapy, which could increase tolerability.

PD-0325901 (Pfizer) is also a second-generation MEK1/2 inhibitor and a structural analog of CI-1040; it has significantly improved potency, solubility and bioavailability and is 100-fold more active in inhibiting MEK (Barrett et al., 2008; Sebolt-Leopold and Herrera, 2004). Unfortunately, in a Phase II study in non-small-cell lung cancer patients, PD-0325901 did not meet its primary efficacy end point (Haura et al., 2010), and its clinical development as an antitumor compound was stopped (Fremin and Meloche, 2010). First experiments demonstrated that PD-0325901 shows antiviral activity against influenza virus in MDCK cells (Droebner et al., 2011). In a second study, these investigations were extended demonstrating an EC_{50} value against H1N1pdm09 influenza virus of 0.6 nM in A549 cells, which was in the same range as oseltamivir (0.4 nM) for this virus strain and this cell line. Moreover, the combination of PD-0325901 with oseltamivir resulted in an increased antiviral effect, with a strong synergism (Haasbach et al., 2013). The same study identified three more MEK-inhibitors that are orally available and are at least in Phase I clinical trials against cancer.

AZD-6244 (Astra Zeneca) is another second-generation potent inhibitor of both MEK1 and MEK2 that was advanced into clinical development against cancer (Adjei et al., 2008; Yeh et al., 2007). The EC_{50} value of AZD-6244 against H1N1pdm09 of 750nM demonstrated reduced anti-influenza virus activity compared to PD-325901. Combination with oseltamivir increased the antiviral activity of oseltamivir with a strong synergism, even in combinations with reduced amounts of oseltamivir (Haasbach et al., 2013).

Table 2
Antiviral activity against influenza virus and stage of clinical development of MEK-inhibitors.

Name	Chemical structure	EC_{50} against influenza virus	Clinical development
U0126		1.2 μM[a]	No
CI-1040 (Pfizer)		4 nM[b]	Phase II against cancer; development stopped
PD-0325901 (Pfizer)		5 nM[c]	Phase II against cancer; development stopped
AZD-6244 (Astra Zeneca)		0.75 μM[c]	Phase II against cancer; In progress
AZD-8330 (Astra Zeneca)		40 nM[c]	Phase I against cancer; In progress
RDEA-119 (Bayer)		6 nM[b]	In progress

[a] Droebner et al. (2011).
[b] Planz, unpublished data, using the same method as described in Droebner et al. (2011).
[c] Haasbach et al. (2013a).

AZD-8330, another MEK-inhibitor from Astra Zeneca against MEK1/MEK2 that successfully finished a phase I clinical trial to investigate the safety and tolerability in patients with advanced malignancies (Wallace et al., 2009). EC_{50} value against H1N1pdm09 was 40 nM, demonstrating a strong antiviral activity. Combination with oseltamivir resulted in an increased antiviral effect, in a synergistic manner (Haasbach et al., 2013).

RDEA-119 (Bayer) is another MEK-inhibitor that selectively inhibits MEK1 (IC_{50} of 19 nM) and MEK2 (IC_{50} of 47 nM) and inhibits ERK1/2 phosphorylation (IC_{50} of 16 nM) (Iverson et al., 2009). RDEA-119 is under evaluation in different Phase I and Phase I/II studies (Fremin and Meloche, 2010). RDEA-119 is very potent as a single compound in inhibiting progeny influenza virus production (EC_{50} of 6 nM against H1N1pdm09), and it significantly increased the antiviral activity of oseltamivir (Haasbach et al., 2013).

3. Development of NFκB inhibitors against influenza virus infection

3.1. The NFB signaling pathway

Another important influenza virus-induced signaling mediator and target for antiviral intervention is the nuclear factor-kappa B (NF-κB) transcription factor, which controls the expression of a variety of genes involved in physiological responses, including immune and acute phase inflammatory responses, cell adhesion, differentiation, oxidative stress responses, apoptosis and antiviral responses (Pahl, 1999). The NF-κB transcription complexes consists of a group of homo- and heterodimers that belong to the Rel family, which encompass five subunits: p50, p52, c-Rel, RelA (p65) and RelB (Gilmore, 2006). Dimers of these NF-κB subunits bind to DNA regulatory sites called kappaB sites. Dimers containing RelA, RelB or c-Rel are transcriptional activators, whereas homodimers of p50 and p52 lack a transcription activation domain and function as repressors.

Although NF-κB subunits are ubiquitously expressed, their target gene specificity depends on a number of considerations, including cell type- and stimulus-specificity, different protein–protein interactions and posttranslational modifications. Distinct κB target site binding specificities of different NF-κB complexes can be induced by so-called 'canonical' (classical) and 'non-canonical' (alternative) signaling pathways (Bonizzi and Karin, 2004; Gilmore, 2006; Hoffmann et al., 2006; Perkins, 2006). NF-κB dimers are located in the cytoplasm in an inactive form, through association with inhibitor-of-kappa-B proteins (IκB). After stimulation of the pathway, IκB is phosphorylated, ubiquitinated and degraded by the proteasome, leading to the release of NF-κB dimers, which then translocate to the nucleus, where they modulate specific biological functions (Bonizzi and Karin, 2004; Karin and Ben-Neriah, 2000). The IκB kinase (IKK) complex mediates the phosphorylation and degradation of IκB. IKK contains two kinase subunits, IKKα and IKKβ, and an associated scaffold-like regulatory protein called

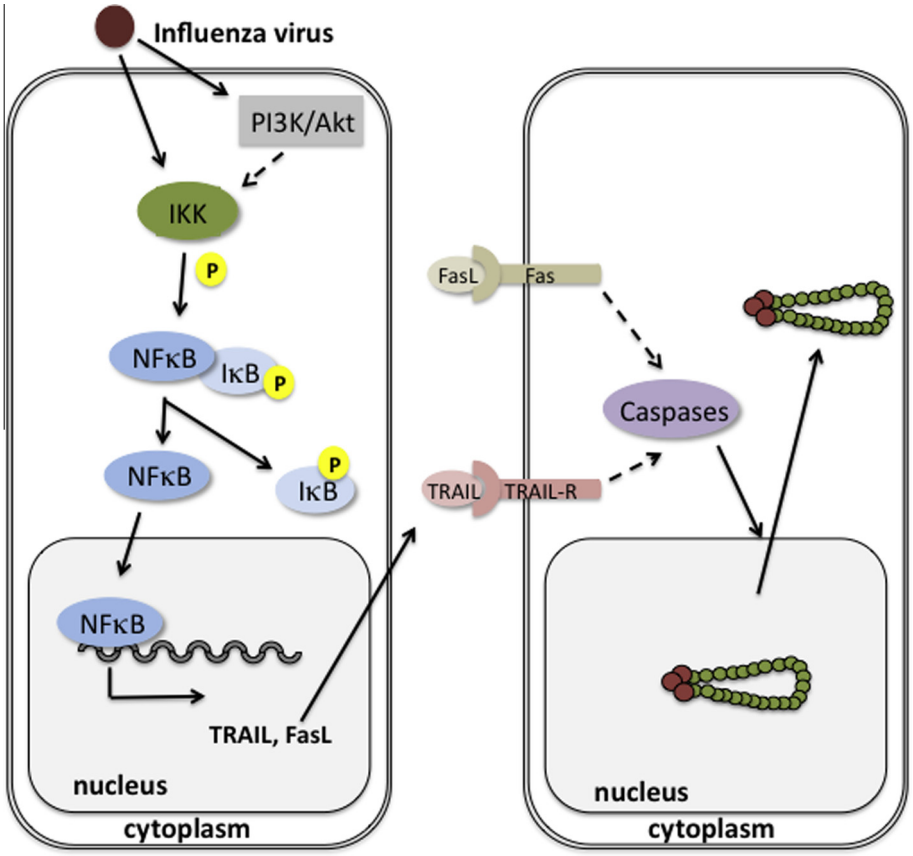


Fig. 3. NF-κB signaling in influenza virus-infected cells. Infection leads to activation of the IKK/NFκB complex and of PI3K/Akt, which acts as a co-regulator of NF-κB. After activation, NF-κB regulates the expression of a large number of genes, including pro-apoptotic factor TRAIL, Fas and FasL. TRAIL and FasL induce autocrine and paracrine activation of caspases. Caspase-mediated disruption of nuclear pore complexes allows the migration of ribonucleoprotein complexes from the nucleus into the cytoplasm. Note: Some regulatory factors, especially those involved in innate immune responses against influenza virus, have been omitted for the sake of clarity. See text for details and abbreviations.

NEMO (IKKγ). In response to a wide array of stimulatory agents such as TNF-α, interleukin-1 (IL-1) or various pathogens, the IKK complex is activated in part by phosphorylation of specific serine residues. The activated complex can then phosphorylate IκB, leading to its ubiquitination and degradation by the 26S proteasome. NF-κB can now translocate to the nucleus (Scheidereit, 2006).

3.2. NF-B signaling pathway and influenza virus

Although NF-κB is considered a central factor and regulator of innate immune defenses (Chu et al., 1999), two independent studies demonstrated for the first time in 2004 that blocking NF-κB signaling in MDCK, Vero and the human lung cell lines A549 and U1752 impaired, rather than enhanced production of progeny influenza viruses (Nimmerjahn et al., 2004; Wurzer et al., 2004). At least three molecular mechanisms are associated with the virus-supportive functions of NF-κB. During virus infection, NF-κB regulates the expression of a large number of genes, including those involved in innate antiviral immune regulation, such as IFN-β, and the induction of pro-apoptotic factors, such as TNF-related apoptosis-inducing ligand (TRAIL). Fas and FasL lead to subsequent activation of caspases (Wurzer et al., 2003).

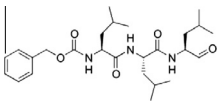
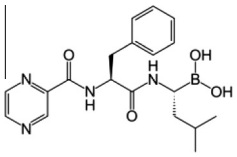
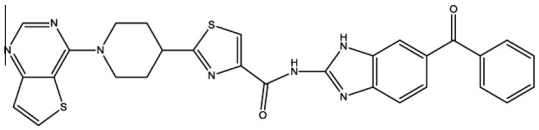
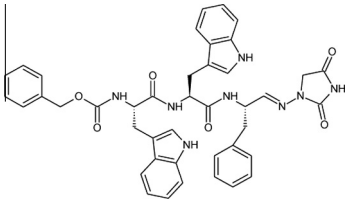
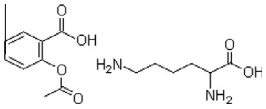
This activation of caspases presumably results in specific cleavage of nuclear pore proteins, allowing the enhanced nuclear export of viral RNPs into the cytoplasm (Faleiro and Lazebnik, 2000; Kramer et al., 2008). Inhibition of the NFκB pathway consequently leads to retention of viral RNPs in the nucleus (Mazur et al., 2007) (Fig. 3), and is the principal target for pharmaceutical intervention. A second mechanism that supports influenza virus replication involves NF-κB-dependent counteraction of type I IFN-induced gene (ISG) expression, either through up-regulation of the suppressor of cytokine signaling-3 (SOCS-3) and/or by direct suppression of ISG promoter regions (Pauli et al., 2008; Ruckle et al., 2012; Wei et al., 2006). It was also demonstrated that NF-κB is involved in the regulation of viral RNA synthesis (Kumar et al., 2008). Each of these mechanisms is required to a different extent for effective influenza virus production (Fig. 1, Fig. 3, Table 1), making NF-κB a promising target for antiviral intervention.

3.3. Compounds inhibiting the NF-κB pathway and influenza virus production

More than 800 compounds that inhibit NF-κB or activation of the pathway have been reported in the medical literature (Gilmore, 2006; Gilmore and Herscovitch, 2006), but only a few are in clinical development or licensed. The main clinical targets for NF-κB inhibitors are cancer therapy and chronic inflammatory diseases (Gilmore and Garbati, 2011). They can be categorized into four groups, targeting different parts of the NF-κB network:

- NF-κB signaling upstream of IKK (e.g., at a receptor or adaptor level), or
- directly at the IKK complex or IκB phosphorylation, or
- at the level of ubiquitination or proteasomal degradation of IκB, or

Table 3
Antiviral activity against influenza virus and clinical development of NFκB-inhibitors.

Name	Chemical structure	Clinical development	EC_{50} against influenza virus
MG132		No clinical development	n.d.[a]
PS-341; Bortezomib; Velcade® (Millenium)		Approved for treating multiple myeloma	n.d.[a]
SC75741 (4SC)		No clinical development	0.3 ng/ml[b]
VL-01 (Virologik; 4SC)		No clinical development	0.8–2.4 μM[c]
LASAG		In progress	40 nM[b]

[a] n.d. = not determined.
[b] Planz, unpublished data obtained using the method described in Droebner et al. (2011).
[c] Haasbach et al. (2011).

- downregulation of NFκB nuclear functions (Gilmore and Herscovitch, 2006).

The development of NF-κB inhibitors as antivirals against influenza might make use either of compounds that until now have not been described in pre-clinical or clinical investigations, or which are already under clinical investigation or in use for other applications. Similar to the Raf/MEK/ERK pathway, NF-κB inhibition may also indirectly influence the pathogenesis of influenza virus infection, because in severe influenza in particular, NF-κB regulates the hyperinduction of cytokines/chemokines during infection with highly pathogenic viruses (de Jong et al., 2006; Pahl, 1999). A summary of compounds that have been tested for antiviral activity against influenza virus is found in Table 3.

Acetylsalicylic acid (ASA), also known as aspirin, is a nonsteroidal anti-inflammatory drug widely used clinically. As regards using NF-κB inhibitors for the therapy of influenza, we were previously able to demonstrate *in vitro* and in mice that ASA functions as an antiviral against influenza virus (Mazur et al., 2007; Wurzer et al., 2004). ASA is an efficient and quite selective inhibitor of the NF-κB-activating kinase IKK, acting directly on the IKK complex and consequently inhibiting phosphorylation and degradation of IκB (Shi et al., 1999; Yin et al., 1998). It efficiently blocked the replication of various influenza viruses, including H5N1 strains, in MDCK or A549 cells by several orders of magnitude, in a concentration range that was not toxic for host cells (Mazur et al., 2007). In animal studies it was demonstrated that aerosol, but not oral administration of ASA reduced virus titers in the lung and significantly promoted the survival of lethally infected mice (Mazur et al., 2007). Treatment was well tolerated, and did not exhibit harmful side effects. Strikingly, the study also showed that ASA, in contrast to the neuraminidase-inhibitor oseltamivir or the M2 ion-channel blocker amantadine, did not lead to the generation of resistant virus variants in multipassaging experiments in cell culture (Mazur et al., 2007). Salicylic acid (SA) and DL-Lysine acetylsalicylate (LASAG) also demonstrated antiviral properties against influenza virus.

LASAG (DL – lysine acetylsalicylate + glycine; Aspirin i.v., Bayer; Aspegic, Sanofi aventis) is a water-soluble aspirin complex that can be administered by intramuscular and intravenous injection. In unpublished studies, we have observed potent activity of ASA and LASAG against highly pathogenic H5N1 and H7N7 avian influenza viruses. In the light of these data, it is surprising that the antiviral action of ASA has neither been observed previously in animal models nor in epidemiological studies in humans, but this may simply be due to the fact that ASA is not usually inhaled, but is given orally or by injection, which does not lead to sufficiently deposition in the lung. Topical treatment with aerosolized ASA is therefore the mandatory application route. Moreover, it is discussed that Reye's syndrome, a very rare but serious acute encephalopathy, has been linked to the usage of aspirin in children and teenagers (Glasgow, 2006; Orlowski et al., 2002), but this has not been observed for LASAG treatment. It was recently demonstrated in a Phase I clinical study that aerosol delivery of LASAG is suitable to supply the amount of drug needed for antiviral activity directly into the lung, without causing adverse effects. A Phase II clinical study in adult hospitalized patents to evaluate the safety and efficacy of thrice-daily inhaled LASAG is now recruiting (https://www.clinicaltrialsregister.eu/ctr-search/search?query=2012-004072-19). LASAG therefore represents the first compound to date that targets an intracellular signaling pathway and is in clinical development for antiviral therapy against influenza viruses.

SC75741 (4SC) *N*-(6-benzoyl-1H-benzo[d]imidazol-2-yl)-2-(1-(thieno[3,2-d]pyrimidin-4-yl)piperidin-4-yl)thiazole-4-carboxamide

is a novel NF-κB inhibitor that was discovered through screening with a whole-cell reporter gene assay. The IC_{50} value of this compound to inhibit NF-κB is in the nanomolar range (Leban et al., 2007). Its anti-influenza activity was demonstrated on A549 and MDCK cells against various virus strains, including H5N1 and H1N1pdm09. Mode-of-action studies revealed that SC75741 blocks DNA binding of NF-κB and κB site-dependent gene expression, leading to impaired expression of pro-apoptotic factors and subsequent inhibition of caspase activation, resulting in retention of caspase-mediated nuclear export of RNPs (Ehrhardt et al., 2013). Another unpublished study showed that the EC_{50} of SC75741 was 0.3 ng/ml against H1N1pdm09 influenza virus. SC75741 also significantly protected mice against highly pathogenic avian influenza viruses with different treatment schedules.

Ubiquitination of IκB, followed by the rapid degradation of ubiquitinated IκB by the 26S proteasome, is the final step before NF-κB leaves the cytoplasm (Scheidereit, 2006). Inhibitors of different steps in the ubiquitin–proteasome pathway therefore suppress activation of NF-κB by stabilizing IκB. The antiviral effect of proteasome inhibitors has been described for different RNA viruses (Ma et al., 2010; Ott et al., 2003; Schubert et al., 2000).

PS-341 (Bortezomib; Velcade; Millennium Pharmaceuticals) is the most effective compound among a class of proteasome inhibitors that block the chymotrypsin-like site in the 20S subunit core (Adams, 2004a; Grisham et al., 1999; Iqbal et al., 1995). PS-341 has significant efficacy against multiple myeloma and several other hematologic and solid tumors (Adams, 2004b; Adams and Kauffman, 2004; O'Connor et al., 2005; Orlowski et al., 2005; Papandreou et al., 2004; Richardson et al., 2003; San Miguel et al., 2008). It is the only drug in this class which has been approved for clinical use in different Phase I and II clinical trials against cancer (Mackay et al., 2005; Russo et al., 2010). The antiviral effect of PS-341 against influenza virus has been shown recently in A549, MDCK, HEK293, HUVEC, HBEpC, U937 and other cell types. Treatment of infected cells with PS-341 resulted in a significant reduction of progeny virus titers. As expected, treatment resulted in an induction of IκB degradation, but also in activation of NF-κB as well as the JNK/AP-1 pathway, along with enhanced expression of type I interferon genes. Thus, the authors concluded that PS-341 blocks influenza virus replication by inducing an antiviral state, mediated by the NF-κB-dependent expression of antiviral gene products (Dudek et al., 2010; Pahl, 1999). EC_{50} values were not provided in this study.

MG132 is a commercially available proteasome inhibitor that interferes with the chymotrypsin-like activity of the proteasome complex. In contrast to the serine protease inhibitor PS-341, MG132 is a cysteine protease inhibitor, which has been described in many publications in basic research, but has never made it to clinical development (Grisham et al., 1999; Jobin et al., 1998; Palombella et al., 1994). In the only report of the antiviral effect of MG132 against influenza virus, the authors showed that inhibition of proteasome activity interferes with influenza A virus infection at a post-fusion step, and that viral RNA synthesis is dependent on the ubiquitin–proteasome system. (Widjaja et al., 2010). Entry was not affected. Treatment resulted in retention of viral particles in the cytoplasm, as observed in earlier studies (Widjaja et al., 2010; Wurzer et al., 2004). There was no significant difference in the antiviral efficacy of MG132 and PS-341 (Widjaja et al., 2010).

VL-01 (Virologik) is another inhibitor of the 20S and 26S proteasome with antiviral properties against influenza virus. The detailed mechanism of action has not been investigated in detail. Treatment with VL-01 led to reduction of replication of different influenza virus strains in A549 cells, with EC_{50} values between 0.8–2.4 mM. Mice treated with aerosolized VL-01 showed reduced viral titers and enhanced survival, without adverse effects. The study also demonstrated that, besides its direct antiviral effect, the compound also reduced the hyperproduction of cytokines such as IL-1αβ, IL-6, MIP-1-β, RANTES and TNF-α after infection with the highly pathogenic avian H5N1 virus (Haasbach et al., 2011).

4. Inhibitors targeting PI3K signaling pathways

The phosphoinositide-3 kinase/protein kinase-B/mammalian target of rapamycin (PI3K/AKT/mTOR) pathway has recently been added to the growing list of signaling pathways that are activated by influenza virus (Ehrhardt and Ludwig, 2009). It has been identified as a key pathway for important cellular functions such as differentiation, metabolism and translation initiation, and it is involved in cross-talk with many other signaling pathways, including the Raf/MEK/ERK and NF-κB networks (Vanhaesebroeck et al., 2005).

PI3K activation is required early in influenza virus infection, for virus uptake, and at a later stage, for localization of RNP complexes (Fig. 1; Table 1) (Ehrhardt et al., 2006; Shin et al., 2007b). Activation of the PI3K/Akt/mTOR pathway also supports virus replication by inhibiting premature cellular apoptosis, through the phosphorylation of caspase 9 (Table 1) (Shin et al., 2007a,b; Zhirnov and Klenk, 2007). Because PI3K/AKT/mTOR, together with Raf/MEK/ERK, plays a key role in the regulation of cell growth and differentiation (Carracedo and Pandolfi, 2008; Castellano and Downward, 2011), several proteins in the pathway are valuable targets for anticancer therapy, and some mTOR are licensed and administered in routine practice. Some PI3K inhibitors have recently become available that display low toxicity, and are under investigation in clinical trials (Kurtz and Ray-Coquard, 2012).

Wortmannin, a viridin soil bacteria product, and LY294002, a morpholino derivative of quercetin, were the first generation of PI3K inhibitors, but they failed to reach clinical investigations because of limited pharmacological properties (Maira et al., 2010). Nevertheless, they have been widely used as tools to investigate the role of the PI3K pathway in various biological systems, including influenza virus infection (Ehrhardt et al., 2006; Ehrhardt et al., 2007; Maira et al., 2010; Zhou et al., 2009). Derivatives of wortmannin and LY294002 with better pharmacokinetic properties are now undergoing clinical investigation as anticancer drugs. In this regard, a dual PI3K/mTOR inhibitor, NVP-BEZ235 (Novartis), which was recently described as a novel treatment strategy for acute myeloid leukemia (Chapuis et al., 2010), has recently entered several Phase II trials for cancer therapy. Unfortunately, none of the PI3K/AKT/mTOR pathway inhibitors that have proven safe in clinical investigations and are now widely used in clinical trials for cancer have been investigated for their ability to inhibit influenza virus infection.

5. Inhibitors targeting PKC

The designation "protein kinase C" (PKC) refers to a family of serine/threonine kinases that are involved in cell signaling, leading to proliferation, differentiation, apoptosis and angiogenesis. One might therefore consider PKC to be a target for cancer therapy, but its function is unfortunately very complex, as individual enzyme isoforms play different roles within the cell, including some antagonistic functions, and the selectivity of many early inhibitors against these isoforms was very poor. A number of small-molecule inhibitors of PKC have recently become available, including peptides, antisense oligonucleotides and natural compounds, but due to the extreme complexity of PKC family isoforms and the incomplete understanding of their function in different cell types, there has been a delay in clinical development with drugs targeting PKC (Bosco et al., 2011).

Table 4
Overview of the most common adverse events for MEK-inhibitors during cancer therapy, compared to adverse events during antiviral therapy of influenza with oseltamivir.

	AZD-6244[a]	CI-1040[b]	PD-0325901[c]	AZD-8330[d]	Oseltamivir[e]
Dosage	50–300 mg BID	800 mg BID	15 mg BID	0.5–60 mg[f]	75 mg BID
Number of patients	$n = 57$	$n = 67$	$n = 21$	$n = 82$	$n = 1057$
Adverse events (%)					
Rash	74[g]	25	33	16[h]	<1
Nausea	44	52	29	18	8
Diarrhea	58	57	76	13	6
Fatique	39	48	47	13	1
Vomiting	25	21	33	11	11

[a] Adjei et al. (2008).
[b] Rinehart et al. (2004).
[c] Haura et al. (2010).
[d] Cohen et al. (2013).
[e] Smith et al. (2011).
[f] 0.5–60 mg/OD (once daily); 20 mg/BID (twice daily).
[g] The term "rash" includes "dermatitis acneiform, rash, rash erythematous, rash maculopapular, and rash pruritic."
[h] Dermatitis acneiform.

Hoffmann and colleagues showed that treatment with the commercially available PKC inhibitor rottlerin at a concentration of 12.5 μM significantly reduced influenza virus replication in A549 cells, while activation of PKC led to enhanced virus production (Hoffmann et al., 2008). Taking a similar approach, the commercially available PKC inhibitor Gö6976, which was known to inhibit influenza virus replication by reducing viral entry (Sieczkarski et al., 2003), also had a post-entry effect, by blocking the PKC-specific phosphorylation of the viral PB1 and NS1 proteins, which appears to be functionally relevant for viral RNA polymerase activity and efficient replication (Mahmoudian et al., 2009). In addition to PB1 and NS1, PKC also mediated phosphorylation of the viral PB1-F2 protein (Table 1) (Mitzner et al., 2009).

The studies described above of the effect of inhibiting PI3K/AKT/mTOR or PKC signaling on influenza virus replication were performed to investigate the biology of influenza virus, rather than to assess the antiviral activity of these compounds. Detailed preclinical investigations should therefore be performed to determing their antiviral effects, especially with inhibitors that are already under clinical evaluation for other targets.

6. Adverse effects of intracellular signaling inhibitors

All of the MEK inhibitors, and most of the other compounds that have been described in this review, are either under clinical evaluation as anticancer drugs, or are already licensed. While it may be tempting to make use of these inhibitors for their anti-influenza activity, concern arises about their diverse side effects. Even though it is known that MEK-inhibitor treatment of various cancers produces only moderate side effects, so that the compounds are well tolerated, these points need to be scrutinized in more detail. In this regard, it is worth noting that cancer patients are more likely to tolerate grade 2 (moderate) and grade 3 (severe) adverse events that would never be acceptable in the treatment of influenza.

In 2011, Smith and colleagues reviewed 10 years of clinical experience with oseltamivir treatment of influenza, including cumulative safety and tolerability data from 1067 study participants in randomized controlled trials (Table 4) (Smith et al., 2011). Significant adverse events associated with oseltamivir therapy include nausea (11%) and vomiting (8%) as single events, – beginning on the first or second treatment day. A wide variety of symptoms and complications typical of influenza were seen in both the treatment and placebo groups, so that they could not be associated with oseltamivir. In contrast, a variety of adverse neuropsychiatric events have been observed during oseltamivir administration, mainly in children and adolescents (Smith et al., 2011).

Several reports have described adverse events in clinical trials of cell signaling inhibitors. In a Phase I trial to assess the tolerability of the MEK-inhibitor AZD-6244 in patients with advanced cancer, rash was the most frequent (74%) and dose-limiting toxicity; other side effects of treatment included diarrhea (58%), nausea (44%) and fatique (39%) (Adjei et al., 2008). These findings are consisted with adverse events described in trials with PD-0325901 and CI-1040 (Lorusso et al., 2005; Rinehart et al., 2004). The frequency of adverse events was dose-dependent; the maximum tolerated dose 50% (MTD 50%) was 100 mg twice a day and was well tolerated (Table 4). In a Phase II study, 67 cancer patients received 800 mg of CI-1040 twice daily; adverse events were mainly diarrhea (57%), nausea (52%) and fatique (48%), while rash was found in a lower frequency (25%), compared to AZD-6244 treatment (Table 4) (Rinehart et al., 2004).

In another study, 15 mg of PD-0325901 given twice daily on an intermittent schedule (3 weeks on/ one week off) was not tolerated, but the same dose given for 5 days on/ two days off for three weeks, followed by one week off, was tolerable to the patients. Diarrhea (76%), fatique (48%), rash (33%), vomiting (33%) and nausea (29%) were the most common treatment-related toxicities (Table 4) (Cohen et al., 2013; Haura et al., 2010). Adverse events were mostly grade 1–2, but it was not clear how soon toxicity began after starting therapy. A recent study assessed the safety, tolerability, pharmacokinetics and pharmacodynamics of AZD-8330 in 82 patients with advanced malignancies. At the MTD of 20 mg twice daily, the most frequent adverse events were acneiform dermatitis (16%), fatigue (13%), diarrhea (13%) and vomiting (11%), all grade 1–2 (Table 4). AZD-8330 therefore showed reduced adverse events, compared to other MEK-inhibitors (Cohen et al., 2013).

For the NF-κB inhibitors presented in this review, safety data are only available for Velcade. The drug was initially developed for the treatment of multiple myeloma and mantle cell lymphoma, and it is now under investigation, either singly or in combination with other drugs, for solid cancers and stem cell transplantation (Cao et al., 2012; Utecht and Kolesar, 2008; Zeng et al., 2013). It can be given intravenously and subcutaneously. The most common serious adverse events observed during Velcade therapy were diarrhea, fatigue, thrombocytopenia, nausea and constipation; pneumonia, renal failure, pyrexia, dehydration and vomiting also occurred with some treatment schedules (Berenson and Yellin, 2008). The percentage and grade of adverse events were strongly dependent on the treatment schedule. (Kane et al., 2006; Zeng

et al., 2013). A number of different adverse events have been reported for systemically administered LASAG, but there is no information available regarding its safety via inhalation. Adverse event information is also lacking for the PI3K/mTOR inhibitor NVP-BEZ235.

Are cell signaling inhibitors suitable as antivirals, from the safety point of view? Phase I/II studies should be conducted to answer this question, and find out whether safety data from cancer trials can be transferred to the influenza situation. In this regard, one should keep in mind that more than 90% of cancer patients received surgery and chemotherapy, and more than 60% received radiation, before initiating treatment with the cell signaling inhibitors described above, which could increase the likelihood and severity of adverse events (Adjei et al., 2008). Another critical factor is the duration of treatment. Cancer therapy usually lasts at least 2–3 months, with some weeks "off treatment," while antiviral treatment for influenza usually lasts only five days. This drastic difference in the duration of therapy may well have an impact on the incidence of adverse events and their severity. The question also needs to be answered whether the same drug dosage is needed for cancer and for antiviral therapy, or if the amount of compound could be reduced when treating influenza, which would presumably reduce the frequency and severity of side effects. It also appears that the new generations of signaling inhibitors, such as the MEK inhibitor AZD-8330, show much better tolerability, with a reduced percentage of adverse events in patient cohorts, compared to trials with earlier versions of MEK inhibitors (Table 3).

Another striking point regarding the adverse effects of signaling inhibitors used as antivirals is the fact that most signaling pathways are involved in regulation of the immune system (Dev et al., 2011; Nakayama and Yamashita, 2010; Visekruna et al., 2012). In particular, NF-κB is a major regulator of cytokine responses, and since severe influenza is often associated with "cytokine storm," treatment with NF-κB inhibitors could target both viral replication and the pro-inflammatory cytokine response. An important question is whether such therapy would also be suitable against seasonal influenza viruses that induce a milder illness, which is controlled through activation of antibody-producing plasma cells, and in which virus-infected cells are eliminated by activated $CD8^+$ T cells. Investigations are needed to determine the effect of cell signaling inhibitors on the immune response against influenza viral infection in greater detail.

7. Future prospects

The greatest potential for the development cell signaling inhibitors as novel influenza therapies is by targeting the Raf/MEK/ERK cascade, for which a vast number of inhibitors are available with good pharmacological data, and that have been shown to be safe, effective and suitable for oral usage in clinical trials. The NF-κB pathway might also be a suitable target for the treatment of severe influenza, especially for patients suffering from virus-induced "cytokine storm." A prerequisite for such treatment would be a compound that can be administered intravenously.

Whether inhibitors of cellular signaling pathways are suitable for the treatment of common forms of seasonal influenza remains to be determined. The main bottlenecks to such an approach are clearly the adverse events, which could be minimized by giving a short course of therapy, compared to long-term cancer treatment. A second strategy to prevent adverse events would be to reduce the dose, while preserving antiviral activity. One elegant way to do this would be to combine a signaling inhibitor with a direct-acting antiviral, such as oseltamivir, as it has recently been shown that this combination resulted in strong anti-influenza activity *in vitro*, even when the amounts of the individual drugs were reduced (Haasbach et al., 2013).

Almost all of the compounds described in this review have been used for cancer therapy, but the development of some of them was stopped because of insufficient anticancer potential. There may therefore be opportunities for pharmaceutical companies that have both cancer and infectious disease programs to investigate the antiviral potential of Raf/MEK/ERK inhibitors that failed in development for cancer therapy. The first step would be to obtain validated preclinical data demonstrating the antiviral efficacy of the selected compounds against influenza virus. In this regard, it would be also worth investigating combinations of Raf/MEK/ERK inhibitors with approved antivirals, such as neuraminidase inhibitors, both to increase their antiviral potential and to prevent the development of resistance to the licensed drugs. As described above, the NF-κB inhibitors also appear to have potential as antivirals, as pre-clinical data have demonstrated their *in vitro* anti-influenza activity. In contrast, this is not yet the case for inhibitors of the PI3K/AKT/mTOR or PKC signaling pathways, making it difficult to estimate their prospects as antivirals.

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References

Adams, J., 2004a. The development of proteasome inhibitors as anticancer drugs. Cancer Cell 5, 417–421.
Adams, J., 2004b. The proteasome: a suitable antineoplastic target. Nat. Rev. Cancer 4, 349–360.
Adams, J., Kauffman, M., 2004. Development of the proteasome inhibitor Velcade (Bortezomib). Cancer Invest. 22, 304–311.
Adjei, A.A., Cohen, R.B., Franklin, W., Morris, C., Wilson, D., Molina, J.R., Hanson, L.J., Gore, L., Chow, L., Leong, S., Maloney, L., Gordon, G., Simmons, H., Marlow, A., Litwiler, K., Brown, S., Poch, G., Kane, K., Haney, J., Eckhardt, S.G., 2008. Phase I pharmacokinetic and pharmacodynamic study of the oral, small-molecule mitogen-activated protein kinase kinase 1/2 inhibitor AZD6244 (ARRY-142886) in patients with advanced cancers. J. Clin. Oncol. 26, 2139–2146.
Barrett, S.D., Bridges, A.J., Dudley, D.T., Saltiel, A.R., Fergus, J.H., Flamme, C.M., Delaney, A.M., Kaufman, M., LePage, S., Leopold, W.R., Przybranowski, S.A., Sebolt-Leopold, J., Van Becelaere, K., Doherty, A.M., Kennedy, R.M., Marston, D., Howard Jr., W.A., Smith, Y., Warmus, J.S., Tecle, H., 2008. The discovery of the benzhydroxamate MEK inhibitors CI-1040 and PD 0325901. Bioorg. Med. Chem. Lett. 18, 6501–6504.
Beigel, J.H., Farrar, J., Han, A.M., Hayden, F.G., Hyer, R., de Jong, M.D., Lochindarat, S., Nguyen, T.K., Nguyen, T.H., Tran, T.H., Nicoll, A., Touch, S., Yuen, K.Y., 2005. Avian influenza A (H5N1) infection in humans. N. Engl. J. Med. 353, 1374–1385.
Berenson, J.R., Yellin, O., 2008. New drugs in multiple myeloma. Curr. Opin. Support. Palliat. Care 2, 204–210.
Bonizzi, G., Karin, M., 2004. The two NF-kappaB activation pathways and their role in innate and adaptive immunity. Trends Immunol. 25, 280–288.
Bosco, R., Melloni, E., Celeghini, C., Rimondi, E., Vaccarezza, M., Zauli, G., 2011. Fine tuning of protein kinase C (PKC) isoforms in cancer: shortening the distance from the laboratory to the bedside. Mini Rev. Med. Chem. 11, 185–199.
Cao, B., Li, J., Mao, X., 2012. Dissecting bortezomib: development, application, adverse effects and future direction. Curr. Pharm. Des. 2012 Nov 2. [Epub ahead of print].
Carracedo, A., Pandolfi, P.P., 2008. The PTEN-PI3K pathway: of feedbacks and cross-talks. Oncogene 27, 5527–5541.
Castellano, E., Downward, J., 2011. RAS interaction with PI3K: more than just another effector pathway. Genes Cancer 2, 261–274.
Chan, M.C., Cheung, C.Y., Chui, W.H., Tsao, S.W., Nicholls, J.M., Chan, Y.O., Chan, R.W., Long, H.T., Poon, L.L., Guan, Y., Peiris, J.S., 2005. Proinflammatory cytokine responses induced by influenza A (H5N1) viruses in primary human alveolar and bronchial epithelial cells. Respir. Res. 6, 135.
Chapuis, N., Tamburini, J., Green, A.S., Vignon, C., Bardet, V., Neyret, A., Pannetier, M., Willems, L., Park, S., Macone, A., Maira, S.M., Ifrah, N., Dreyfus, F., Herault, O., Lacombe, C., Mayeux, P., Bouscary, D., 2010. Dual inhibition of PI3K and mTORC1/2 signaling by NVP-BEZ235 as a new therapeutic strategy for acute myeloid leukemia. Clin. Cancer Res. 16, 5424–5435.

Cheng, X.W., Lu, J., Wu, C.L., Yi, L.N., Xie, X., Shi, X.D., Fang, S.S., Zan, H., Kung, H.F., He, M.L., 2011. Three fatal cases of pandemic 2009 influenza A virus infection in Shenzhen are associated with cytokine storm. Respir. Physiol. Neurobiol. 175, 185–187.

Chu, W.M., Ostertag, D., Li, Z.W., Chang, L., Chen, Y., Hu, Y., Williams, B., Perrault, J., Karin, M., 1999. JNK2 and IKKbeta are required for activating the innate response to viral infection. Immunity 11, 721–731.

Cohen, R.B., Aamdal, S., Nyakas, M., Cavallin, M., Green, D., Learoyd, M., Smith, I., Kurzrock, R., 2013. A phase I dose-finding, safety and tolerability study of AZD8330 in patients with advanced malignancies. Eur. J. Cancer.

de Jong, M.D., Simmons, C.P., Thanh, T.T., Hien, V.M., Smith, G.J., Chau, T.N., Hoang, D.M., Chau, N.V., Khanh, T.H., Dong, V.C., Qui, P.T., Cam, B.V., Ha do, Q., Guan, Y., Peiris, J.S., Chinh, N.T., Hien, T.T., Farrar, J., 2006. Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. Nat. Med. 12, 1203–1207.

Dev, A., Iyer, S., Razani, B., Cheng, G., 2011. NF-kappaB and innate immunity. Curr. Top. Microbiol. Immunol. 349, 115–143.

Droebner, K., Pleschka, S., Ludwig, S., Planz, O., 2011. Antiviral activity of the MEK-inhibitor U0126 against pandemic H1N1v and highly pathogenic avian influenza virus in vitro and in vivo. Antiviral Res.. 92, 195–203.

Dudek, S.E., Luig, C., Pauli, E.K., Schubert, U., Ludwig, S., 2010. The clinically approved proteasome inhibitor PS-341 efficiently blocks influenza A virus and vesicular stomatitis virus propagation by establishing an antiviral state. J. Virol. 84, 9439–9451.

Ehrhardt, C., Ludwig, S., 2009. A new player in a deadly game: influenza viruses and the PI3K/Akt signalling pathway. Cell Microbiol. 11, 863–871.

Ehrhardt, C., Marjuki, H., Wolff, T., Nurnberg, B., Planz, O., Pleschka, S., Ludwig, S., 2006. Bivalent role of the phosphatidylinositol-3-kinase (PI3K) during influenza virus infection and host cell defence. Cell Microbiol. 8, 1336–1348.

Ehrhardt, C., Wolff, T., Pleschka, S., Planz, O., Beermann, W., Bode, J.G., Schmolke, M., Ludwig, S., 2007. Influenza A virus NS1 protein activates the PI3K/Akt pathway to mediate antiapoptotic signaling responses. J. Virol. 81, 3058–3067.

Ehrhardt, C., Ruckle, A., Hrincius, E.R., Haasbach, E., Anhlan, D., Ahmann, K., Banning, C., Reiling, S.J., Kuhn, J., Strobl, S., Vitt, D., Leban, J., Planz, O., Ludwig, S., 2013. The NF-kappaB inhibitor SC75741 efficiently blocks influenza virus propagation and confers a high barrier for development of viral resistance. Cell Microbiol. 2013 Jan 16. doi: 10.1111/cmi.12108. [Epub ahead of print].

Eisenberg, S., Shvartsman, D.E., Ehrlich, M., Henis, Y.I., 2006. Clustering of raft-associated proteins in the external membrane leaflet modulates internal leaflet H-ras diffusion and signaling. Mol. Cell Biol. 26, 7190–7200.

Faleiro, L., Lazebnik, Y., 2000. Caspases disrupt the nuclear-cytoplasmic barrier. J. Cell Biol. 151, 951–959.

Fremin, C., Meloche, S., 2010. From basic research to clinical development of MEK1/2 inhibitors for cancer therapy. J. Hematol. Oncol. 3, 8.

Gilmore, T.D., 2006. Introduction to NF-kappaB: players, pathways, perspectives. Oncogene 25, 6680–6684.

Gilmore, T.D., Garbati, M.R., 2011. Inhibition of NF-kappaB signaling as a strategy in disease therapy. Curr. Top. Microbiol. Immunol. 349, 245–263.

Gilmore, T.D., Herscovitch, M., 2006. Inhibitors of NF-kappaB signaling: 785 and counting. Oncogene 25, 6887–6899.

Glasgow, J.F., 2006. Reye's syndrome: the case for a causal link with aspirin. Drug Safety 29, 1111–1121.

Grisham, M.B., Palombella, V.J., Elliott, P.J., Conner, E.M., Brand, S., Wong, H.L., Pien, C., Mazzola, L.M., Destree, A., Parent, L., Adams, J., 1999. Inhibition of NF-kappa B activation in vitro and in vivo: role of 26S proteasome. Methods Enzymol. 300, 345–363.

Haasbach, E., Pauli, E.K., Spranger, R., Mitzner, D., Schubert, U., Kircheis, R., Planz, O., 2011. Antiviral activity of the proteasome inhibitor VL-01 against influenza A viruses. Antiviral Res.. 91, 304–313.

Haasbach, E., Hartmayer, C., Planz, O., 2013. Combination of MEK inhibitors and oseltamivir leads to synergistic antiviral effects after influenza A virus infection in vitro. Antiviral Res.. 98, 319–324.

Haura, E.B., Ricart, A.D., Larson, T.G., Stella, P.J., Bazhenova, L., Miller, V.A., Cohen, R.B., Eisenberg, P.D., Selaru, P., Wilner, K.D., Gadgeel, S.M., 2010. A phase II study of PD-0325901, an oral MEK inhibitor, in previously treated patients with advanced non-small cell lung cancer. Clin. Cancer Res. 16, 2450–2457.

Hoffmann, A., Natoli, G., Ghosh, G., 2006. Transcriptional regulation via the NF-kappaB signaling module. Oncogene 25, 6706–6716.

Hoffmann, H.H., Palese, P., Shaw, M.L., 2008. Modulation of influenza virus replication by alteration of sodium ion transport and protein kinase C activity. Antiviral Res.. 80, 124–134.

Hynes, N.E., Lane, H.A., 2005. ERBB receptors and cancer: the complexity of targeted inhibitors. Nat. Rev. Cancer 5, 341–354.

Iqbal, M., Chatterjee, S., Kauer, J.C., Das, M., Messina, P., Freed, B., Biazzo, W., Siman, R., 1995. Potent inhibitors of proteasome. J. Med. Chem. 38, 2276–2277.

Iverson, C., Larson, G., Lai, C., Yeh, L.T., Dadson, C., Weingarten, P., Appleby, T., Vo, T., Maderna, A., Vernier, J.M., Hamatake, R., Miner, J.N., Quart, B., 2009. RDEA119/BAY 869766: a potent, selective, allosteric inhibitor of MEK1/2 for the treatment of cancer. Cancer Res. 69, 6839–6847.

Jobin, C., Hellerbrand, C., Licato, L.L., Brenner, D.A., Sartor, R.B., 1998. Mediation by NF-kappa B of cytokine induced expression of intercellular adhesion molecule 1 (ICAM-1) in an intestinal epithelial cell line, a process blocked by proteasome inhibitors. Gut 42, 779–787.

Kane, R.C., Farrell, A.T., Sridhara, R., Pazdur, R., 2006. United States food and drug administration approval summary: bortezomib for the treatment of progressive multiple myeloma after one prior therapy. Clin. Cancer Res. 12, 2955–2960.

Karin, M., Ben-Neriah, Y., 2000. Phosphorylation meets ubiquitination: the control of NF-[kappa]B activity. Annu. Rev. Immunol. 18, 621–663.

Kramer, A., Liashkovich, I., Oberleithner, H., Ludwig, S., Mazur, I., Shahin, V., 2008. Apoptosis leads to a degradation of vital components of active nuclear transport and a dissociation of the nuclear lamina. Proc. Natl. Acad. Sci. USA 105, 11236–11241.

Kumar, N., Xin, Z.T., Liang, Y., Ly, H., 2008. NF-kappaB signaling differentially regulates influenza virus RNA synthesis. J. Virol. 82, 9880–9889.

Kurtz, J.E., Ray-Coquard, I., 2012. PI3 kinase inhibitors in the clinic: an update. Anticancer Res 32, 2463–2470.

Leban, J., Baierl, M., Mies, J., Trentinaglia, V., Rath, S., Kronthaler, K., Wolf, K., Gotschlich, A., Seifert, M.H., 2007. A novel class of potent NF-kappaB signaling inhibitors. Bioorg. Med. Chem. Lett. 17, 5858–5862.

Lorusso, P.M., Adjei, A.A., Varterasian, M., Gadgeel, S., Reid, J., Mitchell, D.Y., Hanson, L., DeLuca, P., Bruzek, L., Piens, J., Asbury, P., Van Becelaere, K., Herrera, R., Sebolt-Leopold, J., Meyer, M.B., 2005. Phase I and pharmacodynamic study of the oral MEK inhibitor CI-1040 in patients with advanced malignancies. J. Clin. Oncol. 23, 5281–5293.

Ludwig, S., 2009. Targeting cell signalling pathways to fight the flu: towards a paradigm change in anti-influenza therapy. J. Antimicrob. Chemother. 64, 1–4.

Ludwig, S., Planz, O., 2008. Influenza viruses and the NF-kappaB signaling pathway - towards a novel concept of antiviral therapy. Biol. Chem. 389, 1307–1312.

Ludwig, S., Planz, O., Pleschka, S., Wolff, T., 2003. Influenza-virus-induced signaling cascades: targets for antiviral therapy? Trends Mol. Med. 9, 46–52.

Ludwig, S., Wolff, T., Ehrhardt, C., Wurzer, W.J., Reinhardt, J., Planz, O., Pleschka, S., 2004. MEK inhibition impairs influenza B virus propagation without emergence of resistant variants. FEBS Lett. 561, 37–43.

Ludwig, S., Pleschka, S., Planz, O., Wolff, T., 2006. Ringing the alarm bells: signalling and apoptosis in influenza virus infected cells. Cell Microbiol. 8, 375–386.

Ma, X.Z., Bartczak, A., Zhang, J., Khattar, R., Chen, L., Liu, M.F., Edwards, A., Levy, G., McGilvray, I.D., 2010. Proteasome inhibition in vivo promotes survival in a lethal murine model of severe acute respiratory syndrome. J. Virol. 84, 12419–12428.

Mackay, H., Hedley, D., Major, P., Townsley, C., Mackenzie, M., Vincent, M., Degendorfer, P., Tsao, M.S., Nicklee, T., Birle, D., Wright, J., Siu, L., Moore, M., Oza, A., 2005. A phase II trial with pharmacodynamic endpoints of the proteasome inhibitor bortezomib in patients with metastatic colorectal cancer. Clin. Cancer Res. 11, 5526–5533.

Mahmoudian, S., Auerochs, S., Grone, M., Marschall, M., 2009. Influenza A virus proteins PB1 and NS1 are subject to functionally important phosphorylation by protein kinase C. J. Gen. Virol. 90, 1392–1397.

Maira, S.M., Finan, P., Garcia-Echeverria, C., 2010. From the bench to the bed side: PI3K pathway inhibitors in clinical development. Curr. Top. Microbiol. Immunol. 347, 209–239.

Marjuki, H., Alam, M.I., Ehrhardt, C., Wagner, R., Planz, O., Klenk, H.D., Ludwig, S., Pleschka, S., 2006. Membrane accumulation of influenza A virus hemagglutinin triggers nuclear export of the viral genome via protein kinase Calpha-mediated activation of ERK signaling. J. Biol. Chem. 281, 16707–16715.

Marjuki, H., Yen, H.L., Franks, J., Webster, R.G., Pleschka, S., Hoffmann, E., 2007. Higher polymerase activity of a human influenza virus enhances activation of the hemagglutinin-induced Raf/MEK/ERK signal cascade. Virol. J. 4, 134.

Mazur, I., Wurzer, W.J., Ehrhardt, C., Pleschka, S., Puthavathana, P., Silberzahn, T., Wolff, T., Planz, O., Ludwig, S., 2007. Acetylsalicylic acid (ASA) blocks influenza virus propagation via its NF-kappaB-inhibiting activity. Cell Microbiol. 9, 1683–1694.

Mitzner, D., Dudek, S.E., Studtrucker, N., Anhlan, D., Mazur, I., Wissing, J., Jansch, L., Wixler, L., Bruns, K., Sharma, A., Wray, V., Henklein, P., Ludwig, S., Schubert, U., 2009. Phosphorylation of the influenza A virus protein PB1-F2 by PKC is crucial for apoptosis promoting functions in monocytes. Cell Microbiol. 11, 1502–1516.

Mizumura, K., Hashimoto, S., Maruoka, S., Gon, Y., Kitamura, N., Matsumoto, K., Hayashi, S., Shimizu, K., Horie, T., 2003. Role of mitogen-activated protein kinases in influenza virus induction of prostaglandin E2 from arachidonic acid in bronchial epithelial cells. Clin. Exp. Allergy 33, 1244–1251.

Nakayama, T., Yamashita, M., 2010. The TCR-mediated signaling pathways that control the direction of helper T cell differentiation. Semin. Immunol. 22, 303–309.

Nimmerjahn, F., Dudziak, D., Dirmeier, U., Hobom, G., Riedel, A., Schlee, M., Staudt, L.M., Rosenwald, A., Behrends, U., Bornkamm, G.W., Mautner, J., 2004. Active NF-kappaB signalling is a prerequisite for influenza virus infection. J. Gen. Virol. 85, 2347–2356.

O'Connor, O.A., Wright, J., Moskowitz, C., Muzzy, J., MacGregor-Cortelli, B., Stubblefield, M., Straus, D., Portlock, C., Hamlin, P., Choi, E., Dumetrescu, O., Esseltine, D., Trehu, E., Adams, J., Schenkein, D., Zelenetz, A.D., 2005. Phase II clinical experience with the novel proteasome inhibitor bortezomib in patients with indolent non-Hodgkin's lymphoma and mantle cell lymphoma. J. Clin. Oncol. 23, 676–684.

Olschlager, V., Pleschka, S., Fischer, T., Rziha, H.J., Wurzer, W., Stitz, L., Rapp, U.R., Ludwig, S., Planz, O., 2004. Lung-specific expression of active Raf kinase results in increased mortality of influenza A virus-infected mice. Oncogene 23, 6639–6646.

Orlowski, J.P., Hanhan, U.A., Fiallos, M.R., 2002. Is aspirin a cause of Reye's syndrome? A case against. Drug Safety 25, 225–231.

Orlowski, R.Z., Voorhees, P.M., Garcia, R.A., Hall, M.D., Kudrik, F.J., Allred, T., Johri, A.R., Jones, P.E., Ivanova, A., Van Deventer, H.W., Gabriel, D.A., Shea, T.C., Mitchell, B.S., Adams, J., Esseltine, D.L., Trehu, E.G., Green, M., Lehman, M.J., Natoli, S., Collins, J.M., Lindley, C.M., Dees, E.C., 2005. Phase 1 trial of the

proteasome inhibitor bortezomib and pegylated liposomal doxorubicin in patients with advanced hematologic malignancies. Blood 105, 3058–3065.
Ott, D.E., Coren, L.V., Chertova, E.N., Gagliardi, T.D., Nagashima, K., Sowder 2nd, R.C., Poon, D.T., Gorelick, R.J., 2003. Elimination of protease activity restores efficient virion production to a human immunodeficiency virus type 1 nucleocapsid deletion mutant. J. Virol 77, 5547–5556.
Pahl, H.L., 1999. Activators and target genes of Rel/NF-kappaB transcription factors. Oncogene 18, 6853–6866.
Palombella, V.J., Rando, O.J., Goldberg, A.L., Maniatis, T., 1994. The ubiquitin-proteasome pathway is required for processing the NF-kappa B1 precursor protein and the activation of NF-kappa B. Cell 78, 773–785.
Papandreou, C.N., Daliani, D.D., Nix, D., Yang, H., Madden, T., Wang, X., Pien, C.S., Millikan, R.E., Tu, S.M., Pagliaro, L., Kim, J., Adams, J., Elliott, P., Esseltine, D., Petrusich, A., Dieringer, P., Perez, C., Logothetis, C.J., 2004. Phase I trial of the proteasome inhibitor bortezomib in patients with advanced solid tumors with observations in androgen-independent prostate cancer. J. Clin. Oncol. 22, 2108–2121.
Pauli, E.K., Schmolke, M., Wolff, T., Viemann, D., Roth, J., Bode, J.G., Ludwig, S., 2008. Influenza A virus inhibits type I IFN signaling via NF-kappaB-dependent induction of SOCS-3 expression. PLoS Pathog. 4, e1000196.
Perkins, N.D., 2006. Post-translational modifications regulating the activity and function of the nuclear factor kappa B pathway. Oncogene 25, 6717–6730.
Pinto, R., Herold, S., Cakarova, L., Hoegner, K., Lohmeyer, J., Planz, O., Pleschka, S., 2011. Inhibition of influenza virus-induced NF-kappaB and Raf/MEK/ERK activation can reduce both virus titers and cytokine expression simultaneously in vitro and in vivo. Antiviral Res. 92, 45–56.
Planz, O., Pleschka, S., Ludwig, S., 2001. MEK-specific inhibitor U0126 blocks spread of Borna disease virus in cultured cells. J. Virol. 75, 4871–4877.
Pleschka, S., 2008. RNA viruses and the mitogenic Raf/MEK/ERK signal transduction cascade. Biol. Chem. 389, 1273–1282.
Pleschka, S., Wolff, T., Ehrhardt, C., Hobom, G., Planz, O., Rapp, U.R., Ludwig, S., 2001. Influenza virus propagation is impaired by inhibition of the Raf/MEK/ERK signalling cascade. Nat. Cell Biol. 3, 301–305.
Richardson, P.G., Barlogie, B., Berenson, J., Singhal, S., Jagannath, S., Irwin, D., Rajkumar, S.V., Srkalovic, G., Alsina, M., Alexanian, R., Siegel, D., Orlowski, R.Z., Kuter, D., Limentani, S.A., Lee, S., Hideshima, T., Esseltine, D.L., Kauffman, M., Adams, J., Schenkein, D.P., Anderson, K.C., 2003. A phase 2 study of bortezomib in relapsed, refractory myeloma. N. Engl. J. Med. 348, 2609–2617.
Rinehart, J., Adjei, A.A., Lorusso, P.M., Waterhouse, D., Hecht, J.R., Natale, R.B., Hamid, O., Varterasian, M., Asbury, P., Kaldjian, E.P., Gulyas, S., Mitchell, D.Y., Herrera, R., Sebolt-Leopold, J.S., Meyer, M.B., 2004. Multicenter phase II study of the oral MEK inhibitor, CI-1040, in patients with advanced non-small-cell lung, breast, colon, and pancreatic cancer. J. Clin. Oncol. 22, 4456–4462.
Ruckle, A., Haasbach, E., Julkunen, I., Planz, O., Ehrhardt, C., Ludwig, S., 2012. The NS1 protein of influenza A virus blocks RIG-I-mediated activation of the noncanonical NF-kappaB pathway and p52/RelB-dependent gene expression in lung epithelial cells. J. Virol. 86, 10211–10217.
Russo, A., Bronte, G., Fulfaro, F., Cicero, G., Adamo, V., Gebbia, N., Rizzo, S., 2010. Bortezomib: a new pro-apoptotic agent in cancer treatment. Curr. Cancer Drug Targets 10, 55–67.
San Miguel, J.F., Schlag, R., Khuageva, N.K., Dimopoulos, M.A., Shpilberg, O., Kropff, M., Spicka, I., Petrucci, M.T., Palumbo, A., Samoilova, O.S., Dmoszynska, A., Abdulkadyrov, K.M., Schots, R., Jiang, B., Mateos, M.V., Anderson, K.C., Esseltine, D.L., Liu, K., Cakana, A., van de Velde, H., Richardson, P.G., 2008. Bortezomib plus melphalan and prednisone for initial treatment of multiple myeloma. N. Engl. J. Med. 359, 906–917.
Scheidereit, C., 2006. IkappaB kinase complexes: gateways to NF-kappaB activation and transcription. Oncogene 25, 6685–6705.
Schubert, U., Ott, D.E., Chertova, E.N., Welker, R., Tessmer, U., Princiotta, M.F., Bennink, J.R., Krausslich, H.G., Yewdell, J.W., 2000. Proteasome inhibition interferes with gag polyprotein processing, release, and maturation of HIV-1 and HIV-2. Proc. Natl. Acad. Sci. USA 97, 13057–13062.
Sebolt-Leopold, J.S., Herrera, R., 2004. Targeting the mitogen-activated protein kinase cascade to treat cancer. Nat. Rev. Cancer 4, 937–947.
Seth, R.B., Sun, L., Chen, Z.J., 2006. Antiviral innate immunity pathways. Cell Res. 16, 141–147.
Shi, X., Ding, M., Dong, Z., Chen, F., Ye, J., Wang, S., Leonard, S.S., Castranova, V., Vallyathan, V., 1999. Antioxidant properties of aspirin: characterization of the ability of aspirin to inhibit silica-induced lipid peroxidation, DNA damage, NF-kappaB activation, and TNF-alpha production. Mol. Cell Biochem. 199, 93–102.
Shin, Y.K., Li, Y., Liu, Q., Anderson, D.H., Babiuk, L.A., Zhou, Y., 2007a. SH3 binding motif 1 in influenza A virus NS1 protein is essential for PI3K/Akt signaling pathway activation. J. Virol. 81, 12730–12739.
Shin, Y.K., Liu, Q., Tikoo, S.K., Babiuk, L.A., Zhou, Y., 2007b. Effect of the phosphatidylinositol 3-kinase/Akt pathway on influenza A virus propagation. J. Gen. Virol. 88, 942–950.
Sieczkarski, S.B., Brown, H.A., Whittaker, G.R., 2003. Role of protein kinase C betaII in influenza virus entry via late endosomes. J. Virol. 77, 460–469.
Smith, J.R., Rayner, C.R., Donner, B., Wollenhaupt, M., Klumpp, K., Dutkowski, R., 2011. Oseltamivir in seasonal, pandemic, and avian influenza: a comprehensive review of 10-years clinical experience. Adv. Ther. 28, 927–959.
Utecht, K.N., Kolesar, J., 2008. Bortezomib: a novel chemotherapeutic agent for hematologic malignancies. Am. J. Health Syst. Pharm. 65, 1221–1231.
Vanhaesebroeck, B., Ali, K., Bilancio, A., Geering, B., Foukas, L.C., 2005. Signalling by PI3K isoforms: insights from gene-targeted mice. Trends Biochem. Sci. 30, 194–204.
Visekruna, A., Volkov, A., Steinhoff, U., 2012. A key role for NF-kappaB transcription factor c-Rel in T-lymphocyte-differentiation and effector functions. Clin. Dev. Immunol. 2012, 239368.
Wallace, E.M., Lyssikatos, J., Blake, J.F., Marlow, A., Greschuk, J., Yeh, T.C., Callejo, M., Marsh, V., Poch, G., Winkler, J.J., Koch, K., Davies, B.R., Wilkinson, R.W., Jones, D.C., Logie, A., McKay, J., Smith, P.D., Robinson, D.T., 2009. AZD8330 (ARRY-424704): Preclinical Evaluation of a Potent, Selective MEK 1/2 Inhibitor Currently in Phase I Trails. AACR 100th Annual Meeting, Denver, USA, 18th–22nd April.
Wei, L., Sandbulte, M.R., Thomas, P.G., Webby, R.J., Homayouni, R., Pfeffer, L.M., 2006. NFkappaB negatively regulates interferon-induced gene expression and anti-influenza activity. J. Biol. Chem. 281, 11678–11684.
Widjaja, I., de Vries, E., Tscherne, D.M., Garcia-Sastre, A., Rottier, P.J., de Haan, C.A., 2010. Inhibition of the ubiquitin-proteasome system affects influenza A virus infection at a postfusion step. J. Virol. 84, 9625–9631.
Widmann, C., Gibson, S., Jarpe, M.B., Johnson, G.L., 1999. Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human. Physiol. Rev. 79, 143–180.
Wurzer, W.J., Planz, O., Ehrhardt, C., Giner, M., Silberzahn, T., Pleschka, S., Ludwig, S., 2003. Caspase 3 activation is essential for efficient influenza virus propagation. Embo. J. 22, 2717–2728.
Wurzer, W.J., Ehrhardt, C., Pleschka, S., Berberich-Siebelt, F., Wolff, T., Walczak, H., Planz, O., Ludwig, S., 2004. NF-kappaB-dependent induction of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and Fas/FasL is crucial for efficient influenza virus propagation. J. Biol. Chem. 279, 30931–30937.
Yeh, T.C., Marsh, V., Bernat, B.A., Ballard, J., Colwell, H., Evans, R.J., Parry, J., Smith, D., Brandhuber, B.J., Gross, S., Marlow, A., Hurley, B., Lyssikatos, J., Lee, P.A., Winkler, J.D., Koch, K., Wallace, E., 2007. Biological characterization of ARRY-142886 (AZD6244), a potent, highly selective mitogen-activated protein kinase kinase 1/2 inhibitor. Clin. Cancer Res. 13, 1576–1583.
Yin, M.J., Yamamoto, Y., Gaynor, R.B., 1998. The anti-inflammatory agents aspirin and salicylate inhibit the activity of I(kappa)B kinase-beta. Nature 396, 77–80.
Zeng, Z., Lin, J., Chen, J., 2013. Bortezomib for patients with previously untreated multiple myeloma: a systematic review and meta-analysis of randomized controlled trials. Ann. Hematol. 23, 1–2, 2013 Mar 2. [Epub ahead of print].
Zhirnov, O.P., Klenk, H.D., 2007. Control of apoptosis in influenza virus-infected cells by up-regulation of Akt and p53 signaling. Apoptosis 12, 1419–1432.
Zhou, Z., Jiang, X., Liu, D., Fan, Z., Hu, X., Yan, J., Wang, M., Gao, G.F., 2009. Autophagy is involved in influenza A virus replication. Autophagy 5, 321–328.