Guanylyl cyclase C (GUCY2C) is a transmembrane-spanning protein expressed on the apical-brush border membrane throughout the entirety of the small and large intestines. This receptor has two endogenous ligands, uroguanylin and guanylin, which regulate fluid secretion and epithelial cell homeostasis in the small and large intestines, respectively, through the catalytic production of the second messenger cyclic guanosine monophosphate (cGMP) upon GUCY2C binding (1). GUCY2C is the only characterized receptor for the exogenous ligand, the heat-stable enterotoxin ST, produced by enterotoxigenic E. coli and responsible for traveler’s diarrhea (2). Currently, the GUCY2C signaling axis is pharmacologically targeted by US FDA-approved peptide therapeutics, linaclotide (Linzess™) and plecanatide (Trulance™), for the treatment of constipation-predominant irritable bowel syndrome (IBS-C) and chronic idiopathic constipation (CIC) (3-5). Beyond therapeutic modulation of intestinal fluid homeostasis, over the last decade, GUCY2C also has emerged as a promising target for cancers of the gastrointestinal (GI) tract.

Homeostatic regulation of the rapidly proliferating intestinal epithelial compartment through the GUCY2C signaling axis, identified GUCY2C as a tumor suppressor (6). However, disruption of GUCY2C tumor suppressing activity during colorectal tumorigenesis results not from abnormal expression or activity of the receptor, but from loss of its ligands, effectively silencing the GUCY2C signaling axis (7,8). Retention of the receptor on dysplastic and neoplastic intestinal epithelia allow for GUCY2C to be targeted by both ligand replacement therapy to restore the signaling axis and immunotherapeutic strategies in preclinical and clinical investigation (9-11). Moreover, upper GI malignancies that undergo preneoplastic metaplasia, adopting an intestinal epithelial phenotype, ectopically express GUCY2C, creating the potential for GUCY2C-targeted immunotherapies to treat not only cancers of the small intestine, colon, and rectum, but also esophagus, gastric mucosa, and even pancreas, which together account for ~20% of all cancer-related deaths (12,13). Of note, the apical restriction of GUCY2C along the intestinal tract, allows for compartmentalization away from the systemic compartment, providing a natural barrier that can be exploited therapeutically to destroy GUCY2C-expressing tumor cells that have formed metastases underlying GI cancer mortality, while preserving normal intestinal tissue (14). Indeed, GUCY2C-targeted vaccines, antibody-drug conjugates, and cellular therapies produce no toxicity in animal models or patients (cellular therapies have not yet been tested in patients), even in the context of colitis models that promote intestinal barrier dysfunction, suggesting that multiple mechanisms may confer protective benefits for healthy tissues during GUCY2C-targeted cancer immunotherapy (15,16).

Cancer immunotherapy has focused on unleashing the cytolytic capacity of T cells on tumor cells. Conventionally, circulating T cells expressing the co-receptor CD8 scan cells for viral peptides in the context of a presentation molecule called major histocompatibility complex (MHC) class I, expressed ubiquitously throughout the body. T-cell receptor (TCR) recognition of its cognate peptide-MHC results in the T-cell forming a synapse with the infected cell and releasing cytolytic granules containing the pro-apoptotic factors perforin and granzyme towards the...
infected cell to promote cell death, limiting viral spread within the body (17). Cancer immunotherapy leverages this cytolytic capacity through biomedical engineering to direct T cells to a tumor-associated antigen (TAA) allowing for the T cell to target the same pro-apoptotic factors at tumor cells, resulting in tumor cell death. This can be achieved by harnessing endogenous T cells with TCRs specific for tumor antigens using vaccines or immune checkpoint blockade, or through approaches in which T cells are genetically modified \textit{ex vivo} to express an engineered TCR recognizing a known TAA peptide-MHC complex or chimeric antigen receptor (CAR). CARs are synthetic receptors that fuse the antigen-recognition domains of a TAA-specific antibody, in a format called the single chain variable fragment (scFv), with the intracellular signaling moieties associated with the TCR (18). In recent years an additional approach has been developed that redirects endogenous T cells to target TAAs, without a requirement for \textit{ex vivo} genetic modification: the Bi-specific T-cell Engager (BiTE).

BiTEs present an alternative strategy for MHC-independent synapsing of T cells with tumor cells. In the simplest format, the BiTE is composed of two discrete scFvs fused into a single molecule. One scFv is directed against the TAA, while the other scFv is directed against the extracellular domain of CD3ε, a signaling molecule that complexes with the TCR. The purpose of the BiTE is to create a stable, molecular bridge between the T cell and tumor cell, independent of TCR specificity, promoting formation of an immunological synapse, degranulation, and tumor cell death (Figure 1) (19). BiTE technology targeting the B-cell antigen CD19 (blinatumomab; Blincyto®) has already received FDA approval after complete responses to treatment were observed in patients with acute lymphoblastic leukemia (20). However, how translatable this approach is to solid GI malignancies remains an open question.

Reflecting the prevalent expression of GUCY2C in various GI malignancies and success of other immunotherapies targeting GUCY2C, Mathur \textit{et al.} developed a bi-specific molecule (PF-07062119) targeting GUCY2C using a variation of the traditional BiTE format called a Dual-Affinity Re-Targeting antibody with a human IgG1 Fc region (DART-Fc) and examined its efficacy and safety in preclinical models (21). This modified format confers numerous advantages, the most important being that the DART design is more compact than the traditional scFv BiTE, allowing for improved cell killing,

**Figure 1** BiTE-mediated tumor cell killing. The Bi-specific T-cell Engager (BiTE) acts as a molecular bridge, linking cytolytic T cells with tumor cells. The BiTE is composed of two, discrete antibody fragments. One fragment binds a tumor-associated antigen (TAA), such as GUCY2C, and the other fragment binds CD3ε, a molecule that forms part of the T cell receptor (TCR) signaling complex. This association promotes the formation of an immunological synapse between the cytolytic T cell and the tumor cell, resulting in morphological changes within the T cell and trafficking of cytolytic granules along microtubules and release at the tumor cell interface. These granules contain the pro-apoptotic molecules perforin and granzyme, which result in destruction of the tumor cell.
which may reflect the spatial constraints of endogenous TCR-peptide MHC interactions (22). Also, the Fc region provides stability and significantly increases the half-life from hours to days, allowing for longer intervals between doses. Although the paper does characterize the expression of GUCY2C in multiple GI cancers, the therapeutic proof-of-concept of PF-07062119 focused solely on models of colorectal cancer.

Importantly, PF-07062119 is able to control tumor growth in multiple cell lines and a patient-derived xenograft model, including orthotopic modeling in the cecum, suggesting that it may have antitumor efficacy in patients, alone or in combination with other immunotherapies, such as checkpoint blockade and anti-angiogenic therapies explored in the study. Surprisingly, however, this antibody-derived biologic produced toxicities not observed in other GUCY2C-targeted immunotherapeutics. In cynomolgus monkeys, PF-07062119 treatment produced emesis, increased body temperature, decreased activity, hunched posture, reduced appetite, dehydration, body weight loss, and soft or liquid feces. Histopathologic analyses revealed minimal to mild infiltrates and crypt hyperplasia and villous atrophy in the intestines, the primary site of GUCY2C expression in normal tissues. Although the distribution of biologics is markedly different from small-molecule drugs, they still retain the ability to penetrate into peripheral tissues. While some of these toxicities observed in cynomolgus monkeys are observational, therapy-mediated dysfunction of the GUCY2C signaling axis could potentiate these adverse events and warrants further investigation. Indeed, the histopathological observations of villus blunting and expansion of the crypt compartment mimics what has been observed in GUCY2C deficient mouse models (23), suggesting that PF-07062119 may impact GUCY2C function regardless of T-cell engagement. While ulceration and necrosis were not observed, the possibility that GUCY2C-expressing cells were ablated cannot be discounted, especially with the increase in immune infiltrates. Similarly, appetite reduction may reflect dysregulation of the GUCY2C signaling axis in the hypothalamus which promotes satiety signals (24,25). Thus, more work is needed to define the mechanisms underlying these toxicities to better inform management strategies as PF-07062119 moves into clinical testing (ClinicalTrials.gov NCT04171141).

Similar to checkpoint inhibitor therapy, the success of BiTE technology may depend upon T cells already resident within the tumor. Given that the majority of colorectal cancer cases are unresponsive to checkpoint inhibitors, it is unclear how T cells in patient tumors will respond versus experimental models using circulating T cells, a question that will not be resolved until clinical trials are concluded. Nevertheless, the overarching message should not be lost, that GUCY2C can be successfully targeted to re-direct cytolytic T cells for the destruction of cancer cells. Over the next decade, registration trials testing GUCY2C-targeted vaccines, BiTEs, CAR-T cells, and antibody-drug conjugates may significantly increase the armamentarium of therapies available to GI medical oncologists, transforming the management of GI malignancies, and improving outcomes for patients with GI malignancies.

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**Footnote**

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**Ethical Statement:** The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
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