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REVIEW ARTICLE

Norovirus in Cancer Patients: A Review

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Norovirus (NoV) is the leading cause of viral-related diarrhea in cancer patients, in whom it can be chronic, contributing to decreased quality of life, interruption of cancer care, malnutrition, and altered mucosal barrier function. Immunosuppressed cancer patients shed NoV for longer periods of time than immunocompetent hosts, favoring quasispecies development and emergence of novel NoV variants. While nucleic acid amplification tests (NAATs) for NoV diagnosis have revolutionized our understanding of NoV burden of disease, not all NAATs provide information on viral load or infecting genotype. There is currently no effective antiviral or vaccine for chronic NoV infections. Screening for inhibitors of NoV replication in intestinal organoid culture models and creation of NoV-specific adoptive T cells are promising new strategies to develop treatments for chronic NoV in immunosuppressed patients. Herein we summarize data on the epidemiology, clinical manifestations, diagnostic challenges, and treatment of NoV infection in patients with cancer.

Keywords. calicivirus; cancer; chronic diarrhea; diarrhea; gastroenteritis; hematopoietic stem cell transplant; immunocompromised; norovirus.

INTRODUCTION

Diarrhea is a common side effect of cancer therapies, including cytotoxic chemotherapy (eg, fluorouracil and irinotecan) [1], radiation, targeted therapies, such as, tyrosine kinase inhibitors [2], immune check point inhibitors [3], and hematopoietic stem cell transplantation (HSCT; due to mucositis, immunosuppression, and acute and chronic graft-vs-host disease [GVHD]) [4]. Diarrhea can also result from microbiome dysbiosis related to antibiotic therapy or cancer therapy [5], as well as from infection. Due to the frequent use of antibiotics resulting in microbiome disruption, it is no surprise that *Clostridioides difficile* is the most common cause of nosocomial diarrhea [6]. The most common cause of viral-associated diarrhea is norovirus (NoV) [7], and these 2 pathogens frequently occur together in patients with cancer [8]. While cancer patients can experience self-limited diarrhea due to NoV, those with underlying immunosuppression can develop chronic diarrhea with dehydration, weight loss, and malnutrition [9]. NoV can also interfere with cancer care by delaying or altering chemotherapy regimens. While there are several reviews on acute NoV gastroenteritis, there is limited information on chronic NoV disease in cancer patients.

NOROVIRUS BIOLOGY

NoVs are small, nonenveloped RNA viruses that belong to the *Caliciviridae* family [10]. The open reading frames of the virus genome encode 2 structural proteins (VP1, VP2) and 6 nonstructural proteins. NoV particles have an icosahedral structure, with 180 molecules of the capsid viral protein 1 (VP1) arranged as dimers, with each dimer bearing a shell (S) and a protruding domain (P) [10]. The P domain is divided into P1 and P2 subdomains, of which the latter is relevant to immune recognition and receptor binding [11].

The genetic diversity among NoV strains is high. Noroviruses are classified into 10 genogroups, of which genogroups GI, GII, GIV, VIII, and IX are known to cause infections in humans [12]. Genogroups are further subdivided into genotypes, and some genotypes are further classified into variants. Within the 5 genogroups that cause human infections, there are 39 different genotypes; GIs and GIIs are the most prevalent and are divided into 9 and 27 genotypes, respectively [10]. Classification of variants has been primarily used for viruses belonging to genogroup II, genotype 4 (GII.4) pandemic lineages [13]. GII.4 is the most common cause of NoV outbreaks worldwide [14] and has been responsible for 6 major NoV acute gastroenteritis pandemics in the last 2 decades (95/96, 2002, 2004, 2006b, 2009, 2012).

EPIDEMIOLOGY OF NOROVIRUS

NoV is a leading cause of epidemic, acute gastroenteritis across all age groups worldwide, with most outbreaks in the United

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States occurring between November and April [15]. Infections in immunocompetent persons are self-limited, with viral shedding that typically lasts 2–3 weeks. In cotrast , NoV symptoms and viral shedding can be prolonged and without seasonal peaks in immunodeficient people including those with congenital immunodeficiency, solid organ transplant (SOT) or HSCT recipients, patients receiving chemotherapy for cancer, and with HIV [16]. The global burden of NoV-related diarrheal disease results in >$4 billion in direct health care costs and >$60 billion in societal costs [17].

Humans are the major reservoir for NoV, with a few reports of human NoV in pigs and cattle [18, 19]. Antigenic drift and shift are responsible for emergence of new GII.4 NoV variants every 2–3 years, allowing re-infection of hosts who were infected with other strains or variants [13]. A single major contemporaneous genotype dominates in immunocompetent people, whereas immunocompromised patients with chronic NoV can shed variants acquired in previous years and display wider genotype diversity [20]. Given prolonged NoV shedding and reduced immune pressure restricting viral mutations in immunocompromised individuals, it has been speculated that these hosts may be reservoirs for emergence of new NoV variants [21]. In a detailed molecular study, Doerflinger [22] analyzed 186 NoV capsid sequences during a 13-month period from a single immunocompromised host who had been shedding NoV for over 6 years. A multitude of capsid quasispecies belonging to GII.4 were observed, sharing 90% identity with other GII.4 sequences in the database. However, these variants had not been previously reported as causing outbreaks, and immediate family members of the patient did not develop infection during the study period despite NoV viral loads in the patient's stool being similar to viral loads seen in acute infections. Therefore, these variants were thought to have limited transmissibility; on the other hand, it is also possible that family members were immune to re-infection with GII.4 quasispecies based on exposure to NoV during the primary infection. In other studies, transmission of NoV from chronically infected persons has been shown [23], and continuous shedding of infectious virus has been detected based on the ability to replicate in human intestinal enteroid (HIE) cultures in vitro [24]. Molecular epidemiology studies suggest that a substantial proportion of NoV infections in immunocompromised patients originally thought to be nosocomial were acquired in the community, and nosocomial outbreaks where persons with immunodeficiency disorders are the source are rare [25, 26].

IMMUNITY TO NOROVIRUS

Human challenge studies in the 1970s first suggested a role for host genetic factors in susceptibility to NoV infection [27]. The P2 domain of NoV binds to the carbohydrate moiety of histo-blood group antigens (HBGAs) on mucosal cells of the gastrointestinal tract and facilitates viral entry [7]. HBGAs involved in NoV recognition belong to the Lewis, secretor, and ABO families [28]. Susceptibility to human NoV infection is determined by the variation in HBGA alleles. Patients who do not express (1,2) fucosylated HBGAs in saliva or the intestinal epithelium are called nonsecretors (found in 20% of Europeans) and are resistant to infection with the prototype human NoV Norwalk virus (genotype GI.1) and many GII viruses [29]. Secretor status is controlled by the fucosyltransferase 2 (FUT2) gene [30]. Given NoV diversity, people resistant to one strain may be susceptible to another, highlighting the effect of polymorphisms in receptor genes [10]. A metanalysis of 17 articles (2304 participants) suggested that blood types A, B, and AB might not affect susceptibility to NoV infection, but blood type O appeared to be more susceptible [31].

Human challenge studies [32, 33] have shown that the human infectious dose for GI.1 NoV is low (18 viral particles) [32], and 1 study showed that secretor-positive subjects with blood groups A and O were more susceptible to infection, while no one in blood group B became infected. Blood type–specific differences were, however, not noted in studies with other genotypes such as GII.4 [34], demonstrating the role of secretor status and adaptive immunity in acquiring infection.

Reeck et al. [35] showed that subjects with serum HBGA–blocking antibodies (inhibiting NoV virus–like particles from binding to HBGA H-type 1 or 3) were protected from developing clinical illness following infection with the isogenic strain [35–39] and shed less NoV than subjects with no preexisting serum-blocking antibody. This supports the hypothesis that HBGA-blocking antibodies may be used as a surrogate measure of NV serum–neutralizing antibodies. Indeed, with the establishment of virus neutralization assays in HIE cultures, a strong correlation between neutralizing antibody titers and HBGA-blocking antibodies has been observed [37].

HBGA-blocking antibody data from challenge studies and vaccine trials come predominantly from studies in healthy adults. Few studies have investigated immune correlates of protection in children. In a prospective study of 43 Finnish children with NoV acute gastroenteritis in whom secretor status was not addressed, there was a correlation between low acute-phase serum GII.4 New Orleans (NO)–specific immunoglobulin G (IgG) titer and low antibody-blocking potential with susceptibility to GII.4 NO infection [40]. High preexisting GII.4 NO antibody titer, measured by both enzyme-linked immunosorbent assay and HBGA-blocking antibodies, did not protect children from infection with other GII genotypes, suggesting the importance of strain-specific immunity for NoV infection at least in young children.

In terms of mucosal and cellular immune responses, volunteer studies have shown that prechallenge levels of NV-specific salivary IgA correlated with protection from gastroenteritis [38] while prechallenge levels of NV-specific fecal IgA correlated

with a reduced viral load. Prechallenge levels of NV-specific memory IgG cells correlated with protection from gastroenteritis and correlated with preexisting serum HBGA–blocking antibodies.

The role of T-cell-mediated immunity as a correlate of protection against NoV infection remains unclear. In a study investigating cell-mediated immunity to NoV in 10 healthy children, NoV-specific T cells were detected in 8/10 children, with higher response to GII.4 compared with GI.3. These responses were transient, with no correlation between cell-mediated and antibody responses [41]. In a case series of 13 HSCT recipient children with chronic NoV infection who needed enteral/parenteral nutritional support [42], CD3 recovery was associated with clearance of NoV from fecal samples; however, the role of NoV-specific antibodies in clearance of NoV was not evaluated. In a study by Davis et al. [24], NoV infection continued despite white blood cell count recovery due to possible continued use of immunosuppressives. The relevance of T cells in gut-associated lymphoid tissue in controlling NoV infection is unknown [43].

There are limited data on NoV-specific antibody responses following infection, role of secretor status, and blood groups in immunocompromised hosts. In a case of chronic NoV disease following rituximab-bendamustine therapy for non-Hodgkin's lymphoma, stool samples and serum antibodies that block GII.4-2009 interaction with carbohydrate ligand were examined at 6 and 8 months after chemotherapy completion. Serum samples at 6 months lacked blocking antibodies, and the patient continued to have diarrhea. However, 2 months later, blocking antibody titers developed, resulting in reduced need for antidiarrheals [44]. This suggests that in this patient population, NoV protective immunity can be restored following a rituximab-bendamustine regimen as its immunosuppressive effects wear off 6 months or more after the last dose.

NOROVIRUS AND THE MICROBIOME

There is new evidence suggesting a role for the intestinal microbiome in NoV infection. Both commensal and pathogenic bacteria can display HBGA-like molecules that bind NoV and form clusters or resist environmental stressors [45, 46]. In healthy individuals, secretor status and an abundance of *Ruminococcaceae* and *Faecalibacterium* spp. correlate with NoV seronegative status, showing that the microbiome, secretor status, and susceptibility to NoV infection are interdependent [47]. In a challenge study with NoV, the pre-infection microbiomes from subjects with asymptomatic infection are enriched in *Bacteroidetes* and depleted of clostridia relative to symptomatic subjects [48]. In vitro, *Enterobacter cloacae* facilitates NoV B-cell infection [49]. It remains to be seen if these observations are relevant across genotypes and in immunocompromised individuals and patients with cancer and which microbial components facilitate infection or help control infection.

CLINICAL MANIFESTATIONS

Immunocompetent patients with NoV gastroenteritis have a short incubation period (usually 24–48 hours), with illness characterized by vomiting, nausea, abdominal cramps, and diarrhea that typically resolves in <72 hours [50]. Viral shedding usually lasts 2–3 weeks after symptom resolution but can last up to 8 weeks [51]. The clinical course of disease for cancer patients with solid organ tumors with limited or no immunosuppression is similar to that of immunocompetent hosts. In contrast, immunosuppressed patients experience prolonged fecal NoV shedding [52]. In a review of viral-associated diarrhea of 97 patients at a tertiary cancer center from 2005 to 2015, 49 patients had NoV [53]. Of these, only 2 patients had solid organ cancers, whereas most cases had underlying leukemia or lymphoma. Diarrhea for >3 weeks was observed in 8/49 (16%) of patients, with viral shedding ranging from 46 to 270 days.

Patients with leukemia experience functional or absolute neutropenia, disordered B-cell function with reduced production of immunoglobulins, and suppressed T-cell function [54]. The latter 2 immunodeficiencies may predispose patients to NoV infection and can occur as a direct result of leukemia or from chemotherapy received. Drugs used to treat chronic lymphocytic leukemia such as rituximab, an anti-CD20 mAb, or alemtuzumab, an anti-CD52 antibody, which has profound effects on B and T cells, have been associated as a risk factor for NoV gastroenteritis in pediatric allograft recipients [55]. NoV affects 2.9% to 22% of allogeneic HSCT recipients in the first post-transplant year [55–57], partly from T-cell-directed immunosuppressive regimens, and is associated with significant morbidity and mortality [9].

Allogeneic transplant–associated NoV diarrhea can be protracted for months [9], requiring enteral or total parenteral nutritional support (Table 1) [25, 42, 56–60]. Clinical differentiation between NoV gastroenteritis and gastrointestinal GVHD (GI GVHD) is challenging and poses a management dilemma, as GI GVHD entails intensification of immunosuppression, which could worsen NoV infection [42]. In these cases, intestinal biopsy of the upper and lower GI tract could be of use. At the microscopic level, crypt apoptosis, the characteristic histologic feature of GVHD, can also be seen in NoV infection [26]. One study suggested that GI-GVHD is characterized by crypt apoptosis at the base of the crypts, with partial loss of epithelial cells and infiltration of the lamina propria by CD8 + T cells, whereas in NoV gastroenteritis, crypt apoptosis was seen at the luminal surface with more villous atrophy and intraepithelial infiltration of CD8 T lymphocytes [45]. Typically, GI-GVHD affects both the small and large bowel, whereas NoV causes small intestinal enteritis [9]. In a study analyzing intestinal biopsies from

Table 1. Select Studies of Adult and Pediatric Hematopoietic Stem Cell Transplant Recipients With NoV Diarrhea

Patient Population	No.	Concomitant GI Graft vs Host Disease, No. (%)	Duration of Symptoms, Median (Range), d	Need for Total Parenteral Nutrition, No. (%)	NoV Genotypes	Reference
Adult	12	8 (67)	90 (15–420)	6 (50)	GII.4 (var.3,4,6,8), GII.3, GII.7	9
Adult	11	1 (9)	2–36	..	GII.4	25
Pediatric	13	1 (8)	150 (60–380)	12 (92.3)[c]	..	42
Adult	6	3 (50)	61.6[b]	..	GI.3	56
Adult	10	2 (20)	42 (3–135)	..	..	57
Adult, pediatric	34, 29	22 (35)	8 (1–328)	10 (16)	..	58
Adult	6	2 (33)	22.5 (6–33)	..	GI, GII	59
Pediatric	25[a]	3 (12)	12.5 (1–324)	12 (48)	GII.2, GII.3, GII.4, GII.6, GII.7	60

Abbreviations: GI, gastrointestinal; NoV, norovirus.

[a]Includes 9 solid organ transplant recipients.

[b]Mean duration of shedding.

[c]Patient 13 had enteral nutritional support.

NoV-infected and noninfected (control) transplant patients (HSCT and small bowel), NoV was associated with edema, gastric metaplasia, and flattening of the epithelium from loss of villin [61]. NoV antigen VP1 was detected in the affected areas of the duodenum, jejunum (small bowel transplant), and ileum, as well as local macrophages, T cells, and dendritic cells. The nonstructural proteins RdRp and VPg, suggestive of viral replication, were detected in the epithelial cells of the duodenum and jejunum. However, NoV-related histopathological changes in the jejunum and ileum of HSCT recipients can be missed on routine colonoscopy or upper gastrointestinal endoscopy.

Another potential tool to determine the contribution of NoV in cancer patients with overlapping causes of diarrhea is estimation of viral burden. Vomiting and diarrhea have been linked to high viral loads in patients undergoing immunosuppressive therapy, compared with those with asymptomatic shedding [62]. In 152 cancer patients with GII (86%) and GI (14%) NoV diarrhea, dehydration and ICU admission were associated with a higher NoV stool load [63]. Interestingly GII viral loads were 1.2 log higher than GI. Adding complexity, coexistence of other enteropathogens is common in HSCT patients with diarrhea. In a cohort of adults and children, 10/63 patients were diagnosed either with adenovirus (3), *Clostridiodes difficile* (4), cytomegalovirus (2), or rotavirus (1) [58].

Chimeric antigen receptor T cells (CAR-T) are revolutionizing the management of refractory diffuse large B-cell lymphoma, mantle cell lymphoma, and acute lymphoblastic leukemia and are being deployed for the treatment of solid tumors. The use of conditioning cyclophosphamide and fludarabine and the effects of the CAR-T against CD19 result in hypogammaglobulinemia and prolonged cytopenias. CAR-T can cause cytokine release syndrome and neurotoxicity, which may require treatment with high-dose corticosteroids or interleukin-6 antagonists [64]. Patients receiving CAR-T cells have a history of being heavily pretreated with chemotherapy and have accumulated immunosuppression over time, placing them at risk for opportunistic infections. In a case series of 9 CAR-T recipients with NoV diarrhea, 6 patients were HSCT recipients, of whom 5 suffered GI-GVHD [65]. Three patients had diarrhea lasting >14 days with NoV shedding lasting 81–546 days. These patients developed malnutrition warranting parenteral nutritional support.

DIAGNOSTIC APPROACH

Commercial enzyme immunoassays used to detect NoV antigen have poor sensitivity [66]. Therefore, NAATs are being adopted, either in stand-alone or multiplexed platforms. NoV probes are present in several Food and Drug Administration (FDA)–approved multiplex platforms (Biofire's Gastrointestinal panel; FilmArray) [67–71], Luminex xTAG Gastrointestinal Pathogen Panel (GPP) [67–70, 72, 73], Verigene Enteric Pathogens Test [67], and BioCode GPP [74]. While these platforms have excellent sensitivity and specificity (Table 2) for GI and GII NoV, they do not provide viral loads or genotype-specific results [67–73].

Single platform PCRs are simpler in design and implementation and avoid primer–primer competition. FDA-approved single-platform PCRs are the RIDA Gene norovirus GI/GII real-time RT-PCR (RGN-RT PCR) [75] and the Xpert Norovirus assay [73, 76, 77]. However, single platforms are less desirable in immunocompromised patients, as co-occurrence of other enteropathogens could be missed.

TREATMENT

Immunocompromised patients with chronic NoV infection have limited options beyond supportive care. When feasible, immunosuppression should be decreased. Blanco et al. [78] described a double HSCT recipient with chronic NoV diarrhea who suffered from GVHD-related bronchiolitis obliterans. His GVHD therapy was switched from tacrolimus to sirolimus, an mTOR-I inhibitor with improvement of diarrhea and resolution of fecal NoV RNA. There are few data to support the

Table 2. Nucleic Acid Amplification Tests That Include Probes for Norovirus

Test	FDA Approved	Platform and No. of Pathogens Tested	Turnaround Time, h	Genotypes Detected	NoV Sensitivity/ Specificity, %	Study Location	Reference
Biofire FilmArray	2014	Multiplex, 23	1	GI/GII	92.9/99.6	North America, Europe, Asia,	67,68,69,70,71
TAG GPP	2013	Multiplex, 14	6	GI/GII	94.6/88.3–95.3	North America, Europe, Asia	67,68,69,70,72,73
Verigene	2014	Multiplex, 9	2.5	GI/GII	89/100	North America	67
BioCode Gastrointestinal Pathogen Panel	2018	Multiplex, 17	≤5	GI/GII	85.7–100/100	North America	74
RIDA Gene Norovirus GI/GII RT-PCR (RGN-RT PCR)	2018	Single	4	GI/GII	82.8–94.8/98.6–99.1[a]	North America	75
Cepheid Xpert Norovirus Assay	2014	Single	≤1.5	GI/GII	85.2–98.7/97–100	Europe, North America, Asia	73,76,77

Abbreviations: FDA, Food and Drug Administration; GI, gastrointestinal; NoV, norovirus; RGN-RT-PCR, RIDA Gene norovirus GI/GII real-time reverse transcriptase polymerase chain reaction; RT-PCR, reverse transcriptase polymerase chain reaction; TAG GPP, TAG Gastrointestinal Pathogen Panel.

[a]For GI genogroup sensitivity/specificity 82.8/99.1%, for GII sensitivity/specificity 94.8/98.6%.

widespread use of this strategy, but reducing immunosuppression, when feasible, would make clinical sense, allowing the innate and adaptive immune response to control NoV infection.

As seroprevalence rates of NoV among adults are 50%–90%, oral administration of serum-derived human immunoglobulin has been used as an adjunctive treatment, with mixed results [79, 80]. One hypothesis is that oral IgG blocks adhesion of NoV to the intestinal epithelium, preventing replication by forming a complex with the virus [81]. Another possible mechanism involves immunoglobulin-induced increase in anti-inflammatory cytokines and reduction of proinflammatory cytokines [79]. NoV HBGA–blocking activity, neutralizing titers, and genotypes bound by IgG in commercial preparations are unknown. Data supporting the bioavailability of oral IgG in the small bowel have been studied in 3 immunocompromised patients in whom IgG was found in stools as immune complexes with NoV [82]. In a placebo-matched case–control study with 24 cancer and SOT patients, trends toward the resolution of diarrhea and decreased stool output were observed with oral IG (25 mg/kg every 6 hours for 2 days) [81].

Nitazoxanide (NTZ) has activity against anaerobic bacteria, protozoa, and viruses and is FDA approved for the treatment of pediatric cryptosporidiosis and giardiasis. Its antiviral activity is thought to be from potentiation of PKR, a host protein kinase, which then phosphorylates the eukaryotic initiation factor 2 alpha (eIF2α), halting viral protein synthesis [83]. There are limited in vitro data for NTZ inhibition of NoV. In a replicon model examining the antiviral potential of NTZ and its active metabolite tizoxanide on GI.1 NoV, the latter activated cellular antiviral response and stimulated the expression of interferon-stimulated genes (ISGs), such as interferon regulatory factor 1 (IRF-1), in both infected and uninfected human intestinal organoids [84]. Data on NTZ efficacy for NoV diarrhea are limited and anecdotal. In a retrospective report [85], 3 of 5 HSCT recipients with NoV diarrhea improved following treatment with 500 mg of NTZ twice daily for 3 to 18 days, and 3 showed resolution of symptoms along with negative RT-PCR in the stool after completion of therapy. In a placebo-controlled clinical trial [86] of 50 subjects with viral gastroenteritis due to adenovirus, rotavirus, or NoV, patients were randomized to either NTZ 500 mg or placebo twice daily for 3 days. Duration of illness was significantly reduced in the entire population and in subsets of patients with NoV; however, the number of patients with NoV only was small (n = 13, 6 actives, 7 placebo). The Nitazoxanide for NoV in Transplant Patients Study (NNITS) is an ongoing phase 2 multicenter, double-blind, placebo-controlled study to determine the clinical and virologic efficacy and safety of NTZ for the treatment of symptomatic NoV diarrhea in SOT and HSCT recipients [87–89].

Favipiravir has been studied in a single patient case report, where it offered symptomatic improvement but was associated with rapidly developing viral variants and required dose interruption due to side effects [90]. Other treatments including interferons, monoclonal antibodies, and antivirals in development have been recently reviewed by others [91, 92].

Adoptive T-cell therapy with ex vivo expanded virus-specific T cells has been used in treating viral infections after HSCT such as cytomegalovirus, adenovirus, BK polyomavirus, and most recently progressive multifocal leukoencephalopathy (PML) [93]. These therapies are being developed for NoV from seropositive donors with promising preclinical results [94]. In a pilot study, peripheral blood mononuclear cells stimulated with NoV peptide mixes spanning the entire open reading frame were cultured for 10 days. After stimulation, a mean 4.2-fold increase in cell yield was noted, and T cells were polyclonal ($CD4^+$, $CD8^+$ populations) with reactivity to multiple NoV antigens. The specificity of these T cells against NoV antigens was further studied using an IFN-γ ELISpot assay. NoV-specific T-cell responses were highly cross-reactive against different strains and variable epitopes. This potential strategy could be

tried in HSCT and CAR-T recipients with chronic NoV diarrhea in the near future.

Given the need for antimicrobial prophylaxis during episodes of neutropenia, the intestinal microbiome undergoes profound alterations during HSCT and CAR-T therapy. In the case of HSCT, loss of microbiome diversity and richness impacts post-transplant immune reconstitution and clinical outcomes such as risk of bacteremia [95], relapse of hematologic malignancy [96], onset of GVHD [97], and death [98]. Fecal microbial transplantation (FMT) has been shown to reverse intestinal dysbiosis following HSCT [99] and appeared to be safe and effective in treating HSCT-associated *C. difficile* infection [100]. In a recent case of a 68-year-old renal transplant recipient with chronic NoV diarrhea of 2 months' duration, FMT was performed with complete symptom resolution with negative NoV testing on serial stool samples over a follow-up period of 5 months [101]. Further studies are needed to determine if FMT, probiotics, or complex microbial communities with glycans with affinity to NoV could potentially be an approach to treat chronic HSCT-associated NoV.

INFECTION PREVENTION FOR IMMUNOCOMPROMISED PATIENTS

Transmission of NoV occurs primarily by person-to-person, foodborne, and waterborne routes [102], with some studies suggesting transmission through aerosolized vomitus particles. Some data suggest that NoV GI.7 and GII.12 are more likely associated with foodborne disease, and GII.4 with interperson spread [103]. Spread is facilitated by thermal stability, relative resistance to alcohol sanitizers [104], persistence on multiple surfaces, presymptomatic viral shedding, and a long shedding period. NoV outbreaks involve people of all ages and occur in a wide variety of settings (eg, hospitals and long-term facilities, restaurants and catered events, schools and day care centers, military, prisons, and commonly cruise ships).

Patients at risk for severe NoV infection should wash their hands with soap and water for at least 20 seconds, especially while handling food or after using the restroom. Complete inactivation of 3 GII.4 strains was seen with 50 ppm of chlorine and was higher in HIEs [105]. Hand sanitizers cannot substitute for hand washing and can only be an adjunct. Hand hygiene is crucial as NoV can be found in vomitus or stool before symptom development and can remain in stool for 2 weeks or longer.

Inpatients with NoV gastroenteritis need to be placed in contact precautions (gowns and gloves for entry) for a minimum of 48 hours after symptom resolution [106]. This becomes challenging in the immunocompromised population, which has prolonged NoV shedding. All efforts must be made to ensure single occupancy rooms for such patients. It is recommended that patients with symptomatic NoV have limited movements in and around the ward and avoid group activities, especially in the setting of an outbreak. Adherence to hand hygiene with soap and water [107] is paramount among patients, health care personnel, and visitors of patients with symptoms.

Table 3. NoV Vaccine Clinical Trials That Have Completed Recruitment

Vaccine Candidate	National Clinical Trial No.	Country	Study Phase	Genotype, Dose, & Route	Objective	Study Population	Reference
Bivalent recombinant virus–like particles	02153112	Columbia, Finland, Panama	Phase II	GI.1, GII.4 (15/15 µg, 15/50 µg, 50/150 µg), IM	Safety, immunogenicity	Children (4–<9 y) Toddlers (1–<4 y) Infants (6 mo–<1 y	109
	01609257	USA	Phase I–II	GI.1, GII.4 50 µg each IM	Safety and efficacy	Adults, 18–49 y	39, 110,111
	02661490 NOR-204	USA	Phase II	GI.1, GII.4 15/50 µg IM	Safety and efficacy	Adults, 60–102 y	112
	02038907	Belgium	Phase II	GI.1, GII.4 (15/15 µg, 15/50 µg, 50/50 µg)	Safety and immunogenicity	Adults, 18–64 y	113
	02142504	USA	Phase II	GI.1, GII.4 15/50 µg, 50/50 µg, 15/15 µg, IM	Safety and immunogenicity	Adults, 18–49 y	114
	01168401	USA	Phase I	GI.1, GII.4 5/5 µg, 15/15 µg, 50/50 µg, 150/150 µg, IM	Safety and immunogenicity	Adults, 18–85 y	115
	00806962	USA	Phase I	GI.1, 50 µg, 100 µg intranasal	Safety and immunogenicity	Adults, 18–50 y	116
	02669121	USA	Phase IIb	GI.1/GII.4 15/50 µg, IM	Efficacy and immunogenicity	Adults, 18–49 y	117
	02475278	USA	Phase II	GI.1, GII.4 15/50 µg IM	Evaluate serologic assays to assess postvaccination immune response	Adults, 18–49 y	118
Recombinant adenovirus	02868073	USA	Phase I	GI.1, 1 x 10^{10} and 1 x 10^{11} IU, Oral	Safety and immunogenicity	Adults, 19–49 y	119
	03125473	USA	Phase Ib	GI.1, 1 x 10^{10} and 1 x 10^{11} IU, Oral	Safety of different dosing regimens	Adults 19–49 y	119,125

Abbreviations: GI, gastrointestinal; IM, intramuscular; IU, infectious units; NoV, norovirus.

Table 4. Key Challenges and Questions Related to NoV in the Immunocompromised

How should a positive NoV NAAT result be interpreted in the setting of overlapping clinical conditions that cause diarrhea in immunocompromised and cancer hosts?
Can fecal viral load and/or cycle threshold distinguish between diarrhea due to NoV and diarrhea from other causes?
Do mutations that occur in those who shed virus chronically result in quasispecies that possess differences in virulence?
What are the risks of secondary spread of infection in those who shed norovirus chronically?
What is the role of co-occurrence of other pathogens in the pathogenesis of NoV diarrhea?
Do NoV genotype, genogroups, variants, and viral load play a role in response to treatment?
Does the presence of NoV quasispecies affect disease pathogenesis and treatment outcome?
Do NoV quasispecies display differences in infectivity? How is this relevant to secondary spread in health care facilities and the community?

Abbreviations: NAAT, nucleic acid amplification test; NoV, norovirus.

VACCINES IN DEVELOPMENT

NoV display wide antigenic diversity, and infections with 1 genogroup generally do not confer protection to other genogroups. It is unclear how long effective immunity against a genotype lasts [108]. While people lacking functional FUT2 enzyme are resistant to GI.1 and GII.4 NoVs, they remain susceptible to infections from other NoV genotypes. Several immune correlates of protection against NoV have been postulated, but the aforementioned factors make NoV vaccine development challenging. One challenge is a decision on which genotypes to include in the vaccine formulation. Considering that the first detected NoV was GI.1, a vaccine containing this virus was first developed. Following the identification of GII.4 as the most common cause of NoV acute gastroenteritis and the low level of cross-reactivity between GI.1 and GII.4, a combined vaccine was developed. Thus far, 2 NoV vaccines are in human clinical studies: nonreplicating virus-like particles (VLPs) [38, 39, 109–118] and recombinant adenoviruses (Table 3) [119]. There are no data on NoV vaccinations in patients with cancer.

AREAS OF UNCERTAINTY

Given the burden of NoV infections in cancer patients, several key challenges and questions remain to be answered (Table 4). Answers to some questions will be aided using next-generation sequencing and availability of HIEs as in vitro culture models [120]. HIEs, derived from stem cells in intestinal tissues, support the growth of multiple GI/GII NoV strains [121]. Studies in HIEs have demonstrated the requirement of bile acids for some strains [122] and have confirmed HBGA restriction [123], described previously in epidemiological studies [103]. HIEs [37] have also allowed the direct evaluation of virus neutralization assays and demonstrated strong correlation between serum-neutralizing antibodies and HBGA-blocking antibodies to GII.4 VLP in healthy adults who received bivalent NoV vaccines.

A bivalent (GI.1, GII.4) NoV vaccine study in healthy US adults described cross-protection to GII.2, but there are limited data overall on cross-protection to different circulating NoV strains as well as duration of protection [124]. Nitazoxanide and oral immunoglobulin need further study to determine efficacy, and it is unclear whether differences in treatment outcomes correlate with virus genotype or viral load. There is a need to identify viral and host druggable targets that can eradicate NoV, to mitigate clinical manifestations, and to better define the role of the microbiome in NoV infection. Effective strategies for NoV infection could have a substantial impact on clinical outcomes and improve quality of life in patients with cancer.

Supplementary Data

Supplementary materials are available at Open Forum Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Potential conflicts of interest. Baylor College of Medicine (M.K.E. and R.L.A. as inventors) has a patent for norovirus cultivation in human intestinal enteroids. M.K.E. has a patent on methods and reagents to detect and characterize Norwalk virus and related viruses. M.K.E. and R.L.A. have received grant support from Takeda Vaccines Business Unit. P.C.O. reports grants from Summit Pharmaceuticals, grants from Deinove, grants and personal fees from Napo Pharmaceuticals, grants from Merck & Co., personal fees from Ferring Pharmaceuticals, personal fees from Singulex, and grants from Melinta Therapeutics, outside the submitted work. D.K. and S.R. have no competing interests. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Patient consent. This manuscript does not include factors necessitating patient consent.

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