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CLINICAL

Update on gastric cancer treatments and gene therapies

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Abstract
Gastric cancer is an active topic of clinical and basic research due to high morbidity and mortality. To date, gastrectomy and chemotherapy are the only therapeutic options for gastric cancer patients, but drug resistance, either acquired or primary, is the main cause for treatment failure. Differences in development and response to cancer treatments have been observed among ethnically diverse GC patient populations. In spite of major incidence, GC Asian patients have a significantly better prognosis and response to treatments than Caucasian ones due to genetic discordances between the two populations. Gene therapy could be an alternative strategy to overcome such issues and especially CRISPR/Cas9 represents one of the most intriguing gene-editing system. Thus, in this review article, we want to provide an update on the currently used therapies for the treatment of advanced GC.

Keywords Gastric cancer · Chemotherapy · Metastases · Gene therapy · CRISPR

1 Introduction

Gastric cancer (GC) is actually the fifth most frequently diagnosed cancer and the third leading cause of cancer death according to GLOBOCAN 2018, demonstrating to be a considerable welfare problem especially in Western Asian countries [1]. Being, the main risk factors the Helicobacter pylori infection [2], an unbalanced diet [3], alcohol consumption, and tobacco smoking [4], we might be commonly inclined to identify GC as an environmental-induced disease, underestimating the role of the genetic components. The majority of GC are classified as adenocarcinomas (80–90%) [5]. While the WHO classified GCs into several subgroups including papillary, tubular, mucinous, signet-ring cell, and mixed carcinoma, the most common Laurén classification is composed by only intestinal, diffuse, and indeterminate type [6]. Thanks to the advances in the diagnostic tools and the deeper knowledge of the genetic profile of cancer, we may currently approach cancers, and in particular GCs, from a molecular point of view. Many different signaling pathways, such as Wnt [7] or miR-126 [8], just to report two of them, have been demonstrated to be aberrant regulated in GCs. Although surgical resection is currently the best method to treat gastric cancer, chemotherapy (CT) is still used as elective therapy, especially when surgery is not possible or in presence of metastatic lesions. However, the most common phenomenon responsible for the treatment failure is the acquisition of resistance to chemotherapy agents. The emerging genome editing technologies, ever more precise and quicker to perform, enable the design of new targeted cancer therapies, being CRISPR the spearhead among them. CRISPR allows for effective gene knockout and gene modifications aimed to repair defective oncogenic pathways, albeit with low *in vivo* efficiency. Better results were obtained in cancer immunotherapy where

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cancer-specific T cells can be engineered in order to be potentiated as anticancer weapons [9]. In this context, GC does not make an exception [10]. Indeed, somatic cells have to overcame a number of obstacles and acquire additional abilities to become tumor cells, among these, the ability to avoid immunogenicity that occasionally arise as a result of DNA rearrangement. Many immunotherapy methods can be used to potentiate immunosurveillance or avoid the immunoescape of GC. The strategies comprise adoptive therapy *via* Chimeric Antigen Receptor T Cells (CAR-T) using engineered lymphocytes from patient expressing chimeric receptors toward tumor antigens, immune checkpoint inhibitors aimed to interfere with negative regulatory pathways of the immune response, and cancer vaccines aimed at inducing APCs, like dendritic cells, to present tumor antigens. Also, some approaches of immunotherapy could be considered an indirect gene therapy that benefit from DNA editing technologies and high throughput transcriptome analysis albeit its efficacy in solid tumors is waiting to be clearly demonstrated [11]. In particular, CRISPR/Cas9 gene editing could be applied in T cell-based immunotherapy in order to generate CAR-T lymphocytes [12]. In GC research and therapy, many, if not all the immunotherapy-based approaches are being under scrutiny, from adoptive immunotherapy using CAR-T, to cancer vaccines (for a comprehensive review about immunotherapy in GC, see [13]). Based on the above considerations, we can envision a future where highly efficient gene-editing approaches will be pivotal in gastric cancer therapy. Another promising field is the regaining of tumor suppressor function, silenced by epigenetic modifications. Artificial transcription factors engineered with transcriptional repressor or activator molecules have been developed and demonstrated to effectively activate and repress gene expression in mammalian cells [14]. Tumor suppressor gene classes such as MASPIN and REPRIMO have been reactivated in GC cell lines using these tools in combination with CRISPR, furnishing a proof of concept to exploit such technologies [15]. Studies on GC at a molecular level provide ever increasing opportunities for therapeutic intervention on GC by gene therapy. An example comes from the long noncoding RNAs (lncRNAs): PANDAR, a lncRNA responsible for blocking the expression of a cyclin-dependent kinase inhibitor (CDKN1A) by competitively binding to p53, was knocked out by CRISPR/Cas9 technology determining GC growth inhibition *in vivo* [16]. In this review article, we report an overview on the state of art of GC therapies.

2 Chemotherapy treatments for advanced gastric Cancer

In Western countries, about 50% of patients present with locally advanced or metastatic gastric cancer (AGC) at diagnosis, while about 40–60% of those radically resected relapses after surgery. In patients with AGC, palliative systemic treatment is the gold standard, with a median survival uncommonly exceeding 12 months. CT demonstrated to prolong survival and improve quality of life compared with best supportive care. However, median overall survival (OS) does not exceed 9–11 months with a 5-year OS of about 5–10% [17]. In patients with human epidermal growth factor receptor 2 (HER2) positive AGC, the addition of the anti-HER2 monoclonal antibody trastuzumab to CT, as demonstrated by the ToGA trial, achieved a survival benefit over CT alone (mOS 13.8 *vs.* 11.1 months, HR 0.74; 95% CI 0.60–0.91; $p = 0.0046$), but the treatment is possible only in a limited subset of patients (15–20%) [18]. After the positive experience of the ToGA trial, first-line treatment with other targeted therapies failed to improve the outcome of AGC, while in second line, anti-VEGFR agent ramucirumab obtained a gain in terms of OS when used in monotherapy with respect to the best supportive care and in addition to CT *versus* CT alone. For first-line approach, international guidelines recommend regimens containing a platinum agent (cisplatin or oxaliplatin) and fluoropyrimidines (5-fluorouracil or capecitabine) in patients with HER2 negative disease. In case of HER2 positivity, trastuzumab is added to CT with cisplatin and fluoropyrimidines. In Eastern countries, S-1 (tegafur-gimestat-otastat potassium), an oral fluoropyrimidine, is used as mono CT or in combination, based on favorable results in trials involving Asiatic patients [19–21]. The addition of taxanes to first-approach CT, as in DCF (docetaxel, cisplatin and 5-fluorouracil) or FLOT (5-fluorouracil, oxaliplatin, and docetaxel) schedules resulted in higher response rates (RR) without impact on OS, but with relevant rate of toxicities: so triplets are reserved to young selected patients with good performance status to whom the goal of treatment is the downstaging (i.e., the neoadjuvant approach in limited disease, bulky and symptomatic disease, oligometastatic AGC) [22, 23]. Globally, after progression of disease to first-line CT, only about 40% of patients with AGC in Europe are able to receive second-line therapy [24]. Until the results with ramucirumab were presented, the standard of care for second line was mono CT, as confirmed by a meta-analysis of 10 randomized trials that demonstrated that doublet CT does not improve OS *versus* monotherapy, at the price of more toxicities [25]. In the REGARD trial, Ramucirumab *versus* placebo obtained an improvement in both OS (5.2 *vs.* 3.8 months, HR = 0.776, $p = 0.047$) and progression-free survival PFS (2.1 *vs.* 1.3 months, HR = 0.48, $p < 0.0001$). The efficacy of ramucirumab alone was comparable to the results of other phase III trials of second-line CT, with favorable toxicity profile. In the RAINBOW trial, the addition of ramucirumab to weekly paclitaxel significantly prolonged m OS (9.6 *vs.* 7.4 months, HR = 0.807, $p = 0.017$) and PFS (4.4 *vs.* 2.9 months, HR = 0.635, $p < 0.0001$) when compared with

paclitaxel monotherapy [26, 27]. When not contraindicated, ramucirumab is recommended by major International guidelines as second-line treatment as mono-CT or in combination with paclitaxel. Third-line treatment is not currently sustained by phase III evidences, so is reserved to selected patients. Trials with immunotherapy are ongoing in this setting of disease. It is also important to underline that nutritional support is a fundamental part of active treatment for patients with AGC, because an impaired nutritional status is associated with increased morbidity and mortality during anticancer treatment with major toxicities, dose reductions, delays, or treatment discontinuation. With a good support, we are able to increase the proportion of patients receiving second- and third-line treatments. We report in Fig. 1 the diagram of the therapeutic algorithm for AGC in Western countries.

3 Molecular insight for metastatic lesions

Metastatic GC is usually associated with incurable conditions. When anticancer therapies for primitive cancer other than surgery fail, interest in molecular mechanism related to metastasis development falls. This is the case of GC advanced disease, where CT can only slightly affect the patient survival. Indeed, until recently, GC databases reported incomplete information about GC spreading, restricting the field to Y or N, regardless of primary and secondary metastasization sites. An extensive study about GC spreading performed on Swedish registers reports that the most common distant metastasization sites are the liver (48%), lung (15%), and bones (12%), while proximal peritoneal dissemination represents 32% [28]. Anyway, distribution of GC metastases sites can vary in different stratification GC patients' studies, probably depending on the cohort considered and heterogeneity in data acquisition. For example, peritoneal localization of GC cells by direct invasion from the gastric wall is elsewhere reported to be the most recurring, in comparison to distant metastatic settlements [29, 30]. Recently, whole-genome sequencing techniques that allowed to identify molecular subtypes in primitive gastric cancer cohorts have been applied to study the molecular signature of GC metastasis from different sites [31]. Recent evidences showed that CCNE1 amplification was found associated with liver metastasis in GC of the TP53 subtype [32] while mutations activating or inactivating specific genes were identified in peritoneal metastasis [33]. Circulating tumor DNA (ctDNA) detection is another innovative approach to analyze GC disease. Fang et al. showed that high ctDNA levels were associated with peritoneal recurrence and advanced GC [34]. Lastly, ctDNA harboring the same mutations of primitive GC were associated with late-stage GC. ctDNA can also furnish a tool to track data about the response to therapy in advanced GC, as in the case of amplification of the HER2 gene in trastuzumab-resistant metastatic HER2-positive GC [35].

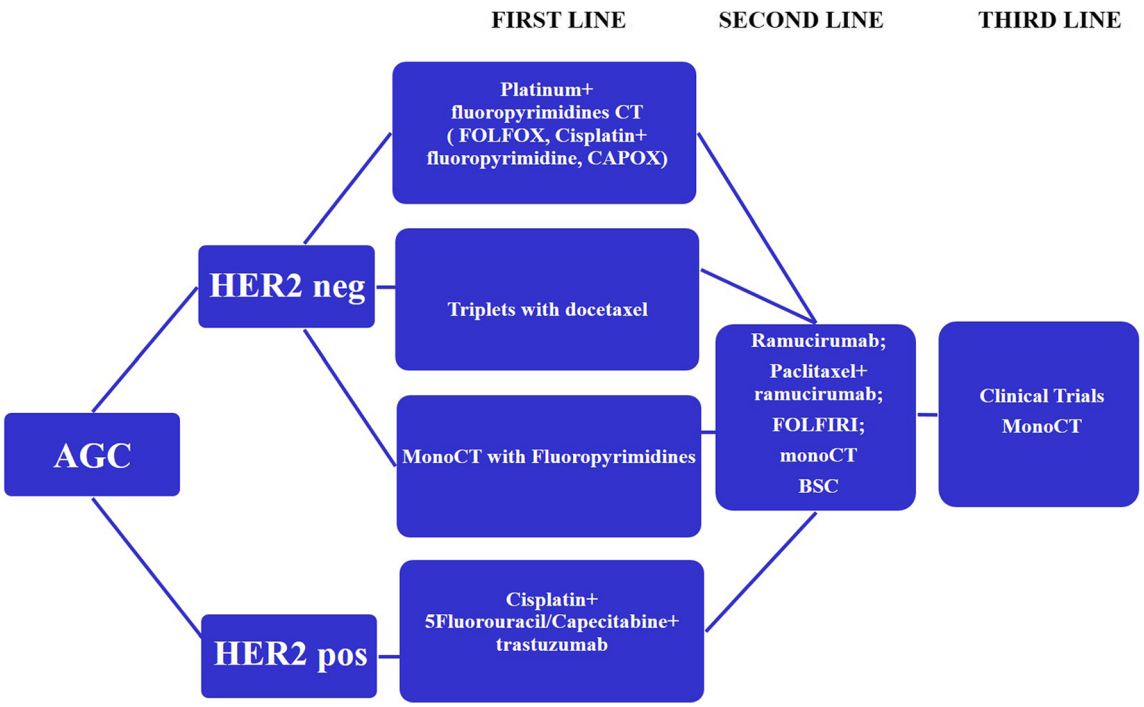

Fig. 1 A diagram reporting the current therapeutic algorithm for advanced gastric cancer in Western countries. *AGC* advanced gastric cancer, *FOLFOX* fluorouracil + oxaliplatin, *Fluoropyrimidines* capecitabine or 5-fluorouracil, *mono CT* irinotecan or paclitaxel, *FOLFIRI* 5-fluorouracil + irinotecan

4 Genetic discordances between Asian and Caucasian GC patients

Differences in development and response to treatments have been observed among ethnically diverse populations. In spite of major incidence, GC Asian patients have a better prognosis and response to treatments than Caucasian ones [36] with a 5-year survival rate of 90% in Japan and 10–30% in Europe [37, 38]. Cristescu et al. [31] classified GC from ethnically different cohorts into four distinct subtypes, on the basis of a pre-defined set of known cancer-related gene expression signatures, suggesting that oncogenic pathways are broadly conserved between populations. On the other hand, studies minimizing differences (clinical management, environmental habits) using SEER data of GC patients treated in the USA showed that the survival advantage of Asians over non-Hispanic whites would continue to hold after control for other well-known prognostic factors [39]. Differing tumor biology has been proposed as a potential explanation for the observed racial disparities in survival [40]. Most of the studies on GC have been conducted on Asian patients; however, even if results are promising, it remains questionable whether they can be fully extrapolated to Caucasian populations, as assessed, *i.e.*, by difference in the somatic mutation rates of several driver genes. Considering the increasing level of understanding of the molecular basis of tumor biology, several biomarkers have been identified for many types of tumors [41]. The frequencies of somatic mutations found between Asian and Caucasian populations were: APC (Asian: Caucasian 6.06 *vs.* 14.40%, $p = 0.0076$), ARIDIA (20.7 *vs.* 32.1%, $p = 0.01$), KMT2A (4.04 *vs.* 12.35%, $p = 0.003$), PIK3CA (9.6 *vs.* 18.52%, $p = 0.01$), and PTEN (2.52 *vs.* 9.05%, $p = 0.008$) [39]. Unfortunately, there are few defined biomarkers of prognostic and diagnostic use for gastric tumors [42]. Moreover, inter-individual variability of drug response together with tumor heterogeneity are particularly hard to overcome when treating GC. The ability to predict whether patients will respond to specific therapies would be of particular value and would allow for stratifying patients for personalized treatment strategies. Currently, only the status of HER2 for therapy with trastuzumab is approved [43]. The standard treatment for advanced GC is CT and the most widely accepted first-line regimen is a combination of fluoropyrimidine plus platinum with or without trastuzumab [44]. To better design new personalized therapies, especially gene targeting ones, it is indispensable to know in depth the genetic discordances between Caucasian and Asian populations.

5 Genome editing mechanisms and ways of action

In order to edit the human genome, to correct or knock out a specific gene expression, a discontinuity should be generated in the DNA strands. Historically, the first studies and trials were conducted by the use of zinc finger nucleases (ZFNs) [45], meganucleases [46], or transcription activator-like effectors nucleases (TALENs) [47] which were able to generate double strand breaks (DSBs) in specific DNA target genes. Once the nick is created, cells start to repair such gap commonly exploiting the Non-Homologous End Join (NHEJ) repair system, inducing indel mutations and introducing a frameshift effect within the coding region of a gene [48]. As a result, the generation of a premature stop codon will interrupt the expression of the target gene. In case a single-stranded oligonucleotide is provided, acting as a donor template, the cell will repair the created nick by Homologous Directed Repair (HDR) allowing the insertion of new genetic material in the host DNA [49]. However, such nucleases needed to be specifically designed and engineered to recognize a single motif on the DNA double helix. The new approach to the gene editing with CRISPR has recently revolutionized the use of the nucleases. Such system is composed by two RNA elements called CRISPR RNAs (crRNAs), which is designed to be complementary to a specific target, and transactivating CRISPR RNA (tracrRNA) [50]. The hybrid of crRNA and tracrRNA binds a nuclease and helicase protein called CRISPR associated protein 9 (Cas9) commonly derived by *Streptococcus pyogenes*. The crRNA element guides the Cas9 to a specific target site exploiting the standard RNA-DNA complementarity Watson-Crick base-pairing rules, if adjacent to short sequences known as protospacer adjacent motifs (PAMs). Indeed, the single guide RNA (sgRNA) having to precede an NGG site, unlike the other nucleases described, it is not suitable to target any site in the genome. However, generally Cas9 is easier and faster to use than any other nucleases. Cas9 is a bi-lobed protein with the sgRNA nestled between the alpha-helical lobe and the nuclease lobe. There are two main domains located in the nuclease lobe: the RuvC, characterized by an RNase H fold structure [51], which cleaves the non-target DNA strand, and the HNH nuclease domain, which cleaves the target strand of DNA [52]. The nuclease also consists of a recognition lobe (REC) that matches the target sequence in the host DNA. A wild-type Cas9 performs a double strand break in the DNA site, while introducing D10A or H840A mutation into the RuvC- or HNH-like domains will make it incapable to perform a DSB, generating only a single cut per strand instead. These mutants also known as Nickase are extremely useful to improve the specificity of the cut, reducing off-target effects, albeit with a reduced efficiency, using two sgRNA instead of only one [53]. The easiest approach to CRISPR-mediated cancer treatment is to completely knockout a target gene exploiting the NHEJ repair system. In such a way, the nuclease should be directed toward genes involved in chemoresistance, proliferation, migration, invasion, and apoptosis resistance in order to inhibit metastasis and tumor growth.

6 CRISPR advancements in GCs

CRISPR is currently revolutionizing the world of gene therapy and even for GC there are some interesting applications of such technology. Yi-Qiang Zhang et al. recently focused on the role of the Prostate-derived Ets factor (PDEF) which is a member of the Ets family of transcription factors, playing an important role in tumorigenesis but with unclear function in GC [54]. They used CRISPR to stably knockout PDEF in the AGS cell line and such depletion inhibited cell proliferation, migration, and invasiveness, demonstrating that the PDEF gene may be involved in the initiation and development of GC and thus might be considered a possible therapeutic target. Benjamin Garcia-Bloj et al. while still using the CRISPR system, exploited a different approach waking up dormant tumor suppressor genes thanks to a mutant version of the Cas9 [15]. Indeed, mutation of two amino acids into the catalytic sites (RUVc and HNH) has generated an inactive form, or so called "deactivated" Cas9 (dCas9), which can be linked to different effector domains, such as p300 [55], an H3K9 acetylase core catalytic domain and VPR [56] for gene activation [57]. Exploiting such molecular mechanism, they reactivated two dormant class II tumor suppressor genes MASPIN and REPRIMO. Indeed, the last one, a novel tp53 dependent G2 arrest mediator candidate, has been proposed to have tumor suppressive functions in gastric [58, 59]. As reported above, CRISPR could also be used to potentiate the effect of primary T cells, disrupting PD-1 expression, upregulating cytokine production, and enhancing cytotoxicity [10]. Such an approach could be promising for future therapies, using immunotherapies of CRISPR-activated cytotoxic T lymphocytes in combination with standard radiotherapy [60].

7 Hereditary diffuse gastric cancer

Even if the majority of GCs are sporadic, 1–3% of GCs arise as a result of inherited cancer syndromes. It has now been widely established that germline mutations in the CDH1 gene, encoding the cell-cell adhesion protein E-cadherin, is one of the main genetic causes of the so called hereditary diffuse GC (HDGC) [61]. Heterozygous germline CDH1 mutations increase lifetime risk of developing diffuse GC [62]. HDGC is an autosomal dominant susceptibility illness causing a poorly differentiated adenocarcinoma that infiltrates into the stomach wall causing thickening of the wall (linitis plastica) without forming a distinct mass. In this particular condition, the use of a CRISPR/Cas9-based gene therapy might be an advantageous approach, designing an sgRNA specific for the CDH1 mutation and delivering the machinery *via* lentivirus or adenovirus [63]. However, such kind of therapies are often shown to produce immunogenic effects, inhibiting or nullifying the efficacy of the treatment [64, 65].

8 Clinical trials and biomarkers

Albeit CRISPR is rapidly revolutionizing the world of gene therapy, cancer, being a multigenic disease with genetically different cell subpopulations, is hard to be treated with such a system to date. However, gene-editing technologies could be applied to basic research to generate an accurate genetic profile of GC, in order to improve the available personalized medicine treatments, striking cancer-specific biomarkers. It is also important to remark that currently, most of GC literature is based on Asian patients and, as reported above, there are many genetic discordances reported between Asian and Caucasian populations [40]. The lack of specific markers affects not only gene therapies but also chemotherapies. Currently, the most common biomarker reported is HER2, which is overexpressed in some subset of breast, ovarian, gastric, colorectal, pancreatic, and endometrial cancers, and can be targeted by trastuzumab [66]. Many randomized controlled clinical trials with several CT mix have been performed in patients with GC but with little significant improvement [67–70]. In the era of biomarker-directed therapy, evaluating expression of gene signatures or multiple genes associated with patient clinical response and outcome in randomized clinical trials is an important challenge [71, 72]. Genomic sequencing of gastric tumors suggests that exploring molecular aberrations in selected patients may offer new avenues for targeted therapeutic opportunities [18, 73]. New genomic technologies such as the NanoString nCounter system have been used in several clinical trial studies for prognostic and predictive markers [74]. This could improve our understanding of the biological processes and also identify the predictors of CT prognosis and response and improve overall treatment outcomes. One of the most important tumor suppressor genes is the TP53 gene, which encodes p53, and plays a critical role in cell cycle regulation and apoptosis in response to cellular stress [75]. High frequency of TP53 mutations and overexpression of the p53 protein is an important biomarker for predicting prognosis and response to CT in GCs [76]. Some studies have shown that high levels of p53 expression are correlated with multidrug resistance in gastric cancer cell lines [77]. Yong Zha et all have studied a specific codon and have observed that a polymorphism can be used to identify a subgroup of patients most likely to benefit from the regimens of paclitaxel plus capecitabine [78]. Kakoli Das et al. found a strong association between the higher expression level of the Wnt5A gene and the unresectable gastric tumors [79]. Additionally, low Wnt5A gene expression was a predictor of good response and better progression-free survival in S-1-treated GC patients. Another important theme was studied by Denil L. Fontes et al. which have focused on MET gene amplification that is one of the well-recognized mechanisms of c-MET overexpression and constitutive activation of the MET/HGF pathway and has been reported in 2 to 10% of gastroesophageal (GE) adenocarcinomas [80]. MET-amplified tumors display a higher

Table 1 A short list of the most commonly mutated genes in GC by analyzing the data from The Cancer Genome Atlas Program (www.cancer.gov)

Symbol	Name	Number affected cases in Cohort	Number of mutations	Description
TTN	Titin	240/404 (59.41%)	783	Central role in contraction of striated muscle. Responsible for muscle passive elasticity. Also known as Connectin
TP53	Tumor protein p53	201/404 (49.75%)	150	Tumor suppressor. Binds to DNA and regulates gene expression to prevent genome mutations
MUC16	Mucin 16, cell surface associated	161/404 (39.85%)	290	Member of mucin family, plays an important role in forming a barrier protecting epithelial cells from pathogens
SYNE1	Spectrin repeat containing nuclear envelop1	133/404 (32.92%)	227	Nuclear envelope protein found in human myocytes and synapses that is involved in maintenance of nuclear organization and structural integrity
CSMD3	CUB and sushi multiple domain3	124/404 (30.69%)	169	Integral component of cells membrane. Biochemical properties and functions are unknown
LRPB1	Low-density lipoprotein receptor-related protein 1B	123/404 (30.45%)	182	Belongs to the LDL receptor family. Potential cell surface proteins that bind and internalize ligands in receptor-mediated endocytosis
FLG	Filaggrin	112/404 (27.72%)	166	Intermediate filament-associated protein that aggregates keratin intermediate filaments in mammalian epidermis
ARID1A	AT-rich interactive domain 1A (SWI-like)	111/404 (27.48%)	121	Nuclear protein that regulate genes transcription by chromatin structure alteration. Has been reported to act as tumor suppressor
FAT4	FAT atypical cadherin 4	111/404 (27.48%)	180	It is a member of the protocadherin family and plays a fundamental role in regulating planar cell polarity
OBSCN	Calmodulin and titin-interacting RhoGEF	100/404 (24.75%)	184	It belongs to the family of giant sarcomeric signaling proteins that includes titin and nebulin and may have a role in the organization of myofibrils
HMCN1	Hemicentin 1	93/404 (23.02%)	138	Plays a role in maintaining the architectural integrity of vertebrate tissues and organs. Serves a role in mitotic cytokinesis cleaving furrow maturation
ZFHX4	Zing finger homeobox4	92/404 (22.7%)	118	May play a role in neural and muscles differentiation. May be involved in transcriptional regulation
FAT3	FAT atipycal cadherin 3	92/404 (22,77%)	124	May play a role in the interaction between neurites derived from specific subset of neurons during development
CSMD1	CUB and sushi multiple domains 1	90/404 (22.28%)	132	Has been proposed to have an active role in cell cycle regulation and controlling apoptosis. It may act as a potential tumor suppressor and reduce cell transformation and invasion
PCLO	Piccolo presynaptic cytomatrix protein	88/404 (21.78%)	135	It is part of the presynaptic cytoskeletal matrix and is involved in establishing active synaptic zones and in synaptic vesicle trafficking
DNAH5	Dynein, axonemal, heavy chain 5	87/404 (21.53%)	115	It is part of a microtubule-associated motor protein complex. It functions as a force-generating protein with ATPase activity
RYR2	Ryanodine receptor 2 (cardiac)	85/404 (21.04%)	120	It is the major mediator for sarcoplasmic release of stored calcium ions in the process of cardiac calcium-induced release. Mutations lead to heart failure
SPTA1	Spectrin, alpha, erytrocytic 1	84/404 (20.79%)	116	Links the plasma membrane to the actin cytoskeleton. It has a role in the determination of cell shape and organization of organelles. Mutations lead to hereditary red blood cell disorders
RIMS2	Regulating synaptic membrane exocytosis 2	82/404 (20.30%)	86	It is involved in synaptic membrane exocytosis and in neurotransmitter release
DST	Dystonin	81/404 (20.05%)	125	It is a cytoskeletal linker protein belonging to the plakin family of adhesion junction plaque proteins

We decided to analyze a large cohort including stomach and small intestine adenomas and adenocarcinomas comprising cases of both gender patients and without any filter based on race or ethnicity

pathologic grade and present at a more advanced stage [81]. In the phase I Clinical Trials Program who had MET amplification/mutation testing, a trend for a worse overall survival (OS) for MET positive GE cancers was detected [82]. Unfortunately, this difference was not statistically significant because MET abnormalities can be found in a small group of patients with GE adenocarcinoma and further studies are necessary to better characterize the prognostic and predictive impact of MET alterations [83]. Michael Stahl et al. investigated whether patients with gastric cancer may particularly benefit from EGFR-targeted therapy, since overexpression and high gene copy number of the epidermal growth factor receptor (EGFR) have been observed in a therapeutically relevant number of gastric carcinomas and therefore, it appeared to be a potentially valuable target in the context of perioperative therapy [84, 85]. They found that activation of cancer-driving pathways such as EGFR [86], fibroblast growth factors receptors (FGFR) [87], MET [88], and c-Kit [89] leads to shorter survival, but any significant difference in progression-free survival between the treatment arms was not found. Fibroblast growth factors (FGFs) and their receptors FGFRs are instrumental in a number of normal biologic processes and their dysregulation by mechanisms including activating gene mutations, gene amplification, and gene fusions is believed to drive human cancers, including GCs [90–92]. Van Cutsem et al. have hypothesized that AZD4547 has the potential to provide clinical benefit in patients with advanced gastric adenocarcinoma with tumors displaying FGFR2 polysomy or gene amplification since AZD4547 is a selective FGFR-1, 2, 3 tyrosine kinase inhibitor that has displayed potent activity in preclinical studies [93]. However, there was no clear correlation between the extent of sub-clonal heterogeneity and tumor shrinkage in response to AZD4547 and treatment with AZD4547 did not improve progression-free survival compared with paclitaxel in the overall population or in patients with FGFR2 amplification or polysomy according to FISH selection. Moreover, Y. Yamada et al. have studied the excision repair cross-complementation group 1 (ERCC1), which is an important component of the nuclear excision repair pathway which repairs DNA intrastrand, inter-strand, and DNA-protein crosslinks caused by cisplatin [94]. High mRNA levels of ERCC1 in primary gastric cancer may be associated with a lower response to cisplatin and poor survival. They observed that low ERCC1 expression was a significant independent favorable prognostic factor in patients with advanced gastric cancer who were receiving first-line CT regardless of the treatment regimen [95]. Furthermore, Min Deng et al. studied the role of miR-26a, suggesting that it might play a critical role in GC suppressing growth and metastasis by regulating FGF9 [96]. Ji Mei et al. focused on gene polymorphisms of GSTP1 G/G, XRCC1 A/A, and 5,10-MTHFR T/T and observed a clinical value for predicting the response to the doxorubicin, cisplatin, and 5-fluorouracil treatment for advanced gastric cancer [97]. Furthermore, an important role is played by the epigenetic silencing of tumor suppressor genes (TSGs) that is commonly observed in GE cancer and is believed to be involved in oncogenesis, metastasis, and CT resistance [98–103]. Bryan J. Schneider et al., in a phase I study, observed that pre-treatment with a DHA can hypomethylate tumor DNA and induce expression of TSGs in cancer cells, thereby sensitizing them to cytotoxic CT [104]. Many genes have been identified as potential biomarkers for personalized cancer-targeted therapy but these studies are frequently limited by a small number of patient samples and usually focused on Asiatic patients. In Table 1 is reported a list of commonly mutated genes in GC. All these findings are encouraging but further evaluation is needed.

9 Conclusions

To date, gastrectomy and chemotherapy are still the best therapeutic approaches for GC patients even though the research for alternative strategies is more and more fueled by new intriguing molecular technologies. Indeed, being drug resistance the main cause for treatment failure, the deeper knowledge of new molecular targets and mutations is of vital value to better understand the genetic differences between ethnicity and thus to boost the personalized medicine treatments [105]. However, many issues need to be faced in order to make a step forward. First is the choice of the gene-editing technology. Indeed, very few clinical trials were successful for GC gene therapy and currently no therapies are available. Among the above cited methodologies, CRISPR represents the most affordable and easy to be used in such a kind of treatment due to the simplicity to design the sgRNA and the possibility to target any genes even the ones in heterochromatic loci. The other side of the coin, it is represented by the possibility to produce off-target effects and the need to use a safe way to deliver the molecular machinery into the tumor site avoiding healthy tissues. In such a way, of key importance is the choice of a surface antigen target to selectively deliver the CRISPR complex. One of the most probable targets might be the CarcinoEmbryonic Antigen (CEA) which is commonly expressed in many kinds of GCs [106] as well as the oncofetal antigen 5T4 [107] which has been linked with disease progression and poor clinical outcome. As a delivery system, the best ones available are Adenoviruses (AVs), Adeno-Associated Viruses (AAVs), and RiboNucleoProteins (RNPs) [108]. Indeed, while a virus-based approach is useful to maximize the infection efficacy, and thus the KO efficiency, it may generate a significant immune response neutralizing the biological effect. The RNPs are commonly well tolerated but they have a lower transfection efficiency so they need to be optimized for the use. Moreover, given the fact that CRISPR application *in vivo*, and especially in humans, has a very low working efficiency, more work needs to be done to make such approach

practicable. The most common gene-editing strategies to treat GC are "tumor-suppressor actions," by which an irreversible gene KO leads to cell growth inhibition or to lose metastatic ability, "suicide gene therapy," *i.e.*, the transduction of a gene processing a non-toxic "pro-drug" into a toxic one and the "immunomodulation" by which we can potentiate the host immune response against cancer cells. Being p53 one of the master regulator of the genome integrity and resulting mutated in about 60% of gastric cancer, its replacement with a wild-type version is fascinating and in 1999, Ohashi M et al. reported that such reintroduction, through AVs, led to a significant growth inhibition showing to be of clinical interest [109], while the two most common suicide gene therapies are based on the conversion of 5-fluorocytosine to 5-fluorouracil (5-FU), through expression of cytosine deaminase and the phosphorylation of ganciclovir, throughout the herpes simplex virus thymidine kinase, both used in combination with a CEA promoter to make their expression cancer-specific [110, 111]. It is our opinion that currently, molecular research needs to take several steps forward to use gene therapy constantly in clinical practice, especially by uncovering specific surface molecular targets to make such therapies more selective and avoiding any off-target effects. Lastly, we want to report an extremely interesting tool for the discovery of new biomarkers: the Genome-Wide Pooled CRISPR libraries. Such libraries, included in lentiviral plasmids, are used to knockout thousands of genes at the same time and randomly, thus once the cells are exposed to a selective pressure, *i.e.*, anoikis resistance, chemotherapies, hypoxia, *etc.*, we can identify which genes are fundamental for a certain phenotype [112].

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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