Repurposing of Kinase Inhibitors as Broad-Spectrum Antiviral Drugs

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The high cost of drug development and the narrow spectrum of coverage typically provided by direct-acting antivirals limit the scalability of this antiviral approach. This review summarizes progress and challenges in the repurposing of approved kinase inhibitors as host-targeted broad-spectrum antiviral therapies.

Keywords: kinase inhibitors, antiviral drugs, repurposing, broad-spectrum antivirals

Introduction

THERE IS AN URGENT need for antiviral strategies to combat hundreds of human disease-causing viruses. Currently approved antiviral drugs treat fewer than ten viral infections. A majority of these drugs are direct-acting antivirals (DAAs) that target proteins encoded by individual viruses. As such, this approach provides a narrow spectrum of coverage and therefore cannot address the large clinical need. The high average cost (over two billion dollars) and long timeline (8–12 years) to develop a new drug (Tufts center for the Study of Drug Development, 2014), further limit the scalability of the DAA approach to drug development, particularly with respect to emerging viruses. Lastly, the inability to provide adequate global health protection and national security preparedness against newly emerging viruses and the emergence of viral resistance further challenge conventional DAAs.

The host-targeted broad-spectrum antiviral strategy represents an attractive potential solution to overcome these limitations. Viruses are dependent on cellular proteins for each step of their life cycle. Targeting host proteins required by multiple viruses can thus provide a broad-spectrum coverage with a possible added benefit of a high genetic barrier to resistance. Moreover, a broad-spectrum therapeutic could be administered even before a viral threat has been accurately diagnosed, thereby increasing protection.

A particularly cost- and time-effective strategy for the development of broad-spectrum antivirals is to repurpose already approved drugs that target host functions required by multiple viruses. By lowering the development cost, this approach has the potential to increase drug access for patients. Moreover, it shortens the path to clinic by enabling utilization of available data (e.g., toxicity, pharmacokinetics, dosing, etc.). Lastly, it can promote preparedness for future outbreaks of newly emerging pathogens by facilitating off-label use of approved broad-spectrum antivirals against new viral indications.

Host kinase inhibitors represent one category of compounds with a great potential to be repurposed as broad-spectrum antivirals. Viruses hijack a large number of host kinases at distinct steps of their life cycle (Supekova *et al.*, 2008; Li *et al.*, 2009; Keating and Striker, 2012; Jiang *et al.*, 2014). Some of these host kinases are broadly required and thus represent attractive targets for broad-spectrum therapy (Table 1 and Fig. 1). These findings, combined with the development and approval of a large number of kinase inhibitors for the treatment of cancer (Gross *et al.*, 2015) and inflammatory conditions (Ott and Adams, 2011) have sparked efforts aimed to determine the therapeutic potential of such drugs to combat viral infections. In this review, we summarize recent efforts to determine the therapeutic potential and biological rationale of repurposing already approved kinase inhibitors as antivirals.

Targeting the NAK Family of Kinases

Targeting the NAK (Numb-associated kinases) family of Ser/Thr kinases by approved kinase inhibitors is one approach showing promise as a broad-spectrum antiviral strategy. We discovered a requirement for the host kinases adaptor protein 2 (AP2)-associated protein kinase 1 (AAK1) and cyclin G-associated kinase (GAK) in the regulation of intracellular viral trafficking during entry, assembly, and release of unrelated viruses (Neveu *et al.*, 2012, 2015; Bekerman *et al.*, 2017).

We recognized that the already approved anticancer drugs sunitinib and erlotinib potently inhibited AAK1 and GAK, respectively, and demonstrated activity against viruses from six viral families, including dengue (DENV) and Ebola viruses (EBOV) in cultured cells (Neveu *et al.*, 2012, 2015; Bekerman *et al.*, 2017). Sunitinib/erlotinib combinations protected against morbidity and mortality in murine models of dengue and Ebola infection (Bekerman *et al.*, 2017). Moreover, we discovered that inhibition of AP-mediated

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TABLE 1. CLASSIFICATION, ANTIVIRAL ACTIVITY, AND MECHANISM OF ACTION OF APPROVED KINASE INHIBITORS

Kinase family	Kinases	Inhibitors	EC50	In vitro	In vivo	Stages of viral lifecycle	Antiviral target validation (siRNA)		Mechanism of action	Refs.
							target validation	Molecular target validation		
NAK	AAK1 GAK	Sunitinib	0.12–12.9 μ M	Flaviviridae (HCV, DENV, WNV, ZIKV)	DENV (mouse)	Entry	+	+	Inhibition of AP-mediated intracellular membrane trafficking	Bekerman <i>et al.</i> (2017)
		Erlotinib	0.12–4.67 μ M	Filoviridae (EBOV) Togaviridae (CHIKV) Arenaviridae (JUNV) Retroviridae (HIV) Paramyxoviridae (RSV)	EBOV (Mouse)	Assembly Release				Neveu <i>et al.</i> (2012) Neveu <i>et al.</i> (2015)
ErbB	EGFR	Erlotinib	0.276–0.6 μ M	Flaviviridae (HCV, DENV, WNV, ZIKV)	HCV (Mouse)	Entry	+	+	Inhibition of receptor-mediated endocytosis	Diao <i>et al.</i> (2012) Neveu <i>et al.</i> (2015)
		Lapatinib Gefitinib	0.18 μ M 4.93 μ M	Herpesviridae (HCMV) Poxviridae (Orthopoxvirus)	HCMV (Guinea pig)					Langhammer <i>et al.</i> (2011) Lupberger <i>et al.</i> (2011) Schleiss <i>et al.</i> (2008)
MAPK/ERK	MEK1/2 ERK1/2	Trametinib	0.1–1 μ M	Coronaviridae (MERS, SARS)	ND	ND	ND	ND	ND	Kindrachuk <i>et al.</i> (2015)
		Selumetinib	1–10 μ M	Flaviviridae (DENV)	ND	RNA Replication	+	ND	Inhibition of actin motility	Chu and Yang (2007)
Src	SRC FYN	Dasatinib	12.2 μ M*			Assembly				de Wispelaere <i>et al.</i> (2013)
		Sarcatanib	4.7 μ M*			Entry Release	+	ND	Inhibition of actin motility	Clark <i>et al.</i> (2016) Dyall <i>et al.</i> (2014) Garcia <i>et al.</i> (2012) Reeves <i>et al.</i> (2011)
Abl	ABL1	Dasatinib	12.2 μ M*	Filoviridae (EBOV)	Vaccinia (Mouse)					
		Imatinib Nilotinib	9.8–17.7 μ M –	Flaviviridae (DENV) Coronaviridae (MERS, SARS) Poxviridae (Vaccinia)						
CDK	CDK1 CDK2 CDK5 CDK4/6 CDK9	Palbociclib	0.016–0.02 μ M	Retroviridae (HIV)	HSV-1	ND	+	+	Inhibition of phosphorylation of viral proteins and reduction of host intracellular dNTPs	Badia <i>et al.</i> (2016) Chao <i>et al.</i> (2000) Guendel <i>et al.</i> (2010) Habran <i>et al.</i> (2005) Pauls <i>et al.</i> (2014a) Perwitasari <i>et al.</i> (2015) Schang <i>et al.</i> (2006) Xu <i>et al.</i> (2016) Yamamoto <i>et al.</i> (2014)
		Dinaciclib	0.02–0.355 μ M	Orthomyxoviridae (IAV)	(Mouse)					
PI3K/Akt/mTor	mTor Akt	PHA-690509	0.9–1.72 μ M	Flaviviridae (ZIKV)						
		Alvociclib	0.01–0.24 μ M	Herpesviridae						
PI3K/Akt/mTor	mTor Akt	Selaciclib	0.024–0.04 μ M	(HCMV, HSV)						
		Everolimus	1–10 μ M	Coronaviridae (MERS)	HCMV (Rat)	ND	+	ND	Disabling of viral block of cell stress response and apoptosis	Kindrachuk <i>et al.</i> (2015) Jaw <i>et al.</i> (2017) Jung <i>et al.</i> (2013) Liacini <i>et al.</i> (2010) Waldman <i>et al.</i> (1999) Williams <i>et al.</i> (2005)
PI3K/Akt/mTor	mTor Akt	Miltefosine	>10 μ M	Herpesviridae (HCMV)	BKV (Human)					
		Teriflunamide	–	Polyomaviridae (BKV)						
PI3K/Akt/mTor	mTor Akt	Leflunomide	–							

* = EC90.

FIG. 1. Approved kinase inhibitors with broad-spectrum antiviral activity. Examples of classes of kinase inhibitors with broad-spectrum antiviral activity and the specific steps of the viral life cycle they target (if known) are denoted in *colored boxes*. Blunt arrows connect these compounds to the corresponding targeted cellular process thought to mediate their antiviral effect.

intracellular membrane trafficking regulated by AAK1 and GAK represents an important mechanism by which sunitinib and erlotinib inhibit viral infection *in vitro* and *in vivo* (Bekerman *et al.*, 2017). The safety and efficacy of sunitinib/erlotinib combinations will be evaluated in dengue patients in the future and potentially in patients with EBOV disease in future outbreaks (ClinicalTrials.gov NCT02380625).

Targeting the ErbB Family of Kinases

Epidermal growth factor receptor (EGFR), a member of the ErbB family of receptor tyrosine kinases, is another host factor broadly required by viruses (Zheng *et al.*, 2014). EGFR is utilized as an entry coreceptor by human cytomegalovirus (HCMV) (Wang *et al.*, 2003) and adeno-associated virus serotype 6 (AAV6) (Weller *et al.*, 2010). EGFR-mediated signaling is implicated in the endocytosis of hepatitis C virus (HCV) and influenza A virus (IAV) (Eierhoff *et al.*, 2010; Lupberger *et al.*, 2011; Diao *et al.*, 2012; Neveu *et al.*, 2012,

2015; Zona *et al.*, 2013) and in postentry events, as in HCMV nuclear trafficking through regulation of F-actin dynamics (Wang *et al.*, 2005). IAV and other viruses hijack EGFR to evade host immune responses (Ueki *et al.*, 2013).

Altogether, these studies validate EGFR as a broad antiviral target. Indeed, erlotinib and gefitinib, approved anticancer drugs targeting EGFR, and lapatinib, an anticancer drug targeting EGFR and another member of the ERBB kinase family, ERBB2, demonstrated *in vitro* activity against a number of viruses, including HCV (Lupberger *et al.*, 2011; Diao *et al.*, 2012; Neveu *et al.*, 2015), HCMV (Schleiss *et al.*, 2008), and poxvirus (Langhammer *et al.*, 2011). Notably, erlotinib and gefitinib inhibited HCV and HCMV infections in a mouse and guinea pig model, respectively (Schleiss *et al.*, 2008; Lupberger *et al.*, 2011).

Targeting the MAPK/ERK Pathway

Several kinases in the MAPK/ERK (mitogen-activated protein kinases/extracellular signal-regulated kinases) pathway are essential for viral replication. ERK1 and ERK2 phosphorylate

HIV-1 proteins and enhance viral infectivity (Yang and Gubza, 1999). IAV activates this pathway and relies on it for effective virus production and export of the viral ribonucleoprotein complexes from the nucleus (Pleschka *et al.*, 2001). Activation of this pathway mediates EBOV entry into target cells and enhances cytokine production and cellular toxicity, thereby possibly contributing to disease pathogenesis (Kolkov *et al.*, 2009) (Zampieri *et al.*, 2007; Johnson *et al.*, 2014).

Moreover, infection with Middle East respiratory syndrome coronavirus (MERS-CoV) activates this pathway and is inhibited by two anticancer compounds, trametinib, an Food and Drug Administration-approved MEK1/2 inhibitor, and selumetinib, an investigational (Phase III) MEK1/ERK1/2 inhibitor (Kindrachuk *et al.*, 2015). The broad-spectrum antiviral potential of these compounds in cultured cells and animal models remains to be established.

Targeting the Src and Abl Families of Kinases

The Src family of kinases (SFKs) is implicated in the life cycle of multiple viruses (Pagano *et al.*, 2013). The replication of viruses from the Flaviviridae family is dependent on the activation of various SFKs, such as Fyn, Lyn, c-Src, and Csk (DENV) (Chu and Yang, 2007; de Wispelaere *et al.*, 2013; Kumar *et al.*, 2016), c-Yes (West Nile Virus, WNV) (Hirsch *et al.*, 2005), and Csk (HCV) (Supekova *et al.*, 2008). The unrelated HIV-1 also activates SFKs to effectively replicate (Narute and Smithgall, 2012).

The approved drug dasatinib and the investigational (Phase III) compound saracatinib, with activity against SFKs, inhibited DENV assembly *in vitro* (de Wispelaere *et al.*, 2013). This antiviral activity was initially attributed to inhibition of c-Src (Chu and Yang, 2007), yet the effect on assembly was subsequently shown to be non-Src mediated, whereas an independent effect of the drug on DENV RNA replication was found to be Fyn-mediated (de Wispelaere *et al.*, 2013).

Another host kinase targeted by both dasatinib and saracatinib is c-Abl (cellular Abelson tyrosine kinase), a member of the Abl family of nonreceptor tyrosine kinases. C-Abl is implicated in the budding or release of poxviruses (Reeves *et al.*, 2011) and EBOV (Garcia *et al.*, 2012), the entry of coxsackievirus (Coyne and Bergelson, 2006) and polyomavirus (Swimm *et al.*, 2010), and in DENV infection (Clark *et al.*, 2016). Imatinib and/or nilotinib, approved anticancer c-Abl inhibitors lacking anti-SFK activity, inhibited replication of EBOV (Garcia *et al.*, 2012), DENV (Clark *et al.*, 2016), MERS-CoV and/or SARS-CoV (Dyall *et al.*, 2014) in cultured cells. While dasatinib also inhibited MERS-CoV and SARS-CoV, it remained uncertain which molecular target(s) (Abl only or Abl plus Src) mediated its activity against these viruses (Dyall *et al.*, 2014).

In a murine model of vaccinia virus, imatinib effectively reduced viral load, viral spread, and mortality (Reeves *et al.*, 2011). In contrast, dasatinib did not protect mice, but rather induced immunosuppression, raising a concern about its utility in controlling viral infections *in vivo*. It is thus important to investigate the antiviral activity and safety of drugs targeting either Src and/or Abl kinases in animal models of other viral infections. Moreover, while inhibition of actin motility was proposed as a mechanism of antiviral action of Src and/or Abl inhibitors (Reeves *et al.*, 2011), additional mechanisms remain to be uncovered.

Targeting the Cyclin-Dependent Kinases

Numerous viruses regulate the host cell cycle by modifying the expression level and activity of members of the host cyclin-dependent kinases (CDKs) family (Schang, 2003). To promote its replication, adenovirus increases CDK2 phosphorylation, thereby forcing progression into late-G1/S-phase (Cheng *et al.*, 2013), whereas hepatitis B virus increases activity of the G₁-phase kinase CDK4 (Gearhart and Bouchard, 2010). CDK6 and CDK9 have both been validated as potential antiviral targets in HIV-1 infection (Nemeth *et al.*, 2011; Pauls *et al.*, 2014b), and CDK9 in herpes simplex virus type 1 (HSV-1) infection (Durand and Roizman, 2008).

Various CDK inhibitors with variable selectivity have demonstrated antiviral activity (Schang *et al.*, 2006). Palbociclib, an approved CDK4/6 inhibitor suppressed HIV-1 reverse transcription and HSV-1 replication *in vitro* (Pauls *et al.*, 2014a; Badia *et al.*, 2016). Moreover, four investigational CDK inhibitors, dinaciclib (CDK1/2/5/9 inhibitor, Phase III), seliciclib (CDK2/5 inhibitor, Phase II), alvociclib (CDK9 inhibitor, Phase II), and PHA-690509 (CDK2 inhibitor, Phase I), inhibited multiple viruses, including HIV-1 (Chao *et al.*, 2000; Guendel *et al.*, 2010), IAV (Perwitasari *et al.*, 2015), and Zika in cultured cells (Xu *et al.*, 2016). Alvociclib and a novel CDK9 inhibitor, FIT-039, demonstrated broad *in vitro* activity against DNA viruses and efficacy in a murine model of HSV-1 (Yamamoto *et al.*, 2014). In addition to suppression of viral replication, targeting CDKs in the context of oncogenic viral infections may also suppress viral persistence and/or transformation (Li *et al.*, 2003).

While grossly undertested, several studies have provided some insight about the potential mechanism of action (MOA) underlying the antiviral effect of CDK inhibitors. Inhibition of phosphorylation of viral proteins plays a role in the case of IAV and varicella zoster virus, (Habran *et al.*, 2005; Perwitasari *et al.*, 2015), whereas reduced intracellular dNTP availability and viral mRNA transcription through inhibition of a cellular protein phosphorylation is one mechanism by which palbociclib exerts its anti-HSV-1 and HIV-1 activities (Pauls *et al.*, 2014a; Badia *et al.*, 2016). A better understanding of the MOA of these compounds in other viral infections remains an important area of investigation.

Targeting the PI3K/Akt/mTOR Pathway

Multiple viruses activate the phosphatidylinositol 3'-kinase-Akt-mammalian target of rapamycin (PI3K/Akt/mTOR) pathway to support their replication and escape cellular stress responses (Buchkovich *et al.*, 2008). HCV (Mannová and Beretta, 2005), WNV (Shives *et al.*, 2014), and IAV (Ehrhardt *et al.*, 2006; Hale *et al.*, 2006) activate this pathway to promote distinct steps in their life cycle and control apoptosis. Although this pathway was shown to be activated in cells infected with MERS-CoV, everolimus, an mTOR inhibitor approved to treat cancer and prevent transplant rejection, demonstrated only mild *in vitro* activity against MERS-CoV, and miltefosine, an approved AKT inhibitor, demonstrated no activity in this infection model (Kindrachuk *et al.*, 2015).

In contrast, leflunomide's active metabolite teriflunomide, which suppresses Akt phosphorylation and is approved for rheumatoid arthritis treatment, has shown potent anti-HCMV

activity both *in vitro* and in a rat model (Waldman *et al.*, 1999). Furthermore, treatment with leflunomide and the two approved immunosuppressant mTOR inhibitors everolimus and sirolimus, either individually or in combination, reduced BK viremia and disease progression in patients with BK virus nephropathy (Williams *et al.*, 2005; Jung *et al.*, 2013; Jaw *et al.*, 2017). Direct suppression of polyomavirus replication was proposed as one underlying MOA of this approach based on *in vitro* studies (Liacini *et al.*, 2010). These findings underscore the importance of further exploring the broad antiviral activity of inhibitors of this pathway.

Summary and Perspectives

While repurposing of approved drugs as host-targeted broad-spectrum antivirals offers several important advantages over development of new DAAs, it also raises concerns. One obvious concern when targeting host functions is toxicity. Nevertheless, the protective effect of sunitinib/erlotinib combinations in murine models of DENV and EBOV (Bekerman *et al.*, 2017) suggests that for some drugs it may be feasible to find a therapeutic window where the drug level is sufficient to inhibit viral replication with minimal cellular toxicity. Shortening the duration of therapy from months or years required to treat cancer to several days sufficient to treat most acute viral infections should further limit toxicity.

Emergence of viral resistance is another potential challenge of broad-spectrum antiviral drugs (de Wispelaere *et al.*, 2013; Haqqani and Tilton, 2013). Nevertheless, targeting host kinases that are not under the genetic control of viruses is more likely to have a higher barrier to resistance than classical DAAs. Moreover, simultaneous inhibition of several kinases or pathways by the same drug or drug combination could increase the effectiveness while minimizing viral resistance, as previously shown in cancer (Knight *et al.*, 2010). Indeed, while DENV overcame inhibition by a DAA with the emergence of a resistance mutation, it remained susceptible to sunitinib and erlotinib, supporting the higher genetic barrier of the host-targeted antiviral approach (Bekerman *et al.*, 2017).

Lastly, understanding the MOA is challenging since cellular kinase function in a complex network of interactions and their inhibitors are often not selective. For example, erlotinib's effect on HCV entry was first attributed solely to its effect on EGFR, its known cancer target (Lupberger *et al.*, 2011; Diao *et al.*, 2012). We later demonstrated that ectopic expression not only of EGFR, but also GAK, which is inhibited by erlotinib with a comparable potency to EGFR (Kd of 3.4 nM vs. 1 nM) (Karaman *et al.*, 2008), reversed erlotinib's anti-HCV effect (Neveu *et al.*, 2015).

Similarly, the anti-DENV effect of dasatinib was attributed to the inhibition of SFKs, particularly Fyn (Chu and Yang, 2007) (de Wispelaere *et al.*, 2013). Nevertheless, the finding that c-Abl, another dasatinib target, is essential for DENV infection (Clark *et al.*, 2016) suggests that c-Abl inhibition likely represents yet another mechanism through which dasatinib mediates its antiviral activity. These examples underscore the importance of validating not only the antiviral targets but also the molecular targets underlying the antiviral effect of such inhibitors.

In summary, these examples provide a proof-of-concept for the potential feasibility of repurposing of approved kinase

inhibitors as host-targeted broad-spectrum antiviral therapies to combat viral infections. While not yet approved, compounds targeting other kinases, such as the phosphatidylinositol 4-kinase family and I κ B kinase- α , have already demonstrated antiviral activity (Altan-Bonnet and Balla, 2012; Li *et al.*, 2013), suggesting that the repertoire of kinase inhibitor classes available for repurposing is likely to grow. Such approaches may find utility in combination with other strategies being developed to combat viruses.

Acknowledgments

This research was supported by grants from the NIH (1U19 AI10966201), DoD/CDMRP (PRMRP, PR151090), American Cancer Society (RSG-14-11 0-01-MPC), Stanford Bio-X, and Stanford SPARK program to S.E. The authors acknowledge all the contributions in the field that could not be included in the review.

Disclosure Statement

No competing financial interests exist.

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Received for publication October 24, 2017; accepted
October 24, 2017.