MINIREVIEW

BJC

British Journal of Cancer (2016) 115, 403–410 | doi: 10.1038/bjc.2016.219

Keywords: Barrett's oesophagus; high-grade dysplasia; biomarkers; oesophageal adenocarcinoma; genome instability; aneuploidy; p53; SMAD4

Genetic progression of Barrett's oesophagus to oesophageal adenocarcinoma

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Barrett's oesophagus (BE) is the premalignant condition associated with the development of oesophageal adenocarcinoma (OAC). Diagnostically, p53 immunohistochemistry remains the only biomarker recommended clinically to aid histopathological diagnosis. The emerging mutational profile of BE is one of highly heterogeneous lesions at the genomic level with many mutations already occurring in non-dysplastic tissue. As well as point mutations, larger scale copy-number changes appear to have a key role in the progression to OAC and clinically applicable assays for the reliable detection of aneuploidy will be important to incorporate into future clinical management of patients. For some patients, the transition to malignancy may occur rapidly through a genome-doubling event or chromosomal catastrophe, termed chromothripsis, and detecting these patients may prove especially difficult. Given the heterogeneous nature of this disease, sampling methods to overcome inherent bias from endoscopic biopsies coupled with the development of more objective biomarkers than the current reliance on histopathology will be required for risk stratification. The aim of this approach will be to spare low-risk patients unnecessary procedures, as well as to provide endoscopic therapy to the patients at highest risk, thereby avoiding the burden of incurable metastatic disease.

There are two main subtypes of oesophageal cancer, squamous cell carcinoma and oesophageal adenocarcinoma (OAC), that are distinct from one another both in terms of the cell of origin and from an epidemiological perspective. OAC is the more common form in the West and showed a three-fold increase in incidence since 1971 in England and Wales (Lepage *et al*, 2008). The susceptibility of OAC has been linked to a history of chronic and severe reflux of acid and bile through development of the pre-cancerous condition Barrett's oesophagus (BE) (Lagergren *et al*, 1999). Other risk factors include Caucasian ethnicity, male sex, obesity (both in relation to a propensity for reflux and as an independent risk factor) and smoking. The survival rate for OAC remains poor, predominantly due to late clinical presentation with advanced disease.

Upper gastrointestinal endoscopy combined with biopsy sampling of the distal oesophagus remains the current gold standard for diagnosing BE. According to the most recent British Society of Gastroenterology (BSG) guidelines (Fitzgerald *et al*, 2014), BE is characterised by the replacement of normal squamous epithelium of the distal oesophagus by columnar epithelium that is visible endoscopically and confirmed histologically. The guidelines of the American Gastroenterological Association (Spechler *et al*, 2011) require the presence of intestinal metaplasia (IM) in order to diagnose Barrett's. This is due to the body of research evidence that BE with IM is biologically more unstable than columnar epithelium without IM and, therefore, more likely to progress towards dysplastic or neoplastic lesions (Bhat *et al*, 2011). However, there is always the concern that IM may be missed due to sampling bias. For these reasons, the BSG guidelines do not require IM for Barrett's diagnosis but suggest that its presence or absence should be taken into consideration for patient management in terms of frequency of follow-up endoscopies (Figure 1) (Fitzgerald *et al*, 2014).

Overall, BE confers a low absolute risk of progression to OAC of 0.2–0.7% per patient per year (Hvid-Jensen *et al*, 2011; Desai *et al*, 2012). The risk of progression to cancer, however, increases with the diagnosis of epithelial dysplasia. Duits *et al* have reported an incidence rate of high-grade dysplasia (HGD) or invasive cancer of 9.1–13.4% per patient per year for patients confirmed to have low-grade dysplasia (LGD) by expert consensus; in contrast with 0.5–0.6% in patients who had no dysplasia at their initial biopsy (Curvers *et al*, 2010; Duits *et al*, 2015). However, the progression rate increases substantially to 25% when HGD is present (Kastelein *et al*, 2015).

National guidelines for the ongoing management of BE in the UK and the United States recommend repeat endoscopies in regular surveillance intervals (Figure 1) (Spechler *et al*, 2011;

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Received 22 April 2016; revised 8 June 2016; accepted 14 June 2016; published online 21 July 2016

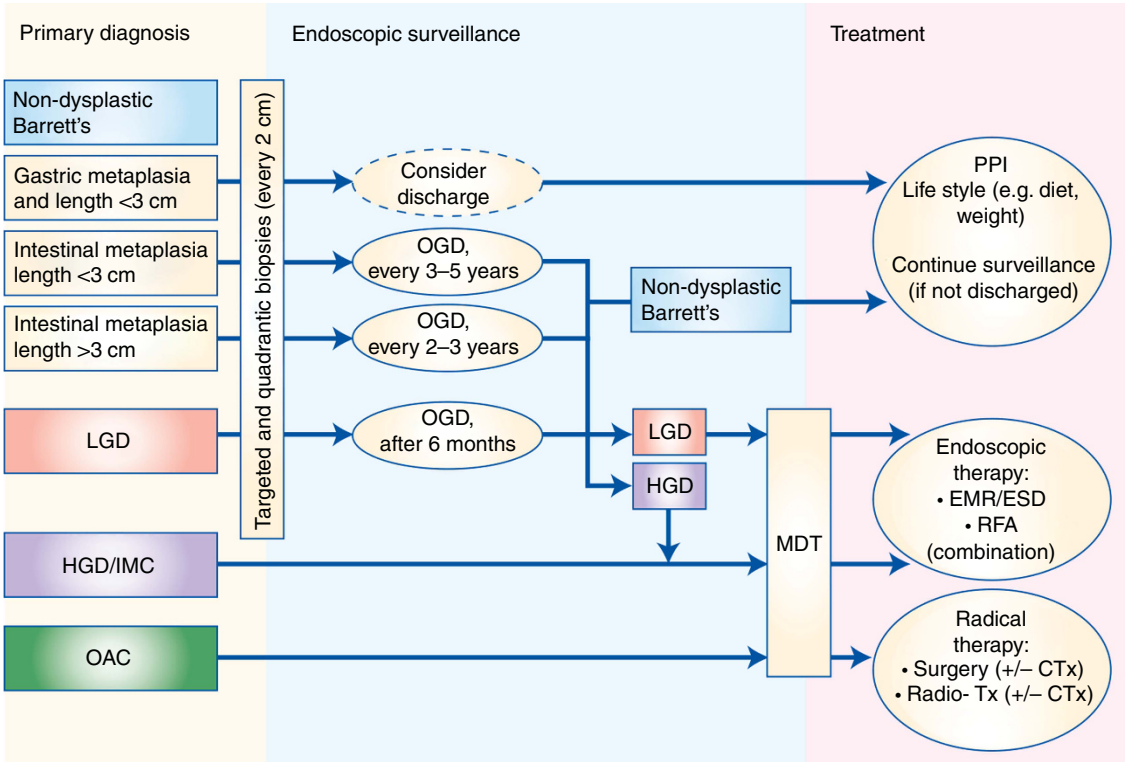

Figure 1. Algorithm for the clinical management of patients with diagnosis of Barrett's oesophagus. Displayed is a summary of the currently recommended algorithm for surveillance and treatment of patients with Barrett's oesophagus according to the latest BSG guidelines. Patients with non-dysplastic Barrett's oesophagus should be included in endoscopic surveillance programmes at clearly defined intervals, apart from patients with short-segment Barrett's and only gastric metaplasia, which can be considered for discharge from surveillance. Patients with HGD and more advanced lesions should be discussed for therapeutic intervention at the local MDT. Patients with LGD need short-term follow up for confirmation. If the degree of dysplasia is confirmed by two independent pathologist treatment can also be discussed at the local MDT. EMR, endoscopic mucosal resection; ESD, endoscopic submucosal dissection; HGD, high-grade dysplasia; LGD, low-grade dysplasia; MDT, multidisciplinary team meeting; OAC, oesophageal adenocarcinoma; RFA, radio frequency ablation.

Fitzgerald *et al*, 2014). Surveillance aims to increase the proportion of patients in which neoplastic lesions are detected at early stages (HGD or intramucosal carcinoma) so that curative, endoscopic treatment can be given. Over the years, there has been contradictory evidence as to the benefits of surveillance. In a study reflecting everyday clinical practice, surveillance was not found to be associated with significantly reduced risk of death from OAC (Corley *et al*, 2013). On the other hand, a meta-analysis of 51 studies including more than 11 000 patients demonstrated that endoscopic surveillance of patients with non-dysplastic BE increases the likelihood for early detection of neoplastic lesions and therefore reduces mortality by more than 61% (mortality risk 0.386; 95% confidence interval (CI): 0.242–0.617) (Qiao *et al*, 2015). These data were supported by the results of a prospective multicentre cohort study from The Netherlands (Kastelein *et al*, 2016). It is, therefore, recommended that in all patients with BE, targeted biopsies should be taken from visible lesions suspicious for dysplastic changes of the mucosa, as well as four 'random' quadrantic biopsies at 2 cm intervals over the entire extent of the Barrett's segment – the so-called Seattle protocol (Levine *et al*, 2000).

Endoscopic treatment entails mucosal resection of any visible lesion, followed by ablation of the entire Barrett's segment, which can be achieved using several methods including radio frequency ablation and argon plasma coagulation. This multi-modal approach has been demonstrated to be both effective and safe (Shaheen *et al*, 2009; Haidry *et al*, 2013). Whereas in the past, treatment was reserved for patients with HGD or intramucosal carcinoma, on the basis of new randomised controlled trial evidence treatment is now being offered to patients with LGD. It must be confirmed by two independent pathologists, given the subjectivity of this diagnosis and to avoid overtreatment in benign disease (Phoa *et al*, 2014). Patients diagnosed with invasive disease extending beyond the mucosa should be treated according to best practice guidelines, which generally involves peri-operative systemic chemotherapy (and/or radiotherapy) followed by surgery if it is deemed to be curative.

GENOMIC LANDSCAPE OF OAC

In order to understand the molecular genetic progression from BE to OAC, it is helpful to consider the genomic landscape of invasive disease. The genomic profile emerging from sequencing studies as part of the International Cancer Genome Consortium and The Cancer Genome Atlas is one of a highly mutated cancer with a mutation burden of around 10 single-nucleotide variations (SNVs) per megabase (Ross-Innes *et al*, 2015a). This rate is approaching as seen in those cancers with a well-defined carcinogen such as melanoma and lung cancer. Furthermore, it is a very heterogeneous disease with only a small number of genes that are recurrently mutated across multiple cases which are thus likely to be causative (so-called driver mutations). These recurrently mutated genes include tumour suppressors like *TP53* and *SMAD4*, which had previously been identified as key in this disease, as well as *MYO18B, SEMA5A, ARID1A* and other members of the SWI/SNF chromatin remodelling complex (Dulak *et al*, 2013; Weaver *et al*, 2014). The lack of driver events in oncogenes is a particular challenge for identifying actionable targets to add to classical chemotherapy agents for molecular-targeted therapy.

The analysis of the pattern of base substitutions together with the exact base either side enables patterns or signatures to emerge. This analysis has aided identification of the causative mutagens, such as a signature associated with exposure to ultraviolet light in melanomas (Alexandrov *et al*, 2013). In OAC, a common mutational signature of T : A > G : C transversions in a CTT setting has been described, and it has been suggested that this could be a mutation pattern caused by acid exposure in the context of gastroesophageal reflux although this remains to be proven (Dulak *et al*, 2013; Nones *et al*, 2014; Weaver *et al*, 2014).

As well as singe-base substitutions Nones *et al* categorised 22 tumour samples based on larger chromosomal rearrangements termed structural variants (SV) into three classes: unstable genomes with $\geqslant$450 SVs present across the genome ($n=6$), scattered alterations where $<$450 events were evenly distributed across the genome ($n=2$) and complex localised changes where SVs cluster only in a single or a few chromosomes ($n=14$) (Nones *et al*, 2014). The latter category could be explained by chromothripsis, a phenomenon of chromosomal shattering due to errors in chromosomal segregation during mitosis. So-called breakage-fusion-bridge (BFB) events define a further category of large-scale re-arrangements involving telomeric loss, chromosomal fusion and disrupted separation during anaphase. In OAC, a number of oncogenes were found to be amplified as a result of chromothripsis and BFB events including the oncogene *myc* (Nones *et al*, 2014). Such chromothripsis, or catastrophic events, occurring in a proportion of patients has a bearing on how we understand the evolution of OAC, which had hitherto been thought to be a gradual process.

GENOMIC LANDSCAPE OF BARRETT'S AND PROGRESSION TO DYSPLASIA AND OAC

The overall mutation rate in non-dysplastic Barrett's is around 5.4–6.8 SNVs per megabase which, while being lower than in OAC, is higher than reported for many other invasive cancers including multiple myeloma and breast cancer (Stachler *et al*, 2015; Ross-Innes *et al*, 2015a). Stachler *et al* observed a significant difference in the mutation burden between non-dysplastic and dysplastic Barrett's when examining coding (exonic) mutations, which was not observed to the same degree by Ross-Innes *et al* where the pathology grade correlated poorly with the mutation rate observed across the entire genome (whole-genome sequencing). These findings underscore the difficulties in providing a histopathological grade that is a phenotypic readout from a complex genetic architecture, which becomes abnormal early on in the pathogenesis of this disease.

In terms of understanding the predilection of mutations for specific genes we have known for some time from candidate-gene studies that loss of the tumour suppressor p16 occurs commonly regardless of progression status, in comparison with p53 loss, which tends to occur later in the progression sequence (Reid *et al*, 2001; Leedham *et al*, 2008). The extent to which mutations can occur across a plethora of genes involved in cancer, even in patients with non-dysplastic Barrett's, has become apparent since the advent of genome-wide sequencing data. In one such study, whole-genome sequencing data in OAC was used to derive a custom panel of 26 genes and patients with stable, non-dysplastic Barrett's with many years of follow up (66 samples) were compared with cases with HGD and OAC (43 and 90 samples, respectively). They showed that similar mutation frequencies were observed across the disease states apart from the tumour suppressor genes *TP53* and *SMAD4*. *TP53* was recurrently mutated in HGD (72%) and OAC (69%) but only in one sample of non-dysplastic Barrett's in samples from patients who never progressed to HGD or OAC ($P < 0.0001$). *SMAD4* was mutated at a lower frequency (13%) but was only found at the stage of invasive cancer ($P=0.0061$). Based on these observations, Weaver *et al* proposed that from the panel of genes examined, mutations in *TP53* and *SMAD4* mark the boundaries between non-dysplastic and dysplastic BE, and invasive cancer, respectively (Figure 2) (Weaver *et al*, 2014). Other sequencing studies observe p53 mutations in non-dysplastic tissue adjacent to cancer (Streppel *et al*, 2014; Stachler *et al*, 2015). Immunohistochemistry studies following patients over time also find aberrant p53 expression in non-dysplastic tissue in a low percentage of BE patients: 7% of non-progressors and 18% of progressors (Kastelein *et al*, 2013). Clinically, p53 is recognised as an adjunct to dysplasia diagnosis (Fitzgerald *et al*, 2014).

In a smaller study using the AmpliSeq Cancer Hotspot panel (which is a generic cancer panel), no mutations were seen at the baseline biopsies for the non-progressors (Del Portillo *et al*, 2015). This is likely due to the use of archival, formalin-fixed paraffin-embedded (FFPE) material and the difference in the respective gene panels as there were only three overlapping genes: *TP53, SMAD4* and *CDKN2A* (Weaver *et al*, 2014). It is surprising that in the Del Portillo study *CDKN2A* wasn't found to be mutated in non-progressors, as this has previously been identified in a number of studies as an early event (Reid *et al*, 2001; Galipeau *et al*, 2007).

In addition to point mutations, chromosomal copy-number changes, called aneuploidy as well as gene-centric focal gains and deletions, have an important role in the progression to OAC (Dulak *et al*, 2012). Genome-wide sequencing data demonstrates that copy-number profiles of the tumour harbour significantly more gains and losses than the adjacent Barrett's (Ross-Innes *et al*, 2015a). Li *et al* previously investigated copy number using SNP arrays on biopsies from the index endoscopy (i.e. at first diagnosis of BE) and the penultimate endoscopy from OAC diagnosis for progressors. These were compared with similar time points for non-progressors (Li *et al*, 2014). While there were some focal changes seen in both patient cohorts, the copy-number profile of non-progressors remained relatively quiet over time, whereas the percentage of the genome harbouring somatic chromosomal alterations in progressor patients increased rapidly from 0 and 50% at baseline to approaching 100% within 48 months of the cancer diagnosis. These copy-number changes included genome doubling in some cases and increased genetic diversity (Li *et al*, 2014). The same authors have proposed a panel of 29 specific chromosomal alterations to predict the risk of BE progression (area under receiver operator curve = 0.94) (Li *et al*, 2015). The samples from these retrospective studies were not histologically annotated; therefore, the relationship between these aberrations and the histological grade of dysplasia cannot be determined. The panel was tested in six OAC samples but has yet to be validated in an independent Barrett's cohort.

Clonal evolution. Inevitably, many of the studies in BE have relied on limited endoscopic biopsies and therefore the number of clones and their spatial relationship has not been forthcoming. Maley *et al* identified clones by differences in flow cytometric DNA content, pattern of loss of heterozygosity (LOH) as well as point mutations in *CDKN2A* (p16) and *TP53* from multiple samples taken from within the same patient (Maley *et al*, 2006). They found that genetic diversity between the samples was strongly predictive of neoplastic progression of the Barrett's mucosa, and this was corroborated in 2010 in a follow-up study by the same group (Merlo *et al*, 2010; Li *et al*, 2014).

Maley *et al* proposed that a founder clone containing a p16 mutation providing a growth advantage to these cells leading to a clonal selective sweep across the Barrett's segment. Subsequent generation of dysplasia occurred via further clonal selective sweeps with loss of additional tumour suppressor genes conferring a selective advantage (Maley *et al*, 2006). Leedham *et al* in 2008 investigated regions of LOH as well as p16 and p53 point mutations on an individual crypt level. Their results suggested that

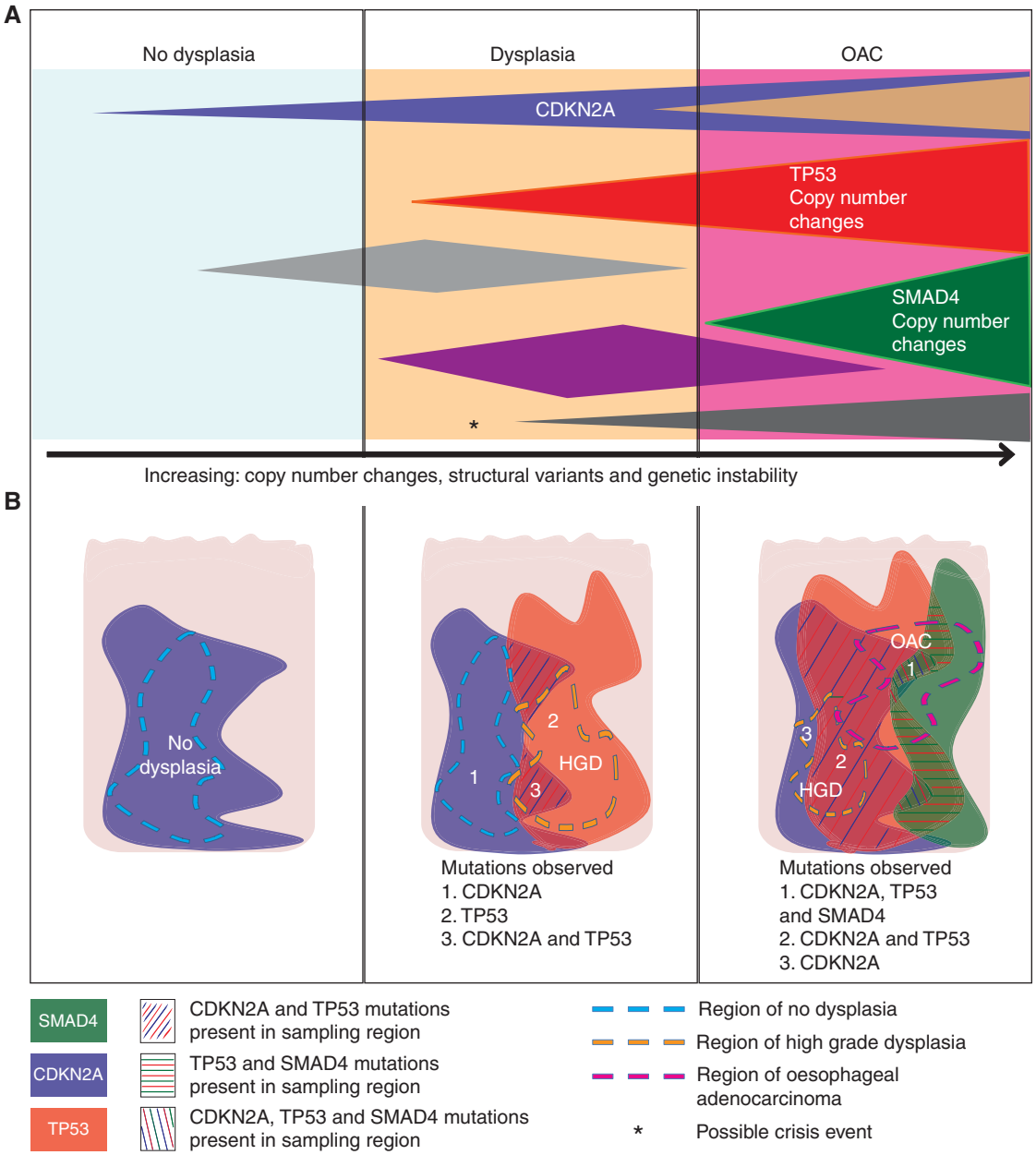

Figure 2. Genetic events in the progression of Barrett's oesophagus. (**A**) Illustration of progression to oesophageal adenocarcinoma (OAC) from multiple clones with accumulation of mutations, with a predominance of tumour suppressor genes over time. CDKN2A loss of function is shown as an early event, p53 mutation likely to mark the boundary to dysplasia and SMAD4 mutations seen uniquely in the cancer. Copy-number changes, structural variants and genetic instability increase over time. A crisis event (denoted by *) may occur to promote rapid genomic instability and progression to cancer. Neutral clones may regress over time (e.g., grey and purple clones). (**B**) A diagram to show issues with sampling bias in a segment of Barrett's over time. Regions of the Barrett's lesion harbouring example mutations are highlighted. Heterogeneity within an individual segment of Barrett's means that endoscopic samples may be from tissue containing all, some or none of the mutations present in the entire lesion. No dysplasia is denoted by the blue dashed line and high-grade dysplasia (HGD) or oesophageal adenocarcinoma (OAC) in orange and pink, respectively. Even in regions described histologically as HGD and OAC, different combinations of mutations may be seen depending on sampling. For example, in panel 2, sequencing of sample 1 would show a CDKN2A mutation, sample 2 a p53 mutation and sample 3 would exhibit both mutations.

heterogeneity in Barrett's lesions arose from multiple independent clones that evolved separately, resulting in a polyclonal mosaic of selective sweeps (Leedham *et al*, 2008). Ross-Innes *et al* assessed 1443 SNVs in 73 samples from a single patient's 10 cm BE segment, which had been collected over a period of 3 years (Ross-Innes *et al*, 2015a). In the non-dysplastic samples, six clones were identified with an initial clonal sweep but with just three SNVs common to all samples. These were three stochastic variants and not in known driver genes. Furthermore, dysplasia was derived from six distinct clones that did not correspond strictly to the dysplasia map generated by histopathological analysis (Ross-Innes *et al*, 2015a). Taken together, the polyclonal and thus heterogeneous nature of Barrett's explains the wide spectrum of the degree of mutational overlap between adjacent BE and OAC samples (Ross-Innes *et al*, 2015a). It should also be remembered that genetic mutations found in non-dysplastic Barrett's sampled adjacent to a cancer is not comparable to those found in non-dysplastic Barrett's in a patient who never progressed. This explains apparently conflicting evidence of the mutation spectrum observed between studies.

The prevailing view for the clonal evolution of OAC is that it occurs gradually through sequential loss of tumour suppressors culminating in loss of *TP53* and cancer development. However, the whole-genome data suggests that in some cases *TP53* mutation can lead to more rapid progression to cancer via chromosomal catastrophe (chromothripsis) or genome doubling and genetic instability (Figure 2) (Nones *et al*, 2014; Stachler *et al*, 2015).

Microenvironment. As well as characterising the epithelial cells of the tumour, it is also important to understand the contribution of interactions between different cellular components of the direct tumour microenvironment (He *et al*, 2013). Mechanisms that lead to an impairment of DNA damage repair mechanisms in the tumour can show a field effect on the surrounding mucosa, which is facilitated by the adjacent inflammatory processes (He *et al*, 2013). This is partly regulated by an increased population of regulatory T cells that can occur at the stage of reflux-related changes of the distal oesophagus, as well as by the activation of myeloid dendritic cells (Somja *et al*, 2013). The resulting cytokine milieu supports epithelial mesenchymal transition in the mucosa of the distal oesophagus, a process that is further maintained by cancer-associated fibroblasts and activation of PI3-Kinase/Akt signalling (Underwood *et al*, 2015). The cell-cell interaction network is also influenced by the formation and activation of cancer-associated adipocytes in the vicinity of tumour tissue (Trevellin *et al*, 2015). This is an area that requires further study.

APPLICATION OF KNOWLEDGE OF SOMATIC MUTATIONS FOR BIOMARKERS OF DISEASE PROGRESSION

Research is ongoing to identify individual markers, or marker panels that allow accurate detection of epithelial dysplasia and stratify patients for treatment, key studies are summarised in Table 1. This is particularly important given the rapid advances in endoscopic therapies, which have replaced oesophagectomy for patients with early disease. However, these therapies still have some risks and costs associated with them and hence it is important to stratify patients appropriately and avoid overtreatment.

In the context of Barrett's, p53 alone and in combination with other markers has been investigated both as a diagnostic biomarker and a marker of progression (Reid *et al*, 2001; Weston *et al*, 2001; Murray *et al*, 2006; Kaye *et al*, 2009; Kastelein *et al*, 2013). As well as the possibility of sequencing, inactivating mutations of *TP53* are also frequently detectable by immunohistochemistry providing a more clinically applicable test. Most mutations lead to stabilisation of the protein and hence increased the levels of expression, but a loss of staining can also be observed for truncating mutations (Kaye *et al*, 2009). In a nested case-control study of 197 patients with BE, the odds ratio (OR) for progression was 11.7 (95% CI: 1.93–71.4) in patients with higher p53 staining scores (Murray *et al*, 2006). A retrospective study of 635 patients confirmed this with a relative risk (RR) of 5.6 (95% CI: 3.1–10.3) (Kastelein *et al*, 2013). The risk for progression was even higher in cases with complete absence of p53 staining (RR 14.0; 95% CI: 5.3–37.2), and the risk of progression for patients with LGD increased from 15 to 33% if aberrant p53 staining was taken into account (Kastelein *et al*, 2013).

Assessment of p53 staining has also been shown to decrease the inter-observer variability between pathologists concerning the diagnosis of dysplastic lesions (Kaye *et al*, 2009, 2016). Therefore, taking all this evidence into account, immunohistochemistry for p53 has been suggested as an adjunct to aid the histological analysis of Barrett's biopsies in the recent BSG guidelines (Fitzgerald *et al*, 2014).

Marker panels. Galipeau *et al* built on previous work from their group (Reid *et al*, 2001) and reported that a panel of *TP53* LOH, *CDKN2A* LOH and presence of tetraploidy indicated a RR for Barrett's progression of 38.7 (95% CI: 10.8–138.5) (Galipeau *et al*, 2007). In a more recent study, the LOH and microsatellite instability status of 10 specific gene loci were combined into a risk score to predict the progression towards HGD (Eluri *et al*, 2015).

In an approach designed to enable analysis of FFPE material, a combination of abnormal DNA ploidy and expression of the novel biomarker *Aspergillus oryzae* lectin resulted in a three-fold increased risk for progression (OR 3.31; 95% CI: 1.81–6.05), or nearly four-fold in patients with baseline LGD (OR 3.90; 95% CI 2.39–6.37) (Bird-Lieberman *et al*, 2012a, b).

Varghese *et al* identified a predominantly MYC-regulated 90 gene signature to distinguish between HGD and non-dysplastic Barrett's ($P<0.0001$) using RNA microarray technology. In an independent validation cohort from the UK and The Netherlands, dysplastic samples were identified with an area under the curve of 0.87 (95% CI: $0.82-0.93$). This panel is particularly useful given its ability to distinguish between patients with LGD who are likely to progress. Indeed, using this panel, the 64% of LGD categorised as being high risk had a significantly higher rate of progression ($P=0.047$) (Varghese *et al*, 2015).

Gene expression regulation by epigenetic factors has also been investigated in BE progression. Array data were used to generate a panel of four hypermethylated genes (*SLC22A18*, *PIGR*, *GJA12* and *RIN2)*, validated by pyrosequencing in an independent cohort, to risk stratify patients into high-, low- and intermediate-risk groups with 94% sensitivity and 97% specificity (Alvi *et al*, 2013). An 8-gene hypermethylation panel consisting of *p16*, *RUNX3*, *HPP1*, *NELL1*, *TAC1*, *SST*, *AKAP12* and *CDH13* has also been investigated in the context of BE progression (area under receiver-operating characteristic curve (AUC) = 0.843 at 2 years, AUC = 0.829 at 4 years and AUC = 0.840 for the combined model) (Jin *et al*, 2009).

These biomarker studes have relied on the acquisition of endoscopically obtained biopsies. However, brush cytology techniques are increasingly being used to reduce the sampling error inherent from forceps biopsies and these methods can also be combined with molecular biomarkers to predict progression of non-dysplastic Barrett's (Timmer *et al*, 2015). Brush sampling, however, still requires direct endoscopic vision and non-endoscopic techniques like the Cytosponge are being developed. The Cytosponge is a densely folded sponge packed into a capsule that rapidly dissolves when entering the stomach. Once dissolved, the sponge can be retracted using the string. Cells of the oesophageal mucosal surface stick to the sponge that can then be assessed for biomarkers using immunohistochemistry and sequencing, such as p53 (Kadri *et al*, 2010; Ross-Innes *et al*, 2015b).

Combination of advanced endoscopic imaging modalities with molecular biomarkers. An alternative approach to reducing the number of random biopsies that are necessary according to the Seattle protocol is to perform a more sophisticated endoscopic assessment of the Barrett's mucosa using techniques such as narrow band imaging or auto-fluorescence imaging. This allows biopsies to be directed towards suspicious areas and then histopathological assessment can be combined with biomarker panels, which have the advantage of a more objective readout (e.g. p53, p16, cyclin A and altered ploidy); (Di Pietro *et al*, 2015). Similarly, confocal laser endomicroscopy allows the real-time detection of intestinalised epithelium and even dysplastic changes and this can also be combined with tissue-based biomarkers, resulting in excellent performance compared with standard histopathology (Tofteland *et al*, 2014; Di Pietro *et al*, 2015). An alternative approach to classical tissue-based assessment of biomarkers is the application of molecular probes that

Table 1. Biomarkers of progression of Barrett's oesophagus

	Reference	Study design	Sample size	Baseline histology	Endpoint histology	Statistical findings	Validation cohort?
P53 Immunohistochemistry	Murray *et al*, 2006	Nested case-control study	197	ND	OAC	OR 11.7 (95% CI 2.37–30.0)	Yes – Kastelein *et al*, 2013
	Kaye *et al*, 2009	Samples prospectively collected, retrospectively analysed	143	LGD	HGD/OAC	RR progression with consensus LGD: 1.56	Yes – Kastelein *et al*, 2013
	Kastelein *et al*, 2013	Case-control study	635	ND	HGD/OAC	p53 overexpression: RR 5.6 (95% CI 3.1–10.3) loss of p53 expression: RR 14 (95% CI 5.3–37.2)	No
17p LOH	Reid *et al*, 2001	Samples prospectively collected, retrospectively analysed	256	ND, ID and LGD	OAC	RR 16 (95% CI 6.2–39)	Yes – Galipeau *et al*, 2007
Aneuploidy/tetraploidy	Reid, 2000	Samples prospectively collected, retrospectively analysed	322	ND, ID and LGD	OAC	RR 11 (95% CI 5.5–21)	Yes – Galipeau *et al*, 2007; Bird-Lieberman *et al*, 2012a, b
17p LOH (p53), 9p LOH (p16) and abnormal DNA ploidy	Galipeau *et al*, 2007	Samples prospectively collected, retrospectively analysed	243	ND	OAC	RR 38.7 (95% CI 10.8–138.5)	Yes – Di Pietro *et al*, 2015
LGD, abnormal DNA ploidy and *Aspergillus oryzae* lectin	Bird-Lieberman *et al*, 2012a	Population-based nested case-control study	380	ND, ID and LGD	OAC	With baseline LGD: OR = 3.90 (95% CI 2.39–6.37) Without baseline LGD: OR = 3.31 (95% CI 1.81–6.05)	No
8-gene methylation panel	Jin *et al*, 2009	Retrospective double blinded	195	ND	HGD/OAC	AUC = 0.843 at 2 years, AUC = 0.829 at 4 years and AUC = 0.840	No
4-gene methylation panel	Alvi *et al*, 2013	Validated in retrospective and prospective cohorts	46	ND	OAC	AUC = 0.988 97% specificity and 94% sensitivity Risk stratification: low risk: <2 genes, intermediate: 2 and high: >2	Yes – Alvi *et al*, 2013
10 loci panel	Eluri *et al*, 2015	Case-control study	69	ND	OAC	AUC = 0.95 (95% CI 0.89–1)	No
90 gene panel	Varghese *et al*, 2015	Samples prospectively collected, retrospectively analysed	150	ND	OAC	Significantly higher rate of progression in patients regarded as high risk ($P = 0.047$)	Yes – Varghese *et al*, 2015

Abbreviations: AUC = area under receiver-operating characteristic curve; CI = confidence interval; HGD = high-grade dysplasia; ID = indefinite for dysplasia; LGD = low-grade dysplasia; LOH = loss of heterozygosity; ND = non-dysplastic Barrett's; OAC = oesophageal adenocarcinoma; OR = odds ratio; RR = relative risk.

can be viewed during endoscopy in real time. One such approach takes advantage of the altered glycosylation patters of the mucosal surface during the progression towards cancer that can be imaged with a fluorescent lectin (Bird-Lieberman *et al*, 2012b). A similar approach using labelled peptides that bind specifically to dysplastic or neoplastic lesions in the oesophagus showed promising results and is under evaluation for clinical practice (Sturm *et al*, 2013).

CONCLUSIONS

Oesophageal adenocarcinoma is frequently precipitated by the loss of p53 and the resultant copy-number changes with a high frequency of heterogeneous genomic alterations. During its evolution, point mutations occur as early as non-dysplastic BE. It is, therefore, challenging to predict progression from Barrett's to OAC using biomarker approaches. However, the advent of increasingly cost-effective sequencing and array technologies mean that it is possible to develop predictive tests, for example copy-number alterations, without necessarily needing an approach limited to a small number of candidate genes. This is still an area for research. For clinical management of patients with BE undergoing surveillance, there is agreement by specialist societies that there should be consensus-based assessment of dysplasia assisted by p53 immunostaining and discussion of the management for individual cases within specialist multidisciplinary teams. The more that biomarkers are coupled with sampling methods that limit the bias inherent in random biopsies, the more accurate we can be at predicting progression and preventing OAC development.

CONFLICT OF INTEREST

RCF developed the Cytosponge technology and is named on related patents. This technology has been licensed by the MRC to Covidien GI Solutions, now Medtronic. The remaining authors declare no conflict of interest.

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