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Antiviral targets of human noroviruses

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Human noroviruses are major causative agents of sporadic and epidemic gastroenteritis both in children and adults. Currently there are no licensed therapeutic intervention measures either in terms of vaccines or drugs available for these highly contagious human pathogens. Genetic and antigenic diversity of these viruses, rapid emergence of new strains, and their ability to infect a broad population by using polymorphic histo-blood group antigens for cell attachment, pose significant challenges for the development of effective antiviral agents. Despite these impediments, there is progress in the design and development of therapeutic agents. These include capsid-based candidate vaccines, and potential antivirals either in the form of glycomimetics or designer antibodies that block HBGA binding, as well as those that target essential non-structural proteins such as the viral protease and RNA-dependent RNA polymerase. In addition to these classical approaches, recent studies suggest the possibility of interferons and targeting host cell factors as viable approaches to counter norovirus infection. This review provides a brief overview of this progress.

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Introduction

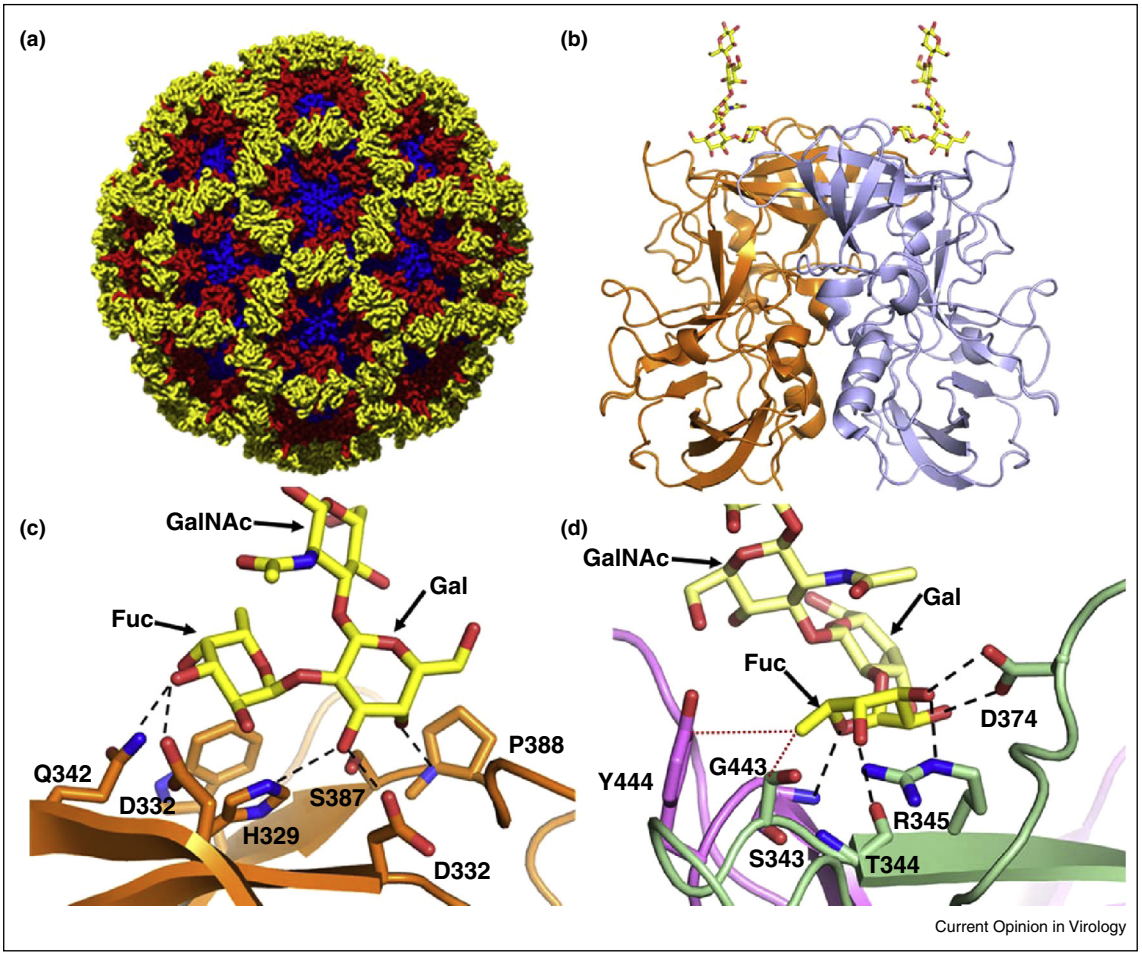
Human noroviruses (HuNoVs) are the most common cause of epidemic and sporadic cases of acute gastroenteritis worldwide [1]. In the US alone, HuNoVs cause approximately 19–21 million cases of acute gastroenteritis annually in all age groups [2•,3]. HuNoV infection can be life-threatening, especially in the elderly and immunocompromised transplant patients [4,5] who are at high risk for serious and prolonged chronic illness. In recent years, with the success of rotavirus vaccination in young children, HuNoVs have replaced rotaviruses as the most common cause of gastroenteritis in this age group [6,7•]. The economic burden of HuNoV infection in the US is estimated to be ~$5.5 billion [8]. In developing countries HuNoVs are estimated to cause more than 1 million hospitalizations and 218 000 deaths in children under 5 years of age occurring annually [9].

HuNoVs belong to the genus *Norovirus*, one of the five major genera in the *Caliciviridae* family. These ~400 Å icosahedral viruses have a positive-sense, single-stranded RNA genome. They exhibit enormous genetic diversity and are phylogenetically divided into at least six genogroups (GI–GVI). The GI, GII and GIV genogroups contain human pathogens. Each of these genogroups is further divided into several genotypes [10]. The HuNoVs belonging to genogroup II and genotype 4 (GII.4) are the most prevalent, and account for the majority of global outbreaks [11]. Epidemiological studies suggest that the GII.4 strains undergo epochal evolution with a new variant emerging every 2–4 years [12,13]. Recent studies also show outbreaks involving GI strains are becoming increasingly prevalent worldwide, with certain GI genotypes predominating in different geographical regions. The preponderance of global HuNoV outbreaks with periodic emergence of new variants poses a major health concern. Currently, there are no effective vaccines or antivirals available to counter HuNoV infection.

Vaccines against HuNoV infections

The genetic and antigenic diversity of HuNoVs and the lack of naturally-occurring longstanding immunity are possible significant challenges for the development of effective vaccines that can offer widespread cross-protection. However, significant effort has led to development of a bivalent vaccine, based on genotype GI.1 and a consensus GII.4 recombinant virus-like particles (VLPs) [14], which is in phase II clinical trials [15,16,17••]. The GII.4 VLP was designed by obtaining a consensus sequence from three GII.4 variants (Henry_2001, Yerseke_2006a, and Den Haag_2006b) using the Houston virus (Henry_2001 variant) as the backbone [18]. Point mutations were made to alter the amino acids into a consensus sequence. The consensus GII.4 VLP elicits antibody

Figure 1



NoV-HBGA binding site, a potential target for antivirals. **(a)** Structure of the Norwalk virus-like particle (PDB ID: 1IHM) comprised of 90 VP1 dimers. The VPI S domain, P1 and P2 subdomains are shown in blue, red and yellow respectively. **(b)** Cartoon representation of P-domain dimer (PDB ID: 2ZL6) bound to H type HBGA. The HBGA binding site is located on the top of the P domain. The individual subunits of the dimer are shown in orange and blue, respectively, and the H type HBGA is shown in yellow as a stick model. **(c)** Close up of HBGA binding site in GI HuNoV showing the galactose dominant nature of HBGA binding. All the residues involved in hydrogen bond interactions with H type HBGA (yellow) are contributed by the individual subunits of the dimer shown in orange stick models with oxygen (red) and nitrogen (blue) atoms shown; hydrogen bonds are shown as black dashed lines. **(d)** Close up of the HBGA binding site in GII.4 bound to H type HBGA (PDB ID: 3SLN) showing the fucose dominant nature of HBGA binding. The HBGA binding site in GII NoVs lies on the dimeric interface with both subunits of the dimer (green and pink) contributing to HBGA binding. Residues involved in hydrogen bond interactions (dashed lines) with HBGA (yellow) are labeled and bound HBGA is shown as a stick model following the same coloring scheme as in (c).

responses that recognize a wide array of GII.4 variants, including those that have yet to emerge [19•]. The HuNoV VLPs are produced by the expression of the major capsid protein VP1, which as 90 dimers forms the T = 3 icosahedral capsid (Figure 1) [20,21]. VP1 is encoded by the open reading frame (ORF) 2 of the HuNoV genome. A second minor structural protein, VP2, not present in the vaccine construct, is encoded by ORF3, whereas the ORF1 encodes a polyprotein that is processed by the virally-encoded protease into 6 non-structural proteins (NSPs). The VP1 exhibits a modular domain organization consisting of an S domain, formed by the N-terminal residues, that provides a scaffold for the protruding P domain, which is further subdivided into P1 and P2 subdomains (Figure 1a and b). The distally located and surface-exposed P2 subdomain, which can be considered as a large insertion in the P1 subdomain, harbors the most sequence variations across the genogroups and genotypes and is responsible for many virus–host interactions. Recombinant VLPs are morphologically and antigenically similar to the authentic HuNoV capsid and are highly immunogenic. Such VLPs can be made from any HuNoV genotype [22], suggesting the possibility of designing multivalent vaccines from selected multiple genotypes. In addition to the VLPs, recombinant P domain by itself elicits a strong immune