Molecular Biology of Gastroesophageal Cancers: Opportunities and Challenges

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Abstract: Gastroesophageal (GE) malignancies make up a significant and growing segment of newly diagnosed cancers. Approximately 80% of patients who have GE cancers die within 5 years of diagnosis, which means that effective treatments for these malignancies need to be found. Currently, targeted therapies have a minimal role in this disease group. Intensive study of the molecular biology of GE cancers is a relatively new and ongoing venture, but it has already led to a significant increase in our understanding of these malignancies. This understanding, although still limited, has the potential to enhance our ability to develop targeted therapies in conjunction with the ability to identify actionable gene mutations and perform genomic profiling to predict drug resistance. Several cell surface growth factor receptors have been found to play a prominent role in GE cancer cell signaling. This discovery has led to the approval of 2 agents within the last few years: trastuzumab, an anti–human epidermal growth factor receptor 2 (HER2) monoclonal antibody used in the first-line treatment of HER2-positive GE cancers, and ramucirumab, an anti–vascular endothelial growth factor receptor 2 (VEGFR2) monoclonal antibody that is currently used in later lines of therapy. This review discusses the current state of molecular testing in GE cancers, along with the known molecular biology and current and investigational treatments. The development of trastuzumab and ramucirumab represents a significant advance in our ability to make use of GE tumor molecular profiles. As our understanding of the impact of molecular aberrations on drug effectiveness and disease outcomes increases, we anticipate improved therapy for patients with GE cancers.

Introduction

Gastroesophageal (GE) malignancies account for a significant and growing segment of newly diagnosed cancers and cancer-related deaths worldwide. An estimated 455,800 new cases of esophageal and GE junction cancers were diagnosed globally in 2012, and the diseases caused 400,200 deaths in the same year.[1] In addition, an interesting phenomenon with regard to histologic subtype has arisen in recent decades. The United States has seen a marked decrease in the incidence of squamous cell carcinoma, whereas a parallel

increase has occurred in non-Western nations.[2] Regardless of subtype, patient survival remains poor. Although the 5-year survival rate has increased since the 1970s, when it was only 5%, it currently hovers at approximately 20% worldwide.[1]

In recent years, scientists have undertaken intensive study of the molecular biology of GE cancers, and this has led to a significant improvement in our understanding of their pathogenesis. As with many other tumor types, an effort has been made to discover actionable genetic mutations and to develop targeted therapies so that patients with these cancers can be treated selectively. In this review, we discuss the current state of testing in GE cancers, along with their molecular biology and the available treatment options.

Advanced Testing

The ability to target specific cancer cell mutations has grown, and a variety of tools have been developed to test specific cancers for relevant molecular markers. Immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) play predominant roles in the molecular testing of tumors. The use of these techniques for the identification of various tumor characteristics, such as hormone sensitivity and pathologic overexpression of cellular receptors, is well established.

Next-generation sequencing (NGS) has been gaining relevance in standard clinical practice. NGS allows the capture of a large amount of cancer genomic information, which can be used to detect known actionable mutations. As this technology has become more widely available, efforts have been made to determine its feasibility and reliability in comparison with IHC/FISH. A recent study evaluating the upfront use of NGS demonstrated that 89% of patients who underwent NGS testing had at least 1 actionable mutation, including mutations of the human epidermal growth factor receptor 2 (*HER2*) gene. Previous studies in patients with breast cancer have shown a concordance rate between NGS and IHC/FISH for *HER2* amplification of greater than 95%. However, this more recent study demonstrated an 84% concordance rate.[3] Although current data demonstrate uncertainty regarding concordance and although other concerns exist, including cost and turnaround time, the potential benefit of identifying individual molecular characteristics and resistance patterns is a source of continued motivation to improve NGS methodology and incorporate it into standard care.

Demonstrating efficacy in live models is an important step before testing is undertaken in human trials. The development of tumor xenografts has resulted in a complete paradigm shift in the preclinical testing of potential therapeutics. Patient-derived tumor xenografts (PDTX), which are tumors directly engrafted from human tissue into mice, offer a complex and accurate environment in which to test potential therapies. Although PDTX models have been established for a variety of malignancies, recent developments have led to the first PDTX models for GE malignancies. Going forward, these models will provide the opportunity to study therapeutic agents in a more representative manner.[4] Additional methods for the more precise monitoring of disease progression include measurement of circulating tumor DNA, which has been demonstrated to correlate with disease status. Potential implications for this technology include dynamic measurement of the treatment response, including measurement of genetic markers, to specific agents.[5]

Molecular Pathways

The ability to personalize treatment based on the individual molecular characteristics of cancers is still in its infancy. However, a significant amount of information has been obtained regarding the genetic changes involved in GE malignancies. The Cancer Genome Atlas (TCGA) has allowed a more comprehensive understanding of the molecular makeup of GE cancers and the key pathways driving oncogenesis.[6] Several cell surface growth factor receptors play a prominent role in the most clearly understood of the GE cancer signaling pathways, including cell surface receptors that result in the activation of RAS, phosphoinositide 3-kinase (PI3K)-AKT, and signal transducer and activator of transcription 3 (STAT3). What follows is a brief overview of the prevalence and potential treatment of these potent pathways.

Human Epidermal Growth Factor Receptor 2

HER2 is a member of the ERBB family of growth factor receptors. When bound by a growth factor, the receptor dimerizes, leading to autophosphorylation in the intracellular domain and subsequent activation of downstream pathways. The result is gene expression that drives cell survival, proliferation, and cycle progression.[7] Approximately 30% of GE adenocarcinomas exhibit overexpression of HER2. Although HER2 overexpression may be more common in GE adenocarcinomas than in breast cancers, its effect on prognosis is not as clear in GE adenocarcinomas.[8] Janjigian and colleagues demonstrated that HER2 status is not an independent prognostic factor in gastric carcinoma.[9] However, an assessment of control groups in several trials of patients with GE cancers did indicate that patients with HER2-positive tumors have a favorable prognosis in comparison with those with HER2-negative tumors, even when targeted therapy is not initiated.[8,9] Most recent expert guidelines now recommend upfront testing of HER2 status in patients with advanced GE

malignancies, and combination treatment if patients are HER2-positive.[10]

Anti-HER2 monoclonal antibodies. Trastuzumab (Herceptin, Genentech) is effective in the treatment of patients with HER2-positive breast cancer. In the landmark ToGA (Trastuzumab for Gastric Cancer) trial, patients with unresectable or metastatic gastric or GE junction cancer were screened for HER2 overexpression. A total of 22% of tumors were HER2-positive. Patients were randomly assigned to treatment with chemotherapy (cisplatin plus 5-fluorouracil [5-FU] or capecitabine) with or without trastuzumab. Patients in the trastuzumab group had significantly longer overall survival (OS; 13.8 vs 11.1 months) and progression-free survival (PFS; 6.7 vs 5.5 months) than did those in the chemotherapy-alone group. These findings, combined with the lack of difference in safety profiles between the 2 groups, resulted in the approval of trastuzumab as first-line therapy in the treatment of HER2-positive GE cancers.[11]

A subsequent phase 2/3 randomized study called GATSBY (A Study of Trastuzumab Emtansine Versus Taxane in Participants With Human Epidermal Growth Factor Receptor 2-Positive Advanced Gastric Cancer), which examined the benefit of continuing targeted HER2 therapy in the second-line setting, found no significant improvement in OS with use of the antibody-drug conjugate trastuzumab emtansine (T-DM1; Kadcyla, Genentech).[12] In response to concerns that a difference in metabolism may have led to underdosing of trastuzumab in a subset of patients, the HELOISE trial (A Study of Herceptin in Combination With Cisplatin/Capecitabine Chemotherapy in Patients With HER2-Positive Metastatic Gastric or Gastro-Esophageal Junction Cancer; NCT01450696) examined the potential impact of increasing trastuzumab dosing. Although blood plasma trough levels did increase with higher doses, no improvement in survival was noted.[13] Multiple studies are also examining the role of trastuzumab in the neoadjuvant setting, as well as with various combinations of chemotherapies.

Pertuzumab (Perjeta, Genentech) is a monoclonal antibody that binds to subdomain 2 of the HER2 receptor and prevents receptor dimerization. Studies have suggested that the actions of trastuzumab and pertuzumab may be synergistic, providing a more complete blockade of HER2 and its subsequent downstream signaling.[14] In addition, PFS and OS were improved in patients with metastatic breast cancer when they received pertuzumab with trastuzumab and docetaxel.[15] The international phase 3 JACOB trial (A Study of Pertuzumab in Combination With Trastuzumab and Chemotherapy in Participants With Human Epidermal Growth Factor Receptor 2-Positive Metastatic Gastroesophageal Junction or Gastric Cancer; NCT01774768), which is currently in progress, is randomly assigning patients to first-line cisplatin, 5-FU, and trastuzumab with or without pertuzumab.[16]

Tyrosine kinase inhibitors and HER2. In addition to extracellular ligands of the HER2 receptor, several intracellular tyrosine kinase inhibitors (TKIs) have been studied. However, to date, TKIs have not demonstrated superiority to trastuzumab. One such study, called TRIOLOGIC (A Phase III Study for ERBB2 Positive Advanced or Metastatic Gastric, Esophageal, or Gastroesophageal Junction Adenocarcinoma Treated With Capecitabine Plus Oxaliplatin With or Without Lapatinib), studied the efficacy of lapatinib (Tykerb, Novartis), a TKI that interrupts the HER2 and endothelial growth factor receptor (EGFR) pathways. Lapatinib was added to capecitabine and oxaliplatin in the first-line treatment of advanced or metastatic HER2-positive upper gastrointestinal adenocarcinomas. Although the addition of lapatinib produced significant improvements in certain subgroups, including Asian patients and those younger than 60 years, OS did not increase.[17] Consistent with these results, the TyTAN trial (Lapatinib Plus Paclitaxel Versus Paclitaxel Alone in the Second-Line Treatment of HER2-Amplified Advanced Gastric Cancer in Asian Populations) compared weekly paclitaxel with or without lapatinib in the second-line setting and found no significant increase in OS or PFS with the addition of lapatinib.[18]

Although the results of studies examining lapatinib have been disappointing so far, the wide breadth of active research into a variety of agents and regimens suggests that anti-HER2 targeted therapies hold promise.

Epidermal Growth Factor Receptor

EGFR is a receptor tyrosine kinase that is overactive in various malignancies. When not bound by activating ligands, EGFR remains in an inhibited state. If bound, however, the receptor dimerizes, and dimerization results in autophosphorylation of the intracellular aspect and subsequent downstream signaling. When inappropriately activated, EGFR signaling has been demonstrated to lead to increased cell proliferation, angiogenesis, metastasis, and apoptosis resistance. Overexpression of EGFR has been noted in as many as 40% to 80% of patients with esophageal cancer.[19]

Two classes of agents targeting EGFR are currently available: extracellular monoclonal antibodies and intracellular TKIs. Although monoclonal antibodies such as cetuximab (Erbitux, Lilly) and panitumumab (Vectibix, Amgen) have been used somewhat successfully in the treatment of other cancers, results in patients with GE cancers have been disappointing. The EXPAND trial (Capecitabine and Cisplatin With or Without Cetuximab

for Patients With Previously Untreated Advanced Gastric Cancer) studied capecitabine/cisplatin with or without cetuximab in advanced GE cancers. No benefit in PFS or OS was found with the addition of cetuximab.[20] In the Radiation Therapy Oncology Group (RTOG) 0436 trial (A Phase III Trial Evaluating the Addition of Cetuximab to Paclitaxel, Cisplatin, and Radiation for Patients With Esophageal Cancer Treated Without Surgery), investigators studied weekly concurrent cisplatin, paclitaxel, and daily radiation with or without cetuximab. Again, no OS benefit was found with the addition of cetuximab.[21] The SCOPE1 trial (A Phase II/III Trial of Chemoradiotherapy in Esophageal Cancer Plus or Minus Cetuximab) investigated the effects of cisplatin/5-FU chemoradiation with or without cetuximab. The cetuximab group had worse outcomes and more toxicities compared with the chemoradiation-alone group.[22] The REAL3 trial (A Randomised Open-Labelled Multicentre Trial of the Efficacy of Epirubicin, Oxaliplatin and Capecitabine With or Without Panitumumab in Previously Untreated Advanced Oesophago-gastric Cancer) studied panitumumab with or without epirubicin, oxaliplatin, and capecitabine in previously untreated patients. This research demonstrated inferior results in the panitumumab group.[23] Further studies have tested nimotuzumab in similar settings. In one randomized phase 2 study, inferior outcomes were observed with the addition of nimotuzumab to cisplatin/S-1 chemotherapy vs chemotherapy alone.[24]

Among the intracellular TKIs, gefitinib (Iressa, AstraZeneca) and erlotinib (Tarceva, Genentech/Astellas) have been tested in advanced GE cancers. The phase 3 COG trial (Gefitinib for Oesophageal Cancer Progressing After Chemotherapy) of patients whose disease had progressed after first-line chemotherapy demonstrated a minimal difference in PFS between patients treated with gefitinib and those treated with placebo. OS was unchanged.[25] A small subgroup of patients had a marked and durable response. To further elucidate which patients might benefit from gefitinib, the TRANS-COG study (Results of a Biomarker Analysis of a Phase III Trial of Gefitinib Versus Placebo) evaluated EGFR copy number gain in 295 patients who had participated in COG. A total of 15% of patients had evidence of copy number gain, and these patients were noted to have improved OS and PFS.[26]

Although multiple phase 2 studies demonstrated promising benefit after the use of EGFR-targeted therapies, the available data from phase 3 studies indicate no benefit and a significant increase in adverse events. EGFR-targeted therapy currently has no role in the treatment of patients with GE cancers.

Vascular Endothelial Growth Factor

The vascular endothelial growth factor (VEGF) pathway is important in the growth of blood vessels from existing vasculature.

VEGF overactivity has been shown to increase tumor cell invasion and metastasis. The VEGF receptor 2 (VEGFR2) is thought to be particularly important when bound by VEGF-A.[27] Multiple studies have associated VEGF overexpression with increased aggressiveness of GE tumors and poorer outcomes.[28]

VEGF inhibitors have been tested in patients with multiple cancer subtypes, including colorectal cancers, in whom they have been shown to improve outcomes.[29] Extracellular monoclonal antibodies as well as intracellular TKIs exist that target the VEGF pathway. Bevacizumab (Avastin, Genentech), an anti-VEGF monoclonal antibody, has been tested in multiple settings—including colorectal, lung, ovarian, and renal cell cancers—with varying levels of efficacy.[30-33] The phase 3 AVAGAST (Avastin in Gastric Cancer) study added bevacizumab to cisplatin in the first-line treatment of advanced gastric cancers. Although both median PFS (6.7 vs 5.3 months) and overall response rate (ORR; 46.0% vs 37.4%) were improved, OS was not improved.[34] There appeared to be geographic variations in benefit, a finding that raised the possibility of differing tumor biology. Similarly, the AVATAR study (Bevacizumab Plus Capecitabine and Cisplatin in Chinese Patients With Inoperable Locally Advanced or Metastatic Gastric or Gastroesophageal Junction Cancer) demonstrated no benefit in median OS or PFS when bevacizumab was used in China in treatment-naive patients.[35]

Ramucirumab (Cyramza, Lilly), a monoclonal antibody targeting VEGFR2, has produced better outcomes than the previously discussed treatments. In the phase 3 REGARD trial (Ramucirumab Monotherapy For Previously Treated Advanced Gastric or Gastro-Oesophageal Junction Adenocarcinoma), ramucirumab was compared with placebo in the second-line setting. A significant improvement in median OS (5.2 vs 3.8 months) was noted with ramucirumab.[36] This benefit was confirmed in the RAINBOW trial (Ramucirumab Plus Paclitaxel Versus Placebo Plus Paclitaxel in Patients With Previously Treated Advanced Gastric or Gastro-Oesophageal Junction Adenocarcinoma), with an increase in median OS of 2.2 months in the ramucirumab group (9.6 vs 7.4 months).[37] Based on these studies, ramucirumab was approved for use as a single agent in patients with gastric or GE cancers that had progressed on platinum/fluoropyrimidine therapy. Although studies examining the role of ramucirumab in the first-line setting are ongoing, results from a phase 2 study of ramucirumab with or without 5-FU, leucovorin, and oxaliplatin (FOLFOX) did not show improvement in PFS with the addition of ramucirumab.[38]

For the various TKIs that target the VEGF pathway, little evidence of benefit has been shown in several phase 2 trials studying sunitinib (Sutent, Pfizer) and sorafenib (Nexavar, Bayer).[39-42] It is therefore not surprising that there is a lack of ongoing trials studying these 2 agents. Regorafenib (Stivarga, Bayer), which is thought to function similarly to sorafenib, was studied in the phase 2 INTEGRATE trial (Regorafenib for the Treatment of Advanced Gastric Cancer) in the second-line setting. Preliminary results demonstrated a statistically significant increase in PFS when regorafenib was compared with placebo (11.1 vs 3.9 weeks). There also was a trend toward increased OS with regorafenib, but this was not statistically significant.[43] Multiple other studies examining regorafenib in the first- and second-line settings are ongoing. A phase 3 study found that apatinib, an experimental selective inhibitor of VEGFR2, improved OS vs placebo when used as a third-line treatment. Although it is unclear whether these results are relevant outside China, multiple additional trials are ongoing.[44]

Although the negative results with bevacizumab in GE cancers have been disheartening, the success of ramucirumab in the second-line setting represents a new standard of care. Further study of apatinib in the third-line setting also offers an exciting new opportunity. Exploration of VEGF monoclonal antibodies and TKIs, coupled with a further understanding of biological variability, offers another path of great interest in the treatment of patients with GE malignancies

MET-Hepatocyte Growth Factor Receptor Pathway

The MET protein is a tyrosine kinase receptor encoded by the *MET* proto-oncogene and stimulated by the hepatocyte growth factor (HGF) ligand. It is involved in organogenesis, wound healing, and embryonic development. When HGF binds to MET, receptor dimerization and downstream activation of various oncogenic pathways occur.[45] When abnormally activated, MET has been linked to tumor angiogenesis, proliferation, and invasion. MET overexpression has been demonstrated in many different cancers, including gastric cancers.[46] Studies have demonstrated MET overexpression in gastric cancers of greater than 50% by IHC and greater than 20% by FISH.[47] Additionally, *MET* amplification has been linked to worsened prognosis because it is correlated with advanced tumor grade at presentation. Among *MET*, *HER2*, and *EGFR* amplifications, *MET* amplification was most strongly correlated with poor prognosis.[48] Thus, multiple MET inhibitors have been developed and tested in GE malignancies.

Onartuzumab did not improve OS or PFS in the METGastric trial (A Phase III Study of Onartuzumab Plus mFOLFOX6 in Patients With Metastatic HER2-Negative and MET-Positive Adenocarcinoma of the Stomach or Gastroesophageal Junction).[49] Rilotumumab, which binds HGF, improved PFS (5.7 vs 4.2 months) in phase 1/2 studies. However, the phase 3 RILOMET-1 trial (First-Line Treatment for Locally Advanced or Metastatic Mesenchymal Epithelial Transition Factor–Positive Gastric, Lower Esophageal, or Gastroesophageal Junction Adenocarcinoma), which compared epirubicin/cisplatin/capecitabine alone and with rilotumumab, was stopped early owing to inferior results in the rilotumumab arm.[50] Several other TKIs have been extensively tested, including AMG 337, tivantinib, and crizotinib (Xalkori, Pfizer). Although early data on AMG 337 were encouraging, further development has been halted owing to safety concerns.[51]

There has been much speculation regarding the reasons for the failure of MET-directed therapy to date. One simple theory is that IHC may inaccurately identify patients with MET overexpression.

As a result, future studies will use IHC in conjunction with FISH to measure *MET* gene amplification.

Immunotherapy

The remarkable results seen with immune checkpoint therapies, which were first used in melanoma but are now being extended to a variety of malignancies, have led to their study in GE malignancies.[52] Ipilimumab (Yervoy, Bristol-Myers Squibb), a monoclonal antibody against cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4), has been studied as a second-line treatment in gastric and GE junction cancers. However, it was found that ipilimumab did not improve PFS compared with best supportive care (An Efficacy Study in Gastric and Gastroesophageal Junction Cancer Comparing Ipilimumab Versus Standard of Care Immediately Following First Line Chemotherapy; NCT01585987).[53]

The anti–programmed death 1 (PD-1) antibodies nivolumab (Opdivo, Bristol-Myers Squibb) and pembrolizumab (Keytruda, Merck) have been studied independently and in conjunction with ipilimumab. KEYNOTE-012 (Study of Pembrolizumab in Participants With Advanced Solid Tumors), a phase 1b study, showed an ORR to pembrolizumab of 30%.[54] Several anti–PD-1 studies are ongoing, including one of pembrolizumab combined with cisplatin as first-line treatment (A Study of Pembrolizumab in Participants With Recurrent or Metastatic Gastric or Gastroesophageal Junction Adenocarcinoma; NCT02335411) and another of pembrolizumab vs paclitaxel as second-line treatment (Study of Pembrolizumab as First-Line Monotherapy and Combination Therapy for Treatment of Advanced Gastric or Gastroesophageal Junction Adenocarcinoma; NCT02494583). Recent data from the phase 1/2

CheckMate 032 study (A Study of Nivolumab by Itself or Nivolumab Combined With Ipilimumab in Patients With Advanced or Metastatic Solid Tumors) revealed an ORR of 16%: 14% in the nivolumab group, 26% in the nivolumab/high-dose ipilimumab group, and 10% in the high-dose nivolumab/ipilimumab group. The overall disease control rate (ORR plus stable disease) was noted to be 38% (A Study of Nivolumab by Itself or Nivolumab Combined With Ipilimumab in Patients With Advanced or Metastatic Solid Tumors).[55] The identification of subgroups in which benefit might be maximized revealed that increased programmed death ligand 1 (PD-L1) expression and mismatch repair deficiency may correlate with increased response.[56,57] Although the results of these studies are still in the early stages, immunotherapies are an exciting development.

Challenges

A recent meta-analysis noted 91 markers that were reported to be significantly associated with outcome in GE cancers. However, most of the included studies were retrospective, small in size, and of poor quality, indicating a lack of reliability and applicability.[58] In another study, a standardized hotspot mutational analysis was performed in 2000 patients with advanced malignancies to determine the frequency of actionable mutations and subsequent enrollment in clinical trials.[59] Approximately 16% of the patients studied who had GE malignancies had actionable mutations. In all cancer types, 39% of patients had actionable mutations, but only 11% of them were eventually enrolled in matched clinical trials. The SHIVA trial (Molecularly Targeted Therapy Based on Tumour Molecular Profiling Versus Conventional Therapy for Advanced Cancer), which was a phase 2 study seeking to evaluate the efficacy of 11 targeted agents in patients with advanced cancer who underwent NGS, demonstrated no median PFS benefit for patients who were treated with a matched molecularly targeted agent.[60]

These findings demonstrate the challenges we face in the use of sequencing techniques to evaluate patients, and in finding a relevant treatment or trial to fit each patient's molecular profile. Improved methods of molecular testing and interpretation of results are needed to properly translate results into clinical treatment selections. Significant logistical barriers also limit the widespread adoption of costly molecular profiling techniques. However, it is hoped that as these tests become more widely used, competition and scaling will result in a relative reduction in price. The time from tumor biopsy profiling to receiving actionable results that can guide treatment—approximately 3 weeks—is currently a major hindrance. This interval will need to be shortened significantly to facilitate timely and useful clinical decision-making based on profiling results.

Identifying actionable mutations, as clearly demonstrated in the previous studies, is only the beginning of a complex process of developing the personalized treatment plans we seek for our patients. The true impact of specific mutations on treatment outcomes, and the degree to which other mutations as well as environmental and histologic factors affect these mutations and treatment outcomes, are still unclear.

Conclusion

Although numerous molecular markers have been described and studied to various degrees, a very limited portion of this research has yielded clinically significant results in patients with GE malignancies. Except for agents targeting the HER2 and VEGF pathways, currently no agents are approved for treating patients with these cancers beyond the current standard of care: neoadjuvant chemoradiation coupled with resection. However, the ability to analyze patient DNA, RNA, and protein profiles holds significant promise. The rapid expansion of immunotherapies for a variety of malignancies also holds promise for patients with GE malignancies. Although it has become abundantly clear that treating GE cancers with targeted monotherapy is not feasible at present, the variance in individual tumor biology, as well as the interaction between various pathways, is still largely unexplored. As our understanding of the impact of molecular aberrations on the choice of chemotherapeutic and targeted agents increases, and as more targeted agents are developed, the potential for improving outcomes in patients with GE malignancies is on the horizon.

Disclosures

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