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Antibody–drug conjugate directed against the guanylyl cyclase antigen for the treatment of gastrointestinal malignancies

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ABSTRACT

Antibody-directed cancer chemotherapy in the form of antibody–drug conjugates (ADCs) may improve the therapeutic index with the potential to enhance efficacy and decrease systemic toxicity. ADCs consist of three key components including an antibody that specifically binds to the target, a toxic agent and a linker which releases the toxic agent inside tumor cells. A novel ADC, MLN0264 (TAK-264) was recently investigated in patients with gastrointestinal (GI) malignancies. TAK-264 is an anti- guanylyl cyclase C (GCC) antibody conjugated via a protease-cleavable linker to the potent anti-microtubule agent monomethyl auristatin E (MMAE) (linker and toxin licensed from Seattle Genetics). Following binding to GCC, the ADC is internalized and transported to lysosomes where MMAE is released to bind to tubulin, leading to cell cycle arrest and apoptosis. This GCC targeting ADC has been evaluated in clinical studies in patients with advanced gastrointestinal malignancies. The early findings from Phase 1 study have shown preliminary activity signals in gastric, gastroesophageal, and pancreatic cancer. Results from two phase II studies in pancreatic and gastoesophageal adenocarcinoma showed only limited activity.

Antibody–drug-conjugates offer a promising therapeutic modality aimed at providing target-directed cancer chemotherapy. Herein we discuss the GCC target and gastrointestinal malignancies where GCC based targeted therapies could further evolve and offer a significant clinical benefit.

Contents

1. Introduction

Antibody–drug-conjugates (ADCs), or immunoconjugates, are a novel and relatively new class of anti-cancer treatment (Smaglo et al., 2014; Casi & Neri, 2015). This class of drug is comprised of an antibody, a linker, and the therapeutic agent. The antibody is able to recognize cancer antigens with high specificity, while the linker ensures that the therapeutic agent remains attached to the antibody until it reaches the specific tumor target. These drugs are designed to deliver a potent therapeutic entity directly to the tumor sparing the normal tissues.

These agents can be divided into three categories based upon the therapeutic agent conjugated to the antibody; the most frequently explored are ADCs using a cytotoxic agent to be delivered directly to the tumor site. The second class has not been broadly used and consists of radionucleotides (Wong et al., 2004). The third class includes catalytic toxins which have been explored widely with promising results across both hematologic diseases and solid tumors (Kreitman et al., 2005; Hassan et al., 2013). To date, three ADCs have been approved by the

Abbreviations: ADCs, Antibody–drug conjugates; CDX-2, Caudal type homeobox transcription factor-2; DAR, Drug-to-antibody ratio; GC, Guanylyl cyclase; GCC, Guanylyl cyclase C; GEJ, Gastroesophageal junction; GI, Gastrointestinal; MTD, Maximum tolerated dose; MMAE, Monomethyl auristatin E; pH3, phospho-histone H3; RT-PCR, reverse-transcriptase polymerase chain reaction; STa, heat-stable enterotoxin.

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Food and Drug Administration (FDA): gemtuzumab ozogamicin for the treatment of relapsed CD-33 positive acute myeloid leukemia in older patient population, brentuximab vedotin for the treatment of relapsed or refractory Hodgkin's lymphoma and relapsed systemic anaplastic large-cell lymphoma, and T-DM1 for treatment of metastatic HER2-positive breast cancer (Sievers et al., 2001; Younes et al., 2010; Verma et al., 2012).

The successful application of T-DM1 in metastatic HER2-positive breast cancer patient populations has further encouraged the development of ADCs for the treatment of other solid tumors (Verma et al., 2012; Petersdorf et al., 2013). Although gemtuzumab ozogamicin was recently withdrawn from the market when further studies showed no benefit, the significant clinical benefit of by T-DM1 and brentuximab vedotin provides compelling evidence that clinical development of novel immunoconjugates could be greatly beneficial. Currently, several ADCs are being studied including among others, inotuzumab ozogamicin in leukemia and lymphoma, glembatumumab vedotin in breast cancer and melanoma, lorvotuzumab mertansine in CD56 expressing hematological malignancies (NCT02420873).

2. Guanylyl cyclase C (GCC)

GCC is a brush border membrane receptor of guanylin (Currie et al., 2006), uroguanylin (Hamra et al., 1999), lymphoguanylin (Forte et al., 1999), and heat-stable enterotoxin (STa), a peptide of enteric *Escherichia coli*. GCC is expressed by the epithelial cells of the GI tract from the small bowel to the rectum. In the esophagus and stomach, GCC expression was identified only in the intestinal metaplasia of the gastroesophageal junction even without high-grade dysplasia as well as gastric carcinoma. (Schulz et al., 1993). In enterocytes of the intestinal villi, intracellular cGMP elevation caused by STa binding to GCC mediates cGMP elevation and stimulates cGKII, leading to phosphorylation and activation of cystic fibrosis transmembrane conductance regulator (CFTR), which stimulates intestinal fluid secretion and diarrhea (Vaandrager et al., 1998). In animal models a disruption of the guanylyl cyclase-C gene leads to a heat-stable enterotoxin-resistant phenotype (Mann et al., 1997; Schulz et al., 1997). GCC is a member of the guanylyl cyclase (GC) family of proteins which includes at least seven forms, A through G. Endogenous ligands, however, have been identified only for forms A, B and C. GCs are involved in regulation of intracellular cGMP concentrations (Tamura et al., 2001). GCC is involved in the G protein signaling cascade that is activated by low intracellular calcium levels and inhibited by high intracellular calcium levels (Sakurai et al., 2011). On the other hand, GCC is a tumor suppressor and it is regulated by caudal type homeobox transcription factor-2 (CDX-2) and it prevents cancer cells proliferation (Pitari et al., 1997).

GCC has multiple characteristics of an ideal target antigen; High expression level across GI malignancies and an anatomically privileged localization within normal tissue would ensure that only tumor, but not normal tissue, would be reached. GCC also has minimal known antigen shedding that would cause the antibody to bind to its target within the circulation and good internalization of the antibody targeting GCC through receptor-mediated endocytosis.

2.1. Guanylyl cyclase C expression in gastrointestinal malignancies

2.1.1. Gastric and gastroesophageal junction cancer

GCC mRNA overexpression has been found in the peripheral blood of patients with gastric cancer and gastric carcinoma tissue. In addition, the levels of GCC mRNA have been shown to correlate with clinical stage, tumor differentiation degree, depth of invasion, and lymph node metastases (Zhang et al., 2012b). Ectopic expression of GCC also correlates with *Helicobacter pylori* infection in gastric carcinogenesis (Zhang et al., 2012a). In a study performed by Mao et al., the expression of GCC in 30 specimens and 3 human gastric cancer cell lines (including SGC-7901) was evaluated. GCC mRNA was detected in 20 of the 30 specimens and GCC siRNA effectively inhibits the proliferation and invasion of SGC-7901 cells, and induces apoptosis making it a novel biomarker and/or therapeutic target in gastric cancer (Mao et al., 2009).

Although not well characterized, it has been proposed that GCC plays a role in the GE junction tumor progression secondary to exposure to acid and bile acids associated with reflux. In a small study, GCC was expressed in ~80% of adenocarcinoma of the esophagus and stomach samples but not in the normal mucosa. The transformation from normal mucosa to dysplasia and carcinoma was thought to be mediated by NF-KappaB and CDX2 (Debruyne et al., 2006).

2.1.2. Pancreatic cancer

The suppressor role of GCC and its ligand has been well characterized in colon cancer; however, its role in pancreatic cancer is less studied. Guanylin, uroguanylin and GCC, are expressed in normal pancreatic duct cells, but their anti-tumor role is not clear. Using reverse transcriptase-polymerase chain reaction (RT-PCR) analysis, Kulaksiz et al. investigated the expression of GCC in tissues from patients with normal pancreas, chronic pancreatitis and pancreatic tumor cell lines using specific primers. Guanylin, uroguanylin and GCC were expressed in pancreatic cancer and cancer cell lines. GCC expression was significantly higher in pancreatic cancer tissue compared to normal pancreatic tissues ($p < 0.00001$) and chronic pancreatitis ($p < 0.05$), while Guanylin and uroguanylin were not expressed in pancreatic cancer. In cell lines, uroguanylin inhibits pancreatic cancer cell line proliferation in a dose-dependent fashion. Panc1 and Capan1 cell lines were significantly inhibited by lower concentration (2 nM, $p < 0.05$). The study concluded that uroguanylin can play a role in the treatment of pancreatic cancer via GCC-dependent mechanisms. In addition, determination of GCC expression might be a useful marker for differentiation between pancreatic cancer and chronic pancreatitis. In another study of tumor samples derived from 218 patients with primary and metastatic pancreatic carcinoma GCC was expressed in 137 cases (Zhang et al., 2013).

2.1.3. Colon cancer

GCC is expressed in colon cancers in the primary tumor and metastatic lesions regardless of tumor location, stage and grade (Parkinson et al., 1997). GCC binds to paracrine hormones guanylin and uroguanylin, which are lost in the course of early colon carcinogenesis (Pitari et al., 1997). More recently GCC has been shown to play a role in intestinal tumor suppression and in maintaining genomic integrity as well as restricting proliferation (Pitari et al., 1997).

Since GCC is uniquely expressed in the gastrointestinal tract, detection of GCC RNA in negative lymph nodes removed in the course of colon tumor resection was thought to be indicative of sub-clinical or molecular LN metastasis. Following that initial observation several studies examined the association between GCC positive occult lymph node metastases and recurrence or survival in patients with colon and rectal cancer (Waldman et al., 2009) as assessed by using the reverse transcriptase–polymerase chain reaction.

In a prospective study of 257 patients from North America with N0 colorectal cancer it has been found that patients with lymph nodes negative for GCC had a recurrence rate of 6.3% (2 out of thirty-two patients). In contrast, patients who had lymph nodes positive for GCC had a recurrence rate of 20.9%. The difference between the two groups was statistically significant ($P = .006$). Multivariate analyses revealed that GCC positivity in lymph nodes was an independent marker of prognosis. Patients who had expression of GCC in the lymph nodes had shorter recurrence free survival ($P = .03$) (Waldman et al., 2009). GCC has been also detected in intestinal metaplasia and dysplasia in the gastrointestinal tract (Birbe et al., 2005) which suggests that the expression of GCC is maintained in the course of tumor progression process.