Human enterovirus 71 (EV-A71) is the main etiological agent of hand, foot and mouth disease (HFMD). Typical clinical symptoms of HFMD are fever, rashes on the palms and soles and oral ulcers. Unlike other enteroviruses that cause HFMD, EV-A71 is more frequently associated with severe neurological complications, such as brainstem encephalitis and aseptic meningitis in children below 6 years of age. The first outbreak was reported in 1969 and subsequently many large outbreaks have been reported in Japan, Hungary, Bulgaria, Malaysia, Taiwan, Singapore, Australia, Vietnam, China and Cambodia [1]. The recent outbreak in China affected half a million people with more than 500 deaths. Fatalities are predominantly seen in Asian outbreaks, but neither a vaccine nor an antiviral is presently available for clinical use.

EV-A71 could emerge as the next significant enteroviral health threat after eradication of polioviruses. Owing to the potential severe neurological complications in EV-A71 infection, there is a pressing need to develop effective antiviral therapeutics. Our growing knowledge in EV-A71 viral–host receptor interactions provides opportunities to identify new targets for rational design of attachment or entry inhibitors.

**Keywords:** attachment • drug target • enterovirus 71 • entry • inhibitor • receptor
and CD155 for the polioviruses. Cryo-electron microscopy reveals that these receptors interact with viral capsids through penetration into the canyon, a depression that encircles the fivefold axis, and trigger capsid structural transition to allow virus entry [4]. The two EV-A71 entry receptors, scavenger receptor class B2 (SCARB2) and P-selectin glycoprotein ligand-1 (PSGL-1) are different from the known enterovirus receptors. SCARB2 is a type III double transmembrane protein that is widely expressed in different cell types. In most cell types, EV-A71 binds to the SCARB2 receptor and is then internalized via clathrin-mediated endocytosis [5,6]. The canyon around the EV-A71 VP1 Gln172 has been showed to interact with the amino acids between 144 and 151 of SCARB2 [7]. Interestingly, desialylation of SCARB2 abolished the EV-A71–SCARB2 interactions, implying that the sialic acids present on the SCARB2 are critical [3]. Involvement of PSGL-1 as an EV-A71 receptor has also been demonstrated, but this receptor is only expressed in neutrophils and lymphocytes [8]. Recent studies have demonstrated that SCARB2 is functionally more important than PSGL-1 as a receptor. At pH <6.0, EV-A71 uncoating was observed when SCARB2 was present, but not PSGL-1 [9].

“The major obstacle is delivering a sufficient amount of the inhibitor to the targeted site early enough to delay disease progression to neurological involvement or to prevent the spread of infection to others.”

Recent developments of receptor inhibitors for EV-A71 involve targeting the virus capsid proteins (such as hydrophobic pocket binders) or host-receptor binding sites (host-receptor inhibitors). Small synthetic capsid hydrophobic pocket binders are known to be effective inhibitors against picornaviruses through stabilizing the capsid against receptor-induced conformational changes and thereby preventing uncoating. The mechanism of the capsid-binding drug WIN51711 (5-[7-[4-(4,5-dihydro-2-oxazolyl)phenoxyl]heptyl]-3-methylsulfoxazole) against EV-A71 uncoating was recently resolved by x-ray crystallography [10]. BPROZ-194, a pyridyl imidazolidinone, is a novel class capsid binder that exhibited antiviral activity against EV-A71 with EC_{50} between 2.13 and 4.67 µM [11]. The key factor that determines drug efficacy is its ability to fit into the hydrophobic pocket of the VP1 capsid protein and stabilize the virus. Inhibitors of host attachment factors include SP40, a VP1-derived synthetic peptide that blocks viral attachment to cell surface heparan sulfate with IC_{50} between 6 and 9 µM [12]. Similarly, bovine lactoferrin that binds to heparan sulfate and VP1 also inhibits EV-A71 infection in vitro [13]. Pre-incubation of cells with anti-SCARB2 antibodies or monoclonal antibody targeting the N-terminal of PSGL-1 inhibits EV-A71 infection in a dose-dependent manner, but does not completely protect the cells from infection [5,8,9]. The soluble form of cellular receptors could act as molecular decoys of cell-associated receptors. Soluble forms of highly negatively charged heparin, dextran sulfate and suramin reduces viral infectivity, possibly due to disruption of the integrity of the EV71 capsid structure or steric hindrance of receptor interactions [2].

Virus receptors for therapeutics: the success stories of HIV entry inhibitors

A successful example of entry inhibitors is the HIV fusion inhibitor enfuvirtide, which obtained US FDA approval in March 2003. Enfuvirtide is used in combination with other antiretroviral agents in the treatment of experienced patients with resistance to other antiretroviral drugs. Enfuvirtide is a 36-amino acid peptide derived from the heptad repeat region-2 sequence of the HIV transmembrane protein GP41. Enfuvirtide interacts with the CD4+ T-cell receptor and prevents the critical fusion step of the viral entry process. As a result of low bioavailability and short half-life, second- and third-generation peptide-based fusion inhibitors with improved stability and potency have been developed [14]. These include sifuvirtide, which has a more stable secondary structure of the α-helix structure and prolonged plasma stability of up to 22 h [15]. This will result in lower dosage and frequency of administration. Another successful entry inhibitor has been maraviroc, which was approved by the FDA in 2007. Maraviroc binds to the chemokine co-receptor CCR5, blocking the binding of HIV-1 virus envelope glycoprotein GP120, hence preventing HIV-1 from entering and infecting immune cells [16].

Challenges of developing EV-A71 entry inhibitors

Cost, efficacy, route of administration and safety will continue to be the barriers to the success of taking antivirals targeting receptors from benchside to bedside. A possible use for attachment and entry inhibitors is for prophylaxis; for example, during an outbreak of HFMD associated with severe neurological disease in a kindergarden. However, this approach is unlikely to be cost-effective for resource-limited countries in Asia where large outbreaks frequently occur. The effectiveness of the attachment or entry inhibitor would be highly dependent on the timing of the treatment provided. The major obstacle is delivering a sufficient amount of the inhibitor to the targeted site early enough to delay disease progression to neurological involvement or to prevent the spread of infection to others. Bioavailability is often a determinant of drug efficacy. Although WIN compounds exhibit significant in vitro activity against rhinovirus, it has been unsuccessful due to their poor bioavailability. Similarly, clinical trials of intranasal anti-ICAM-1 antibodies targeting the rhinovirus receptor only delays onset of infection, but does not eliminate it [17]. EV-A71 peptide-based inhibitors such as the SP40 peptide currently have limited bioavailability and stability in plasma. Further chemical modifications, such as addition of N-terminal pyroglutamate and C-terminal homoserine lactone to the peptide, could improve the resistance to peptidase [18].

An additional challenge of small synthetic inhibitors such as the WIN compounds and peptides is the development of resistant mutants. RNA viruses exist as quasispecies, a cloud of virus variants carrying different mutations within a virus population. This can eventually lead to selection of mutant viruses resistant to the inhibitors. A single Val192Met mutation in VP1 is sufficient to confer resistance to BPROZ-194, the capsid binder [19]. EV-A71 mutants with VP1 Glu98Gln and Lys244Arg mutations conferred resistance to the suramin analog, NF449 [20]. Based on
lessons from HIV combination therapy, targeting multiple stages in viral replication can significantly reduce the emergence of resistant mutants. Understanding the molecular mechanisms of resistance may lead to design of improved inhibitors.

**Outlook for the future**

Limited antivirals targeting HIV, influenza, herpesviruses and hepatitis viruses are available on the market. Emerging infections with epidemic potential such as EV-A71 warrant greater attention. Development of antivirals based on understanding of virus-host receptor interactions show promise, exemplified by the success of enfuvirtide. However, further chemical modifications to improve the potency, efficacy, oral administration, safety and cost will be needed for EV-A71 antivirals, such as SP40 peptide, BPROZ-194 and WIN51711. Combination therapy with more than one inhibitor may yield more promising results. There should be more concerted efforts to screen other targets involved in the virus life cycle (e.g., polyprotein processing, capsid assembly and virus release) and host cellular pathways (e.g., apoptosis and autophagy). Additionally, several EV-A71 inactivated vaccines are currently in clinical trials and showing promising results.

While waiting for a new antiviral or vaccine, preventive measures, such as early detection of infection, social distancing, hand hygiene and effective supportive clinical treatment remain the cornerstones to combating EV-A71 infection.

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