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Strategy for prevention of cancers of the esophagus

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The following, from the 12th OESO World Conference: Cancers of the Esophagus, includes commentaries on the animal reflux–inflammation models for Barrett's esophagus and esophageal adenocarcinoma; genomic/epigenomic analyses; eflornithine-based combinations; the molecular derangements that promote neoplastic transformation; the role of COX-2 inhibitors, proton pump inhibitors, and phase II trials in Barrett's adenocarcinoma; statins in chemoprevention and treatment of esophageal cancer; and biomarkers as potential targets in Barrett's adenocarcinoma.

Keywords: animal reflux–inflammation models; Barrett's adenocarcinoma; elofrnithine; COXIBs, PPIs; statins; biomarkers; OESO

Concise summary

An animal model of Barrett's esophagus (BE) and esophageal adenocarcinoma (EAC) could provide better understanding of the pathogenesis and could be used for investigating new prevention and treatment strategies. The Levrat *in vivo* model is widely accepted and adapted since it mimics the stepwise event of esophagitis, BE, and EAC, showing immunohistochemical characteristics resembling human BE. The use of rat models for mechanistic studies is limited owing to a lack of genetically modified rat strains. On the other hand, the mouse has a well-characterized genome, and transgenic or knockout mice can be used to investigate the functional role of specific genes. Although none of the models offers an ideal system for the complex study of environmental exposure, genetic risk, and prevention strategies, the rodent models are the best options that we have to improve our understanding of the pathogenesis of BE.

The science of genomics and epigenomics seems to have successfully identified promising targets for the prevention and treatment of esophageal cancers. It is critical to identify not only the common and diverse categories but also to distinguish the drivers from the passenger mutations at the early stages of carcinogenesis. The Cancer Genome Atlas has revealed copy number abnormalities and gene amplification events at distinct loci in the two broad esophageal cancer types. There are some copy number abnormalities with similar frequencies in both histologies and there are some genes with copy number abnormalities with different frequencies in the two histologies. Some are amplified and interventions are available, and a number of genes are deleted with similar frequencies in both histologies: hence, possible targeted interventions for amplified genes in esophageal cancer. Beyond these targets, additional efforts are underway in the form of a pan-cancer initiative comparing genomic data across

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12 tumor types to identify drivers instead of passenger genes for carcinogenesis.

Chemoprevention using safe agents as a primary approach or as an adjunctive approach to endoscopic therapy remains an attractive option to reduce neoplastic progression.

Ornithine decarboxylase (ODC) is a key enzyme that is involved in the synthesis of polyamines, which also seems to play important roles in tumorigenesis. Accumulation of polyamine could increase oxidative stress and DNA damage, and these mechanisms could be operational during neoplastic transformation of BE. Polyamine synthesis could therefore be targeted individually or in a combinatorial manner to prevent EAC. Some studies showed that difluoromethylornithine prevented growth of Barrett's epithelial cells, reduced the incidence of esophageal tumors, and inhibited esophageal cell proliferation induced by dietary zinc deficiency in rats. However, no correlation was found between polyamine levels and difluoromethylornithine activity in patients. Alternate genetic approaches, such as overexpression of IL-1β or loss of p63, emphasize that the pro-inflammatory and developmental pathways can drive carcinogenesis and metaplasia.

Understanding the molecular derangements that promote neoplastic transformation is important for identifying cancer prevention targets. Preclinical models have significantly contributed to our understanding of the pathogenesis of carcinogenesis in BE. Interpretation of results, however, requires consideration of the phenotypic changes in the cell lines, and it is pertinent to characterize these cells over time so that they are as relevant to human models as possible. An alternative to organotypic culture is the use of denuded tracheal models to grow epithelial cells in the trachea that can be manipulated into transdifferentiating into BE. Animal models are useful from a pharmacological point of view in terms of identifying the effects of anti-inflammatory medications on reducing inflammation and oxidative injury and studying the metaplastic *in vivo* changes in esophageal cells. The activation of certain pro-inflammatory pathways by bile acid refluxates may induce genetic changes, cytokeratin modulation, and changes in mucin production that promote transdifferentiation of the epithelium. During activation of the pro-inflammatory pathways, many cytokines are produced, and some studies emphasize the roles IL-1β and IL-6 play in triggering transdifferentiation and carcinogenesis in normal squamous esophageal epithelium. Another study showed that bile acids induce CREB- and AP-1–mediated COX-2 expression in cells, and the formation of reactive oxygen radicals within esophageal cells that induce PI3K/AKT and ERK 1/2, a pathway that has been implicated in carcinogenesis. There is a prospective benefit from incorporating COX-2 inhibitors in the prevention of Barrett's carcinogenesis. Another molecular mechanism found to be of significance in the carcinogenetic pathway involves GLI1, a hedgehog-regulated transcription factor. GLI1 upregulates the transcriptional activity of a key cell cycle regulator, *CDK2*. Overexpression of GLI1 increases CDK2 levels, thus promoting proliferative activity of cancer cells. Chromosomal instability forms the basis of most human cancers, including esophageal cancer. The *CDKNA2* gene encodes for the p16 inhibitor, a cell cycle cyclin D/cyclin-dependent kinase 4 regulator. Global hypomethylation and promoter hypermethylation of the *p16* promoter region leads to inactivation of *p16*, allowing unregulated cell proliferation to occur. Epigenetic dysregulation also appears to use the extracellular signaling glycoprotein ligand Wnt family to drive metaplastic and neoplastic changes. The role of oxidant damage to DNA in the form of double-strand breaks on exposure to nitrosating species has also been implicated in triggering epigenetic changes that ultimately promote tumorigenesis.

For prevention of Barrett's dysplasia and cancer in the clinic, proton pump inhibitor (PPI) therapy is a necessity in BE, mostly for symptoms and mucosal damage control, but the effective PPI dose is uncertain. Despite their early promise, COX-2 inhibitors (coxibs) are of no clinical value at this point. On the other hand, aspirin may be beneficial, but this has not yet been proven.

A considerable research focus has been placed on the potential of statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors) as both chemopreventive and adjuvant agents for many malignancies, including esophageal cancer. In a number of epidemiological studies, statin use appears consistently inversely associated with risk of high-grade dysplasia (HGD) or EAC in patients with BE. *In vitro* studies have demonstrated that statins limit cellular proliferation and promote apoptosis in BE and EAC cell lines. In epidemiological investigations of

statins as chemopreventive agents in BE populations, significant dose and duration responses were reported in individual included studies, thus supporting a causal inverse association. In esophageal cancer populations, statin use was associated with improved cancer-specific survival.

Biomarkers serve as a means of enhancing the efficacy of treatments. The central role of the *p53* gene in the control of cell growth, DNA repair, and apoptosis makes it a potentially powerful biomarker and a unique target for cancer therapy. Three phases of biomarker studies are designed to obtain answers to specific questions on their potential clinical value: in phase I, testing prevalence and specificity, the robustness of the biomarker *TP53* has been amply proven. In phase II, addressing the reproducibility of a marker test, the first standardized gene-specific sequencing protocol for the *TP53* gene has been successfully evaluated in clinical studies. For the second aim of phase II biomarker trials, the determination of the marker type, it is still not generally known whether *TP53* is predictive or prognostic. In phase III, addressing the magnitude of the effect of a biomarker, the Pancho trial (*p53*-adapted neoadjuvant chemotherapy for operable esophageal cancer) conducted by the p53 research group is the first prospective randomized trial evaluating the magnitude of the predictive effect of the biomarker *TP53* in esophageal cancer.

Screening and early detection of precursor lesions may significantly contribute to reducing cancer-associated mortality. While precursor lesions may serve as targets for both screening and chemoprevention, randomized controlled trial data supporting the effectiveness of either approach are lacking in both EAC and squamous cell cancers. Population-based endoscopic screening, although potentially warranted by its prevalence in high-risk settings of the so-called esophageal cancer belt, is likely not feasible, and surgical or endoscopic ablative therapies form the basis of current clinical management for patients with progressive HGD. Promising preliminary data exists for many agents for chemoprevention, but none have been translated effectively into the clinic. Well-designed phase III randomized trials serve as an example of what is required to advance the use of chemopreventive agents in esophageal cancer risk management. A method to risk stratify asymptomatic individuals could significantly enhance the prevention and early detection of esophageal cancer, as could biomarkers predictive of the progressive or responsive nature of neoplastic precursors. Molecular imaging could lead to a substantially refined and more precise approach to the early detection of esophageal neoplastic lesions and cancers. Secondary prevention will evolve from endoscopic therapy based on histology to therapy guided by predictive/therapeutic response biomarkers, and may include chemoprevention as an adjuvant to ablation; more precise molecularly targeted therapies may eventually replace ablative techniques.

1. Animal models for BE and EAC

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BE is an acquired disorder in which, through chronic gastroesophageal reflux disease (GERD), the normal squamous epithelium is replaced by columnar epithelium. This metaplastic lesion is associated with an increased risk for developing EAC. In Western countries, the prevalence of this cancer is increasing dramatically, while the overall 5-year survival of these patients, despite therapies, is <20%. Currently, the only possible way to improve patient outcome is by detecting the disease in an early stage. To this end, all patients diagnosed with the precursor lesion, BE, have to undergo periodic endoscopy to check biopsies for development of early cancer.[1] An ideal strategy to improve patient outcome would be if we could cure the BE before even dysplasia or cancer occurs. Therefore, it is essential that we understand the molecular mechanisms involved in the development of BE. An animal model of BE and EAC could provide better understanding of the pathogenesis and could be used for investigating new prevention and treatment strategies.

The concept of acid-induced reflux esophagitis was introduced when ligation of the pyloris in rats resulted in acute acid reflux. To study the physiology and pathology of acid-induced reflux esophagitis, chronic reflux was induced by surgical pyloric stenosis.[2] Total gastrectomy, as an antireflux treatment in patients, still resulted in

esophagitis, suggesting that duodenal contents might also be harmful.[3] Subsequently, different components of refluxate were tested individually or combined with external esophageal perfusion. In this setting, the exact amount and concentration of the separate components can be controlled. Later on, Levrat developed a surgical procedure to induce gastroduodenojejunal reflux.[4] In the majority of studies, esophagoduodenal anastomosis was combined with administration of chemical carcinogens to induce cancer, which resulted in esophageal squamous cell carcinoma (ESCC) rather than EAC.[5] This *in vivo* model has been a widely accepted and adapted, since it mimics the stepwise event of esophagitis, BE, and EAC, and shows immunohistochemical characteristics resembling human BE.

Unfortunately, the use of rat models for mechanistic studies is limited, owing to lack of genetically modified rat strains. The mouse, on the other hand, has a well-characterized genome, and transgenic or knockout mice can be used to investigate the functional role of specific genes. P63-deficient mice show well-developed columnar epithelium rather than normal squamous epithelium,[6] which may model acid-reflux damage. This columnar epithelium resembles BE, with comparable gene expression, although without the characteristic goblet cells. A disadvantage of this model is that this deletion is lethal. BE-like metaplasia and neoplasia were found in IL-1ß–overexpressing mice.[7] Columnar metaplasia started around 12–15 months at the squamocolumnar junction (SCJ) and further developed into HGD in 22 months. Both bile acids and carcinogens enhanced the development of BE and dysplasia. Gene expression profiles of these mice closely resemble gene expression found in human BE and EAC.

Recently, the surgical model has also been introduced in mice,[8] allowing researchers to study the functional role of specific genes in the development of EAC. However, given their size, operating on mice is technically challenging, resulting in highly variable outcomes with sometimes severe morbidity and high mortality rates. Buttar recently developed a novel approach in which the anastomosis between the esophagus and jejunum is created by implanting neodymium micromagnets, causing pressure necrosis between organs to produce a fistula and thereby inducing reflux.[9] This new improved suture-less method overcomes the disadvantages of traditional microsurgical methods and results in significantly less morbidity and mortality of animals. However, no goblet cells were described in the area of metaplasia after 12 weeks, and it remains possible that the columnar epithelium comes directly from progenitor cells in the intestine that is now in contact with the esophagus.

Nowadays, the most common animal models that are used to study BE include mice and rats. An important thing to keep in mind is that both rats and mice differ from humans in the basic biology of the esophagus. The rat esophagus is covered by keratinizing squamous epithelium, compared to nonkeratinizing squamous epithelium in humans. Also, rodents do not have submucosal glands. Both Wang and Quante suggest that the origin of BE lies at the SCJ.[6,7] However, this does not exclude the possibility that, in humans, BE is derived from esophageal submucosal glands. Instead, dogs would be more suitable for the study of development of BE and EAC since they mimic the human situation more closely, although BE development can take up to 3 years.[10]

In the past decades, exciting progress has been made in our understanding of reflux esophageal injury thanks to the use of animal models. However, it seems that all models used so far have their own limitations. Although none of the models offers an ideal system for the complex study of environmental exposure, genetic risk, and prevention strategies, the rodent models are the best options that we have to improve our understanding of the pathogenesis of BE. Clearly translating results from these studies to the human situation should be done carefully.

2. How might we identify the most promising targets/agents going forward?

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The current state of the science for genomics and epigenomics is reaching its ultimate potential and seems to have successfully identified the most promising targets for prevention and treatment of EAC.[11] The technologies for these techniques are such that one is able to process thousands of samples from tumors or precancerous lesions for systemic discovery of these targets, and knowledge from these endeavors can be systematically and empirically applied to interventions that have tremendous potential to be effective. This technology is

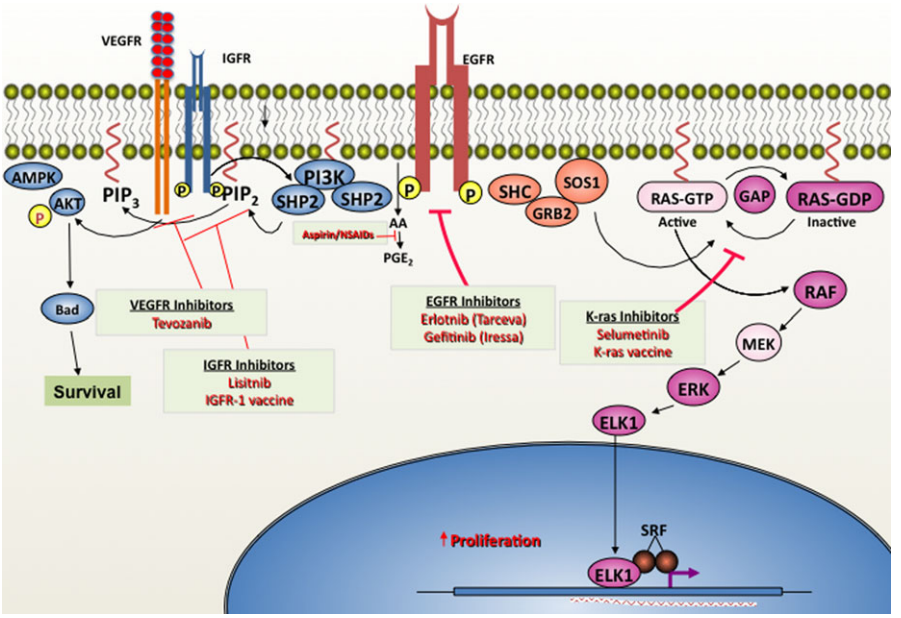

Figure 1. Possible targeted interventions for amplified genes in esophageal cancer.

further helped by several other related fields, especially bioinformatics and the use of algorithms to apply to the data sets that are obtained in these endeavors.

Cancer is a compilation of hundreds of diseases in itself, which when examined at the single-cell level provides us with tools for classification in a very different light than previously possible.[12] It is critical to identify not only the common and diverse categories but also to distinguish the drivers from the passenger mutations at the early stages of carcinogenesis. Here, an example is provided from recent published data of the genomic and epigenomic landscape to simplify this information into major pathway dysfunctions in esophageal cancers. In addition, possible promising targets for interventions, especially at an early stage of disease, are outlined.

There are two major histologic types of esophageal cancer: ESCC, predominant globally; and EAC, which has a higher incidence in most Western countries. Five-year overall survival is 15%. ESCC and EAC are not only two distinct histologic types, they are different diseases, having different genetics and epigenetics as well as distinct origins. Hence, the discussion of targeting these different diseases requires different strategies.

Genomics and epigenomics of esophageal cancer

The Cancer Genome Atlas (TCGA) and others have revealed copy number abnormalities and gene-amplification events at distinct loci in these two broad esophageal cancer types.[13] These genomic analyses have outlined the following parameters to consider for the treatment and prevention of both types of esophageal cancer. There are some copy number abnormalities with similar frequencies in both histologies: *CDKN2A, EGFR, KRAS, MYC, CDK6,* and *MET.* There are some genes with copy number abnormalities with different frequencies in the two histologies. The following genes are amplified and interventions are either available or in clinical trials: *VEGFA, ERBB2, PIK3CA,* and *FGFR1.* Similarly, there are a number of genes that are deleted with similar frequencies in both histologies, including *FHIT*, *PDE4D*, and *PTPRD*, or are deleted with different frequencies in the two types, such as *SMAD4*, *DCC*, and *MACROD2.*

Hence, possible targeted interventions for amplified genes in esophageal cancer (Fig. 1) include gefitinib (Iressa®) and erlotinib (Tarceva®) for epidermal growth factor receptor (EGFR); tevozanib (Astellas®) for the vascular endothelial growth factor (VEGF); selumetinib (AZD®) and deltarasin,

a novel novel PDEδ inhibitor that disrupts oncogenic KRAS signaling or HSP90 inhibitors, for K-RAS; and linsitnib and IGF-1R vaccine for the insulin growth factor 1 receptor (IFG1R).

Beyond these targets, there are additional efforts underway in the form of a pan-cancer initiative that is comparing genomic data across 12 tumor types to identify drivers instead of passenger genes for carcinogenesis. One may ask if there is a common set of driver mutations across a variety of cancers and then deduce the information for EAC and ESCC. Initial sets of data show that most tumor types have two to six driver mutations that can be further organized into three core cellular processes; cell survival, cell fate, and genome maintenance.[2] Expanded data sets and larger number of tumors, including subtypes and whole-genome sequencing, will provide a more comprehensive picture and further insight into identifying the most promising targets for esophageal malignancies.[14]

3. Eflornithine-based chemopreventive approaches in esophageal cancer

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Esophageal cancer (EC) incidence has been on the rise, particularly within North America and Europe. Five-year survival rate for EAC has not improved in the past 20 years.[15] Though endoscopic surveillance is utilized to detect and treat dysplasia and early malignancy, it may not detect early cancers in nearly half of patients, and endoscopic therapy has durable response in only up to 75% of cases.[16] Chemoprevention using safe agents as a primary approach or as an adjunctive approach to endoscopic therapy, therefore, remains an attractive option to reduce neoplastic progression.

The polyamines putrescine, spermidine, and spermine are produced during cellular metabolism and are signaling molecules for cell growth and differentiation that play important roles in tumorigenesis. ODC is a key enzyme that is involved in the synthesis of polyamines. Patients with BE have markedly increased ODC activity,[17] and this could promote neoplastic changes in BE. Although regulation of polyamine metabolism in Barrett's mucosa remains to be investigated, in colonic epithelium APC suppresses transcription of *MYC*, which is an activator of *ODC* transcription. APC also regulates ODC antizyme (OAZ), which targets ODC for degradation. Tumor suppressor peroxisome proliferator–activated receptor-γ (PPAR-γ) activates spermidine/spermine N1-acetyltransferase (SSAT) transcription to acetylate cellular polyamines, which helps to reduce intracellular polyamine levels. Oncogenic mutations in *KRAS* downregulate PPAR and could therefore prevent polyamine catabolism. Accumulation of polyamine could increase oxidative stress and DNA damage. These mechanisms could also be operational during neoplastic transformation of BE, and polyamine synthesis could therefore be targeted individually or in a combinatorial manner to prevent EAC. α-Difluoromethylornithine (DFMO) is an irreversible inhibitor of ornithine decarboxylase and is commonly used to treat facial hirsutism and African trypanosmiasis. Polyamine synthesis can also be inhibited by nonsteroidal anti-inflammatory drugs (NSAIDs) or nitric oxide inhibitors that are known to curtail pro-inflammatory processes associated with carcinogenesis. Finally, antioxidants and dietary or pharmacological changes in PPAR-γ activity targeting polyamine metabolism could also prevent carcinogenesis in BE.

Garewal *et al.* report that DFMO prevented growth of Barrett's epithelial cells in both colony count and viable cell number metrics.[17] Reduction in cell count was noted at all concentrations of DFMO (0.05–5 mM). However, they report no correlation between polyamine levels and ODC activity in 18 patients. They acknowledge that they only tested patients with nondysplastic BE, and higher ODC activity is typically seen in dysplastic tissue. Sinicrope *et al.* report that in a cohort of patients ($n = 10$) with BE and LGD who were administered DFMO (0.5 g/m^2/day) for 6 months, significant reductions in levels of putrescine, spermidine, and the spermidine/spermine ratio were observed, which persisted at 6 months following cessation of treatment.[18] A partial increase in putrescine levels was found 12 months after drug cessation. Furthermore, modulation of genes was observed after DFMO treatment; downregulation was observed for *RPL11*, which is known to activate the p53 pathway, directly involved in tumor suppression. KLF5, a transcription factor that promotes cell proliferation, was also downregulated. Furthermore, RFC5, a protein critical for cell proliferation known to

interact with proliferating cell nuclear antigen (PCNA), was suppressed. Cyclin E2, which is involved in cell cycle regulation and Plexin1, which is involved in cell adhesion and invasion, were upregulated. One patient demonstrated regression of LGD and two demonstrated reduction of extensive to focal LGD. Unfortunately, LGD diagnosis is highly variable with marked inter-observer variation, and in many patients spontaneously regresses.

In preclinical investigation, Fong *et al.* placed rats ($n = 108$) into four groups: $Zn^+/DFMO^-$, $Zn^+/DFMO^+$, $Zn^-/DFMO^-$, and $Zn^-/DFMO^+$.[19] Zn deficiency was used to promote oxidative stress and neoplasia. One percent DFMO was administered through drinking water. A subgroup of animals completed the study at 5 weeks to detect cells in S phase, and the remaining animals were administered *N*-nitrosomethylbenzylamine (NMBA; 2 mg/kg). After 12 weeks, DFMO treatment in animals reduced putrescine and spermidine levels 48–82% in rat esophagus, colon, and liver. Furthermore, DFMO reduced the incidence of esophageal tumors from 80% to 4% in Zn-deficient rats. DFMO also effectively inhibited the increased esophageal cell proliferation induced by dietary zinc deficiency. However, Chen *et al.* report that in a study of the effectiveness of sulindac, nordihydroguaiaretic acid, and DFMO as chemopreventive agents on esophagogastroduodenal anastomosis (EGDA) rat models (which mimicked the staged process of EAC), DFMO by itself was not observed to have any effect on reducing adenocarcinogenesis.[1] Furthermore, use of 1% DFMO resulted in significant toxicity, with animals presenting with dermal lesions on the head, neck, and abdomen, which resulted in a decrease of dosage to 0.5%. The use of sulindac (an NSAID that acts on COX1 and COX2 to inhibit prostaglandin production) in combination with 0.5% DFMO reduced tumor incidence from 57.7% to 20%. However, use of sulindac alone reduced tumor incidence to 26.9%, indicating that DFMO's impact was marginal at best.

In summary, eflornithine-based chemopreventive approaches have mechanistic rationale, but only limited combinatorial chemoprevention approaches involving DFMO have been tested. Potential phase I/II studies involving DFMO plus aspirin, DFMO plus NO-releasing aspirin, or DFMO plus antioxidants or PPAR-γ activators will be reasonable.

4. Preclinical systems to identify and test cancer prevention targets in Barrett's esophagus

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Neoplastic transformation in BE is a protracted process that, in a subset of patients, turns into highly lethal adenocarcinoma. Understanding the molecular derangements that promote neoplastic transformation is important for identifying cancer-prevention targets. An ideal preclinical model to identify and test cancer-prevention targets should have molecular, biochemical, morphological, and biological alterations that resemble human BE. Although no single model meets these criteria, various *in vitro* and *in vivo* models can be used to complement each other.

Preclinical models have significantly contributed to our understanding of the pathogenesis of carcinogenesis in BE. These models range from *in vitro* studies that involve utilization of 2-D and 3-D cultures to *ex vivo* and organotypic cultures and *in vivo* animal models. Numerous cell lines extracted from human biopsy samples, such as esophagus-derived normal squamous, metaplastic Barrett's, and EAC cells, have been utilized in developing preclinical *in vitro* and *ex vivo* models for studying the process of carcinogenesis. While primary squamous or Barrett's epithelial cell cultures retain the unaltered genetic makeup and have the potential to provide insight into colonial expansion, they unfortunately exhibit limited life span and patient-to-patient variation. To extend the life span and to improve consistency, various cell lines have been established including Het-1A (derived from normal squamous epithelial cells transfected with SV40 LTA), BAR-T and CP-A-C (hTERT immortalized non-dysplastic and dysplastic Barrett's epithelial cells), and FLO-1, SKGT-4, OE33, and OE19 (from EAC). The strength of these models is that they allow for the derivation of clear-cut experimental data concerning viability and apoptosis, the tracking of intracellular molecular mechanisms, and the quantification of RNA transcripts and proteins. However, the use

of primary cultures and monolayer cell lines has the disadvantage of lacking the conditions and interactions seen in the natural environment. Their growth behavior is also influenced by the immortalization procedures. Another novel approach uses 3-D culture or *ex vivo* esophageal cell cultures obtained from biopsy specimens. These models mimic the natural environment of the cells, and through their exposure to pathophysiological intervention such as acidic pH and or bile salts, one could uncover cell–cell interactions and molecular derangements that promote carcinogenesis. Problems with these models relate to artificial selection pressures and a limited time span of experimentation. The employment of organotypic cultures, where multilayered cultures are performed using a monolayer of esophageal cells derived from patients on specialized matrices rich in collagen and fibroblasts, have also been incorporated in the studies on Barrett's carcinogenesis. This model is predominantly employed to study the interactions between epithelial cells and the underlying stroma that are indicative of carcinogenesis. Cells may be exposed to acid, bile, and other relevant agents and studied over time to ascertain morphological changes, stromal invasiveness, and genetic changes consistent with oncogenesis. The disadvantage of having an inadequate stromal environment, lack of glandular differentiation, and increased phenotypic heterogeneity demonstrated by the BE cell lines limit its repertoire in providing conclusive results. Interpretation of results, therefore, requires consideration of the phenotypic changes in these cell lines, and it is pertinent to characterize these cells over time, so that they are as relevant to human models as possible. An alternative to organotypic culture is the use of denuded tracheal models. The goal of these models is to grow epithelial cells in the trachea that can be manipulated into transdifferentiating into BE. Genetic manipulation of donor mice through induction of sonic hedgehog (Shh) in the epithelium and bone morphogenetic protein 4 (Bmp4) in the stroma leads to increased expression of cytokeratins 8–18, which are markers of columnar epithelium, and Sox9 in the epithelium. Our laboratory at the Mayo Clinic has been successful in humanizing rat tracheas extracted from Sprague–Dawley rats that are capable of growing epithelial, stromal, and immune cells. These models hold promise for replicating the *in vivo* metaplastic and consequent oncogenic changes seen in BE, thus enabling us to better comprehend the complex pathophysiology of this condition.

Animal models that employ esophageojejunostomy-treated Sprague–Dawley rats and mice with magnet-induced fistulas to replicate the GERD–BE–EAC sequence are useful from a pharmacological point of view in terms of identifying the effects of anti-inflammatory medications on reducing inflammation and oxidative injury and studying the metaplastic *in vivo* changes in esophageal cells. However, anatomical differences between human and rodent foregut, such as the lack of submucosal glands and the presence of keratinized squamous esophageal epithelium in the latter, as opposed to stratified squamous epithelium in humans, make it difficult to accurately study the pathogenesis of BE in animal models. Alternate genetic approaches, such as overexpression of IL-1β or loss of p63, emphasize that the pro-inflammatory and developmental pathways can drive carcinogenesis and metaplasia. However, these models do not incorporate pathophysiological signals such as acid- and/or bile-reflux injury that may influence these, or multiple other genetic alterations that may cooperate to induce metaplasia and neoplasia. Canine animal models with surgical manipulation to induce columnar metaplasia and adenocarcinoma are not typically used, owing to ethical issues and the need for a prolonged duration of follow-up. With the aforementioned backdrop of strengths and weaknesses of various models, in the following text we will discuss the various pathways that have been identified through these models.

Bile acid refluxate triggers inflammation- and oxidative stress–induced injury in the esophageal squamous epithelium. The activation of certain pro-inflammatory pathways then induces genetic changes, cytokeratin modulation, and changes in mucin production that promote transdifferentiation of the epithelium. Immortalized human esophageal keratinocyte organotypic studies have shown that c-myc and CDX1 play key roles in the earliest transdifferentiation changes. Upregulation of pro-inflammatory NF-κB increases the expression of the Cdx1 and Cdx2 transcription factors, which trigger transdifferentiation of squamous epithelium into columnar-lined Barrett's epithelium. The Hedgehog pathway, which plays a key role in embryonic foregut development, is also upregulated

in the esophageal epithelium in response to bile injury. This ligand activation triggers BMP4 in the underlying stromal tissue, which then activates villin and SOX9, an intestinal cryptic transcription factor, resulting in induction of DMBT-1, which is the human homolog of the columnar cell factor hensin.[20] These findings were corroborated by *in vivo* mice studies that demonstrated upregulation of the same pathway upon induced reflux injury. BMP4 inactivation by its specific antagonist noggin has exhibited inhibition of the above-mentioned downstream cascade.[21]

During activation of these pro-inflammatory pathways, many cytokines are produced. One such cytokine is IL-1β, which activates downstream STAT3, which, during our preliminary investigations, was found to acetylate the *AKT1* promoter region. AKT1 is a serine–threonine protein kinase, and we found increased acetylation and activation during Barrett's carcinogenesis. IL-1β not only activates *AKT1*, but also increases the activity of p300, a histone acetylase that STAT3 employs in activating the promoter region of *AKT1*. IL-1β also inhibits the anti-inflammatory action of transcription factor KLF11, which is responsible for recruiting Sin3-HDAC to deacetylate *AKT1*. The opposing action of IL-1β–STAT3–AKT1 and the KLF11–Sin3-HDAC pathways implies that inflammation plays a key role in carcinogenesis. A study conducted by Quante and colleagues on transgenic mouse models further emphasized the roles IL-1β and IL-6 play in triggering transdifferentiation and carcinogenesis in normal squamous esophageal epithelium. A mouse line was established using the EBV–L2–IL-1 transgene that affects the esophagus and the forestomach mucosa. Mice treated with this transgene exhibited esophagitis and, without any additional manipulations, developed BE by 12 months and spontaneously progressed to adenocarcinoma with advancing age. Treatment with bile acids and nitrosamines accelerated the progression to carcinoma. This study highlights the role of bile acids and IL-1–induced upregulation of IL-6–dependent carcinogenetic changes in esophageal squamous epithelium. Our group also found that bile acids induce CREB- and AP-1–mediated COX-2 expression in cells and the formation of reactive oxygen radicals within esophageal cells that induce PI3K/AKT and ERK 1/2, a pathway that has been implicated in carcinogenesis. This pathophysiological response was demonstrated successfully in immortalized BE and adenocarcinoma cells, as well as mimicked in *in vivo* rat models.[22] We conducted an *in vitro* study on the effect of selective cyclooxgenase inhibition in Barrett's esophageal epithelium, utilizing primary epithelial and fibroblast cell cultures obtained from endoscopic biopsy specimens of established BE patients. COX-2 expression and activity were assessed using a reverse transcription polymerase chain reaction (RT-PCR) and PGE2 enzyme assay. Cell proliferation was estimated by Ki-67 staining. Esophageal epithelial and fibroblast cells were found to express COX-2 mRNA in high amounts, and PGE-2–expressing esophageal epithelial cells treated with the anti-inflammatory compound NS-398 were found to show a downregulation of COX-2 expression. This study highlights the prospective benefit from incorporating COX-2 inhibitors in the prevention of Barrett's carcinogenesis.[23] The results of this study were reaffirmed by another *in vivo* study conducted on esophageojejunostomy-treated rat models. These Sprague–Dawley rats were then randomized to receive treatment with the anti-inflammatory drugs sulindac and MF-tricyclic or a placebo. The animals were then assessed for BE, development of cancer, tumor burden, and the expression and activity of COX-2 enzymes. A definite risk reduction was observed in the development of BE with the use of COX-2 inhibitors.[24] These *in vitro* and *in vivo* studies, therefore, provide clear-cut evidence that anti-inflammatory medications may play significant roles in the prevention of Barrett's carcinogenesis. These concepts have given direction to several chemoprevention trials aimed at reducing the risk of developing adenocarcinoma. A randomized, double-blinded, placebo-controlled phase II trial on the effect of combination therapy involving aspirin and esomeprazole on tissue concentrations of PGE-2 was conducted, and the preliminary results were published in *Gastroenterology* in 2012. Although results showed a reduction in the tissue PGE-2 levels in patients with BE with or without dysplasia, further evaluation needs to be conducted in order to confirm these findings and determine their clinical significance.[25] There is also a progressive increase in expression of pro-inflammatory cytokines (IL-1, IL-8) and NF-κB during the metaplasia–dyplasia–adenocarcinoma sequence, and autocrine VEGF signaling increases Barrett's

epithelial cell proliferation through the PLCK1–PKC–ERK pathway. Through strategic chemopreventive measures, these pro-inflammatory factors may be targeted, and inhibition of VEGF signaling with sunitinib could be utilized in preventing or treating EAC in BE patients.[26,27]

Another molecular mechanism found to be of significance in the carcinogenetic pathway involves GLI1, a hedgehog-regulated transcription factor. GLI1 upregulates the transcriptional activity of the key cell cycle regulator CDK2. Overexpression of GLI1 increases CDK2 levels, thus promoting proliferative activity of cancer cells. We published the pathophysiological significance of this GLI1–CDK2 pathway in carcinogenesis and the effect of a combinatorial chemopreventive strategy involving ursodeoxycholic acid and aspirin on this pathway. We demonstrated that a statistically significant reduction in GLI1 expression is observed upon treatment of human BE-associated adenocarcinoma cell lines SKGT-4 and FLO-1 with these drugs. The findings from this *in vitro* study were then confirmed by *in vivo* studies conducted on esophagojejunostomy-treated rat models. These rats were surgically manipulated to induce GERD, BE, and, eventually, adenocarcinoma. Overexpression of GLI1 was also found to be present in these animal models, and could be effectively suppressed through treatment with ursodeoxycholic acid–aspirin combination therapy. This study emphasizes the potential use of this combinatorial therapy to reduce bile acid production implicated in inducing the primary insult to normal esophageal epithelium and later downregulating CDK2 expression through suppression of the GLI1-mediated oncogenic pathway.[28]

Chromosomal instability forms the basis of most human cancers, including esophageal cancer. The *CDKNA2* gene encodes for the p16 inhibitor, a cyclin D/cyclin-dependent kinase 4 regulator. Global hypomethylation and promoter hypermethylation of the *p16* promoter region leads to inactivation of *p16*, allowing unregulated cell proliferation to occur. These tumor-suppressive mechanisms are further compromised, owing to loss of heterozygosity of 9p21 at *p16* and inactivation of the tumor suppressor *p53* gene in BE. Loss of these important tumor-suppressive mechanisms in the presence of reflux-induced oxidative stress allows the accumulation of cells with chromosomal instability resulting from DNA damage. Typically, repeated exposure to oxidative stress leads to increased activity of glutathione peroxidase 3, which is an extracellular antioxidant in normal cells. However, in Barrett's columnar cells, repeated oxidative stress leads to progressive methylation of the gene coding for glutathione peroxidase. Silencing of this antioxidant-coding gene further aggravates oxidative injury and resultant dysplastic progression. Epigenetic dysregulation also appears to use the extracellular signaling glycoprotein ligand family Wnt to drive metaplastic and neoplastic changes. Wnt signaling is responsible for cell growth, differentiation, and motility. Silencing of the Wnt inhibitor *WIF1* through promoter hypermethylation can lead to increased cellular proliferation. Treatment with a demethylating agent like 5-AZA-2-deoxycytidine can restore WIF1 function and halt proliferation.[29] Epigenetic changes are therefore crucial in Barrett's carcinogenesis. MicroRNAs are short RNA fragments that are also involved in epigenetic regulation of genes involved in cellular processes. Downregulation of miR-143, miR-145, and miR-215 and upregulation of miR-196 have been observed in several studies.[30] Prediction analysis of miRNA targets show that they could regulate developmental and carcinogenetic signaling pathways such as TGF-β and Notch and inflammatory pathways like Toll-like receptor signaling. Genome-wide profiling of miRNA during neoplastic progression demonstrates that the expression of onco-miRs, such as miR-21, miR-25, and miR-223, and tumor suppressor miRNAs, including miR-205, miR-203, let-7c, and miR-133a, is altered from NE to EAC, suggesting that miRNA likely fine tunes epigenetic response during carcinogenesis in BE. The role of oxidant damage to DNA in the form of double-strand breaks on exposure to nitrosating species has also been implicated in triggering epigenetic changes that ultimately promote tumorigenesis.[31] Dietary modifications, through incorporation of polyphenols in the diet, can protect against this oxidant stress. Song *et al.* demonstrated, in preclinical studies, the effects of polyphenon E (poly E) on BE and EAC cell lines and the possible mechanisms through which it alters carcinogenetic changes.[22] Poly E acts through suppression of cyclin D1 protein expression. Cyclin D1 suppression, in turn, leads to dephosphorylation of the retinoblastoma protein in a dose-dependent manner. These changes cause G_1 phase cell cycle arrest. It was therefore found that poly E, through cyclin D1

inhibition, prevents the proliferation of transformed aerodigestive cells. The use of polyphenols as a potential chemopreventive and therapeutic strategy in reversing BE and arresting progression to cancer, therefore, seems plausible.[32] The option to use these agents to modify epigenetic targets, along with early detection of epigenetic changes, may assist in isolating highly susceptible BE patients and enrolling them in intensive surveillance, screening, and treatment. Incorporation of evolutionary biology and bioinformatics in devising novel methods of targeting these cancer mechanisms, in order for us to curb Barrett's carcinogenesis, may show promise in the future.

In conclusion, a comprehensive knowledge of the various models to help understand the disease mechanisms that drive intestinal metaplasia in the esophagus and its progression to adenocarcinoma is essential. These tools can help in devising effective strategies to halt the rapidly increasing incidence of EAC. The important caveat is that we need more robust translational research that links novel molecular mechanisms identified in preclinical models to human trials.

5. Which agents are most promising for the prevention of Barrett's dysplasia and cancer in the clinic now? Coxibs, PPIs?

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The answer is that we do not (yet) know. BE, a significant complication of GERD, is the single most important risk factor for EAC. The strong association between BE and chronic GERD suggests that abnormal esophageal acid exposure plays an important role in this condition. The progression of BE from specialized intestinal metaplasia to dysplasia and finally invasive carcinoma is incompletely understood, but increased and disordered proliferation is a key cellular event.[33] There are several factors that may promote dysplasia and cancer in BE. Reduced esophageal acid sensitivity in BE allows ongoing, poorly recognized, and untreated reflux. In *ex vivo* organ culture experiments, cell proliferation is increased after exposure to short pulses of acid, while proliferation is reduced in BE specimens taken from patients with esophageal acid exposure normalized by antisecretory therapy. In long-term clinical studies,

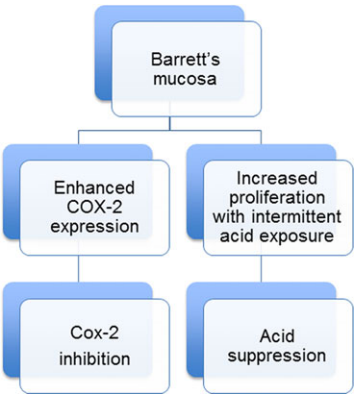

Figure 2. Potential pathways to Barrett's cancer chemoprevention.

consistent and profound intra-esophageal acid suppression with PPIs decreases cell proliferation and increases differentiation in BE, but the clinical importance of such favorable effects on these surrogate markers is not clear. Increased cyclooxygenase-2 (COX-2) expression and activity at baseline and after pulses of acid or bile salts have also been noted in *ex vivo* studies[33] (Fig. 2). In clinical practice, PPIs relieve symptoms and induce partial regression to squamous epithelium, but abnormal esophageal acid exposure and the risk for dysplasia or adenocarcinoma persists in many patients. The ability of PPIs to suppress acid profoundly and consistently may be critical in the long-term management of BE.[34]

Why use PPIs for BE? PPIs control reflux symptoms and heal mucosal damage, they prevent recurrent esophagitis and strictures, and they are known to regress the Barrett's metaplastic surface. In addition, PPIs reduce duodenogastroesophageal (bile) reflux and facilitate the recognition and regression of dysplasia. More recently, PPIs have been universally used as an adjuvant treatment to ablation modalities, such as radiofrequency ablation (RFA).[35] In a recent multicenter prospective cohort study of 540 patients with BE, the investigators collected information on medication use at each surveillance visit, which was cross-checked with pharmacy records. Patients also completed a questionnaire about their use of over-the-counter medication. Incident cases of HGD and EAC were identified during a median follow-up period of 5.2 years. Time-dependent Cox regression models were used to investigate the effect of acid suppression on the risk of neoplastic progression. Forty patients (7%) developed HGD or EAC during the follow-up

period. Use of histamine 2 receptor antagonists did not affect the incidence of neoplastic progression. However, use of PPIs at inclusion in the study or during the follow-up period reduced the risk of neoplastic progression (HR 0.41; 95% CI 0.18–0.93 and HR 0.21; 95% CI 0.07–0.66). Prolonged use of PPIs and good adherence were associated with an additional protective effect. The prevalence of esophagitis decreased during PPI use, but the length of BE was not affected.[36]

In a randomized trial of 58 BE patients using PPIs with or without rofecoxib (25 mg/day), only 28% of the coxib arm had decreased COX-2 expressions at 6 months. The addition of rofecoxib to PPI therapy did not affect cell-proliferation index in Barrett's cells after 6 months of therapy, but it reduced COX-2 and VEGF expression and increased cell apoptosis. Cell proliferation and dysplasia were not affected.[37] The potential chemopreventive effect of celecoxib in dysplastic BE was explored in a phase IIB multicenter randomized placebo-controlled trial. Celecoxib (200 mg twice daily) was given for 48 weeks but did not prevent progression to cancer. There were no significant differences in BE surface area, prostaglandin levels, COX-1/2 mRNA levels, or in methylation of tumor suppressor genes *p16*, *APC*, and *E-cadherin*.[38]

Experimental evidence has suggested that aspirin might reduce the risk of EAC. In a study of deaths due to cancer during and after randomized trials of daily aspirin versus control, performed originally for prevention of vascular events, there was a significant reduction in the 20-year risk of adenocarcinoma death (HR 0.66, 0.56–0.77, $P < 0.0001$). Benefit was unrelated to aspirin dose (75 mg upwards), sex, or smoking, but increased with age: the absolute reduction in 20-year risk of cancer death reaching 7.08% (2.42–11.74) at age 65 years and older.[39]

In conclusion, PPI therapy is a necessity in BE, mostly for symptoms and mucosal damage control, but the effective PPI dose is uncertain. Despite their early promise, coxibs are of no clinical value at this point. On the other hand, aspirin may be beneficial, but this has not yet been proven. A phase III, randomized study of aspirin and esomeprazole in chemoprevention in Barrett's metaplasia (AspECT) has as its primary outcome measure the conversion of BE to HGD or EAC. Its secondary outcome measure is all-cause mortality. The study started in March 2005 in the United Kingdom and it is estimated to be complete in March 2019.[40]

6. Statins in the chemoprevention and treatment of esophageal adenocarcinoma: data from epidemiological studies

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Introduction

The majority of patients with EAC present with advanced disease, and their overall prognosis remains poor despite recent advances in treatment. Endoscopic surveillance of BE used to detect, treat, and prevent progression from non-dysplastic BE to dysplasia or EAC has not led to observable reductions in cancer-related mortality. Novel and acceptable interventions to prevent and treat EAC are required. A considerable research focus has been placed on the potential of statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors) as both chemopreventive and adjuvant agents for many malignancies. While often yielding inconsistent and conflicting results in the majority of cancers, statin use appears consistently inversely associated with risk of HGD or EAC in patients with BE in a number of epidemiological studies. The following summarizes the existing literature that examines the potential of statins as chemopreventive and adjuvant agents against EAC.

Possible mechanisms

In addition to cholesterol production, the mevalonate pathway influences a number of other cellular processes, including cell cycle regulation and survival. *In vitro* studies have demonstrated that statins limit cellular proliferation and promote apoptosis in BE and EAC cell lines.[41] Statins reduce the production of downstream intermediates of the mevalonate pathway, such as farnesyl pyrophosphate, to reduce extracellular signal-related protein kinase and protein kinase B/Akt, which are responsible for cell survival and growth signal transduction. Statins also reduce, in a dose-dependent manner, intracellular adhesion molecule-1 (ICAM-1),[42] a critical adhesion molecule involved in transendothelial tumor cell migration, which promotes metastatic spread.

Epidemiological investigations of statins as chemopreventive agents in BE populations

We updated our previous meta-analysis[43] to examine the association between statin use and risk

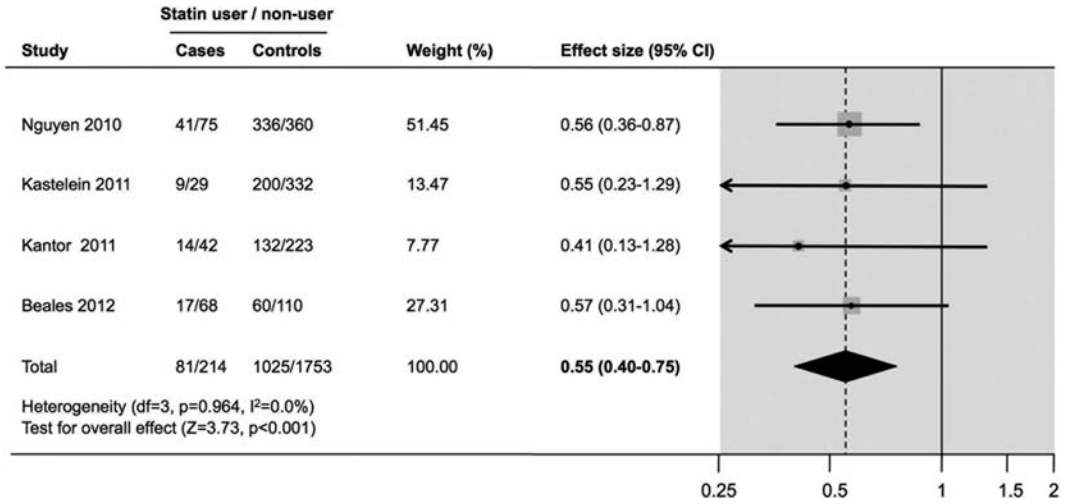

Figure 3. Fixed-effects meta-analysis of statin use and adjusted risk of HGD/EAC in patients with BE.

of progression of BE to HGD or EAC. Using the same search terms and methodology (searched until July 2013), we identified and extracted data from four observational studies.[44–47] STATA version 11 (StataCorp LP, College Station, TX) was used to calculate the pooled effect size (ES) using the inverse-variance method, fixed-effects model. We identified two cohort and two case-control studies including 2048 patients in total: 295 patients with HGD/EAC, and 1753 patients with BE who did not progress. Prior statin use was consistently associated with a significant reduction in risk of progression in both adjusted (adjusted ES 0.55, 95% CI 0.40–0.75, $P < 0.001$, $I^2 = 0\%$) and unadjusted analyses (unadjusted ES 0.56, 95% CI 0.47–0.66, $P < 0.001$, $I^2 = 0\%$; Fig. 3). Significant dose[44] and duration[44,47] responses were reported in individual included studies, supporting a causal inverse association. Not all studies adjusted for body-mass index or smoking, which may lead to an underestimate of the effect size for statin use and risk of malignant progression. While these results are encouraging, residual confounding and healthy user bias are possible, and randomized controlled trials (RCTs) are warranted before statins can be advocated as chemopreventive agents.

Epidemiological investigations of statins in esophageal cancer populations

One large observational study in the entire Danish population examined the effect of pre-diagnosis statin use on cancer-specific survival in 295,925 patients diagnosed with cancer at any site.[48] In a sub-analysis of 4398 cases of esophageal cancer (including any histological subtype), statin use was associated with improved cancer-specific survival (HR 0.81, 95% CI 0.69–0.95). These analyses were adjusted for age at diagnosis, year of birth, sex, cancer stage, radiotherapy, chemotherapy, prior diagnosis of cardiovascular disease or diabetes, and highest level of education. It is not clear here whether statins exert differential effects on survival according to the histological subtype of esophageal cancer. There were no significant dose–response effects on overall cancer mortality; however, the effect of dose–response on esophageal cancer mortality was not specifically presented. Concomitant use of other medication, such as aspirin, that could feasibly affect survival and is likely to be strongly associated with statin use were not included in multivariable analyses. Healthy-user bias is an important consideration whereby behaviors associated with statin use, either on the part of the patient or health professional, could be associated with improved survival. Again, RCTs are required to clarify whether statins are efficacious as adjuvant treatment in patients with EAC.

Conclusion

Statins are among the most widely prescribed medications worldwide and have a highly favorable side-effect profile. Their potential as chemopreventive and adjuvant agents against EAC, as suggested by the experimental and epidemiological literature, deserves further study in the form of RCTs.

7. How does one identify promising biomarkers for esophageal cancer?

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Chemoradiation is commonly used on its own, before or after surgical treatment of esophageal cancer. Many patients do not benefit from cytotoxic agents and/or radiation, but still experience their toxic effects. Biomarkers serve as a means of enhancing the efficacy of treatments. The identification and functional analysis of tumor-specific genetic alterations has disclosed a number of potential biomarkers. The crucial steps of translating potential biomarkers into clinical use are summarized in the present report. Specifically, the use of the biomarker TP53 is addressed. The central role of the *p53* gene in the control of cell growth, DNA repair, and apoptosis makes it a potentially powerful biomarker and a unique target for cancer therapy. Analogous to investigational drugs, the clinical value of potential biomarkers is best demonstrated by clinical trials. On comparing clinical trials, we identified three phases of biomarker studies designed to obtain answers to specific biomarker questions (Table 1).

Phase I biomarker study

In a clinical phase I trial, researchers test an investigational drug with respect to its safety and dosage. A phase I biomarker study addresses the robustness of a biomarker. This means that a potential biomarker is tested with respect to its prevalence and specificity. The testing can be done retrospectively using archived material. A clinically useful biomarker should have a high prevalence and high specificity.

TP53 gene mutations, the most common genetic alterations associated with human cancer, are detected in approximately 50% of human cancers. Data concerning more than 28,000 somatic mutations and 750 germline mutations of the TP53 gene, obtained from peer-reviewed publications, have been included in the IARC *TP53* database R17 (http://p53.iarc.fr/, p53.free.fr).[49] Thus, the robustness of the biomarker *TP53* has been amply proven.

Phase II biomarker study

The main subject of phase II biomarker studies is reproducibility. This is again somewhat comparable to clinical phase II trials, which address the safety and effectiveness of a drug. Phase II biomarker studies address the sensitivity and specificity of a marker test as well as the type of biomarker.

Sensitivity and specificity of the marker test

Evidence of high sensitivity and specificity is crucial for clinical application of a marker test. A number of conditions may affect the performance of a test. Further aspects to be addressed in phase II biomarker studies include standardization of the test and standardized reporting of test results (including scoring). Various assays have been used for *TP53*.[50,51] The absence of a standardized and sensitive marker test brought about inconsistent results and fostered confusion about the true prevalence and value of *TP53*. Currently, the first standardized gene-specific sequencing protocol for *TP53* has been successfully evaluated in three clinical studies encompassing more than 900 patients (MARK53GSS Kit, Mark53 Ltd.,Vienna, Austria). This protocol is the first to do justice to the numerous pitfalls arising from the characteristics of *p53* and its mutations, as well as arising from common technical pitfalls such as FFPE (formalin-fixed, paraffin-embedded) material, low copy numbers of tumor DNA (biopsies), or the presence of normal cells in tumor material.

Table 1. Phases of biomarker studies

Phase	Report	Goal	Study design
Phase I	Robustness	Hypothesis prevalence specificity	Retrospective
Phase II	Reproducibility	Marker-test sensitivity specificity	Prospective
		Marker-type prognostic predictive	Retrospective
Phase III	Relevance	Confirm magnitude of effect	Prospective randomized
		Compare with standard treatment	

Determination of the type of biomarker

The nature or type of biomarker also has to be addressed in phase II (i.e., whether it is prognostic or predictive). Knowledge of the marker type is crucial for planning and interpreting the results of clinical studies involving biomarkers and is also a prerequisite for phase III biomarker studies. Prognostic and predictive markers call for different patient-selection criteria and are employed to answer different questions. A prognostic marker predicts freedom from disease or relapse, whereas a predictive marker predicts response to a treatment or treatment failure. All of these conditions (freedom from disease, relapse, response to treatment, and treatment failure) predict survival; if not, the disease or the treatment is of no clinical relevance. Thus, survival is an appropriate endpoint for studies addressing the type of biomarker.

To determine the type of marker, a biomarker must be analyzed in a homogenous cohort of cancer patients treated with and without chemo- or radiotherapy. If a marker affects survival only in the presence of chemo/radiotherapy but not in its absence, it is a predictive marker. A marker that affects survival in the untreated cohort is a prognostic marker. Despite a vast body of literature, we do not know with certainty whether *TP53* is predictive or prognostic, although the marker type can be easily determined and the information is crucial. Appropriate studies in this regard have yet to be performed for *TP53*.[52,53]

While drug assessment in clinical phase II trials must be performed prospectively, the prospective design is not obligatory for assessing the type of biomarker. This is an important difference because untreated arms are rare in current clinical cancer research.

Phase III biomarker trials

Phase III biomarker trials address the clinical relevance of a biomarker. The magnitude of effect of the biomarker is assessed. The trial design must be randomized and must include a prospective application of the biomarker assay. The individual design is highly dependent on the type of biomarker. Thus, determination of the type of a biomarker is an important phase II biomarker task. Analogous to clinical trials, the final evaluation must include comparison with standard treatment. An ongoing prospective randomized trial (Pancho trial: NCT00525200, clinicalTrials.gov; p53.at), is the first to evaluate the magnitude of the predictive effect of the biomarker *TP53* in esophageal cancer.[54] The Pancho trial (p53-adapted neoadjuvant chemotherapy for operable esophageal cancer) is being conducted by the p53research® group; 168 patients with primarily resectable esophageal cancer were recruited from 2007 to 2012 at 13 centers in Austria. Recruitment of patients has been concluded, and the results of the study are awaited.

8. The future: opportunities to reduce the burden of esophageal cancer and its precursors

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Current issues in esophageal neoplasia management

Early detection of esophageal cancers is critical, as 5-year survival rates are strongly associated with stage of disease at presentation: 38% for localized disease, 20% for regional disease, and just 3% for distant disease. Unfortunately, most cases are clinically diagnosed at advanced stages. Therefore, screening and early detection of precursor lesions may significantly contribute to reducing cancer-associated mortality.

BE is the neoplastic precursor to EAC and proceeds through low- and high-grade glandular dysplasia before culminating in adenocarcinoma. Wide variation has been reported in the progression rates of BE to EAC, with recent studies [55–57] suggesting lower progression rates (0.12–0.33% per year) than earlier estimates of 0.5% per year.[58] ESCC unfolds through a series of dysplastic precursor lesions (i.e., esophageal squamous dysplasia (ESD)) associated with 3-(mild dysplasia) to 30-fold (severe dysplasia) risk for progression to ESCC.[59] While these precursor lesions may serve as targets for both screening and chemoprevention, randomized controlled trial data supporting the effectiveness of either approach are lacking in both EAC and ESCC. Because no effective screening strategy exists for either cancer, case finding is dependent upon symptomatic presentation, often resulting in late diagnoses. In the case of EAC, patients found to have BE typically enter an endoscopic surveillance program in which the frequency of endoscopic examination is determined by the degree of dysplasia.[60] But again, clinical trial data supporting this regimen are lacking, so it is

based on expert opinion. In the case of ESD and ESCC, population-based endoscopic screening, although potentially warranted by its prevalence in the high-risk settings of the "esophageal cancer belt" (from northern China through Iran and parts of Africa), is likely not feasible. Unfortunately, dysplasia remains the only validated risk biomarker, though its inter-observer accuracy and reliability is poor.

Given the substantial clinical needs of high-risk groups, as well as the current limitations of screening and surveillance strategies, surgical and endoscopic ablative therapies form the basis of current clinical management for patients with progressive HGD. RFA is a commonly employed ablative technique for the management of Barrett's dysplasia, having been demonstrated effective in a randomized controlled trial.[61] Chemoprevention offers another experimental option to reduce esophageal cancer morbidity and mortality associated with these precancerous conditions. However, only photodynamic therapy with photofrin is approved by the FDA as a chemoprevention-like agent for risk reduction in those with HGD. Promising preliminary data exists around many agents, including aspirin and NSAIDs, eflornithine, PPIs, statins, and various dietary agents, but none have been translated effectively into the clinic.

Roadmap to the future

A method to risk stratify asymptomatic individuals to reduce those needing endoscopic screening and/or the use of a non-endoscopic method in conjunction with a screening biomarker, as demonstrated in a 2010 study by Kadri *et al.*,[62] could significantly enhance the prevention and early detection of esophageal cancer, as could biomarkers predictive of the progressive or responsive nature of neoplastic precursors. Big data and massive data analytics in conjunction collect and integrate all available information about a patient and, crucially, all other patients like him/her, now or historically, to uncover meaningful patterns within the clinical data derived from their care. Leveraging big data and massive data analytics provides an opportunity to identify factors associated with the development, progression, response to therapy, and prognosis of esophageal cancer and its neoplastic precursors, drawing from current patients and their treatment. Of course, any biomarkers identified through such an observational approach would only suggest promising associations; there remains a need for them to be appropriately tested and validated in randomized controlled trials.

Additionally, recommendations for chemopreventive agents for use either in a primary prevention setting in the general population or in an adjuvant setting in those at high risk undergoing ablative therapies could also lead to significant reductions in morbidity and mortality from esophageal cancer. Such recommendations will be based upon well-designed phase III randomized trials, such as the aspirin and esomeprazole chemoprevention in Barrett's metaplasia (AspECT) trial. The AspECT trial is a multicenter, phase III, randomized, open-label trial testing the ability of the PPI esomeprazole with and without aspirin to prevent esophageal cancer in patients with Barrett's metaplasia.[40] Although results are not expected for several more years, the trial serves as an example of what is required to advance the use of chemopreventive agents in esophageal cancer risk management.

Advanced imaging technologies also underlie future enhancements in the screening and early detection of esophageal precursor lesions and cancers. A number of endoscopic technologies are being tested or are in development, including autofluorescence imaging, confocal laser microscopy, endocytoscopy, and molecular imaging. Molecular imaging allows for the detection of molecular changes in cells that may signal the development of cancer. Bird-Lieberman *et al.* recently demonstrated the feasibility of this approach in BE using fluorescent lectins.[63] Although still in development, molecular imaging could lead to a substantially refined and more precise approach to the early detection of esophageal neoplastic lesions and cancers.

Potential management of esophageal cancer risk in 2030

Primary prevention in the future may endorse the adoption of lifestyle modifications involving tobacco and alcohol avoidance, proper diet, weight management, and physical activity, and additionally incorporate chemopreventive agents to modulate risks. In the clinic, we may see the use of integrative risk modeling based on host genomic factors, biomarkers, clinical data, and sociodemographics. The development of novel non-endoscopic

screening methods may greatly facilitate early detection efforts, particularly for ESD/ESCC in high-risk regions. Secondary prevention will evolve from endoscopic therapy based on histology to therapy guided by predictive/therapeutic response biomarkers, and may include chemoprevention as an adjuvant to ablation; more precise molecularly targeted therapies may eventually replace ablative techniques. In summary, management of esophageal cancer risk in the future is likely to rely upon a combination of reinvigorated public health efforts to advance the understanding of risk factors and the adoption of healthy lifestyles in the general population and more precise estimates of individualized patient risk and interventions in the clinic.

Conflicts of interest

The authors declare no conflicts of interest.

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