The potential role of statins in the treatment and prevention of oesophageal adenocarcinoma

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For Rebecca, Claudia and Gabriel

Declaration

This dissertation and the work presented in it are my own and have been generated by me as the result of my own original research.

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;

2. This work has not been submitted for a degree, diploma or any other qualification at this University or any other institution;

3. Where I have consulted the published work of others, this is always clearly attributed;

4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;

5. I have acknowledged all main sources of help;

6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;

7. Parts of this work have been published before submission as:

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Thesis abstract

Oesophageal adenocarcinoma (OAC) is an aggressive malignancy with a very poor prognosis overall. Barrett's oesophagus (BO) is the only known precursor lesion. Emerging preclinical evidence indicates statins, medications commonly used in the primary and secondary prevention of cardiovascular disease, inhibit proliferation, promote apoptosis and limit invasiveness of OAC. Inhibition of the mevalonate pathway depletes downstream products involved in candidate growth-signalling cascades.

This research aimed to determine: (1) associations between statin use after diagnosis of oesophageal carcinoma (OC) and mortality outcomes; (2) the feasibility of assessing adjuvant statin therapy in patients with operable OAC in a future phase III randomised controlled trial; and (3) associations between statin use and malignant progression to high-grade dysplasia (HGD)/OAC in BO populations.

In a cohort of 4445 patients with OC in a large primary care dataset, the General Practice Research Database, post-diagnostic statin use was associated with significant reductions in OC-specific and all-cause mortality. Significant associations were demonstrated in patients with OAC but not in oesophageal squamous cell carcinoma.

A multi-centre, double-blind, parallel group, randomised, placebo-controlled feasibility trial of adjuvant statin therapy recruited patients with operable OAC. In total, 32 patients were randomised (1:1) to simvastatin (40mg) or matched placebo. Treatment started from the date of discharge following surgery and continued for up to one year. The trial estimated recruitment, retention, drug absorption, adherence, safety, quality of life, generalisability, and mortality outcomes. The feasibility of a future phase III trial was demonstrated; and derived feasibility estimates inform its design and conduct.

A nested case-control analysis of a cohort with BO registered with the United Kingdom National Barrett's Oesophagus Registry (UKBOR) demonstrated no significant associations between statin use and malignant progression. Significant dose and duration-response relationships were not demonstrated.

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Name	Position	Contribution
Dr Allan Clark	Senior Lecturer in Medical Statistics, Norwich Medical School	Input into the statistical design of all three projects. Dataset construction and statistical analysis of the GPRD study (chapter 2). Critical review of dataset construction and analysis of the STAT-ROC feasibility study (chapter 3) and UKBOR study (chapter 4). Critical review of the thesis.
Sharon Walton	Research Practitioner, Cancer Research Team, Norfolk and Norwich University Hospital	Lead research practitioner at NNUH for the STAT-ROC feasibility study. Co-ordinated all research visits at this site and maintained the site file. Data collection. Handled missing data queries.
Mr Michael Lewis	Consultant oesophago-gastric surgeon, Norfolk and Norwich University Hospital	Secondary supervisor. Contribution to the overall design and clinical aspects of the STAT-ROC feasibility study (chapter 3). PI at the Norfolk and Norwich University Hospital for the STAT-ROC feasibility study. Surgery read code selection for the GPRD study (chapter 2).
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Professor Ann Marie Swart	Director of Norwich Clinical Trials Unit (NCTU)	Adoption of the STAT-ROC feasibility study by NCTU. Co-investigator. Oversight of alignment of trial with NCTU processes for safety, quality, monitoring and data management. Protocol development for expansion to multi-centre trial.
Matthew Hammond	Senior Trials Manager, NCTU	Advice and development of trial-specific (STAT-ROC) NCTU working practice documents for management of safety, quality and monitoring. Development of materials for national site initiation visits. Compliance of all trial-specific procedures with good clinical practice and the MHRA.
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		visits. Lead author of the STAT-ROC Data Management Plan.
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List of abbreviations

5-FU	5-fluorouracil
AE	Adverse event
AGA	American Gastroenterology Association
ALT	Alanine Aminotransferase
AR	Adverse reaction
ATC	Anatomical Therapeutic Chemical
BMI	Body Mass Index
BSG	British Society of Gastroenterology
CI	Chief Investigator
CK	Creatine Kinase
COX	Cyclo-oxygenase
CRF	Case Report Form
CRTU	Clinical Research and Trials Unit
CT	Clinical Trials
CT	Computerised Tomography
CTA	Clinical Trials Authorisation
CTCAE	Common Terminology Criteria for Adverse Events
CX	Capecitabine, Cisplatin
CYP	Cytochrome P450
DFS	Disease-free survival
DMEC	Data Monitoring and Ethics Committee
DNA	Deoxyribonucleic acid
ECX	Epirubicin, Cisplatin, Capecitabine;
eGFR	Estimated glomerular filtration rate
EORTC QLQ	European Organization for Research and Treatment Quality of Life Questionnaire
ER	Endoplasmic reticulum
EUS	Endoscopic ultrasound
GCP	Good Clinical Practice
GDP	Guanosine diphosphate
GEF	Gefitinib
GOJ	Gastro-oesophageal junction

GP	General Practitioner
GPRD	General Practice Research Database
GTP	Guanosine Triphosphate
HMG-CoA	3-hydroxy-3-methylglutaryl-CoA
ICF	Informed Consent Form
ICH	International Conference of Harmonisation
IHC	Immunohistochemistry
IMP	Investigational Medicinal Product
IWRS	Interactive Web Responsive System
LDL	Low density lipoprotein
LFTs	Liver function tests
MHRA	Medicines and Healthcare products Regulatory Agency
MI	Myocardial Infarction
MNAR	Missing Not at Random
NHS	National Health Service
NNUH	Norfolk and Norwich University Hospital
NRES	National Research Ethics Service
OAC	Oesophageal Adenocarcinoma
OC	Oesophageal carcinoma
OGD	Oesophagogastroduodenoscopy
OS	Overall survival
PI	Principal Investigator
PIL	Participant/ Patient Information Leaflet
PMU	Pharmacy manufacturing unit
PP	Pyrophosphate
PS	Progression-free survival
QMMP	Quality Management and Monitoring Plan
R&D	NHS Trust R&D Department
RCT	Randomised controlled trial
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SMPC	Summary of Medicinal Product Characteristics
SREPB	Sterol Regulatory Element-binding Protein
SSAR	Suspected Serious Adverse Reaction

SUSAR	Suspected Unexpected Serious Adverse Reactions
TAE	Transcatheter arterial embolization
TMF	Trial Master File
TMG	Trial Management Group
TSC	Trial Steering Committee
ULN	Upper limit of normal
US	United States

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1. Chapter 1 - Introduction

1.1. Barrett's oesophagus and oesophageal adenocarcinoma

Barrett's oesophagus (BO) refers to the replacement of the normal stratified squamous epithelium of the lower oesophagus by columnar epithelium, through the process of metaplasia[1]. BO develops as a complication of chronic oesophageal mucosal injury to gastric reflux[2, 3]. BO is the only known premalignant lesion to oesophageal adenocarcinoma (OAC). This is the most common histological subtype of oesophageal malignancy in the west, an important gastrointestinal epithelial malignancy with a dismal prognosis[4, 5]. The following describes the history of BO as a clinical entity, its epidemiology, pathogenesis, clinical presentation, diagnosis, and clinical management.

1.1.1. History of Barrett's oeosphagus

The origins, nature and existence of the columnar-lined oesophagus, commonly termed Barrett's oesophagus, has been the subject of considerable historical confusion and debate. Norman Barrett (1903-1979), a distinguished consultant thoracic surgeon at St. Thomas' Hospital, is frequently credited with first describing the eponymous condition in 1950; in fact, Tileston, a Harvard pathologist, first characterised the disorder in 1907[6, 7]. In a review of 44 patients with "peptic ulcer of the esophagus" he noted "the close resemblance of the mucous membrane about the ulcer to that normally found in the stomach"[7]. In 1931, Findlay and Kelley, from 9 cases, proposed the columnar-lined organ was not oesophagus but rather intrathoracic stomach; the result of congenital shortening of the oesophagus with resultant herniation and trapping of the tubular portion of the stomach in the chest[8]. This assertion was supported by Barrett in his treatise in 1950[6]. He rejected the conclusions of previous pathologists that the mucous membrane approximating oesophageal ulcers represented gastric heterotopia. Barrett drew a distinction between "reflux oesophagitis": reflux of acidic gastric juices leading to inflammation of the oesophagus potentially complicated by ulceration of squamous mucosa and stricture formation; and the lesion described by pathologists previously as "chronic peptic ulcer of the oesophagus" surrounded by gastric mucosa, which he asserted arose from the stomach and not the oesophagus, and were complicated by "emergencies such as massive bleeding, perforation or carcinoma", typical of classical gastric ulcers[6]. Three years later Alison and Johnston persuasively concluded this columnar-lined structure was in fact the oesophagus and not stomach: "more careful examination of such a specimen shows that it has no peritoneal covering, that the musculature is that of the normal oesophagus, that there may be islands of squamous epithelium

within it, that there are no oxyntic cells in the mucosa, and that in addition to gastric glands there are present typical oesophageal mucous glands."[9]. They proposed the chronic ulcer which develops within the oesophagus lined by gastric mucous membrane be termed "Barrett's ulcer"; to reflect this entity as distinct from gastric ulcers. They asserted their use of the eponym did not imply agreement with Barrett's original view the ulcer arose from intrathoracic stomach; though it seems plausible it was also used to appease him. Indeed, in 1957, Barrett revised his position and conceded in these cases the lower oesophagus was "lined by columnar epithelium" and did not represent stomach[10]. The eponym, Barrett's oesophagus, has remained in common use since, particularly from the late 1960s[11].

The view the disorder was a congenital abnormality was widely held, including by Barrett: "it is probably the result of a failure of the embryonic lining of the gullet to achieve normal maturity"; and Allison and Johnston, despite the recognition of an association with oesophagitis and hiatus hernia in these patients[6, 9]. Indeed, it was Tileston who first correctly recognised the role of GORD in the pathogenesis of the ulceration within the columnar-lined oesophagus: "the first requisite for the formation of the peptic ulcer of the oesophagus is an insufficiency of the cardia"[7]. By the 1970s the acquired nature of Barrett's oesophagus and the role of GORD in its pathogenesis were established[12-15]. While the malignant potential of the Barrett's ulcers had been alluded to in his original treatise, this was only widely recognised two decades later in the 1970s[6].

The subject of the histological features required to make a diagnosis of Barrett's have been the topic of debate and controversy[16, 17]. The earliest the histological features were comprehensively characterised was in 1976, in a study by Pedersen et al. of 11 patients with Barrett's oesophagus from whom biopsies were been taken from different levels guided by oesophageal manometry[18]. These patients were found to have three types of oesophageal columnar epithelium: a specialised columnar epithelium with goblet cells (known commonly today as intestinal metaplasia); a junctional (cardia-type) epithelium that comprised mucus secreting cells; and a gastric-fundic type (with parietal and chief cells). These three types of columnar epithelium were found to be localised to different levels in the oesophagus, respectively proximally to distally, with specialised found in the proximal oesophagus adjacent to squamous epithelium; followed by junctional epithelium and then the gastric-fundic type in continuity with the proximal stomach. The presence of intestinal metaplasia was of particular interest: pathological examination of resected oesophageal adenocarcinomas in case-series revealed the malignancy to be in continuity with this type of columnar epithelium in the majority of patients[19]. Therefore, predominantly this type of

columnar epithelium, rather than the other two types, was believed to harbour potential to undergo dysplasia and hence malignant transformation[17]. This view was particularly held by gastroenterologists in the United States (US), where American Gastroenterology Association (AGA) clinical practice guidelines stipulated, "Intestinal metaplasia of the esophagus is the premalignant lesion for adenocarcinoma of the esophagus", and required its presence in order to make a diagnosis of Barrett's oesophagus[20]. In contrast, the view proposed in the United Kingdom was more relaxed, and required "metaplastic glandular mucosa, whether intestinalised or not"[21]. A number of considerations have recently led to a change in position in the US and adopt this view as reflected in their recent clinical practice guidelines: detection of intestinal metaplasia using biopsies is subject to considerable sampling error, hence potentially leading to under diagnosis; intestinal metaplasia can develop over time within columnar epithelium; and most importantly patients with CLO without intestinal metaplasia have also been shown to still have an appreciable risk of adenocarcinoma.

1.1.2. Diagnosis

The current gold standard modality for diagnosis of Barrett's is high-definition, white light, (transoral as opposed to transnasal) endoscopy[22, 23]. At least 1cm of visible columnar-lined oesophagus measured above the gastro-oesophageal junction with histological confirmation from biopsy is required. The length of metaplastic mucosa should be described using the Prague C & M criteria (see figure 1, adapted from Sharma et al[24]). The current British Society of Gastroenterology (BSG) guidelines recommend a minimum endoscopic dataset when reporting findings of Barrett's oesophagus, which in addition to length of metaplasia are: the presence of Barrett's islands (areas of Barrett's not in continuity with the gastro-oesophageal junction) including length and distance from incisors, presence of hiatus hernia and length, visible lesions including their number, distance from incisors and classification using the Paris classification, and the location and number of biopsies taken[22]. Biopsies should initially be targeted to visible lesions within the Barrett's segment before proceeding with four-quadrant biopsies every 2cm (the Seattle biopsy protocol)[25]. Histopathological findings, required to corroborate an endoscopic diagnosis of Barrett's oesophagus differ between the UK and US guidelines: the BSG guidelines recognise any type of columnar mucosa (expected to be of cardiac, oxyntic or intestinal types), whereas the AGA guidelines restrict the definition to the intestinal type[22, 23]. The presence of native oesophageal structures such as oesophageal submucosal glands, while also supportive of a diagnosis, are not essential as they are present in a minority of samples[26]. Once a diagnosis of Barrett's oesophagus has been established, further management will depend on estimated life expectancy, patient choice, Barrett's length and the presence of dysplasia and/or adenocarcinoma.

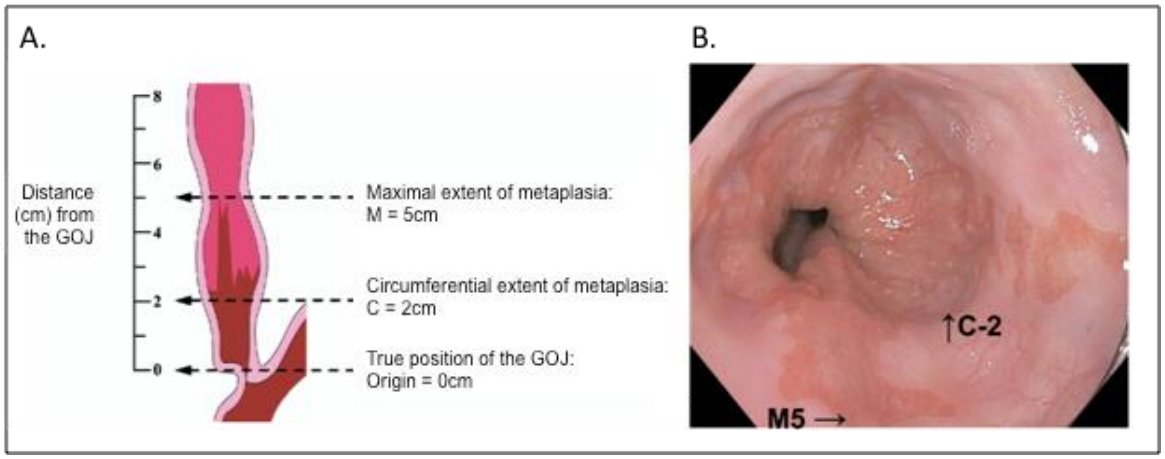

Figure 1: Panel A – diagrammatic representation of the landmarks used to define the endoscopic extent of Barrett's oesophagus using the Prague C and M criteria.

Panel B – corresponding endoscopic image demonstrating the same extent of Barrett's oesophagus. C (cm) refers to the circumferential extent and M (cm) refers to the maximal extent from the gastro-oesophageal junction (GOJ). In this example, the area would be defined as C2M5 (adapted from Sharma et al. 2006).

1.1.3. Management

1.1.3.1. Surveillance of non-dysplastic Barrett's oesophagus

The aim of endoscopic surveillance of patients with Barrett's oesophagus is to aid the early detection of dysplastic or cancerous lesions, which are more readily amenable to curative treatment. The intended benefits of endoscopic surveillance need to be carefully balanced against the risk of complications, which are not insignificant: risk of perforation or death is 0.03% and 0.001% respectively[27]. Large population-based cohort studies have demonstrated strong associations between enrolment in Barrett's surveillance programs and improved outcomes for patients who progress to OAC, in terms of reduced mortality, earlier cancer stage at presentation and reduced need for oesophagectomy[28-30]. However, despite correcting for both lead and length time bias, these observational studies are still potentially susceptible to selection bias: those with favourable characteristics, such as being younger, fitter, and more motivated, or judged by their treating clinician as having a good prognosis, would seem most likely to be selected, agree and adhere to surveillance, (compared to prevalent cases of OAC or those with known prior barrett's who did not receive surveillance) thus confounding associations in favour of improved patient outcomes. Recent studies of cost-effectiveness of surveillance that consider contemporary estimates of malignant progression are conflicting[31, 32]. Results are awaited of a multi-centre randomised-controlled trial, the Barrett's Oesophagus Surveillance Study (BOSS), which aims to definitively establish whether two-yearly endoscopic surveillance is superior to surveillance "at

need only" in terms of overall survival and cost-efficacy has completed recruitment and is the follow-up phase[33]. Despite the lack of current trial data, surveillance of Barrett's oeosphagus is widely advocated by clinical practice guidelines and it is routinely conducted in the western world[22, 23, 34, 35].

The BSG guidelines advocate an algorithm for surveillance of non-dysplastic Barrett's surveillance based on length of Barrett's segment and the presence of intestinal metaplasia on histology: two factors which are consistently associated with malignant progression[28, 36-38]. If the length is less than 3cm and histology confirms gastric metaplasia, a repeat oesophagogastroduodenoscopy (OGD) with quadrantic biopsies is advocated[22]. If these findings are replicated the guidelines recommend considering discharging the patient from active follow-up. If the length is less than 3cm but intestinal metaplasia is confirmed surveillance is recommended every 3-5 years. If the length is at least 3cm then repeat OGD is recommended every 2 to 3 years.

1.1.3.2. Dysplasia and intramucosal cancer

If dysplasia or malignancy is found within a Barrett's segment either at diagnosis or during follow-up, it is categorised using the revised Vienna classification for gastrointestinal mucosal neoplasia (see table 1, adapted from Schlemper et al.[39]) according to the degree of abnormal cellular architecture.

Category 1	Negative for neoplasia/dysplasia
Category 2	Indefinite for neoplasia/dysplasia
Category 3	Non-invasive low grade dysplasia (low grade adenoma/dysplasia)
Category 4	Non-invasive high grade dysplasia
	4.1 High grade adenoma/dysplasia
	4.2 Non-invasive carcinoma (carcinoma in situ)
	4.3 Suspicion of invasive carcinoma
Category 5	Invasive neoplasia
	5.1 Intramucosal carcinoma
	5.2 Submucosal carcinoma or beyond

Table 1: Vienna classification of gastrointestinal epithelial neoplasia (adapted from Schlemper et al. 2000). Non-invasive indicates absence of evident invasion. Intramucosal indicates invasion into the lamina propria or muscularis mucosae.

In patients with indefinite dysplasia a repeat OGD in 6 months with antireflux medical therapy is advised. If no dysplasia is subsequently found, surveillance should follow the recommendations

described above for non-dysplastic Barrett's oesophagus. In patients with low grade dysplasia, radiofrequency ablation (RFA) is now the new standard of care, following the results of Surveillance vs Radiofrequency Ablation (SURF) study[40, 41]. Radiofrequency energy is delivered endoscopically to Barrett's segments with either a specially designed balloon (HALO^{360}) device (to achieve circumferential ablation) or an articulated, cap-based electrode (HALP^{90}) (to achieve focal ablation); with the aim of removing Barrett's mucosa and reducing risk of neoplastic progression[42]. The SURF study was a European multi-centre, parallel group, open-label, randomized controlled trial of radiofrequency ablation (active arm) vs. endoscopic surveillance (control) in patients with Barrett's oesophagus with low grade dysplasia[41]. The primary outcome was neoplastic progression to HGD/adenocarcinoma within three years of randomisation. 68 patients were allocated to receive ablation (receiving focal and or circumferential ablation for a maximum of 5 sessions) and 68 received surveillance only. One patient (1.5%) in the ablation group developed the primary outcome vs. 18 (26.5%) in the control group (risk difference 25% (95% CI 14.1-35.9), p <0.001). In the ablation arm 12% developed oesophageal stricturing requiring endoscopic dilation and there were no adverse events in the control arm. Other adverse events previously reported in the literature are mucosal laceration, bleeding and fever[43]. Following ablation, patients are offered high dose proton pump inhibitors (PPIs) to promote mucosal healing and growth of new squamous (neosquamous) epithelium[44].

High grade dysplasia or early tumours confined to the mucosa (T1a lesions – see table 4, adapted from Edge et al.[45]), were historically treated with radical surgery (oesophagectomy)[46]. However, endoscopic therapy has emerged as the new standard of care, sparing the need for major surgery with its associated adverse impact on quality of life, morbidity and mortality. Current BSG guidelines advocate expert high resolution endoscopy in all patients with high grade dysplasia/suspected intramucosal adenocarcinoma to detect visible abnormalities suitable for endoscopic mucosal resection (EMR)[22]. There exist two methods for endoscopic resection: band ligation and the cap and snare technique. Bland ligation involves suction of the target mucosa into a cap fixed at the distal end of the endoscope and a rubber band is deployed to create a pseudopolyp which is subsequently resected with a snare[47]. The cap and snare technique involves injecting the submucosal space to initially lift the target lesion before its suction into the cap and subsequent resection using a preloaded snare[22]. In addition to the removal of dysplastic Barrett's tissue or intramucosal cancer, EMR has emerged as a valuable staging modality to guide further management: by permitting histological assessment of the whole lesion, crucially mapping its lateral extent and depth[44]. Adequately resected areas of high grade dysplasia or T1a lesions confined to the mucosa should be offered RFA to ablate remaining Barrett's mucosa:

metachronous lesions within the Barrett's segment are common (20%) and observational evidence demonstrate patients receiving RFA (vs. no RFA) after EMR have reduced risk of recurrence[22, 48]. A recent European multi-centre observational study (EURO-II) of 107 patients with HGD (29%) and T1a cancers (71%) demonstrated patients receiving focal EMR followed by RFA, achieved approximately 90% recurrence free survival by 48 months after the first negative endoscopy following primary treatment[43]. In contrast, in patients with T1b lesions (those invading the submucosa – see table 4, adapted from Edge et al.[45]), EMR should be considered non-curative and patients should be offered oesophagectomy if they are surgical candidates[22]. This recommendation is born of the observation that lymph node metastases are common with T1b lesions (up to 44%), and rare with T1a lesions[49-51]. If inspection reveals a flat lining with no visible lesions amenable to endoscopic resection, RFA alone is recommended.

1.1.4. Descriptive epidemiology

Oesophageal carcinoma: global perspective

Worldwide, oesophageal carcinoma is the sixth leading cause of cancer-related mortality and the ninth most common malignancy[52]. In 2012, the number of new cases of oesophageal cancer approximated the number of attributable deaths: 455 800 and 400 200 respectively[53]. The bulk of disease burden, and hence mortality, rests in developing countries, where it is ranked eighth for cancer incidence and fifth for cancer-related mortality; compared to developed countries where it is ranked 20th for cancer incidence and 11th for mortality[52] (see figures 2 and 3, adapted from GLOBOCAN 2012[54]). In developing verses developed countries, overall the age-standardised incidence rates (ASIRs) (per 100 000) are 8.94 vs 3.90, and age-standardised death rates (ASDRs) (per 100 000) are 9.11 vs 3.7. The incidence rates for men and women are highest in East Asia, sub-Saharan Africa and central Asia; and they are lowest in Andean Latin America, eastern and central Europe (particularly in women), North Africa and the Middle East (particularly in men). Worldwide in 2013, oesophageal cancer accounted for 9.8 million disability adjusted life years. Globally, for men and women, oesophageal carcinoma accounts for the 6th highest cancer-related cause of years of life lost, following breast, colorectal, stomach, liver and lung cancer.

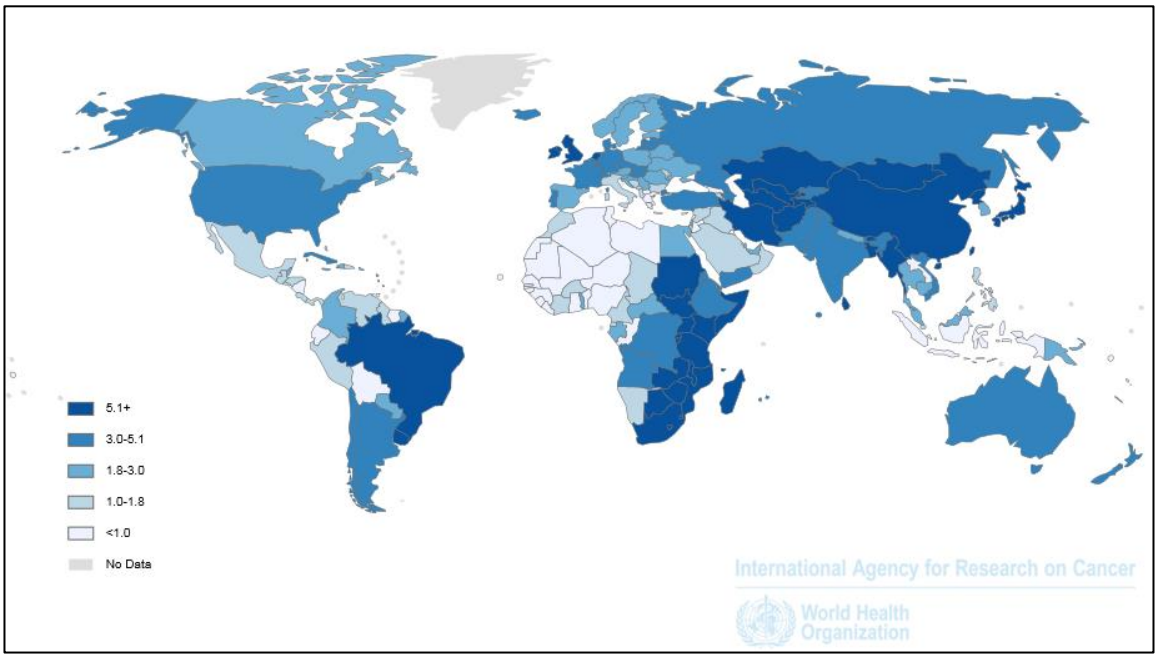

Figure 2: Geographic representation of the age-standardised incidence rates (per 100 000) for oesophageal carcinoma in men and women in 2012. Adapted from GLOBOCAN 2012, International agency for Research on Cancer.

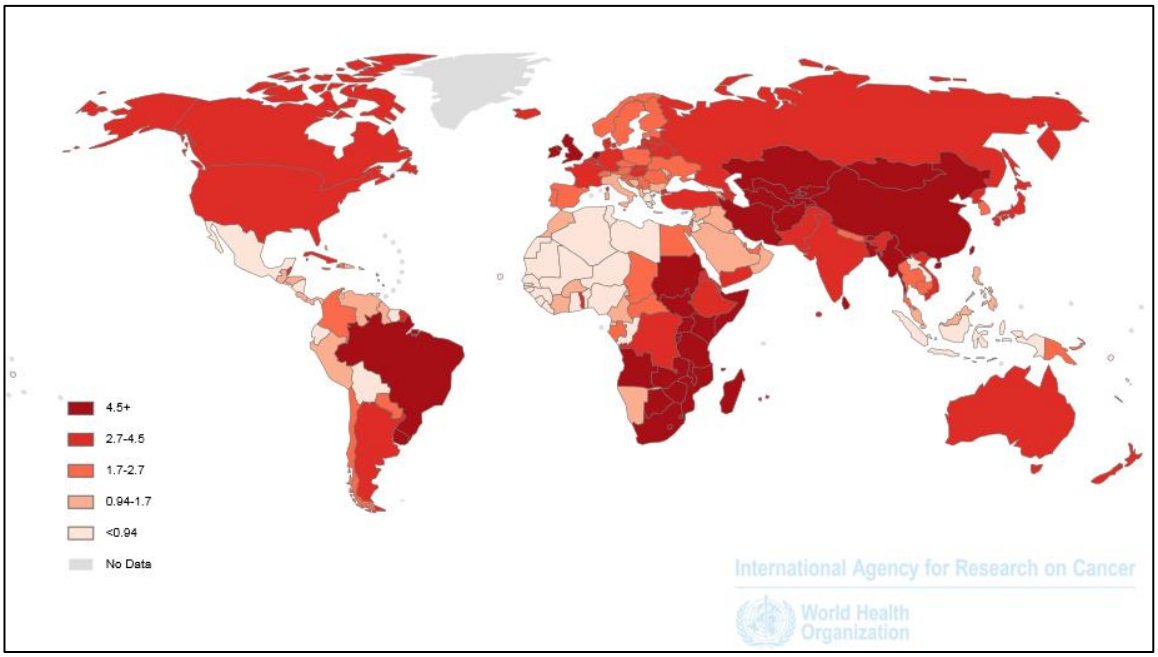

Figure 3: Geographic representation of the age-standardised mortality rates (per 100 000) for oesophageal carcinoma in men and women in 2012. Adapted from GLOBOCAN 2012, International agency for Research on Cancer.

The global variation in incidence rates of oesophageal carcinoma is more than 21-fold[53], and is best understood in the context of the two main histological subtypes: OAC and squamous cell carcinoma (OSCC) (although there are others including small-cell carcinoma, leiomyosarcoma and melanoma). In 2012, of the estimated 455 800 with oesophageal carcinoma, 398 000 were OSCC,

the globally predominant histological subtype, particularly in developing countries, 52 000 were OAC, predominant in western populations, and 6000 were other carcinomas (see figures 4 and 5 which demonstrate the global distribution in oesophageal cancer according to the two main histological subtypes, adapted from Arnold et al.[55]). The highest incidence rates of OSCC are found in the "oesophageal cancer belt" from Northern Iran to North-Central China and runs through the Central Asian republics, where 90% are OSCC. The major risk factors for OSCC are smoking tobacco and alcohol excess; other relevant risk factors are mutations of enzymes involved in the metabolism of alcohol, achalasia, caustic injury, thoracic radiation, low socioeconomic status, poor oral hygiene, certain nutritional deficiencies and non-epidermolytic palmoplantar keratoderma[56]. In contrast, OAC is increasingly predominant in developed Western populations, where OSCC has been observed to be in decline.

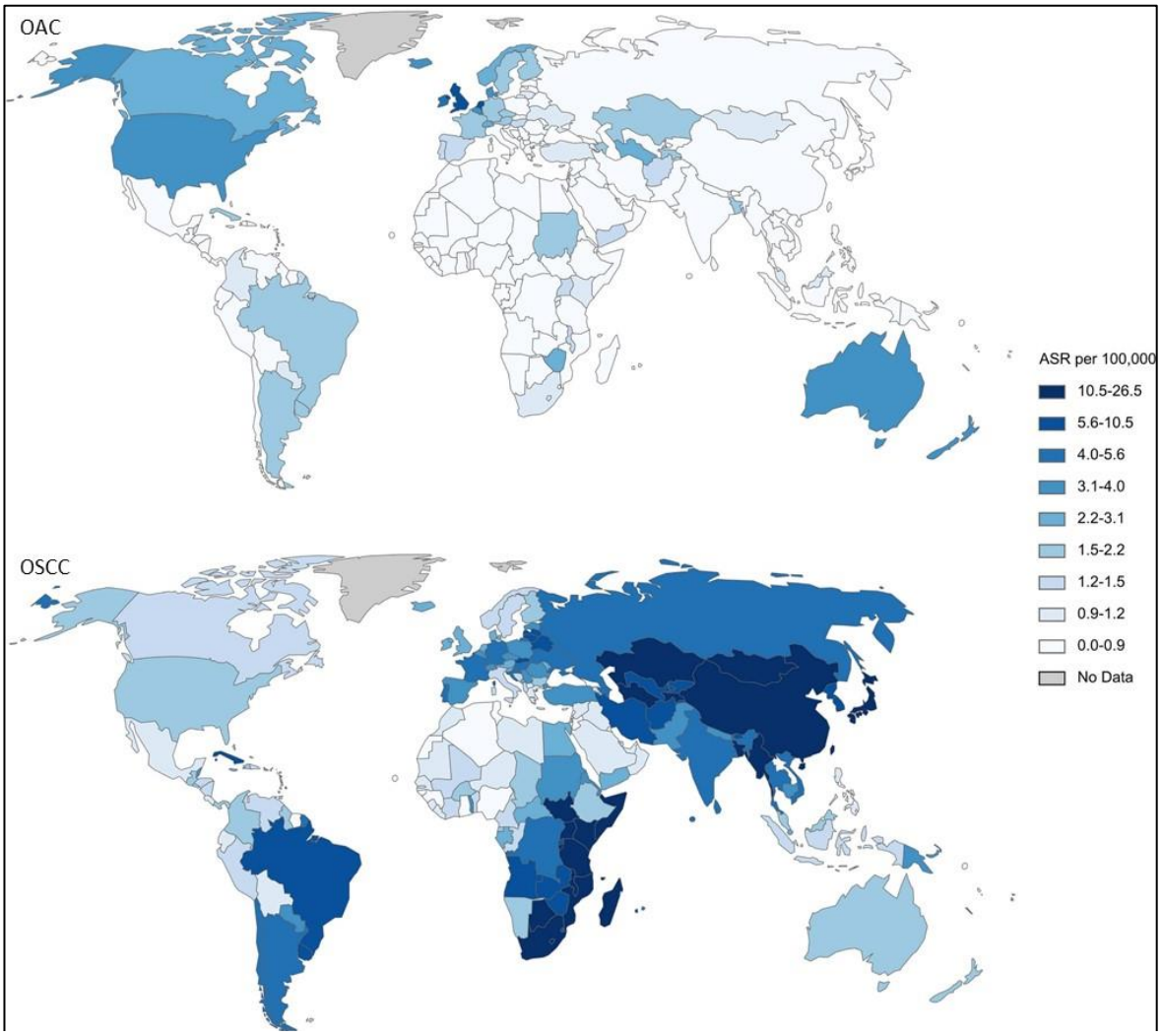

Figure 4: Geographic representation of the age-standardised incidence rates (per 100 000) for oesophageal adenocarcinoma (OAC) and oesophageal squamous cell carcinoma (OSCC) in men 2012. Adapted from Arnold et al. (2014).

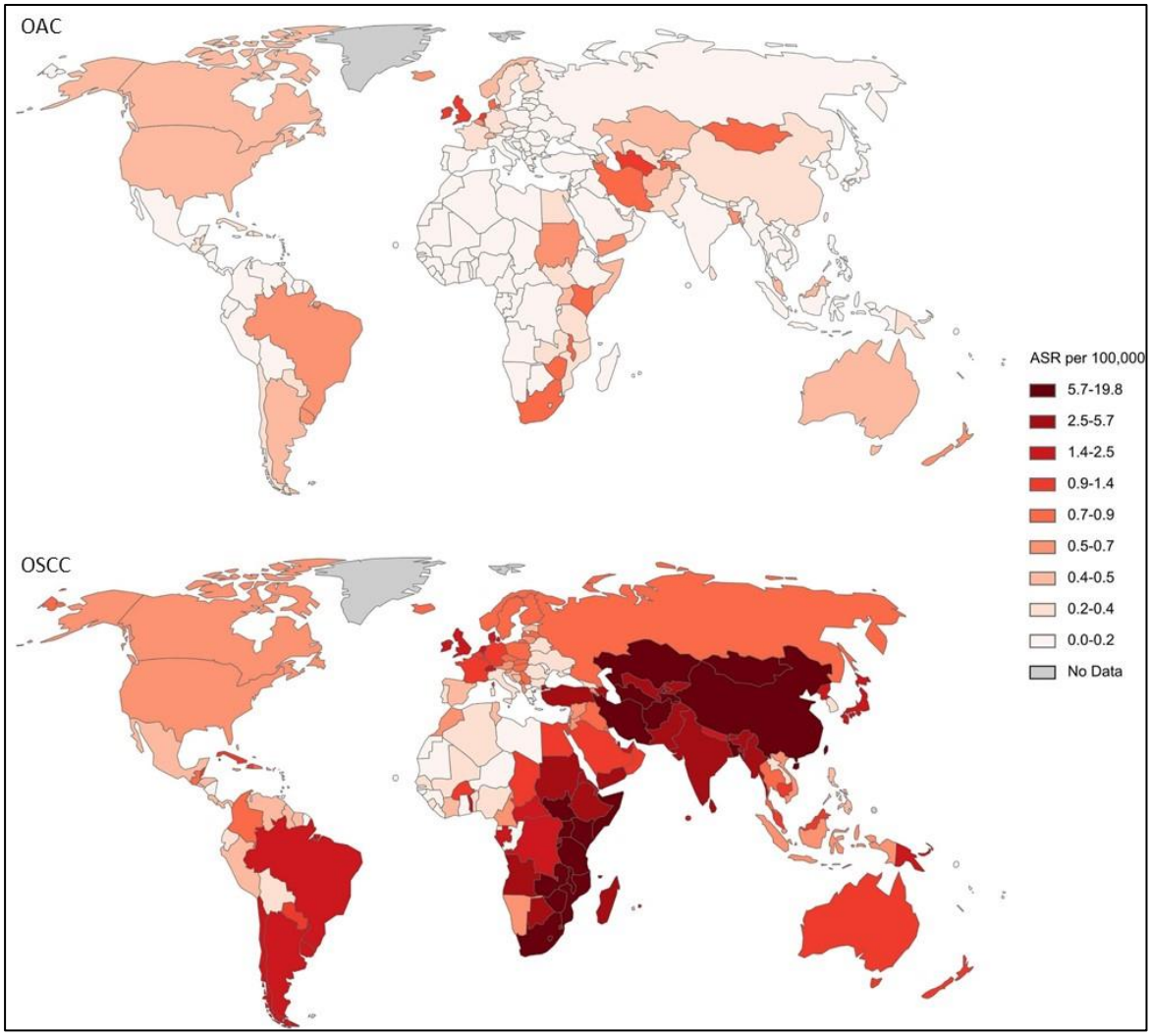

Figure 5: Geographic representation of the age-standardised incidence rates (per 100 000) for oesophageal adenocarcinoma (OAC) and oesophageal squamous cell carcinoma (OSCC) in wonen 2012. Adapted from Arnold et al. (2014).

Oesophageal carcinoma: United Kingdom perspective

In the United Kingdom (UK) in 2013, the number of new cases of oesophageal carcinoma was 8 784, and in the preceding year, the number of attributable deaths was 7 701[57]. In England and Wales, survival rates are among the poorest of all malignancies, similar to lung and pancreatic, with overall 5-year survival rates in 2011 estimated at 15.1%[58]. Of the two main histological subtypes of oesophageal malignancy, adenocarcinoma is predominant, accounting for approximately two thirds of cases[55]. The incidence of OAC has risen rapidly since the early 1970s such that currently the age-standardised incidence of this subtype is higher in the UK than anywhere else in world[4]. Risk of oesophageal cancer increases with age and the main burden of disease rests with older populations: nearly 60% of patients are diagnosed from 70 years of age in the UK[59]. In an analysis of UK cancer registries, in men the age-standardised rates per 100 000/year are estimated to be 77 in 70-74 year olds, 87 in 75-79 year olds and 111 in 80-84 year olds[59].

Oesophageal adenocarcinoma epidemic

A dramatic rise in incidence of OAC, described as an epidemic, has been noted over the last four decades, such that it has overtaken OSCC in incidence in western populations, particularly the UK, US and western European countries[60-62]. The largest population-based study to date conducted using cancer registries in Europe, the US and Australia, involving 117 946 patients with OAC, comprehensively examined its changing incidence[4]. The majority of patients (99%) were over 40 years of age at diagnosis, and were male (87.7%). The time from which the incidence began to rapidly rise varied by country, from 1976 in Denmark to 1991 in Sweden. The rising incidence appeared to have already begun in England by 1971, the earliest point from which the English cancer registry data were available. The changing incidence appeared to follow one of two calendar period patterns: 1) a stable phase with incidence of 1 case per 100 000 person-years, then following an inflection point, an increment of 1-2 cases per 100 000 person-years per decade (observed in New Mexico, San Francisco, Hawaii, Denmark, Sweden and Finland); and 2) a continuous increase throughout the period of observation (through to 2009 at the latest) of approximately 2-3 cases per 100, 000 person years per decade (observed in England, Scotland, the US and Australia) (see figure 6, adapted from Edgren et al.[4]). The second pattern was observed in countries where presumably the inflection point had already occurred prior to the period of observation captured by the registries. Extrapolating the incidence curves seen in the second

pattern of incidence to the point where they would intersect the presumed baseline rate observed in the first pattern indicated the earliest estimated inflection point to be 1960 in Scotland and England, and early or mid-1970s in the US. The change in incidence appeared best explained by a calendar period effect rather than by birth cohort. Furthermore, across all registries examined, there was no clear evidence of an abating trend in incidence. While the incidence of OAC in women was lower than men in absolute terms, the proportional increase was the same overall. Therefore as expected the sex ratio appeared stable over this time and overall the incidence was three to nine fold higher in men, with considerable variation by country. The current incidence of OAC in England is 12 per 100 000.

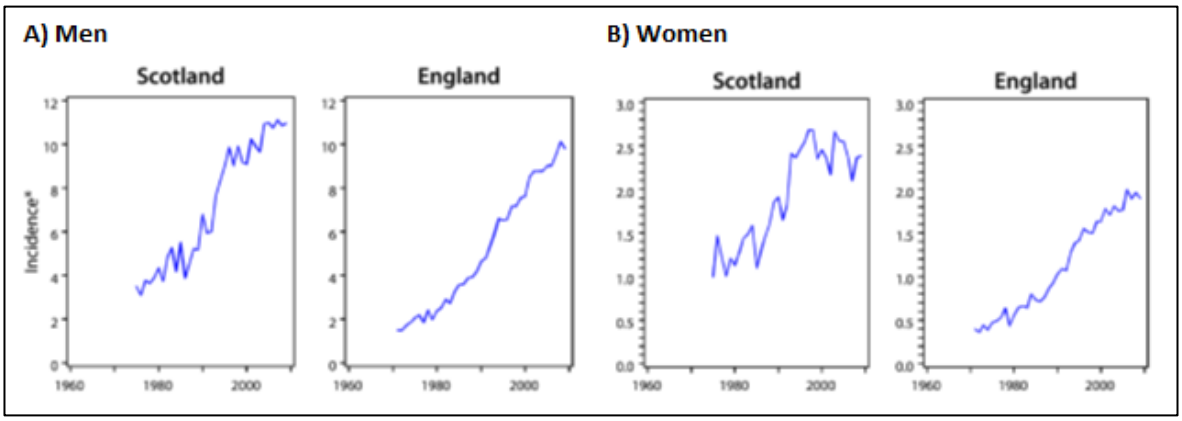

Figure 6: Age-standardised incidence of oesophageal adenocarcinoma per 100 000 by calendar period in A) Men and B) Women in England and Scotland. Note the scales differ between men and women. Adapted from Edgren 2013.

1.1.5. Pathogenesis

Genetic pathogenesis of oesophageal adenocarcinoma

Two genomes which can contribute to the development of cancer are the somatic (evolution to cancer within nuclei of cells of organs and tissues of the body) and inherited (constitutive genomes propagated in the germline)[63].

Somatic genome

The clonal evolution model of neoplastic progression describes the accumulation of genetic abnormalities in initially normal progenitor cell populations which provide a Darwinian selection advantage for aberrant clonal populations, which ultimately give rise to invasive malignancy[64]. Linear models propose this occurs in an ordered, stepwise fashion and this would appear to be the case for mutations in tumours which develop gradually, such as the evolution of colorectal tumours[65]. However, there is evidence that aberrant somatic genome evolution can arise in a branched manner in some solid tumours, including OAC, a process which can occur rapidly[66-68]. Development of an early branch within Barrett's epithelial cell populations may give rise to a stable state, resulting in non-progression as seen in the majority of patients, while for the minority, rapid branching and selection pressures could lead to neoplastic progression, and hence invasive malignancy[63]. The threshold for the required somatic genome alterations which herald malignancy in Barrett's may be reached at very different rates: gradual, slow accumulation of abnormalities may be seen in those who do not progress; while those exposed to a relevant environmental factor or mutational event, may undergo accelerated accumulation and reach this threshold sooner. In contrast, even more rapid, "punctuated", somatic genome alterations can arise, whereby an environmental factor or mutational event leads to chromosome instability (either an increased rate of loss or gain of whole chromosomes or large regions of chromosomes)[63]. Some genomic alterations involving chromosomes are yet more dramatic and can arise from a single cell division – such as chromothripsis (chromosome shattering due to errors in chromosome segregation during mitosis) and whole genome doubling[69].

OAC typically has very high mutational frequencies, with median 26, 161 (IQR 18, 881 – 66, 225) single nucleotide variants (SNVs) per tumour[70]. The only tumours which exceed this frequency are melanoma, and lung cancer, malignancies with a well-defined mutagen[71, 72]. Barrett's oesophagus is surprisingly also highly mutated with 12, 714 (IQR 6, 604 - 21, 559) SNVs, with mutation rates at least double that of multiple myeloma, breast cancer, hepatocellular carcinoma and colorectal adenocarcinoma[68, 73-76]. Owed to its branched evolution, OAC demonstrates marked heterogeneity in the spectrum of mutations observed between tumours from different patients

but also between paired (adjacent) OAC and Barrett's oesophagus samples (<20% SNV overlap)[68]. Despite the high mutational frequency observed in OAC, only a few number of genes are recurrently mutated (summarised in table 2), the majority of which are loss of function mutations to tumour suppressors, whereas no clear oncogenic mutations linked to progression of OAC have been identified[69, 70, 77]. The most commonly mutated gene, tumour suppressor p53, was observed in approximately 70% of samples, high enough to have implications for future strategies for early detection and prevention. This is in contrast to other mutations identified at much lower frequencies thus limiting their future utility, for example, the next most frequently occurring mutations, SMAD4, MYO18B and CDKN2A, were individually observed in only 12% of OAC samples[70, 77]. Indeed similar mutational frequencies of a panel of 26 genes mutated above background rate or in pathways of interest (derived from whole-genome sequencing data from OAC) were observed between samples from non-dysplastic Barrett's oesophagus (from 40 patients whom did not progress during follow-up (over a median of 58 months, range 4-132 months), 39 patients with HGD and 90 with OAC. Recurrent (≥1) mutations in these genes were identified from Barrett's tissue in 21 (53%) patients with non-dysplastic Barrett's oesophagus. The only mutations which defined disease boundaries between non-dysplastic Barrett's oesophagus and HGD, and between HGD and OAC were, respectively, p53 and SMAD4[77]. A mutational signature has been identified, characterised by adenine to cytosine (A>C) transversions (base substitutions) at adenine-adenine (AA) sites, accounting for 29% of the total mutations observed in OAC[70]. This would appear to be unique to OAC and has not been identified in other tumour types to date. This signature has been found in SNVs which occur both early and late in the neoplastic progression of Barrett's oesophagus[68].

Mutated gene	Proportion	Gene function
TP53[70, 77]	70%	Tumour suppressor gene. Plays multiple roles, including regulating cell cycle progression, apoptosis, DNA repair, autophagy, differentiation and senescence[77, 78]. Mutation accurately defines boundary between non-dysplastic Barrett's oesophagus and Barrett's with HGD[77].
SMAD4[77]	12%	Tumour suppressor gene[79]. Mutation results in loss of function. Key mediator of transforming growth factor beta (TGF-β) signal transduction (tumour suppressor pathway). Forms trimer with two R-SMAD molecules to form SMAD4-R-SMAD complexes which bind other DNA binding transcription factors as partners for transcriptional regulation. Mutation accurately defines boundary between Barrett's with HGD and early invasive OAC[77].
MYO18B[77]	12%	Tumour suppressor gene[80]. Encodes myosin XVIIIB which regulates muscle-specific genes when in the nucleus and may influence intracellular trafficking when in the cytoplasm.
SEMA5A[77]	8%	Encodes Semaphorin 5A, a transmembrane bound Semaphorin, a member of a family of axonal growth molecules involved in development of vascular, skeletal, cardiac systems and immune response[81]. Involved in tumour formation, chemotaxis, cell viability, angiogenesis and metastases.
SWI/SNF chromatin modelling complex[70]	20%	ARID1A (8%), SMARCA4 (6%), ARID2 (5%), PBRM1 (3%) and JARID2 (3%)[70]. All loss of function. Evolutionary conserved, consume ATP to mobilise and eject nucleosomes to modulate chromatin compaction[82].
PIK3CA[70]	6%	Gain of function. Encodes the p110α catalytic subunit of Phosphatidylinositide 3-kinases (PI3K). PI3K are a ubiquitous family of lipid kinases which mediate a number of downstream targets which regulate cell proliferation, migration and survival and oncogenic transformation[83].
CDKN2A[70]	12%	Also called P16/INK4α/MTS1. Loss of function. Encodes p16 which regulates progression through G1/S of the cell cycle[84].
ELMO1 & DOCK2[70]	17%	Encodes dimerization partners and intracellular mediators of the Rho family GTPase, RAC1[85]. In cancer models, mutated ELMO1 and other DOCK family members mediate enhanced migration and invasion[86]. ELMO1 mutation suggested gain of function phenotype and enhances invasiveness[70].
KRAS[70]	3%	Gain of function. Mutated RAS leads to constitutively activated Raf/MEK/ERK (MAP Kinase signal transduction pathways)[87].
ABCB1[77]	6%	Also known as the MDR1 gene. Encodes P-glycoprotein, a cell efflux transporter, one of many ubiquitous adenosine triphosphate (ATP)-binding cassette (ABC) pumps[88].

Table 2: Proportion and function of recurrent mutated genes in oesophageal adenocarcinoma**.**

Chromosomal changes which appear to have an important role in the progression of Barrett's oesophagus to OAC include copy-number changes (aneuploidy), and focal gains and losses[68, 89]. Chromothripsis has been observed in a third of OACs, a much higher frequency than in other malignancies (2-5%)[69]. These events may lead to the formation of double-minute chromosomes: extrachromosomal DNA, composed of chromatin which replicates within the cell nucleus, which harbour oncogenes, such as MYC and MDM2 (a known inhibitor of p53)[69, 90]. Further large-scale chromosomal rearrangements have also been observed in OAC which can result from breakage-bridge-fusion cycles[69]. These are initiated by telomere loss, then followed by fusion of unprotected chromosomal ends or sister chromatids and lead to duplications and dramatic copy-number increases[69]. Regions amplified by such events can harbour oncogenes (including RCF3, MDM2, VEGFA, BCAT1 and KRAS) and provide a selective growth advantage for cancer cells.

Inherited genome

Array heritability analysis of genome-wide association studies (GWAS) indicate that 24% of OACs, and 35% of Barrett's oesophagus cases are inherited, with a high genetic correlation and significant polygenic overlap[91, 92]. GWAS have identified eight loci within the constitutive genome within or near CRTC1, FOXP1, BARX1, ALDH1A2, MHC, FOXF1, GDF7 and TBX5 associated with the development of Barrett's oesophagus and/or OAC[93-95]. The largest meta-analysis of GWAS in OAC and Barrett's oesophagus to date, respectively including 4112 and 6167 individuals, with 17159 representative controls, identified nine risk loci, in addition to the eight previously identified, for one or other or both these diseases[96]. This study implicated these previously identified loci with both diseases. These new loci were within or near the following genes: CFTR, MSRA, LINC00208 and BLK, KHDRBS2, TPPP and CEP72, TMOD1, SATB2, HTR3C and ABCC5, and LPA. Pathway analysis to investigate potential causal genetic pathways implicated these genes in the involvement in negative regulation of muscle-cell differentiation, mesenchyme development, and mesenchyme cell differentiation and proliferation[96]. This finding is of particular interest as hiatal hernia and defective relaxation of the lower oesophageal sphincter permit gastro-oesophageal reflux; and therefore the harsh environment in which Barrett's oesophagus and OAC develop. Furthermore, the most strongly associated new variant associated with both OAC and Barrett's oesophagus was located within the cystic fibrosis transmembrane conductance regulator (CFTR), the chloride channel mutated in cystic fibrosis[97]. This could plausibly have functional relevance as reflux is very common in patients with cystic fibrosis (up to 80%)[98]. This study identified the first

risk locus associated with OAC, independent of Barrett's oesophagus, rs9823696, located near HTR3C and ABCC5 (which plays a role in embryonal development of the gastrointestinal tract)[99].

Environmental contribution to malignant progression

The high mutation rate, variable mutation spectra and mutational signature characterised by A>C transversions observed in OAC suggest a causal effect of mutagens in carcinogenesis, potentially attributable to oxidative stress mediated by the harsh mutagenic environment created by gastric and bile refluxate and resulting inflammation[70].

1.1.6. Clinical presentation

Endoscopic surveillance of patients with BO detects only 8% of the total number of cases of OAC[30]. Diagnosis of oesophageal malignancy depends mainly on presentation with symptoms to primary care prior to rapid onward referral to secondary care for diagnostic gastroscopy[100]. The most common symptoms are dysphagia and weight loss, and other less common presenting symptoms are odynophagia (pain when swallowing), haematemesis (vomiting blood), epigastric pain, reflux, haemoptysis (coughing up blood), cough, or symptoms clearly relating to metastatic disease, such as hoarseness (indicating involvement of the left recurrent laryngeal nerve), abdominal swelling secondary to malignant ascites or lymphadenopathy[101, 102]. However, there is considerable symptomatology overlap between malignant and benign disease. The challenge for general practitioners (GPs) is therefore judging when and when not to suspect oesophageal or gastric malignancy and hence refer patients for urgent gastroscopy. Risk of undiagnosed oesophageal malignancy varies considerably according to presenting symptoms. In a nested case-control analysis of the General Practice Research Database (GPRD) of patients of at least 40 years of age which included 7471 cases of oesophago-gastric malignancy (a selected composite endpoint given endoscopy is the diagnostic tool of choice for both) and 32877 controls matched by year of birth, gender and practice, quantified the risk of case status and positive predictive value according to recorded symptoms in the year prior to the index date[103]. The strongest risk factors were dysphagia (OR 139, 95% CI 112-173), weight loss (OR 8.9, 95% CI 7.1-11.2) and epigastric pain (OR 8.8, 95% CI 7.0-11). Unsurprisingly, dysphagia was a dramatically stronger risk factor for oesophageal (OR 230, 95% CI 180-300) than gastric cancer (OR 20, 95% CI 14-29) when analysed separately, while there were no substantial differences in effect sizes for the other listed predictors. The sensitivity of individual symptoms in predicting diagnosis of oesophago-gastric

malignancy is low. For example, even for two "red flag" symptoms, dysphagia and weight loss, the respective sensitivities are 32% and 8%[104]. Therefore in primary care, an approach that relied on a single symptom, such as dysphagia, to determine the need for urgent diagnostic gastroscopy would therefore miss 68% of diagnoses. Current referral pathway recommendations from the National Institute of Clinical Excellence (NICE) advise urgent (within two weeks) direct access for gastroscopy to assess for oesophageal cancer in people with dysphagia or if aged 55 years and over with weight loss and any of: upper abdominal pain, reflux or dyspepsia[105]. Non-urgent direct access gastroscopy is advised in patients over 55 years with at least one of the following: treatment resistant dyspepsia; upper abdominal pain with anaemia; raised platelet count with any of nausea, vomiting, weight loss, reflux, dyspepsia or upper abdominal pain; or nausea or vomiting with at least one of: weight loss, reflux, dyspepsia or abdominal pain. However, the studies on which these symptoms are based were mainly conducted in secondary care[106, 107], and are likely to suffer selection bias and be less relevant to patient population who present to primary care, the main point of referral. Furthermore, validation of these recommendations (particularly the two week wait referral guidelines in oesophago-gastric cancer) with measures of discrimination, calibration and performance are yet to be established. Patients with oesophago-gastric cancer with classical "red flag" symptoms, compared to those without, have more advanced cancer staging (47% vs. 11% International Union Against Cancer stage IV, $p < 0.001$), are less likely to undergo potentially curative surgical resection (50% vs. 95%, $p < 0.001$) and have poorer five-year survival rates (13% vs 42%, $p = 0.005$)[108]. Therefore reliance on such "red flag" symptoms to guide urgent referral for diagnostic gastroscopy selects out patients who are most likely to benefit from rapid diagnosis.

Symptom-based algorithms derived from large UK primary care datasets seem a rational method for determining future guidance on which urgent referral pathways should be based. An algorithm to estimate the absolute risk of the gastro-oesophageal malignancy in primary care from the presence or absence of a number of clinical characteristics has been derived and validated using a large UK primary care database, QRESEARCH. The final variables selected for the model were: smoking status (non-smoker, ex-smoker, current light, moderate or heavy), dysphagia, abdominal pain, appetite loss, haematemesis, weight loss and anaemia (Haemoglobin < 11g/dl in the last year) (see table 3). The receiver operating curve statistics were 0.89 for females and 0.92 for males. This algorithm together with another risk assessment tool[103] has been incorporated into an electronic clinical decision support tool, which is integrated into some GP computer systems to systematically identify those at the highest risk to facilitate early investigation. Its

implementation, effectiveness in impacting cancer-related outcomes and cost-effectiveness is the subject of an ongoing two-arm, multi-centre, cluster-randomised, controlled phase II trial[109].

Current symptoms and anaemia	Adjusted hazard ratio in women (95% CI)	Adjusted hazard ratio in men (95% CI)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Dysphagia	131 (97.5-175.0)	143 (108-189)	32.3	99.5	7.8	99.9
Abdominal pain	4.74 (3.54-6.33)	3.78 (3.32-4.30)	7.5	99.5	2.3	99.9
Appetite loss	10.0 (5.28-19.0)	3.87 (2.82-5.32)	23	90.5	0.3	99.9
Haematemesis	25.2 (14.4-44.2)	7.62 (6.08-9.55)	2.6	99.7	1.1	99.9
Weight loss	3.97 (3.06-5.16)	5.64 (4.67-6.81)	8	99.1	1.2	99.9
Hb < 11g/dl in last year	2.32 (1.84-2.93)	1.79 (1.44-2.23)	8.9	98.9	1.1	99.9

Table 3: adjusted hazard ratios, sensitivity, specificity, positive and negative predictive values for diagnosis of oesophago-gastric cancer according to documented symptoms and anaemia preceding diagnosis. Adapted from Hippisley-Cox and Coupland, 2011.

Abbreviations: CI, confidence interval; Hb, haemoglobin; NPV, negative predictive value; PPV, positive predictive value.

1.1.7. Management

The most recent National Oesophago-Gastric Cancer Audit indicates that 40% of patients with oesophageal cancer are treated with curative intent, while 60% are managed on a palliative pathway[110]. The mainstay of curative treatment for invasive oesophageal malignancy is oesophagectomy (oesophageal resection and reconstruction) with or without chemotherapy/chemoradiotherapy[111]. However, even in patients treated with curative intent, outcomes are still frequently poor: 57% develop recurrent cancer within 5 years of surgery[112], and at best, five-year survival is 45%[113, 114]. Of those patients with oesophageal carcinoma (including both adenocarcinoma and squamous cell carcinoma) who died following discharge after surgery in this trial, 85% were attributable to recurrent disease. Consistent with this, from observational data, 57% of patients with OAC treated with curative intent develop recurrence within five years of surgery[112]. The focus of management of patients on a palliative pathway is aimed at symptom control, improving survival and quality of life with palliative chemotherapy or radiotherapy, and endoscopic stenting to improve swallowing difficulties[110].

Staging

Accurate cancer staging is critical to determining management of patients with oesophageal cancer. Staging initially includes clinical examination, gastroscopy and computed tomography (CT) of the chest, abdomen and pelvis[115]. The focus of initial staging is to establish the presence of obvious metastatic disease[111]. Further staging modalities, such as positron-emission (PET) CT, endoscopic ultrasound (EUS), endoscopic mucosal resection (ER) or laparoscopy are employed to determine precise local staging if potentially curative treatments are being considered[111, 115]. For example, it is important to determine the depth of tumour invasion if endoscopic treatment modalities are being considered, or to determine the presence of peritoneal metastases in locally advanced tumours of the gastro-oesophageal junction for surgical candidates. Clinical guidelines advocate the use of the current staging system, the 7th edition of the American Joint Committee on Cancer (AJCC)/Union for International Cancer Control (UICC) Cancer staging manual[45]. Unlike the 6th edition, the 7th edition was data driven: all-cause mortality for 4627 patients with cancer of the oesophagus or gastro-oesophageal junction who underwent oesophagectomy without pre-operative chemo/radiotherapy from 13 centres across three continents (North America, Europe, Asia) were analysed using random forest methodology to derive stage groupings for which survival was monotonically decreasing, distinctive and homogenous[116]. By convention, stage 0 and IV were pre-determined, and respectively refer to tumour in situ (high grade dysplasia) and

distant metastatic disease, leaving stage I to III and their sub-categories to be derived. The stage groupings are determined from a combination of the extent of the cancer using the tumour, nodal and metastases (TNM) classification, the cancer grade and location (see table 4 and 5).

TNM staging	Description
Primary tumour (T)	
TX	Primary tumour cannot be assessed
T0	No evidence of primary tumour
Tis	Carcinoma in situ/High-grade dysplasia
T1	Tumour invades lamina propria, or submucosa
T1a	Tumour invades mucosa or lamina propria or muscularis mucosae
T1b	Tumour invades sub-mucosa
T2	Tumour invades muscularis propria
T3	Tumour invades adventitia
T4	Tumour invades adjacent structures
T4a	Tumour invades pleura, pericardium, diaphragm or adjacent peritoneum
T4b	Tumour invades other adjacent structures such as aorta, vertebral body or treachea
Regional lymph nodes (N)	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in 1–2 regional lymph nodes
N2	Metastasis in 3–6 regional lymph nodes
N3	Metastasis in 7 or more regional lymph nodes
Distant metastasis (M)	
MX	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

	Adenocarcinoma				Squamous cell carcinoma				
Stage	T	N	M	Grade	T	N	M	Grade	Location
0	is	0	0	1	is	0	0	1	Any
IA	1	0	0	0-1	1	0	0	1	Any
IB	1	0	0	3	1	0	0	2-3	Any
	2	0	0	1-2	2-3	0	0	1	Lower
IIA	2	0	0	3	2-3	0	0	1	Upper, middle
					2-3	0	0	2-3	Lower
IIB	3	0	0	Any	2-3	0	0	2-3	Upper, middle
	1-2	1	0	Any	1-2	1	0	Any	Any
IIIA	1-2	2	0	Any	1-2	2	0	Any	Any
	3	1	0	Any	3	1	0	Any	Any
	4a	0	0	Any	4a	0	0	Any	Any
IIIB	3	2	0	Any	3	2	0	Any	Any
IIIC	4a	1-2	0	Any	4a	1-2	0	Any	Any
	4b	Any	0	Any	4b	Any	0	Any	Any
	Any	3	0	Any	Any	3	0	Any	Any
IV	Any	Any	1	Any	Any	Any	1	Any	Any

Abbreviations: M, metastases; N, nodal involvement; T, tumour stage
Location definitions (distance from incisors): upper thoracic, 20-25cm; middle thoracic 25-30cm; lower thoracic 30-40cm

Prognosis

The overall prognosis of oesophageal malignancy is very poor: survival rates are among the worst of all malignancies, similar to lung and pancreatic, with overall 5-year survival rates in England in 2011 estimated at 15.1%[5]. One of the main driving contributors is advanced disease at presentation: approximately 70% of patients present with non-localised disease (either regional [spread to regional lymph nodes] or distal [metastatic disease])[5]. Even in patients suitable for potentially curative surgery, outcomes are often poor: in those with the earliest tumour stage with regional nodal involvement without metastatic disease (stage IIB), 5 year survival rates are 40% for both OAC and OSCC (see table 4)[116].

Prognostic risk factors

The known prognostic factors for OAC can be divided into demographic, clinico-pathological and molecular risk factors. The clinico-pathological factors TNM staging and grade are established prognostic variables which inform the 7th edition of the American Joint Committee on Cancer (AJCC) Cancer staging manual. In patients undergoing surgery for OAC, response to neo-adjuvant chemotherapy determined by tumour downstaging (defined by reduction in T or N stage of pathological staging [from the resected specimen] compared to clinical staging [initial staging] is independently associated with reduced recurrence (HR 0.49, 95% CI 0.35 – 0.68) and mortality (HR 0.50, 65% CI 0.35 – 0.71)[114]. Similarly, other markers of tumour response to neo-adjuvant chemotherapy, such as the Mandard Score (see figure 7[117]), independently predict survival[118]. Lymphovascular invasion and positive surgical resection margins determined by histological examination of the surgical specimen also independently predict recurrence and mortality[114].

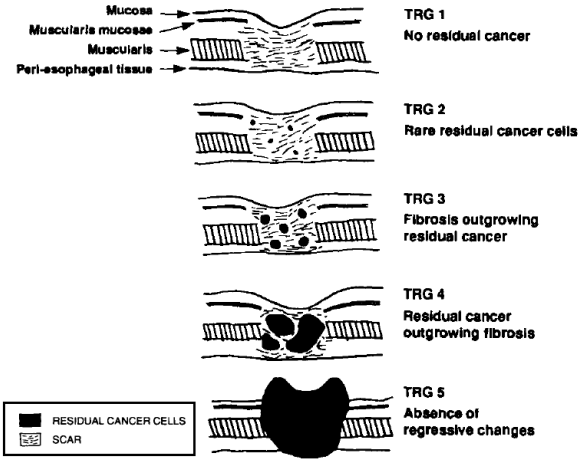

Figure 7: Tumour regression grade (Mandard score).

A number of prognostic molecular markers for OAC have been identified through immunohistochemistry (IHC), whole-genome and whole-exome sequencing. The tumour-suppressor gene tumour protein 53 (p53) is highly mutated in up to 70% with OAC, and plays multiple roles, including regulating cell cycle progression, apoptosis, DNA repair, autophagy, differentiation and senescence [77, 78]. In a meta-analysis of 11 studies of 644 patients with OAC, mutated p53 was associated with higher all-cause mortality (pooled HR 1.46, 95% CI 1.17-1.83) independent of clinical stage[119]. In addition to P53, a recent meta-analysis has identified other candidate biomarkers consistently predictive of mortality in OAC: cyclooxygenase-2 (COX-2), CD3 and CD8+ T cell infiltrate, and epidermal growth factor receptor (EGFR)[120, 121]. COX-2 is the rate-limiting enzyme which catalyses arachidonic acid to potentially mitogenic prostaglandins which promote cell survival, cell proliferation and angiogenesis[122]. In an individual patient level meta-analysis of eight RCTs, including 25 570 patients with 674 cancer-related deaths, (62 were due to oesophageal cancer), demonstrated that allocation to aspirin (a COX-2 inhibitor) significantly reduced cancer-specific mortality from OAC (pooled HR 0.36, 95% CI 0.21-0.63, p=0.0001); although the reduction in mortality very likely reflects a causal reduction in incidence rather than an isolated impact on mortality[123, 124]. COX-2 expression detected by IHC was associated with increased risk of all-cause mortality in three studies which included 382 patients (HR 2.47, 95% CI 1.15-3.79)[120]. There is growing evidence the adaptive immune response influences solid tumour behaviour; and both CD3+ and CD8+ T cell tumour infiltration are associated with reduced mortality for gastric and colorectal cancers[125, 126]. A meta-analysis of two studies which included 203 patients with OAC demonstrated that tumour CD3+ (HR 0.51, 95% CI 0.32-0.70) and CD8+ (pooled HR 0.55, 95% CI 0.32-0.70) T cells infiltration were independently associated with reduced risk of all-cause mortality[120]. The EGFR is a tyrosine kinase receptor which is overexpressed and/or amplified in a number of epithelial malignancies and its associated signalling transduction cascade mediates tumour cell proliferation, cell survival, adhesion and angiogenesis[127]. A meta-analysis of two studies including 642 patients with OAC demonstrated aberrant EGFR expression was associated with higher risk of all-cause mortality (HR 1.65, 95% CI 1.14-2.16)[120]. A number of other potential prognostic molecular markers for OAC have been identified that require validation, including the hepatocyte growth factor receptor (Met), tumour macrophage infiltration, FOXM1 and its target gene PLK1, heat-shock protein and glucose regulated protein expression profiles, and insulin-like growth factor binding protein expression[128-132]. Although none of these biomarkers are currently in routine clinical use, there is clinical interest in their application to not only further prognosticate patients and allow improved risk stratification to guide treatment, but also they may represent novel therapeutic targets[120].

The challenge for improving prognosis in patients is devising strategies to ensure patient presentation at the earliest stage of disease. Oesophago-gastric cancer awareness campaigns are operational in the UK, which seek to educate the public and encourage prompt presentation to primary care with the well characterised “alarm symptoms”. Nevertheless, public education, and symptom-based urgent referral guidelines or algorithms will not address the central driver of poor prognosis in this malignancy: early stage tumours do not typically cause the classical symptoms, which become apparent usually with advanced disease[108, 111]. Similarly to other epithelial, gastrointestinal malignancies, 25% with oesophageal cancer have three or more consultations with their general practitioner prior to referral to hospital for diagnosis[133].

1.2.HMG-CoA reductase inhibitors

Mounting preclinical and observational data indicate that 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) inhibitors, better known as statins, exert anti-neoplastic effects against a number of malignancies. The following outlines the history of statins, current indications, regulation of the mevalonate pathway, its relevance as a potential therapeutic cancer target, preclinical and pharmaco-epidemiological evidence relating statin use with disease incidence and mortality.

1.2.1. History

Interest in the isolation and development of potent compounds to lower serum cholesterol were stimulated by growing evidence for the association between hypercholesterolaemia and cardiovascular morbidity and related mortality[134]. The earliest and most compelling epidemiological evidence was from the Framingham Heart Study, a prospective population-based cohort conducted in Framingham, Massachusetts, initiated in 1948 of 5, 127 men and women aged 30-60 years, followed up for 10 years, which demonstrated the association between serum cholesterol and risk of incident coronary heart disease and death within three weeks of MI[135].

The 3-hydroxy-3-methylglutaryl-coenzyme (HMG-CoA) reductase inhibitors, better known as "statins", were discovered in 1976 by Japanese Biochemist, Akira Endo, with the isolation of ML-236B (mevastatin) from the fungus, *Penicillium citrinum*[136, 137]. Endo demonstrated this compound competitively inhibited HMG-CoA, the rate limiting step of the mevalonate pathway, without affecting other enzymes in the pathway[136, 137]. In the same year, a British group, Brown et al. independently isolated the same compound (they named it compactin) from *Penicillium brevicompactum*, and determined its molecular structure using a combination of spectroscopy, chemical and X-ray crystallography[138]. Subsequently, Endo demonstrated in a number of animal studies that mevastatin significantly and substantially reduced plasma cholesterol in rats, monkeys and dogs[137, 139, 140]. In the earliest human non-randomised clinical study, in 11 patients with heterozygous familial hypercholesterolaemia and combined hyperlipoproteinaemia, mevastatin administration for between 4-8 weeks led to a significant reduction in serum cholesterol (mean reduction 27% [range 11-37%])[141]. Further human clinical trials of mevastatin were initated, but subsequently terminated due to the development of lymphoma observed in dogs that received high doses of this drug[134]. In 1979, another HMG-CoA reductase inhibitor, mevinolin, later known as lovastatin, was isolated from *Aspergillus terreus*[142]. In animal studies this compound was found to be more potent in inhibiting HMG-CoA and reducing plasma

cholesterol[143]. Clinical trials demonstrated its favourable safety profile and tolerability and confirmed its efficacy in lowering cholesterol in healthy "normocholesterolaemic" volunteers, a finding later confirmed in patients with hypercholesterolaemia[144, 145]. In 1987 Lovastatin became the first statin to be approved by the US Food and Drug Administration[146]. This experience provided the stimulus for the development and marketing of the semi-synthetic statins (simvastatin and pravastatin) and synthetic statins (fluvastatin, rosuvastatin, atorvastatin and pitavastatin): derivative compounds with even greater potency[134, 147].

Since these statins have been approved for market use, their efficacy and safety profile have been examined extensively in numerous randomised controlled trials. The Cholesterol Treatment Trialist's (CTT), an international collaboration of investigators which contribute data from large RCTs (with a target of at least 1000 participants per included trial, with at least 2 years' follow-up and examine interventions to modify serum lipid levels for individual participant data meta-analyses) have robustly confirmed the efficacy of statins against a number of cardiovascular endpoints both in patients at risk and not at risk of cardiovascular disease[148-151]. In their first meta-analysis of 90, 056 participants in 14 randomised controlled trials, allocation to a statin (studies included either simvastatin, pravastatin, lovastatin, atorvastatin or fluvastatin as the intervention) was effective in the prevention of non-fatal MI, death attributable to coronary heart disease, first coronary revascularisation (both coronary artery bypass graft and percutaneous transluminal coronary angioplasty) and incident ischaemic stroke[148]. Studies included patients both at risk and not at risk of cardiovascular events. These findings were irrespective of baseline lipid profile and other relevant clinical characteristics. There was an approximate linear relationship between absolute reduction in low density lipoprotein (LDL) cholesterol and the proportional reduction in the event rate for major coronary and vascular events (defined as a composite outcome of myocardial infarction or coronary death, stroke, or coronary revascularisation). Significant effects on the risk of a major coronary events were apparent during the first year of treatment with statins, however effect sizes were even stronger with longer durations of use. In a meta-analysis of 39, 612 individuals from five trials, more intensive LDL cholesterol reduction with higher versus lower dose statin allocation resulted in significantly fewer major coronary events, coronary revascularisations and ischaemic strokes[151]. Even in patients at low risk of major vascular events (lower the 10% five year risk), a group previously not advocated to receive statins, proportional reduction in major vascular events was at least as strong compared with higher baseline risk groups with statin allocation[149].

The statins currently available for clinical use in the UK are simvastatin, atorvastatin, pravastatin, fluvastatin, and rosuvastatin[152]. The current licenced indications for statins are for primary hypercholesterolaemia, post-transplantation hyperlipidaemia (particularly pravastatin), homozygous and heterozygous familial hypercholesterolaemia and for the primary and secondary prevention of cardiovascular events[152].

1.2.2. The mevalonate pathway: function, cascade and regulation

The mevalonate pathway is an essential metabolic pathway present in all eukaryotes and plays a central role in a number of cellular processes[153, 154]. Mevalonate is a precursor for a series of sterol and non-sterol isoprenoid groups which are incorporated into the end-products of the pathway: cholesterol (required for lipoprotein, steroid hormone, vitamin D and bile acid synthesis); haem A and ubiquinone (which participate in the electron transport chain); dolichyl-pyrophosphate (responsible for N-glycosylation of growth-factor receptors); geranylgeranyl pyrophosphate and farnesyl pyrophosphate (responsible for post-translational modification of a number of small guanosine triphosphatases [GTP-ases] which have essential roles in controlling signalling pathways responsible for proliferation, differentiation and carcinogenesis)[155, 156].

At the apex of the mevalonate pathway (figure 8, adapted from Goldstein & Brown and Konstantinopoulos[153, 156]), Acetyl Co-enzyme A (CoA) undergoes cleavage and condensation, catalysed by acetyl-CoA thiolase to acetoacetyl-CoA[157]. Subsequently this enzyme undergoes condensation with acetyl-CoA, catalysed by HMG-CoA synthase to HMG-CoA. HMG-CoA reductase, the rate-limiting step of the mevalonate pathway, reduces HMG-CoA to mevalonate. Subsequently mevalonate kinase, phosphomevalonate kinase then mevalonate pyrophosphate decarboxylase respectively convert mevalonate to mevalonate-5-phosphate, mevalonate-5-pyrophosphate, then isopentenyl pyrophosphate (PP), the first in a series of isoprenoid intermediates produced by the pathway[157]. Isopentenyl-PP is then converted to geranylgeranyl-PP, and subsequently farnesyl-PP by farnesyl-PP synthase. Farnesyl-PP can then either be converted into cholesterol, dolichyl-PP or geranylgeranyl-PP[153, 156].

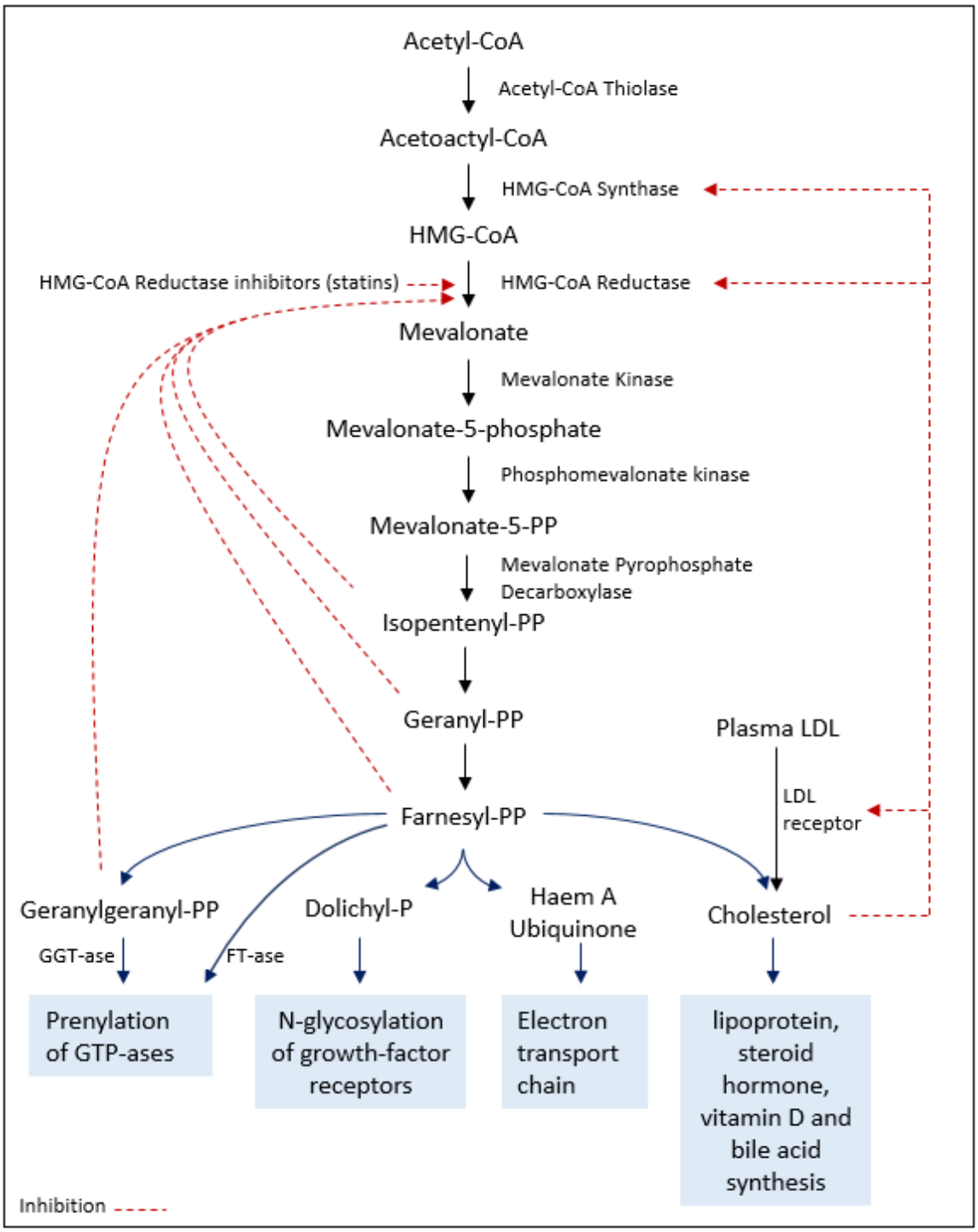

Figure 8: Mevalonate pathway: enzymatic cascade, end-products, functions and regulation. HMG-CoA reductase is the rate limiting step of the mevalonate pathway which is responsible for the production of sterol and non-sterol isoprenoids. Not all enzymes involved in the pathway are shown. Adapted from Goldstein & Brown 1990, and Konstantinopoulos 2007. Abbreviations: CoA, co-enzyme A; FT-ase, farnesyltransferase; GGT-ase, geranylgeranyltransferase; GTP-ases, guanosine triphosphatases; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; LDL, low density lipoprotein; PP, pyrophosphate.

The mevalonate pathway is highly regulated through negative feedback of downstream sterol and non-sterol products. Cellular cholesterol is obtained through two sources: endogenous production via the mevalonate pathway and exogenous uptake from plasma of LDL cholesterol via the LDL receptor. The mevalonate pathway is regulated by intracellular cholesterol through negative feedback to inhibit transcription of HMG-CoA synthase, HMG-CoA reductase and the LDL receptor[153]. Non-sterol components also regulate the pathway through negative feedback, via post-transcriptional control of HMG-CoA reductase through modulating translation of its messenger RNA and by controlling the enzyme's degradation[153, 158]. These feedback mechanisms are mediated the sterol regulatory element-binding protein (SREPB) family of transcription factors[159]. When sterol levels are high, the SREPBs are inactive and are localised to the endoplasmic reticulum (ER). In states of sterol depletion, SREBPs dissociate from the ER and translocate to the Golgi, where they are cleaved by site-1 and site-2 protease, before translocating to the nucleus where they bind sterol regulatory elements (SREs) of the promoters of their target mevalonate pathway genes, therefore restoring sterol and non-sterol components[159, 160].

1.2.3. Malignant modulation of the mevalonate pathway

The hallmarks of cancer proposed by Hanahan and Weinberg in 2000 are sustained proliferative signalling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis and activating invasion and metastasis[161]. This list was subsequently updated in 2011 to also include cancer's ability to evade immune destruction and reprogramming of energetic metabolism[162]. There are numerous examples of cellular mechanisms of malignant modulation of the mevalonate pathway to confer survival advantage[159]. Accumulating experimental data indicate that common recurrent mutations, including p53, can exert gain-of-function properties to upregulate transcription of mevalonate pathway enzymes to provide unrestricted supply of mevalonate pathway products to permit the survival and proliferation of malignant cells[159]. This has been demonstrated in breast cancer previously, where such mutations enabled p53 to interact with nuclear SREBP2 in increase transcription of mevalonate pathway genes[163]. The modulation of the mevalonate pathway by certain p53 gain-of-function mutations underscores its potential as a viable therapeutic target, especially in the context that p53 mutations are very common in OAC[77].

1.2.4. Anticancer mechanisms of statins

The actions of statins which appear to be independent of their cholesterol-lowering properties, termed their pleiotropic effects, have been characterised in the setting of cardiovascular medicine

and include beneficial effects on endothelial function, limiting vascular inflammation, enhancing plaque stability, inhibiting platelet aggregation and promoting myocardial contractility[164]. Further compelling examples of the pleiotropic actions of statins are their disparate and well-documented non-cardiovascular effects as demonstrated from clinical trial evidence[165], including: myopathy[148], diabetes[166], reduced risk of pancreatitis[167] and contrast-induced nephropathy[168]. As the mevalonate pathway is such a ubiquitous metabolic cascade with multiple downstream products including sterol and non-sterol isoprenoids, it follows that competitive inhibition at its apex (of HMG CoA by statins) will exert multiple and a diverse number of actions, some of which are relevant to cancer biology. The following summarises the functional relevance of downstream products of the mevalonate pathway and how through their depletion, statins may plausibly exert anticancer effects.

Cholesterol

Cholesterol is an essential structural component of cellular membranes. Proliferating cancer cells rapidly produce cell membranes and increased cholesterol synthesis contributes to this[159]. Patients with cancer have significantly lower serum LDL cholesterol than age and gender matched controls, a finding not entirely explained by poor nutritional status[169]; and may reflect peripheral absorption of extracellular cholesterol by cancer cells to meet demand. All statins undergo extensive first-pass extraction with relatively low systemic bioavailability, although there are differences between individual statins[170]. Inhibition of cholesterol production by statins, either predominantly in the liver and/or locally within tumour cells, could therefore inhibit tumour growth and metastases. Whether this is a causal mechanism which could reduce the incidence of cancer or improve cancer-related mortality is not clear. Low plasma LDL cholesterol has been associated with an increased risk of cancer at a population-level, however a mendelian randomisation study did not establish a causal association between genetically reduced LDL cholesterol levels (due to polymorphisms that are associated with lifelong reduced plasma LDL cholesterol) and risk of cancer[171]. Hypercholesterolaemia has been shown to be associated with reduced mortality from hepatocellular carcinoma at a population level (HR 0.50, 95% CI 0.37-0.67)[172], however this may be explained in part by reverse causation bias - patients with more advanced cancer may have lower cholesterol levels; and confounding by medication use - hypercholesterolaemic patients would be expected to be more likely to receive statins during follow-up. Cholesterol is also a precursor for downstream steroid hormones which are known to initiate and further the malignant progression of a number of tumours, including breast and prostate cancer[173]. Given the male predominance of Barrett's oesophagus and OAC, and the expression of androgen receptors in OACs it has been proposed that testosterone may play a role in their aetiology[174, 175]**.** A meta-analysis of 11 trials demonstrated that allocation to a statin significantly lowered serum testosterone[176]. While observational data indicate the highest quintile of circulating free testosterone levels are strongly associated with risk of Barrett's oesophagus in men (OR 5.36, 95% CI 2.21-13.03)[175]; to date there are no published data which examine the association between testosterone levels and risk of OAC and subsequent prognosis. Depletion of cholesterol with resultant reduced testosterone could operate as a causal mechanism in conferring chemopreventive and therapeutic effects in patients with BO and OAC respectively, however there is insufficient evidence to substantiate this hypothesis further.

Geranylgeranyl pyrophosphate and farnesyl pyrophosphate

Geranylgeranyl-PP and farnsesyl-PP are responsible for the post-translational modification of a number of members of the RAS superfamily of guanosine-triphosphate-bound proteins (GTPases); the overexpression or mutation of which is established in the aetiology and prognosis of many

solid tumours, including epithelial gastrointestinal malignancies, such as OAC[70, 156]. The RAS superfamily are a functionally diverse group of G proteins involved in many important biological processes. They are able to switch between biologically active (GTP-bound) and inactive (Guanosine diphosphate [GDP]-bound) conformations[177]. They include KRAS, NRAS, HRAS, RHOA, RHOC, RAC1/2, CDC1/2, RAB, ARL5, SARA1/2 and ARF6[156]. RAS proteins mediate transmembrane signal transduction: they activate the RAF/MEK/ERK pathway which mediates cell growth and cell cycle entry by phosphorylation of MAPK family of kinases, and the P13K/AKT pathway modulates cell survival, growth and metabolism. Common RAS mutations stabilise the proteins in a constitutively active GTP-bound conformation. RAS mutations have also been implicated in mediating resistance to chemotherapy agents such as cisplatin[178]. The RHO subfamily of GTPases play important roles in cytoskeleton organisation, cell adhesion and cell motility; and promote cell-cycle progression through G1 by regulating cyclin D1 and cyclin-dependent inhibitors. Consequently RHO GTPases are implicated in invasion and metastasis, and their overexpression is linked to poorer clinical outcomes.

RAS GTPases contain a CAAX motif (c=cysteine, AA=aliphatic amino acid, X=any amino acid) to which hydrophobic FPP or GPP form covalent attachment (specifically with the cysteine residue)[156]. This process (termed “farnsesylation” or “lipidation”) creates a lipidated hydrophobic domain which localises and tethers RAS proteins to cell membranes permitting their proper function. Potent inhibition of the mevalonate pathway with statins depletes geranylgeranyl-PP and farnesyl-PP, preventing RAS localisation and limiting downstream effector pathways. The functional relevance of this mechanism has been demonstrated in OAC (discussed below in more detail)[179].

1.2.5. Preclinical studies of the effects statins in OAC cell lines

To date, the effects of a number of statins on cell viability, proliferation and apoptosis on four verified OAC cell lines, have been determined[179-182]. The functional relevance of downstream products of the mevalonate pathway and linked signalling cascades, which are of relevance to malignant proliferation, have also been demonstrated.

In the OAC cell line, OE33, the effects of three statins – simvastatin, pravastatin, and lovastatin were examined on viable cell numbers, proliferation, apoptosis and respective relevant signalling pathways were elucidated[179]. All three statins produced a dose-dependent reduction in cell viability (cell numbers). Significant reductions for all three statins were observed at a 50 µM concentration when cultured in serum-free media, and at 10 µM concentration when cultured for 24 hours in 10% fetal calf serum (FCS). There was no quantitative difference between the three statins. Separately, the same group demonstrated in FLO-1 cells (another validated OAC cell line) that rosuvastatin even at 0.1 µM concentrations have been shown to significantly reduce cell viability[183]. Reduced cell viability was explained by both decreased proliferation (78%, 67% and 73% reduction respectively with simvastatin, pravastatin and lovastatin) and increased apoptosis (between 43-101%, using two different assays) with consistent increases in caspase-3 activity (by 152-189%) and expression of the pro-apoptotic proteins, Bax and Bad (only pravastatin was used to demonstrate this)[179]. Statins did not affect expression of the anti-apoptotic protein Bcl-2. Through "adding back" intermediates of the mevalonate pathway, the investigators systematically demonstrated the functional relevance of the components of the mevalonate pathway and hence their subsequent depletion by statins. Treatment with mevalonate and farsenyl-pyrophosphate substantially attenuated, but did not completely abrogate the effect of statins. Geranyl-geranyl pyrophosphate did not alter the effects of simvastatin or pravastatin. This would suggest the predominant anti-prolferative/pro-apoptotic mechanism is mediated by farsenylation, rather than geranylgeranylation. This was further corroborated by reduced Ras activation in simvastatin treated cells, while inhibition of Ras activation was not observed using a specific inhibitor of geranylgeranylation. Implicated growth signalling pathways involved in proliferation and cell survival, protein kinase B (AKt) and extracellular signal regulated kinase (ERK) activity were modulated by simvastatin: pre-treatment with simvastatin before stimulation with 10% FCS reduced ERK and Akt activation. P38 MAP Kinase and JNK activity were not affects by statin pre-treatment. Co-treatment with any of the three statins with either cisplatin or 5-fluorouracil did not significantly alter cell viability compared to statin-treated cells without these cytotoxic agents.

The same group showed Rosuvastatin caused pro-apoptotic effects in non-malignant BO cells[183], however, the effects on normal squamous oesophageal mucosa were not determined

In FLO-1 cells, the effects of simvastatin, atorvastatin and pravastatin were examined on cell viability, proliferation, apoptosis and expression of ICAM-1, an adhesion molecule involved in transendothelial tumour cell migration, and metastases[180, 184-186]. Cell viability was attenuated by simvastatin at 30 μM and 50 μM concentrations, with consistent reductions in proliferation, and increased apoptosis, all with dose-dependent effects. In contrast, treatment with atorvastatin or pravastatin did not affect viability, or apoptosis but did attenuate proliferation. All three statins significantly attenuated concentrations of cell-surface ICAM-1 expression. However, the functional relevance of this finding was not further examined.

In OE-19 OAC cell lines, exposure to simvastatin reduced cell viability in a dose-dependent manner with 10 μM and 30 μM concentrations[182]. Cyclo-oxygenase-2 (COX-2) expression when stimulated by tumour necrosis factor alpha (TNFα) was inhibited by co-treatment with simvastatin at 10 μM. Simvastatin treatment increased Bax expression (at 10 μM) and reduced expression of Bcl-2, the latter in contrast to OE33 cells[179].

In SKGT-4 OAC cell lines, lovastatin at 4 μM concentrations significantly reduced cell viability and suppressed cell invasion (measured using matrigel coated chambers)[181]. Lovastatin downregulated ERK, c-jun and COX-2 expression, and upregulated caspase 3. Nude mouse xenografts (SKGT-4 cells treated with and without lovastatin for 3 days were injected into the right flank with 50% matrigel) were treated with and without lovastatin orally for 5 days per week for 30 days. The weight of xenograft tumours was non-significantly less in Lovastatin treated mice (n=5), compared to controls (n=5) assessed at 30 days. There was reduced expression of Ki67, phosphorylated ERK and COX-2 in xenograft explants as assessed by immunohistocytochemisty in the lovastatin treated mice compared with controls.

While there is encouraging evidence that statins inhibit proliferation and stimulate apoptosis in cell lines, and elucidation of plausible candidate pathways has begun, it is not clear whether these findings are of clinical relevance to patients with BO (for the prevention of progression) or OAC to (improve cancer-related outcomes). It is difficult to take findings from *in vitro* studies of malignant cells and draw direct inferences about disease prevention. Similarly, in vitro studies cannot be expected to adequately mimic tumour cell signalling and behaviour, or host interactions; as such in vitro culture conditions differ from the microenvironment that a cell would experience in vivo. Future experiments examining the effects of statins in 3D cell culture models of BO and OAC would be of interest. Although biological mechanisms implicated in aetiology and prognosis are frequently distinct, some do overlap, which may be of relevance to OAC: specific gain-of-function

p53 mutations can regulate the mevalonate pathway to harness cellular metabolism, and p53 mutations confer both higher risk of malignant progression in BO and are associated with worse prognosis in established tumours[77, 119]. An accumulating number of well-conducted observational studies suggest significant inverse associations between statin use and cancer incidence and mortality in patients with malignancy[187-190]. Taken together, supportive preclinical and observational data indicate the potential for statins as novel chemopreventive or therapeutic strategies deserves further research.

1.2.6. Observational evidence for the association between statin use and cancer-related outcomes

The following summarises the wider observational literature which examines associations between statin use and mortality outcomes in patients with malignancy overall. There is a paucity of data on the relationship between statin use and survival in patients with OAC. The following therefore considers the results and methodological considerations for studies conducted in patients with colorectal carcinoma (CRC) for which key tenets may be applicable.

Statin use and cancer-related outcomes overall

Associations between statin use and cancer related outcomes in populations with cancer have been examined extensively across many tumour types in the observational literature, with the weight of evidence suggesting significant inverse associations. The most recent systematic review and meta-analysis of observational studies on the subject included 95 cohorts with over 1.1 million patients diagnosed with cancer[191]. The most common sites to be investigated in individual epidemiological studies were prostate (32.6%), breast (15.8%) and colorectal cancers (9.5%), followed by renal cell carcinoma (5.3%), bladder, hepatocellular, lung and uterine cancers (each 4.2%). Haematological malignancies (lymphoma, leukaemia and myeloma), biliary tract, gastric and neurological malignancies are relatively under-represented, as such accounting for less than 3% of studies each. The total number of participants examined for each site varies substantially: breast cancer (208, 780 participants), prostate cancer (108, 399), colorectal carcinoma (44, 476) and lung cancer (15, 846) account for the majority. Statin exposures measured vary considerably between and within studies, including their measurement pre-diagnosis, at the time of diagnosis and post-diagnosis. Investigators examined cancer-related outcomes include all-cause mortality, cancer-specific mortality, progression-free survival and disease-free survival. Statin use was significantly associated with a reduced risk of all-cause mortality (HR 0.70, 95% CI 0.66-0.74 pooled from 55 studies), cancer-specific mortality (HR 0.60, 95% CI 0.47-0.77 pooled from 32 studies), progression-free survival (HR 0.67, 95% CI 0.56-0.81 pooled from 22 studies), and disease-free survival (HR 0.53, 95% CI 0.40-0.72 pooled from 9 studies). Exposure to statins measured separately pre and post-diagnosis of cancer were each associated with significant improvements in all-cause mortality, cancer specific mortality, progression-free survival and disease-free survival in pooled analyses, with no evidence of significance differences between these subgroups of exposure (pre vs. post diagnosis, all p values > 0.1), see table 6 (adapted from Mei et al.[191]). Evidence of publication bias was demonstrated for studies which reported all-cause mortality (egger's p value < 0.001) but not the other outcomes listed. Subgroup analysis

demonstrated that stratification of studies by quality (the exact method for assessing study quality was not specified), yielded different effect sizes: modest effect sizes (HR 0.81, 95% CI 0.77-0.85, from 29 pooled studies) were observed in studies assessed of higher quality, while larger effect sizes (HR 0.57, 95% CI 0.47-0.68) were observed for studies assessed of lower quality, for the outcomes of all-cause mortality.

Statin exposure and outcome	Number of studies	Hazard Ratio (95% CI)	P for subgroup difference
All cause mortality			
Prediagnosis	31	0.74 (0.68-0.79)	0.133
Postdiagnosis	24	0.65 (0.60-0.72)	
Cancer-specific mortality			
Prediagnosis	21	0.64 (0.55-0.73)	0.809
Postdiagnosis	12	0.65 (0.55-0.76)	
Progression-free survival			
Prediagnosis	11	0.65 (0.49-0.88)	0.734
Postdiagnosis	8	0.73 (0.60-0.91)	
Disease-free survival			
Prediagnosis	5	0.48 (0.26-0.88)	0.539
Postdiagnosis	4	0.60 (0.44-0.81)	

Table 4: Subgroup analyses of statin exposure (measure pre or postdiagnosis) and associations with cancer-related outcomes. Adapted from Mei 2007.

While the pooled estimates of the association between statin use and cancer-related outcomes were significant and remained so when stratified according to tumour site, statin exposure and mortality outcome, there was considerable heterogeneity between studies pooled overall (I^2 92.9%, heterogeneity P < 0.001). This is not unexpected, as there were considerable variations in study characteristics, including those highlighted above, also disease stage investigated, duration of follow-up and the setting and location of the research. Nevertheless, even when stratified for tumour site and mortality outcome, heterogeneity was still significant (I^2 > 50%) for most sites and outcomes tested. Funnel plot inspection revealed asymmetry and suggests possible contributory publication bias.

Therefore, while it is encouraging that statin use overall is associated with significant improvements in cancer-related outcomes, this finding per se is of limited value as it is difficult to

interpret and is not necessarily of direct clinical relevance: the epidemiological evidence base needs to be carefully considered for each cancer site separately before concluding whether a causal association may exist. Design considerations which demonstrate features suggestive of causal associations, such as the Bradford Hill criteria, can be informative[192]. However, it should be noted the absence or presence of any or all nine criteria can neither absolutely confirm nor refute causality. Methodological appraisal is required to identify shortcomings commonly encountered in this field, such as immortal time-bias, confounding and channeling bias/reverse causation bias which may offer alternative, more likely explanations for associations.

Post diagnosis statin use and mortality in patients with colorectal carcinoma

The only other epithelial gastro-intestinal adenocarcinoma in which associations have been extensively examined is CRC. Clearly there are major distinctions between OAC and CRC as disease entities in terms of aetiology, cancer biology and outcomes; nevertheless shared treatment effects exist between the two (as demonstrated by the widespread use of platinum-based chemotherapy regimens in both diseases), and insights may therefore be gained from appraising the evidence relating statin use and outcomes in patients with CRC. To date, 11 studies have examined associations between statin use following diagnosis of CRC and all-cause mortality[187, 193-202]; and four have examined associations with cancer-specific mortality[187, 196, 199, 200]. Cancer-specific mortality, where deaths due to other causes are censored, is an outcome of particular interest in attempting to infer causality with the purported anti-neoplastic effects of statins, as a means of negating the known cardiovascular benefits and considering competing risks of death. The two largest studies were population-based cohort studies which addressed both of these outcomes and were conducted by the same research group, using consistent methodology[187, 199]. The first was conducted using the Clinical Practice Research Datalink (CPRD), a large primary care healthcare dataset (described in detail below), with linkage to the National Cancer Registry and the office for national statistics dataset[187]. In total 7657 patients with incident stage I-III CRC, diagnosed from 1998 to 2009, who survived at least one year following diagnosis were identified. Statin use was modelled as a time-dependent covariate, with exposure lagged by 6 months. Statin use post-diagnosis (prescribed to 53% after diagnosis) was associated with significant reductions in all-cause mortality (HR 0.75, 95% CI 0.66-0.84) and cancer-specific mortality (HR 0.71, 95% CI 0.61-0.84). Analyses were adjusted for year of diagnosis, age at diagnosis, gender, tumour stage and grade, surgery/chemotherapy/radiotherapy within 6 months of diagnosis, smoking, co-morbidity, deprivation, and use of concomitant medications (also measured as time-varying co-variates) including low dose aspirin, angiotensin converting enzyme (ACE) inhibitors and

metformin. Cumulative dose-response relationships were demonstrated with stronger associations observed in patients receiving higher cumulative dosages (accounting for the statin used within class and potency) than patients receiving lower dosages for both all-cause and cancer-specific mortality. The second study was conducted in Scotland, using the Scottish cancer registry, the prescribing information system, the general/acute inpatient and day case dataset, the outpatient attendance dataset and the national records of Scotland death record[187, 196, 199, 200]. A cohort of 8391 incident Duke's A-C CRC patients, diagnosed from 2009-2012, were identified after patients who died in the first year after diagnosis were excluded. Again, a lag of six months was applied to post-diagnosis statin exposure, and adjustment was made for the same factors listed above (except ACE inhibitors and metformin). Statin use (observed in 76%) was not significantly associated with cancer-specific (HR 0.90, 95% CI 0.77-1.05) or all-cause mortality (HR 0.90, 0.80-1.02). New initiation post-diagnosis (excluding prior users) was inversely associated with cancer-specific (HR 0.64, 95% CI 0.42-0.99) and all-cause mortality (HR 0.68, 95% CI 0.48-0.96), however these estimates were informed by relatively few events: 24 and 42 respectively. There was no convincing evidence for either duration-response or cumulative dose-response relationships for either cancer-specific or all-cause mortality.

While these two studies did not yield truly distinct results: there was overlap of reported confidence intervals between both studies for the primary exposures (from 0.77-0.84 for cancer-specific mortality; and 0.80-0.84 for all-cause mortality); the non-significant finding observed in the Scottish cohort study, neither excludes nor confirms an inverse association. Nevertheless, it is of interest estimates from the Scottish cohort did not reach significance, or demonstrate other features consistent with a causal relationship (such as cumulative duration-response relationship). This finding may rest with differences in unmeasured lifestyle factors between the two studies (unmeasured confounders) and advances in detection (the bowel cancer screening programme) and treatment (oxaliplatin-based chemotherapy) which were well established during the study period of the Scottish study, and were introduced during the course of the CPRD study and were potentially associated with statin use; although the former would be expected to have been addressed through adjusting for stage. Systematic differences between populations in adherence to statins would introduce measurement error and potentially bias results to the null. It is not possible to test this hypothesis in these cohorts: routine healthcare datasets accurately define dispensed prescriptions, but do not measure adherence. The use of lagged exposures could also have feasibly biased associations to the null. The practice of lagging exposures is used to subvert reverse causation in the context of cancer recurrence: where a recurrence may influence exposure (statin use)[203]. While these studies did not examine recurrence specifically, disease

progression in non-curative cohorts or recurrence in curative cohorts is a logical precursor to cancer-specific mortality. It is highly probable that these events are of relevance to these cohorts and could in themselves influence statin prescription or adherence. In theory, the duration of lag periods also requires accurate assumptions for the latent period for recurrent disease or progression and the period over which the exposure is expected to exert a plausible biological effect at a population level[203]. In practice, however, these details are not known with any degree of certainty, and the duration of any selected lagged exposure is therefore at best based on assumptions and at worst arbitrary. A lagged exposure would be expected to systematically draw associations to the null, and could therefore bias associations such that null hypotheses are falsely accepted: person-time in exposed participants, after the onset of exposure, is classified as non-exposed for the duration of the lagged period, while periods after are assigned to the exposed groups. This is demonstrated in sensitivity analyses for the CPRD study, with HRs for cancer-specific mortality incrementally approaching the null the longer the lag period applied (HR 0.71, 95% CI 0.61-0.84, for a 6 month lag; HR 0.77, 95% CI 0.66-0.90, for a one year lag; and HR 0.84, 95% CI 0.69-1.02 for a 2 year lag). Furthermore, lagging exposures for all post-diagnostic statin users where many would be expected to have also been prior users (58.7% of post-diagnosis users also used statins pre-diagnosis in the CPRD study), would seem potentially superfluous: patients with prior use would be expected to have still have received ongoing statin exposure while harbouring an undiagnosed tumour.

In a systematic review and meta-analysis of 11 studies, including 21 030 participants, post diagnosis statin use was significantly inversely associated with all-cause mortality (HR 0.84, 95% CI 0.73-0.98), with substantial heterogeneity (I^2 69%, heterogeneity P < 0.001)[199]. In four studies, including 19, 152 patients, post diagnosis statin was not significantly associated with cancer-specific mortality (HR 0.84, 95% CI 0.68-1.04), with evidence of heterogeneity (I^2 67%, heterogeneity P = 0.03). Both pooled estimates included the two large CRC cohorts described above. Heterogeneity may be ascribed to substantial differences in the included studies, which included community and hospital-based studies, early and advanced disease, and not all studies accounted for time-dependent covariates.

Statin use and mortality in patients with oesophageal carcinoma

There are only two previous epidemiological investigations which have examined the association between statin use and mortality in patients with OC[189, 204]. The first, conducted in Denmark,

included 277, 204 patients, 98% of the Danish population diagnosed with cancer from many primary sites, from 1995 to 2007[189]. Participants were followed until December 2009 such that at least 2 years' follow-up per patient was possible. The study used linkage between the Danish Cancer Registry; the Danish Civil Registration System (which uniquely identified all Danish inhabitants and provided complete data on emigrations, date and cause of death, age and gender); and the Danish Registry of Medicinal Products Statistics (containing electronic information on dispensed medications, including the Anatomical Therapeutic Chemical (ATC) Classification System to define the precise statin dispensed, dosage and amount dispensed). All participants were aged over 40 years as statin exposure was expected to be unlikely in younger patients. Statin use was measured prior to the date of cancer diagnosis (to two years previously), with the intention of preventing reverse causation bias. Regular statin use was defined as use within 6 months of the date of cancer diagnosis and separately received two prescriptions within 2 years of diagnosis. This exposure definition was considered a proxy for statin use before and after cancer diagnosis. In total 18, 721 patients (6.7% of the cohort) were defined as statin users prior to diagnosis of cancer. This proportion of statin use is low, but may reflect the time period selected and the inclusion of relatively young patients. The association between pre-diagnosis statin use and cancer-specific mortality was estimated (censoring for deaths due to other causes) to account for competing risk of death. In total, 162, 067 deaths were registered as due to cancer during follow-up. Overall, statin use (compared to patients who had never used statins) was significantly inversely associated with cancer-specific mortality (HR 0.85, 95% CI 0.82-0.87) and all-cause mortality (HR 0.85, 0.83-0.87). Analyses were adjusted for gender, ethnicity, age at diagnosis, cancer stage (using the TNM classification), chemotherapy, radiotherapy, diagnosis of cardiovascular disease and diabetes preceding cancer diagnosis, year of birth, educational attainment, and size of residential area. A clear dose-response relationship was not evident for categories of dosage based on the penultimate statin prescription dose prior to diagnosis (HR 0.82, 95% CI 0.81-0.85, for low dose; HR 0.87, 95% CI 0.83-0.89, for the standard dose; and HR 0.87, 95% CI 0.81-0.91, for the higher dose category). This definition would not necessarily account for the actual cumulative dose received and would not be expected to sensitive to changes in dose over time (although this is a lesser concern)**.** A separate matched propensity score analysis (patients were matched for the probability for statin initiation based on prescriptions of other medications for chronic illnesses – diuretics, calcium channel blockers, anti-hypertensives, warfarin, beta blockers, bronchodilators and anti-depressants) yielded very similar estimates to the full cohort study. Aspirin use was not adjusted for and could plausibly have confounded associations[123]. There were considerable missing data on covariates – particularly cancer staging (missing in 34%) and chemotherapy and radiotherapy (each missing in 72%). Missing data was handled using the "missing indicator method", an approach which is not advised

and which can readily lead to biased estimated associations even when data are assumed to be missing completely at random[205]. Multiple imputation (if assumptions of missingness were justified) or complete case analysis may therefore have been preferable. In the subgroup of 4398 with oesophageal malignancy (a composite diagnosis, not identified by histological subtype) of whom 3328 died during follow-up from their index cancer, statin use was significantly inversely associated with cancer-specific mortality (HR 0.81, 95% CI 0.69-0.95). Dose response analyses were not repeated in individual cancer cohorts, and therefore it is not known whether such relationship existed for OC. New statin use, initiated after diagnosis of cancer, was not assessed and therefore through measuring statin use pre-diagnosis exclusively (while attempting to avoid reverse-causation bias) could have underestimated treatment effects if they existed. Furthermore, defining regular statin use in the two years until the date of cancer diagnosis may not fully address reverse-causation bias: patients with more advanced OC (compared to those with less advanced disease) may have a lower propensity to be prescribed statins if symptomatic management or further investigation is more likely to take priority over primary prevention of cardiovascular disease immediately prior to diagnosis of cancer.

The second study was a single-centre cohort study based at the MD Anderson Centre, Texas, US[204]. In total, 1174 patients with OC (78% OAC, 20% OSCC), including surgical and non-surgical cohorts (560 and 614 patients respectively), with stage I-IV disease were included. Patients treated at the centre between 1998 and 2012 were included. The aim of the study was to examine associations between seven common comorbidities and 18 medication groups and the outcomes of all-cause mortality, cancer specific survival and Non-cancer specific survival. In total, 400 (34%) of the cohort were classified as statin users, measured at baseline as documented in the medical notes, although a more detailed exposure definition was not provided. Non-significant inverse associations were observed between statin use and all-cause mortality (HR 0.92, 95% CI 0.76-1.11) and cancer-specific survival (HR 0.87, 95% CI 0.70-1.07). Analyses were adjusted for age, race, histology, tumour location, BMI, smoking, performance status, clinical stage, radiation modality and surgery for all-cause mortality. In addition, for cancer-specific mortality analyses were adjusted for grade and tumour length. The main strength of this study is the comprehensive collection of data on clinical and tumour characteristics which could feasibly otherwise confound associations. With up to 75 potential comparisons, the risk for type 1 error was substantial. Furthermore, statin exposure was not the primary focus of this study. Therefore further analyses which may help infer causality were not conducted, such as the evaluation of dose-response relationships. Statin use was also only assessed at a single point in time, and would

not have considered treatment effects for new use. Associations were not stratified by histological subtype.

In summary, while studies which have examined the relationship between statin use (measured pre or post-diagnosis) and outcomes in patients with cancer overall have generally estimated significant inverse associations, uncertainty remains over whether causal relationships could exist for particular individual tumour sites[189, 191]. Such is the nature of observational research, that competing explanations for significant associations may operate, such as immortal-time bias and confounding, specifically reverse causation bias[206]. Some of the most rigorous and well-conceived observational studies in patients with CRC which employ advanced approaches to avoid these sources of bias, may have unintentionally underestimated statin treatment effects. There is a relative paucity of data on the association between statin use and survival in patients with OC and, to date, aside the current study there are no other published cohorts to date which examine associations between statin use measured after diagnosis and mortality in patients with oesophageal cancer or more importantly, according to histological subtypes[189, 204, 207].

1.2.7. Trial evidence of statin as therapeutic agents

Randomised controlled trials

Cancer-related mortality has been assessed in patients allocated to statin therapy/high dose compared to placebo/low dose use, in the cholesterol treatment trialists' collaboration (see table 7 for summary of current trial evidence), an individual patient data meta-analysis on 27 RCTs, which demonstrated no significant difference when used for a median of 5 years[208]. While this study provides reassurance at a wider population level that statin use does not markedly increase the incidence of malignancy, following concerns raised by the PROSPER[209] and CARE[210] trials (which respectively showed increased incidence of gastrointestinal and breast cancers), it is more difficult to draw inferences about mortality, as all patients with a diagnosis of cancer prior to randomisation (including 164 with OC) were excluded. Therefore, most patients would be expected to be cancer free at the point of randomisation.

Relevance of current clinical trials

Published RCTs which directly assess statins in patients with gastro-intestinal malignancy (including colorectal, gastric and pancreatic cancer) show no evidence of therapeutic benefit[197, 211, 212]. However, these trials are of little direct relevance to curative cohorts with OAC in terms of gauging potential therapeutic efficacy. Studies selected participants with advanced malignancy[197, 211-213], received short durations of allocated statin treatment[197, 211-213], and had small sample sizes[197, 211-213]. Selected patient groups were therefore probably least likely to benefit from statin therapy, and studies were not powered to examine smaller treatment effects. The studies also suffered substantial methodological limitations and risked bias. Reassuringly there is no evidence of increased toxicity, in terms of absolute numbers of adverse events according or their severity, in patients allocated to statins[197, 211, 212]. This is of relevance, as there was considerable overlap between chemotherapy regimens used in these trials and those use in current UK practice as peri-operative agents.

First author (year) Country Design	Tumour site Stage	Number of Patients	Active (n)	Control (n)	Treatment duration	Outcome(s)	Comments
Cholesterol Treatment Trialists' Collaboration[208] (2012) International collaboration Individual patient data meta-analysis of 27 RCTs	Cancer free at randomisation Any site and stage during follow-up 164 with OC	174, 149	In statin vs. control: A, F, L, P, R (67, 258) In more intensive vs. less: A, S (19, 829)	In statin vs. control: placebo (67, 279) In more intensive vs. less: A, S, P (19, 783)	In statin vs. control: median 4.8 years In more intensive vs. less: median 5.1 years	**Cancer incidence** Statin vs. control: RR, 1.00, 95% CI 0.96-1.05 More intensive vs. less: RR, 1.00, 95% CI 0.93-1.07 **Cancer mortality** Statin vs. control: RR 1.00 (0.93-1.08) More intensive vs. less: RR 0.93 (0.82-1.06). **OC deaths:** statin/more (45/87, 087); control/less (55/97062) (p=0.36)	Cancers first diagnosed prior to randomisation excluded (150 with OC excluded). Statin/intensive regimen exposure likely preceded cancer development.
Lim[197] (2015) South Korea Phase III, 5 centres	Colorectal Stage IV	269	S40 plus FOLFIRI/XELIRI (134)	Placebo plus FOLFIRI/XELIRI (135)	Statin exposure: median 6 (1-36) 21 day cycles during which S administered daily	**PFS (primary)** HR 1.03, 95% CI 0.77–1.37, p=0.86 PFS with KRAS mutation p=0.86	Short durations of treatment. No clinically significant increase in toxicity with S.
Kim[211] (2014) South Korea Phase III, 9 centres	Gastric (64%) or GOJ (36%) Stage IV	244	S40 plus CX	placebo plus CX	In active group: median 4.4 months In control group: median 4.5 months	**PFS (primary)** HR 0.93, 95% CI 0.68-1.26 P=0.66 **OS (secondary)** HR 0.97, 95% CI 0.72-1.3 p=0.82	No clinically significant increase in toxicity with S.
Konings[212] (2010) Netherlands Phase II, 3 centres	Gastric NS. "advanced" not resectable	30	P40 plus ECX	Placebo plus ECX	In active group: mean 3.6 21 day cycles. In control: mean 4.5 21 day cycles	**PFR at 6 months (primary)** 5/15 responders in active arm 7/15 responders in control arm (p=0.47)	No clinically significant increase in toxicity in P40 plus ECX arm.

Hong[213] (2013) South Korea Phase II, 4 centres	Pancreatic Locally advanced (12%) Stage IV (88%)	114	S40 plus G	Placebo plus G	In active group: median two cycles (range 1-25) 21 day cycles. In control: mean four (range 1-22) 21 day cycles	**Time to progression (primary)** 2.4, 95% CI 0.7-4.1 months in active arm 3.6, 95% CI 3.1-4.1 months in control group p=0.90	No clinically significant increase in toxicity with S.
Kawata[214] (2001) Japan Randomised open label trial, Single centre	Hepatocellular carcinoma Unresectable disease Stage II or III (~30%) Stage IV (~70%)	83	P20-40	No placebo	P administered for 16.5 (SD 9.8) months	**All-cause mortality** HR 0.42, 95% CI 0.20-0.83, p=0.02 Median survival in active arm 18 months Median survival in control arm 9 months	Open label. Baseline groups well balanced for known prognostic markers in hepatocellular carcinoma.
Garwood[215] (2009) US Pilot study, 6 centres	Ductal carcinoma in situ or breast cancer Stage I	40	F80	F20	21-50 days	**Ki-67 (proliferative biomarker)** Median 7.2% (IRQ 0-13.4) reduction in active vs control arm (p=0.008) in subgroup of high grade tumours (n=15).	No comparison reported between treatment groups overall. Subgroup analyses at risk of type 1 error.
Han[216] (2011) South Korea Open label, phase II, single centre	Non-squamous cell lung cancer, failed at least one platinum-based chemotherapy. 75% adenocarcinoma. Stage IIIB/IV	106	S40 + Gef	Gef	NS	**Response rate** 31.5%, 95% CI, 19.1–43.9% in active arm 38.5%, 95% CI, 25.3–51.7% in placebo arm (p=0.666) **Progression-free survival** HR 0.89, 95% CI 0.60-1.32, p=0.491	No clinically significant increase in toxicity with S, either in terms of frequency of AEs or severity

Table 5: Summary of randomised controlled trials which assess statins in patients with solid tumours

Abbreviations: A, atorvastatin; F, fluvastatin; L, lovastatin; P, pravastatin; R, rosuvastatin; CX, capecitabine, cisplatin; ECX, epirubicin, cisplatin, capecitabine; FOLFIRI/XELIRI, irinotecan, capecitabine, leucovorin, 5-fluorouracil; G, gemcitabine; GOJ, gastro-oesophageal; OS, overall survival; PFS, progression free survival; RCT, randomised controlled trial; TAE, transcatheter arterial embolization; 5-FU, 5-fluorouracil; Gef, Gefitinib

1.2.8. Observational evidence for association between statin use and oesophageal malignancy

Previous epidemiological studies: general population cohorts

The association between statin use and oesophageal malignancy in the general population has been examined previously. The most recent published observational study based within QRESEARCH (a large anonymised healthcare dataset from contributing general practices throughout the UK which use the EMIS computer system[217]) demonstrated in a nested case-control analysis of 3159 patients with OC (a composite diagnosis) matched to 13041 controls, a non-significant inverse association between statin use and OC (OR 0.88, 95% CI 0.77-1.01, p=0.072)[218]. Statin exposure was considered for all patients until one year prior to the index date for all participants. Analyses were adjusted for Townsend quintile (a proxy for socioeconomic status), BMI, smoking, MI, coronary heart disease, diabetes, hypertension, stroke, rheumatoid arthritis, use of NSAIDs, COX-2 inhibitors, and aspirin. Cumulative duration-response analyses did not suggest evidence of a biological gradient: statin use for < 12 months, OR 0.82, 95% CI 0.67-1.02; 13-24 months, OR 0.91, 95% CI 0.71-1.36; 25-48 months OR 0.82, 95% CI 0.66-1.02; >49 months, OR 1.04, 95% CI 0.83-1.30. This study has a number of strengths: first, it is a suitably large study to examine with sufficient power associations between statin use and risk of OC; second, the use of a nested case-control approach is appropriate given that OC can be considered a rare outcome overall; third, the exposure window excluded the year prior to index therefore excluded recent drug use, a time period where statins may not reasonably be expected to exert a biological effect; and fourth, this study used a representative dataset and should be generalisable to the wider UK population. The most notable limitation is the outcome: OC is recorded as a composite diagnosis (including presumably any histological subtype of oesophageal malignancy), and clearly adenocarcinoma and squamous cell carcinoma (among other rare subtypes) are different disease entities, with differing epidemiology, aetiology, and biology[56, 219]. Therefore a composite diagnosis of OC when examining disease aetiology has diminished value and is difficult to interpret.

To address this specific issue, we conducted a nested case-control study using the GPRD[220]. In total, between January 2000 and December 2009 (a period purposefully selected for the high prevalence of statin use), 4220 cases of incident OC were matched to 15570 controls. All participants required at least 1 year's up-to-standard records within the database. A subset of cases were linked to the national cancer registry to confirm the histological subtype. Regular statin use (the primary exposure definition) was defined as a minimum of 10 months' dispensed

statin prescriptions in the year preceding the index date for both cases and controls. Participants with < 10 months' prescriptions were excluded, leaving 581 cases of OAC matched to 2167 controls; and 332 cases of OSCC matched to 1242 controls. Regular statin use was inversely associated with risk of OAC (OR 0.58, 95% CI 0.39-0.87, p=0.009) with evidence of both dose (p for trend = 0.036) and duration-response relationships (p for trend = 0.005). These associations persisted in sensitivity analyses where follow-up for participants was restricted equally to at least 5 years. Regular statin use was not significantly associated with OSCC (OR 0.61, 95% CI 0.35-1.06, p=0.081), with no significant dose (p for trend=0.057) or duration-response (p for trend=0.249) relationships. However, between 1-5 years' use of statins was inversely associated with OSCC (OR 0.51, 95% CI 0.27-0.98), although caution should be applied in the interpretation of this subgroup as the number of cases and controls were small (13 and 18 participants respectively). Cases were matched for calendar time (using the index date), gender, year of birth (+/- 3 years), and general practice (a proxy for socioeconomic status). Analyses were adjusted for smoking status, (alcohol intake for OSCC), BMI, prescription of aspirin, NSAIDs and PPIs. The main strength of this study was the use of electronic healthcare records to ascertain case and control status, independent to exposure status, and linkage with the cancer registry to determine the histological subtype of malignancy. The main limitation is the exposure definition: while it ensures that exposure duration is potentially sufficient to mediate a biological effect, statin use initiated in the year prior to diagnosis (a period when malignancy would be present, but undiagnosed) would not necessarily satisfy the assumption of a temporal relationship. Nevertheless, the 93% of cases and 96% of controls used statins for > 1 year prior to index, for a median duration of 3 years (IQR 1.78-4.37) for cases and 3.1 years for controls (IQR 1.91-5.19).

While both of these studies would be expected to be generalizable to the wider UK population, they would not necessarily be applicable to patients with Barrett's oesophagus.

Previous epidemiological studies: Barrett's cohorts

There are seven contemporaneous epidemiological investigations (summarized in table 8) which examine associations between statin use and risk of HGD and/or OAC in populations with BO[188, 221-226]. The most recent US nested case-control study within the national Veteran affairs (VA) datasets (fully electronic health record dataset) included 311 cases of OAC (with known prior BO) matched to 856 controls with BO who had not progressed by the index date of cases (date of diagnosis)[188]. This study supersedes two previous smaller studies conducted in the same base-

population[227, 228]. BO diagnoses were ascertained from 2004 to 2010 and cases were identified until 2011. All participants were male and were matched for year of birth (within +/- one year) and date of prior BO (within +/- three years). Statin use was ascertained from electronic pharmacy records from the Veterans Health Administration inpatient and outpatient datasets. Statin exposure was defined when dispensed after diagnosis of BO and until 90 days prior to OAC diagnosis. Statin use was inversely associated with risk of OAC (OR 0.65, 95% CI 0.47-.91) adjusted for age at BO diagnosis, smoking, BMI, medication use (individually, PPIs, NSAIDs, H2As), the number of gastroscopies prior to the date of diagnosis and index date (a means of limiting healthy user bias). There was no clear evidence for a duration response for the association between statin use and malignant progression (< 6 months, OR 0.82, 95% CI 0.52-1.31; 6-18 months, 0.52, 0.32-0.85; and > 18 months 0.64, 0.40-1.01). Significant inverse associations were observed for late stage OAC with statin use (OR 0.44, 95% CI 0.25-0.79), but not for early stage OAC (OR 0.85, 0.54-1.33). Dose-response associations were not reported. This study's main strengths are the use of electronic data systems to capture medication exposures and the implementation of a nested case-control analyses within prospectively collected patient dataset. While it is possible that prescriptions dispensed outside the VA would not be recorded, the prevalence of statin use was sufficiently high (40% of cases, and 50% of controls) and of the order expected, that such prescriptions causing significant measurement error is a lesser concern. Time-dependent exposures would also be adequately controlled for using the fixed exposure window definition. One limitation is this study may not account for latency: short exposures to statins satisfy the definition of statin use, and medication initiated within 100 days of diagnosis of OAC would unlikely seem related to the malignancy's aetiology. Furthermore, such exposure definitions risk selection bias, specifically another guise of reverse causation bias: in the time preceding diagnosis (likely months) patients with malignancy would be expected to have more frequent interactions with their general practitioner or treating clinician than controls[133], and therefore have a greater propensity to receive treatment for unrelated conditions, such as lipid lowering medications; although this would be expected to draw associations to the null. All participants were male, therefore limiting the external validity of the study (albeit the majority of patients with known BO are male).

A prospective cohort study conducted from three university medical centres and 15 endoscopy units in the Netherlands included 570 patients with BO (including non-dysplastic metaplasia and LGD), with 2, 738 person-years of follow-up, of whom 38 progressed (26 with HGD and 12 with OAC)[224]. Consensus for the diagnosis of any grade of dysplasia was made with at least two pathologists, of which at least one was considered expert. Patients with BO were diagnosed between November 2003 and December 2004. Patients with intestinal metaplasia and BO

measuring at least 2cm (M2) were included. Statin use was ascertained from a questionnaire administered at each surveillance visit and was cross-checked with pharmacy records. Any use of statins (the primary exposure) was defined as a prescription of at least one month's duration during follow-up. Using this definition, statin use was inversely associated with risk of progression in a non-time dependent cox regression model (HR 0.46, 95% CI 0.21 – 0.99), adjusted for age, gender, length of BO, baseline histology, and use of NSAIDs, low dose aspirin, and PPIs. This exposure definition and analysis is susceptible to immortal-time bias: the time period between entry to the cohort and exposure to statins (immortal time) is otherwise erroneously considered exposed, therefore biasing statin users to lower incidence rate of progression, and a biased underestimate of the HR for malignant progression[229]. In a subsequent time-dependent Cox regression model (the ideal approach), which enabled patients to move from periods of non-exposure to periods of exposure following initiation of a statin, risk of malignant progression was non-significantly inversely associated (HR 0.55, 95% CI 0.23-1.29)[230]. In non-time dependent cox regression models there was no convincing evidence for a duration-response relationship (≤ 5 years' statin exposure, HR 0.51, 95% CI 0.18–1.47; and > 5 years, HR 0.49, 95% CI 0.18-1.29; p for trend=0.204). The main strengths of this study are its prospective cohort design, the use of pharmacy databases to validate medication exposures, and use of expert GI pathologists to reach consensus for the diagnosis of dysplasia. However, the study is likely to be underpowered given the low number of events, as expected for a cohort of this size and the known low overall rate of malignant progression in patients with no dysplasia[36]. It is not clear whether accurate start dates for medications could be ascertained at follow-up: doing so only at intervals dictated by surveillance risks measurement error when considering time-dependent exposures. Again, recent exposure (to the date of diagnosis of cases, or incident date) as is considered in this study would be expected to have little biological basis for influencing the process of malignant progression (latency).

A UK single centre case-control study included 85 patients with OAC, and 170 controls with BO recruited from September 2009 to July 2011[221]. Cases required confirmation of diagnosis by the upper gastrointestinal MDT by a specialist gastrointestinal pathologist. Cases also included Siewert I and II lesions, and both prevalent and incident cancers. Included patients with controls with BO were at least 3cm in length, with intestinal metaplasia, and were diagnosed at least one year prior to their most recent endoscopy appointment. Drug use was ascertained from patient interviews conducted before or after their endoscopy (which determined case/control status), and cross checked with the referral letter when possible. Statin exposure was defined by patient recall of use for at least 6 months before the date of cancer diagnosis or current surveillance endoscopy. Height and weight were measured at the time of the interview for the controls, and

cases were asked to estimate their weight one year before presentation. Statin use was significantly inversely associated with risk of OAC (OR 0.57, 95% CI 0.28-0.94), adjusted for age, BMI, smoking, alcohol, aspirin, metformin and NSAIDs. Significant dose (≤ 40mg daily, OR 0.59, 95% CI 0.27-0.98; > 40mg OR 0.31, 95% CI 0.05-0.97; p for trend < 0.05) and duration (0.5-2 years, OR 0.77, 95% CI 0.29-1.87; 2-5 years, OR 0.58, 95% CI 0.27-1.43; > 5 years OR 0.41, 95% CI 0.15-0.85; p for trend < 0.05) response relationships were demonstrated. Given the short time period over which participants were recruited (approximately 22 months), calendar time would be expected to have been controlled for to a degree. Differential ascertainment methods of exposures between cases and controls is a significant limitation, as such an approach can readily lead to information bias. This study primarily captured drug exposures by patient interview (potentially soon after cancer diagnosis for cases), such an approach could feasibly lead to differential recall between cases and controls (recall bias) and considerable error, if non-differential; although the authors stated that patient reported exposure "invariably correlated with that seen in the medical records". Although the significant duration and dose-response relationships are consistent with a causal relationship, caution should be used in their interpretation: models were unstable, with wide confidence intervals attributable to the few cases and controls distributed between exposure categories (for example two cases and 14 controls comprised the > 40mg dose category). The exposure definition to statins included use within 6 months before interview, as with the other studies discussed, this would appear not to consider biological latency.

A population-based cohort study in the UK GPRD using primary care data included a large BO cohort (n=9, 660) and followed patients up for a diagnosis of OC (a composite diagnosis as histological confirmation was not possible) using read codes[225]. Progressors diagnosed with OC within 12 months of BO diagnosis (prevalent cancers) were excluded. In total, 103 patients developed OC 12 months after diagnosis of BO. Exposure to statins was ascertained from electronic prescription data and analysed using two distinct methods: first, a "conventional" binary approach whereby exposure was defined by use of statins at baseline analysed with Cox proportional hazard regression; and second, a proportion days covered (PDC) approach whereby the cumulative duration of statin prescriptions dispensed during follow-up was divided by the total duration of follow-up, expressed as a percentage, analysed with time-dependent marginal structural equation models. Using the conventional approach, statin use at baseline was not significantly associated with malignant progression (HR 0.61, 95% CI 0.34-1.10). Using the PDC approach, statin use was significantly inversely associated with risk of malignant progression (HR 0.61, 95% CI 0.45-0.83). Models were adjusted for age, gender, smoking, BMI, hiatal hernia, type 2 Diabetes Mellitus, PPI use, metformin use, insulin use, and "oral anti-diabetic medications". The

main strength of this study is its prospective design, large cohort size and electronic ascertainment of exposures (which optimally identifies statin prescriptions, a routine prescription administered in primary care) and outcomes. The use of time-dependent marginal structural models avoid immortal time bias and theoretically handles time-dependent confounding[231]. This study has several limitations: first, although it may be reasonable to conclude that most oesophageal malignancies which develop during follow-up of patients with BO are adenocarcinomas, this is not known with certainty in this population; and this study would have benefitted from linkage to the National Cancer Registry for confirmation of the histological subtype of OC in a subset. Second, while read codes for OC have been shown to be reliable in the GPRD, when validated against cancer registrations[232], it is not clear whether read codes for BO are: to date there are no published validation studies of this condition.

The Seattle Barrett's Esophagus Study included a baseline cohort of 411 patients with Barrett's oesophagus (80, 19.5% had HGD at baseline) of which 56 (13.6%) progressed to OAC during follow up[223]. Patients with less than five months' follow-up were excluded. Medication use was ascertained by interview at baseline and each subsequent surveillance appointment. Statin use was defined based on recall during follow-up. Statin use was non-significantly inversely associated with progression to OAC (HR 0.68, 95% CI 0.30-1.54) in time-dependent analyses, adjusted for age, sex, smoking and NSAID use. This study's strength is its prospective design and capture of time-dependent variables. This study has several limitations: first, it may be underpowered with only 56 events; second, 19.5% of the cohort had HGD at baseline making comparisons with other studies difficult (as HGD was frequently the outcome of interest in other studies), and generalising the findings to wider populations with BO difficult; third, patients who progressed to adenocarcinoma within one year should be considered as prevalent cases and measurement of statin exposure during this period should not be considered as having played an aetiological role and therefore this study did not consider a plausible window of latency.

A nested case-control study based within the Health Improvement Network (THIN) database included 55 patients with prior BO (diagnosed greater than one year previously) who developed OC during follow-up and 3, 694 patients with BO with no record of progression to cancer within one year of diagnosis[222]. OAC was confirmed in 34 of these cases from a number of sources: free-text entry in THIN, chemotherapy consistent with OAC and death certificates. Medication exposure data were retrieved from the database differentially for cases and controls: for cases, drug prescription data was censored from one year before the first code denoting diagnosis of OC; while the same procedure was not applied to the BO controls (exposure measurement was until the index date). Exposure to the medication groups of interest was classified according to

ever use. Cox proportional hazard regression estimated the association between ever use and malignant progression, adjusted for age, gender and smoking. Statin use was non-significantly inversely associated with risk of malignant progression (HR 0.82, 95% CI 0.43-1.56). This study's main strength is the available routinely recorded drug prescription data. This study has several limitations: first, the study may be underpowered despite a large control population as the absolute number of cases was relatively low; second, given "ever use" definitions, analyses are likely susceptible to immortal time bias; third, differential censorship for exposures between cases and controls would likely bias estimates for drug exposures towards inverse associations; and fourth, OC and BO are diagnoses which are yet to be validated in THIN, nevertheless, given the similarities with other large routine healthcare datasets in the UK, these diagnoses would still be expected to be valid[217].

Study (year) location Design	Source	Time period	Number of patients	Cases with known prior BO	Statin use: definition ascertainment prevalence	Outcomes	Risk estimates of statins (vs. none)	Covariates matched/ adjusted for[a]	Limitations
Nguyen[188] (2015) US Nested case-control study	VA database	2004-2011	OAC: 311 BO controls: 856 Definition of BO and length not provided.	311 (100%)	Filled (dispensed prescription) between 90 days prior to OAC/index date and date of BO. 40.2% cases 54% controls	OAC	Statin use (primary definition), OR 0.65, 95% CI 0.47-.91 Duration responses < 6 months, OR 0.82, 95% CI 0.52-1.31; 6-18 months, 0.52, 0.32-0.85; > 18 months 0.64, 0.40-1.01	Matched: 1, 2, 12 Adjusted: 4-7, 12, 14, 15	Limited account for biological latency. Susceptible to time-window bias.
Kastelein[224, 230] (2011) Netherlands Cohort	Three medical academic centres and 15 endoscopy units	BO: 2003-2004 HGD/OAC: NS	HGD/OAC: 38 BO cohort: 570	38 (100%)	Patient interview, questionnaire, pharmacy records. Ever use (1 month prescription) during follow-up. 36.7% in whole cohort.	HGD/OAC	Ever use (primary definition) in non-time dependent analysis, HR 0.46, 95% CI 0.21 – 0.99; time-dependent analysis: HR 0.55, 95% CI 0.23-1.29. ≤ 5 years' statin exposure, HR 0.51, 95% CI 0.18 – 1.47; > 5 years, HR 0.49, 95% CI 0.18-1.29	Adjusted: 1,2,5, 7, 8, 13	Statin use definition includes potentially short exposure windows. Non-time dependent analyses risk immortal time bias. Drug exposure determined at surveillance intervals. No account of biological latency.
Beales[221] (2012) UK Case-control study	Single centre	2009-2011	OAC: 85 BO controls: 170	5 (5.9%)	Patient interview and referral letter. Use for at least 6 months. 20% cases. 35.3% controls.	OAC	6 month's use (primary exposure), OR 0.57, 95% CI 0.28-0.94; ⩽ 40mg daily, OR 0.59, 95% CI 0.27-0.98; > 40mg OR 0.31, 95% CI 0.05-0.97; p for trend for dose response relationship < 0.05;	Adjusted: 1, 4, 7, 8, 9, 10, 12	Drug exposure ascertainment with patient interviews -recall bias, and measurement error. No account of biological latency. Susceptible to time-window bias.

							0.5-2 years, OR 0.77, 95% CI 0.29-1.87; 2-5 years, OR 0.58, 95% CI 0.27-1.43; > 5 years OR 0.41, 95% CI 0.15-0.85; p for trend for duration response relationships < 0.05		
Iyer[225] (2015) UK Cohort	GPRD (Routine healthcare dataset within primary care)	1991-2010	OC: 103 BO: 9660	103 (100%)	Electronic prescription records. Binary use at baseline. Proportion days covered (PDC). 27.6% in whole cohort.	OC	Use at baseline, HR 0.61, 95% CI 0.34-1.10; PDC, HR 0.61, 95% CI 0.45-0.83.	Adjusted: 1, 2, 4, 5, 9, 14, 18, 19.	No histological confirmation of subtype of OC. No account of biological latency. BO is yet to be validated in the GPRD. Time periods investigated include periods of negligible statin prescriptions.
Kantor[223] (2012) US Cohort	Seattle Barrett's Esophagus Study Single centre	1999-2009	EAC: 56 BO: 411 (80 had HGD at baseline)	56 (100%)	Interview at surveillance intervals. 13.6% in whole cohort.	OAC	HR 0.68, 95% CI 0.30-1.54	Adjusted: 1, 2, 4, 7	Drug exposure determined at surveillance intervals. HGD at baseline in 19.5%. No account of biological latency.
Cooper[222] (2014) UK Nested case-control	THIN (Routine healthcare dataset within primary care)	1988-2004	OC: 55 BO: 3694	55 (100%)	Electronic prescription records. Ever use. 30.5% in the whole cohort.	OC	HR 0.82, 95% CI 0.43-1.56	Adjusted: 1, 2, 4	Likely under-powered. No histological confirmation of subtype of OC. Ever use exposure definition risks immortal time bias. Susceptible to time-window bias.
Masclee[226] (2015) UK and	THIN and IPCI	THIN: 1996-2011	<u>THIN</u> OAC: 40 BO: 656	1409 (100%)	Exposure window not defined.	OAC HGD/OAC	>3 years OR, 0.5, 95% CI 0.1-1.7 <0.8 DDD OR 1.0,	Matched: 1, 2, 11, 21 Adjusted:	Multiple testing. Limited adjustment for confounders.

Netherlands Nested case-control		IPCI: 1996-2012	IPCI 5: OAC 12: HGD BO: 753		Electronic prescription records. According to duration and DDD per day 26.3% in cases 25.5% in controls		95% CI 0.5-2.1 ≥0.8 <1.2 DDD OR 0.7, 95% CI 0.2-3.1 ≥1.2 DDD per day 0.8, 95% CI 0.3-2.4	20	No account of biological latency.

Table 6: Observational studies which examine associations between statin use and malignant progression in patients with Barrett's Oesophagus

Abbreviations: DDD, defined daily dose; OAC, oesophageal adenocarcinoma; GPRD, general practice research database; HGD, high-grade dysplasia; IPCI, integrated primary care information database; NS, not stated; OC REF, reference category; THIN, the health improvement network US, United States;

[a]1, Age; 2, gender; 3, year of OAC diagnosis; 4, smoking, 5, PPI use; 6 H2A use; 7, NSAID use; 8, aspirin use; 9, metformin; 10 alcohol use; 11, date of BO diagnosis; 12, age at BO diagnosis; 13 dysplasia; 14, BMI; 15, number of gastroscopies prior to index; 16, hiatus hernia; 17 Type II diabetes; 18, insulin; 19, other oral hypoglycaemic agents; 20, duration of follow-up since BO diagnosis; 21, country

2. Chapter 2 – The association between post-diagnostic statin use and survival in patients with oesophageal carcinoma: a population-based cohort study

2.1. Abstract

Background

Oesophageal cancer (OC) is a significant cause of cancer-related mortality worldwide. Statins have anti-carcinogenic effects in OC cell lines. The aim of this study was to determine whether statin use following diagnosis of OC, including the histological subtypes, is associated with reduced OC-specific and all-cause mortality.

Methods

A cohort of 4445 men and women in the United Kingdom diagnosed with OC between January 2000 and November 2009 and followed-up until November 2011 were identified using the General Practice Research Database. Cox proportional hazard regression analysis with time-dependent exposures estimated the association between post-diagnostic statin use and OC-specific and all-cause mortality.

Results

The median survival of the whole cohort was 9.2 months (IQR 3.7-23.2). The median survival in post-diagnostic statin users was 14.9 months (IQR 7.1-52.3) and in non-users was 8.1 months (IQR 3.3-20). Post-diagnostic statin use was associated with a decreased risk of OC-specific mortality (adjusted Hazard Ratio [HR] 0.62, 95% CI 0.44-0.86) and all-cause mortality (HR 0.67, 95% CI 0.58-0.77) for the full cohort. In patients with oesophageal adenocarcinoma (OAC), post-diagnostic use of statins was associated with decreased risk of OC-specific mortality (HR 0.61, 95% CI 0.38-0.96) and all-cause mortality (HR 0.63, 95% 0.43-0.92). This effect was not observed in patients with oesophageal squamous cell carcinoma (OSCC). There was no evidence of effect modification on associations by pre-diagnostic statin use.

Conclusions

In a large population-based cohort, post-diagnostic statin use in patients with OC was associated with reduced OC-specific and all-cause mortality, specifically in those with OAC but not OSCC.

2.2. Introduction

Oesophageal cancer (OC) is the 5th and 8th most common cause of cancer-related death in men and women respectively worldwide[233]. Of the two main histological subtypes, oesophageal squamous cell carcinoma (OSCC) is globally predominant, while oesophageal adenocarcinoma (OAC), the incidence of which has rapidly risen since the 1970s, is the most common form in the west[4, 233]. Most patients with OC present with advanced disease and are often only amenable to palliative management. Consequently, the overall 5-year survival rate is approximately only 15%[5].

Novel clinical interventions to improve prognosis in patients with OC are required. There has been a considerable research focus on the potential anti-cancer effects of statins (3-hydroxy-3-methylglutaryl coenzyme A [HMG-CoA] reductase inhibitors), which are commonly prescribed for the primary and secondary prevention of cardiovascular disease[234]. A body of basic research has demonstrated that statins promote apoptosis and limit proliferation in OAC and OSCC cell lines[179, 180, 182, 235]. A number of well-conducted epidemiological studies have demonstrated that use of statins post-diagnosis is associated with reduced risk of cancer-specific mortality in a number of malignancies, including prostate, breast and colorectal carcinoma[187, 190, 236]. Furthermore, at a population level their use is inversely associated with development of the histological subtypes of OC[220]. A population-based cohort study in Denmark demonstrated that statin use prior to diagnosis of OC was associated with a 19% decrease in cancer-specific mortality[189]. Whether statin use following diagnosis of OC, a more relevant time period for clinical intervention, improves survival is unknown. Furthermore, whether or not statins exert differential effects on survival for the two main histological subtypes, OAC and OSCC, is unknown. Therefore, the primary aim of this epidemiological study was to determine whether statin use following diagnosis of OC, including the histological subtypes, is associated with reduced OC-specific and all-cause mortality. Secondary aims were to determine whether pre-diagnostic statin use is an effect modifier on the association between post-diagnostic statin use and survival; determine whether a dose-response relationship exists; and determine whether differential effects exist according statin type.

2.3. Methods

Data sources

This study was conducted using three databases: the United Kingdom (UK) General Practice Research Database (GPRD), the UK National Cancer Registry (NCR) and the Office of National Statistics (ONS) database. The GPRD is the world's largest electronic database of prospective demographic, lifestyle and medical data in a primary care setting[237]. At the time of data extraction, 4 million patients were registered at 488 general practices, covering 6% of the UK population. The age and sex distributions of participants in the GPRD are comparable with the National Population Census, and the distribution of participating practices is representative of the UK population[238]. General Practitioners (GPs) prospectively record incident diagnoses and medical procedures using a modified Read/Oxford Medical Information System (OXMIS) classification system. Filled drug prescriptions issued by GPs are automatically recorded and coded using the UK Prescription Pricing Authority Dictionary. Data recorded on diagnostic codes to identify diseases, including OC, and drug prescriptions in the GPRD have been shown to be valid in independent studies[232, 239, 240]. Linkage between databases used a deterministic algorithm based on the patient National Health Service number, postcode, gender and date of birth. The NCR contains information on tumour site (coded using the International Classification of Diseases, 10th Revision [ICD-10]), histology, cancer stage and treatment modalities. A comprehensive list of codes to define the study cohort and covariates have been included in appendix A. Approximately half of GPRD practices were linked to the NCR at the time of data extraction. For patients with data linked to the NCR, ONS data was available to determine cause of death. The GPRD group have obtained blanket approval from a multi-centre ethics committee for observational research conducted within the database. The study protocol was approved by the MHRA Independent Scientific Advisory Committee (approved protocol number: 11_131).

Study cohort

Participants with incident oesophageal or oesophagogastric junction cancers, diagnosed between 1st January 2000 to 30st November 2009, and followed-up until 1st November 2011 were identified from the GPRD. Patients were included with no prior history of cancer. All patients were required to be diagnosed at least one year after the contributing practice had received its "up-to-standard" date: the time from which the practice was considered to generate continuous high quality data fit for research. The histological subtype for a subset of patients was determined through linkage to the NCR. ICD codes were used to confirm oesophageal (C15) and oesophago-gastric junctional

(C16) cancers, and specific morphology codes were used to obtain the histological subtypes: OAC, oesophagogastic junctional adenocarcinoma (OGJA) and OSCC. Follow-up was from the date of diagnosis until death, or until they were transferred out of the GPRD or the date of last data entry, whichever came first.

Statin use

Exposure to the following statins currently in clinical use in the UK were extracted: Simvastatin, Atorvastatin, Pravastatin, Rosuvastatin and Fluvastatin. Post-diagnostic statin use was defined as a prescription of any of these statins recorded in the GPRD at any time after the date of diagnosis. Post-diagnostic statin use was included as a time-dependent covariate in the models to avoid immortal-time bias: whereby a span of cohort follow-up during which death could not occur (i.e. between diagnosis and the first statin prescription) is inappropriately introduced due to the definition of the exposure of interest[206]. Patients were considered unexposed until the first post-diagnosis prescription, from which point they were considered continuously exposed until the end of follow-up. Deeming patients continuously exposed sought to minimize reverse causation bias, whereby ultimately discontinuation could reflect poor prognosis and therefore death may otherwise be more likely inappropriately classified during an "unexposed" period[241]. Exposure to the individual statins listed above was also considered in survival analyses. To investigate the possibility of healthy survivor bias in the statin users post-diagnosis, the intervals between diagnosis and statin initiation for all statin users post-diagnosis were presented using a Kaplan-Meier plot (figure 12).

Pre-diagnosis statin use was also an exposure of interest. It was defined as a prescription of any of the statins recorded above in the GPRD for a minimum of two months between 6 and 18 months prior to diagnosis. This definition sought to minimize reverse causation bias, whereby symptomatic OC (and hence likely more advanced disease) could influence prescribing practice or medication use. Pre-diagnosis statin use was determined for the following three reasons: it was entered as a covariate in models of post-diagnostic statin use to determine whether it modifies the effect of post-diagnosis statin use on survival; in sensitivity analyses the association between pre-diagnosis statin use on survival was determined to consider an exposure to statin use, alternative to post-diagnosis statin use, in which the potential effect of reverse-causation bias would be expected to be minimal; and finally it was used to determine categories for dose-response analyses. Statin users were categorized as low (equivalent to ≤ 20mg simvastatin) or

high (equivalent to > 20mg simvastatin) dose users based on the mean daily dose for statin prescriptions collected between 6-18 months prior to diagnosis. Cumulative statin dose was determined using categories of cumulative defined-daily dose (DDD). The DDD, a standardized measure of drug exposure as defined by the World Health Organization, is the assumed average maintenance dose per day for a drug used for its main indication in adults[242]. For example, 1 DDD is equivalent to a single dose of 30mg Simvastatin or 20mg Atorvastatin. The median cumulative DDD collected between 6-18 months prior to diagnosis in the whole cohort was the threshold for cumulative dose categories. Post-diagnostic mean or cumulative dose-response analyses were not examined *a priori* as the dose categories would be expected to be a function of survival time. In a post-hoc analysis we conducted dose-response analyses using the dose (expressed in DDDs) of the first statin prescribed post-diagnosis to determine the dose category.

Covariates

The following covariates which could plausibly confound associations between post-diagnostic statin use and survival were extracted from the GPRD: age at diagnosis, gender, body mass index (BMI) at the time closest to and preceding diagnosis, smoking status, cardiovascular diseases (coronary artery, cerebrovascular and peripheral vascular disease) and diabetes mellitus, surgery (either esophagectomy, esophago-gastrectomy or extended gastrectomy) recorded within 6 months of diagnosis and medication use (aspirin, angiotensin converting enzyme inhibitors [ACEi] and angiotensin 2 receptor blockers [ARBs], beta-blockers and non-steroidal anti-inflammatory drugs [NSAIDs]). Use of these medications were extracted both post-diagnosis and pre-diagnosis using the same exposure definitions as for statin use. The following covariates were extracted from the NCR: chemotherapy, radiotherapy and surgery (either esophagectomy, esophago-gastrectomy or extended gastrectomy) recorded within 6 months of diagnosis.

Outcome measures

The outcome measures were OC-specific and all-cause mortality. All-cause mortality was determined for all study patients in the GPRD. OC-specific mortality was determined for the subset of participants with data linked to the NCR and ONS datasets where OC was listed in part one of the death certificate.

Statistical analysis

Descriptive statistics were used to summarize the characteristics of the cohort, including the histological subtypes; and separately for pre and post-diagnosis statin users. The characteristics between statin users and non-users were compared using the Chi-squared test for categorical data, two sample t-test for age and the Mann-Whitney U test for survival time. Crude rates of OC-specific and all-cause mortality were calculated which reflect time-dependent exposure to statins. To account for the time-varying nature of drug exposures, Cox proportional hazard regression with time-dependent exposures, estimated the associations between statin use (versus non-use) post-diagnosis on OC-specific and all-cause mortality for the full cohort and the histological subtypes. In OC-specific analyses, deaths due to any other cause were censored, a valid approach to examine causal treatment effects on the cause-specific hazard, and therefore account for competing risks[243, 244]. Concomitant medication use and surgery were included as time-dependent covariates in the models. Surgery was not included in multivariable analyses of the full cohort (total OC, n= 4445) as it was under-recorded in the GPRD, however it was included with cohorts linked to the NCR, where it was more comprehensively recorded. Survival curves according to post-diagnosis statin use were constructed using Cox proportional hazard regression with time-dependent exposures. For analyses of pre-diagnosis statin use, follow-up began from the date of diagnosis, and all included covariates were measured prior to this date. Cancer stage was incomplete for 95.9% of the cohort and was therefore not included in multivariable analyses. Tests for interaction examined for any effect modification of pre-diagnosis statin use on the association between post-diagnostic statin use and mortality. A test for linear trend was applied across dose categories.

Sensitivity analyses

We performed a number of sensitivity analyses to determine the robustness of our findings. As previously outlined, the association between pre-diagnosis statin use on OC-specific and all-cause mortality for the full cohort and the histological subtypes was examined. To determine whether treatment modality (surgery, chemotherapy and radiotherapy) was an important confounder in the relationship between post-diagnostic statin use and OC-specific and all-cause mortality, analyses were repeated with and without these covariates in the model. To explore the potential impact of reverse causation bias on analyses of post-diagnostic statin use and consider latency, the cohort was restricted to those surviving at least three months, all drug exposures were lagged for at least three months and all new prescriptions in the final three months of life were ignored. In a post-hoc analysis, the effect of post-diagnosis statin use on-cause and OC-specific mortality

stratified by pre-diagnosis cardiovascular disease status were performed as effect sizes could differ according to their indication (primary versus secondary prevention). All analyses were performed with STATA version 11 (StataCorp LP, College Station, Texas, USA).

2.4. Results

Cohort

In total, 4676 patients identified from the GPRD with oesophageal or esophago-gastric junctional carcinoma met the inclusion criteria (figure 9). From these, 231 (5%) patients were excluded as they had no follow-up from diagnosis. The main cohort (total OC) comprised 4445 patients of whom 3655 died during follow-up. In total, 1530 (34.4%) patients were linked to the NCR in whom there were 1323 all-cause and 805 OC-specific deaths. Of these 1165 had complete information on both histology and site including 602 with OAC, 221 with OGJA and 342 with OSCC.

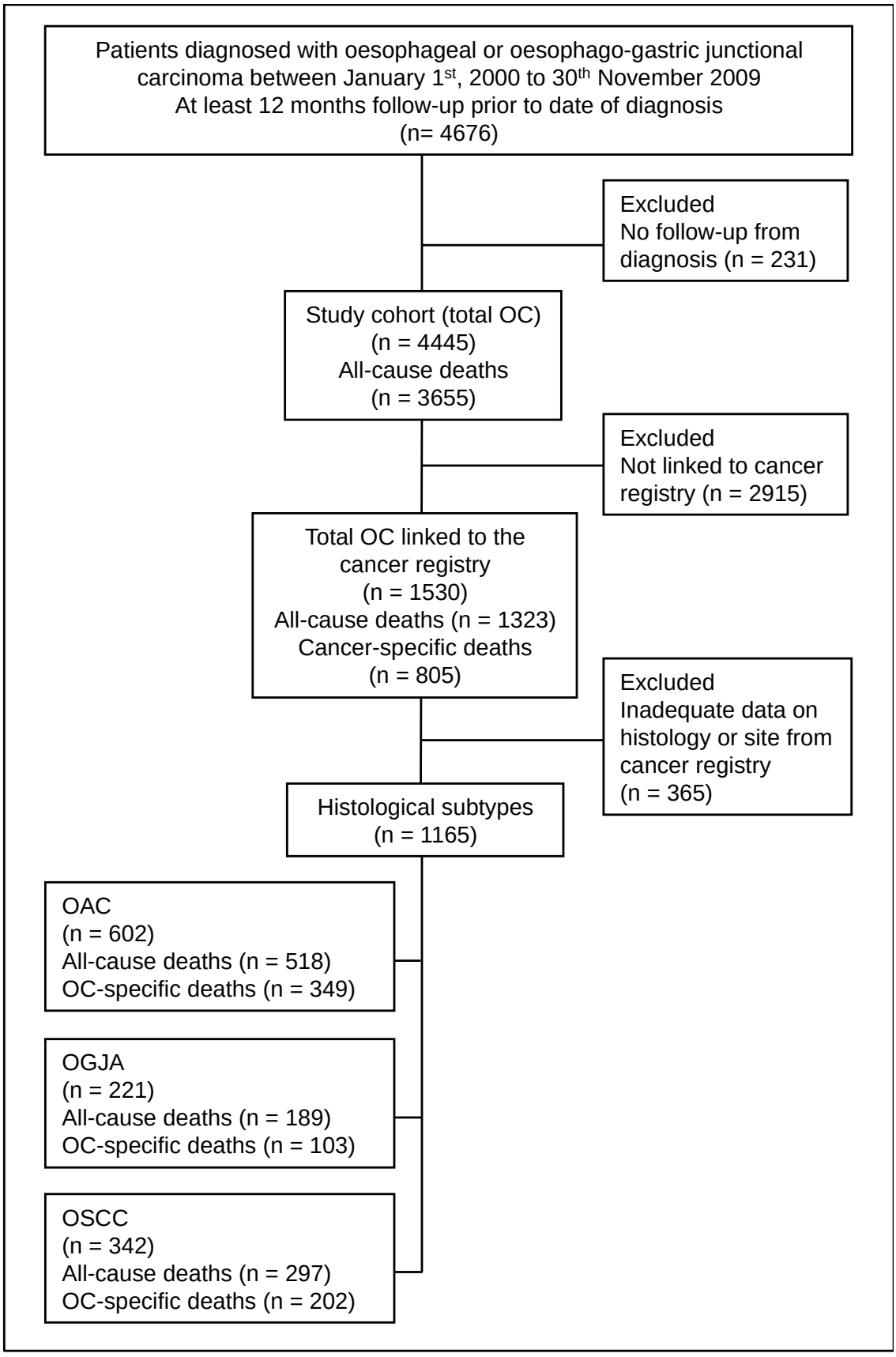

Figure 9: Flow chart of study participants

Abbreviations: OAC, oesophageal adenocarcinoma; OC, oesophageal carcinoma; OGJA, oesophagogastric junctional adenocarcinoma; OSCC, oesophageal squamous cell carcinoma

Clinical characteristics

Overall, patients in the whole cohort were more likely to be male, smokers and overweight or obese (table 9). Median survival for the whole cohort was 9.2 months (inter-quartile range 3.7-23.2). Post diagnosis statin use was observed in 18.7% of patients. Accounting for immortal-time, the median survival in post-diagnosis statin users was 14.9 months (IQR 7.1-52.3) and in non-users was 8.1 months (IQR 3.3-20). Most patients with OAC and OGJA were male and overweight, whereas the majority of patients with OSCC were female and had a normal or low BMI. Data on surgery was more complete for the histological subtypes (as additional surgical data was available from the NCR) than the whole cohort.

Pre and post-diagnosis statin use was more common among patients with OAC and OGJA than for those with OSCC. Pre and post-diagnosis statin users (compared to no pre and no post-diagnosis statin users respectively) were more likely to be older, male, overweight, smokers, have associated cardiovascular diseases or diabetes, and use aspirin, ACEi/ARBs or beta-blockers (all p values < 0.001) (table 10). Post-diagnosis statin users were more likely to have had surgery compared to those who did not use statins post-diagnosis (24.9% vs. 21.2% respectively, $p = 0.018$); whereas pre-diagnosis statin users were less likely to have had surgery than those who did not use statins pre-diagnosis (17.5% vs. 23% respectively, $p < 0.001$). 830 patients were prescribed statins post-diagnosis in the whole cohort, of whom 163 were new users. Of these 117 (72%) were started without a prior history of cardiovascular disease (suggesting their indication for primary prevention), and 46 (28%) were started after a record of cardiovascular disease (suggesting their indication for secondary prevention). Of all patients who used statins following diagnosis, 90% were prescribed within 6 months of diagnosis (figure 12).

Characteristics	Total EC (n=4445)	EAC (n=602)	OGJA (n=221)	ESCC (n=342)
Age (years), mean (SD)	70.8 (11.5)	70.7 (11.3)	68.3 (11.6)	71.8 (12.1)
Male gender, n (%)	2913 (65.5)	468 (77.7)	171 (77.4)	136 (39.8)
Smoking status, n (%)				
Ever	2701 (64.3)	348 (62.0)	127 (60.2)	187 (59.2)
Unknown[1]	244 (5.5)	41 (6.8)	10 (4.5)	26 (7.6)
Body mass index (kg/m^2), n (%)				
< 25	1460 (40.2)	168 (35.0)	58 (31.4)	160 (59.0)
≥ 25 < 30	1435 (39.5)	215 (44.8)	76 (41.1)	80 (29.5)
≥ 30	737 (20.3)	97 (20.2)	51 (27.6)	31 (11.4)
Unknown[1]	813 (18.3)	122 (20.3)	36 (16.3)	71 (20.8)
Comorbidities, n (%)				
Diabetes mellitus	347 (7.8)	52 (8.6)	17 (7.7)	18 (5.3)
Cardiovascular diseases	771 (17.3)	99 (16.4)	28 (12.7)	57 (16.7)
Oesophageal cancer treatment, n (%)				
Surgery	973 (21.9)	196 (32.6)	98 (44.3)	92 (26.9)
Chemotherapy	325 (7.3)	146 (24.3)	60 (27.1)	68 (19.9)
Radiotherapy	231 (5.2)	88 (14.6)	9 (4.1)	78 (22.8)
Median survival, months (IQR)	9.2 (3.7-23.2)	9.6 (4.0-23.3)	10.6 (4.2-24.8)	8.6 (4.0-18.7)
Prior medication use				
Statin prescription, n (%)	908 (20.4)	101 (16.8)	30 (13.6)	39 (11.4)
Aspirin prescription, n (%)	998 (22.5)	130 (21.6)	44 (19.9)	74 (21.6)
Beta-blocker prescription, n (%)	685 (15.4)	96 (15.9)	36 (16.3)	51 (14.9)
ACEi or ARB prescription, n (%)	947 (21.3)	117 (19.4)	31 (14.0)	58 (17.0)
NSAID prescription, n (%)	391 (8.8)	51 (8.5)	16 (7.2)	29 (8.5)
Post diagnosis medication use				
Statin prescription, n (%)	830 (18.7)	104 (17.3)	35 (15.8)	31 (9.1)
Aspirin prescription, n (%)	839 (18.9)	116 (19.3)	39 (17.6)	48 (14.0)
Beta-blocker prescription, n (%)	709 (16.0)	99 (16.4)	32 (14.5)	43 (12.6)
ACEi or ARB prescription, n (%)	889 (20.0)	109 (18.1)	38 (17.2)	33 (9.6)
NSAID prescription, n (%)	793 (17.8)	128 (21.3)	41 (18.6)	65 (19.0)

Table 7: Baseline demographic and clinical characteristics of the cohort stratified by histological subtype and site

Abbreviations: ACEi, angiotensin conversing inhibitor; ARB, angiotensin receptor blocker; OC, oesophageal cancer; OAC, oesophageal adenocarcinoma; OGJA, oesophagogastric junctional adenocarcinoma; OSCC, oesophageal squamous cell carcinoma; NSAID, non-steroidal anti-inflammatory drugs.

[1]Percentages presented for unknown categories reflect overall proportion of missing data for the relevant covariate; while percentages presented for known categories refer to complete data only

Characteristics	No pre-diagnostic statin use (n=3537)	Pre-diagnostic statin use (n=908)	p-value	No post-diagnostic statin use (n=3615)	Post-diagnostic statin use (n=830)	p-value
Age (years), mean (SD)	70.4 (12.0)	72.3 (9.3)	<0.001	70.7 (11.9)	71.3 (9.2)	0.137
Male gender, n (%)	2249 (63.6)	664 (73.1)	<0.001	2291 (63.4)	622 (74.9)	<0.001
Smoking status, n (%)						
Ever	2037 (61.6)	664 (74.4)	<0.001	2102 (62)	599 (73.7)	<0.001
Unknown[1]	229 (6.5)	15 (1.7)		227 (6.3)	17 (2.0)	
Body mass index (kg/m^2), n (%)						
< 25	1202 (43.1)	258 (30.7)		1246 (43.3)	214 (28.4)	
≥ 25 < 30	1085 (38.9)	350 (41.7)		1109 (38.5)	326 (43.2)	
≥ 30	505 (18.1)	232 (27.6)	<0.001	523 (18.2)	214 (28.4)	<0.001
Unknown[1]	745 (21.1)	68 (7.5)		737 (20.4)	76 (9.2)	
Comorbidities, n (%)						
Diabetes Mellitus	180 (5.1)	167 (18.4)	<0.001	236 (6.5)	111 (13.4)	<0.001
Cardiovascular diseases	419 (11.8)	352 (38.8)	<0.001	491 (13.6)	280 (33.7)	<0.001
Oesophageal cancer treatment, n (%)						
Surgery	814 (23.0)	159 (17.5)	<0.001	766 (21.2)	207 (24.9)	0.018
Chemotherapy	282 (8.0)	43 (4.7)	0.323	280 (21.4)	45 (20.5)	0.786
Radiotherapy	202 (5.7)	29 (3.2)	0.265	203 (15.5)	28 (12.8)	0.302
Median survival, months (IQR)	9.2 (3.7-23.3)	9.5 (4.0-23.1)	0.913	8.1 (3.3-20.0)	14.9 (7.1-52.3)	<0.001
Prior medication use						
Statin prescription, n (%)	0 (0)	908 (100)	N/A	262 (7.2)	646 (77.8)	<0.001
Aspirin prescription, n (%)	470 (13.3)	528 (58.1)	<0.001	572 (15.8)	426 (51.3)	<0.001
Beta-blocker prescription, n (%)	371 (10.5)	314 (34.6)	<0.001	417(11.5)	268 (32.3)	<0.001
ACEi or ARB prescription, n (%)	483 (13.7)	464 (51.1)	<0.001	567 (15.7)	380 (45.8)	<0.001
NSAID prescription, n (%)	296 (8.4)	95 (10.5)	0.047	306 (8.5)	85 (10.2)	0.103
Post diagnosis medication use						
Statin prescription, n (%)	184 (5.2)	646 (71.1)	<0.001	0 (0)	831 (100)	NA
Aspirin prescription, n (%)	456 (12.9)	383 (42.2)	<0.001	390 (10.8)	449 (54.1)	<0.001
Beta-blocker prescription, n (%)	419 (11.8)	290 (31.9)	<0.001	385 (10.7)	324 (39.0)	<0.001
ACEi or ARB prescription, n (%)	493 (13.9)	396 (43.6)	<0.001	437 (12.1)	452 (54.5)	<0.001
NSAID prescription, n (%)	635 (18.0)	158 (17.4)	0.698	598 (16.5)	195 (23.5)	<0.001

Table 8: Baseline demographic and clinical characteristics of the whole cohort stratified by statin use

Abbreviations: ACEi, angiotensin conversing inhibitor; ARB, angiotensin receptor blocker; OC, oesophageal cancer; OAC, oesophageal adenocarcinoma; OGJA, oesophagogastric junctional adenocarcinoma; OSCC, oesophageal squamous cell carcinoma; NSAID, non-steroidal anti-inflammatory drugs

[1]Percentages presented for unknown categories reflect overall proportion of missing data for the relevant covariate; while percentages presented for known categories refer to complete data only

Post-diagnosis statin use and survival

In the full cohort post-diagnosis statin use was associated with decreased OC-specific (HR 0.62, 95% CI 0.44-0.86) and all-cause mortality (HR 0.67, 95% CI 0.58-0.77) (table 11, figure 10 and 11). Post-diagnosis statin use was associated with reduced OC-specific (HR 0.61, 95% CI 0.38-0.96) and all-cause mortality (HR 0.63, 95% CI 0.43-0.92) in patients with OAC only, but not for the other subtypes. There was no significant interaction of pre-diagnosis statin use on the effect of post-diagnostic statin use on OC-specific or all-cause mortality for whole cohort or the subtypes. Post-diagnosis use of Simvastatin and Atorvastatin, but not the other statins, was associated with reduced OC-specific mortality (table 12). Post-diagnosis use of each of the individual statins investigated was associated with decreased all-cause mortality.

Cohort	Statin exposure	Number of patients, n (%)	Person-Years	Mortality rate (95% CI) (per 100 person-years)	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	p for interaction[3]
OC-specific mortality							
Total OC	No post-diagnosis statin use	1311 (85.7)	1848.3	38.9 (36.2-41.9)	1.00 (reference)	1.00 (reference)	
(n=1530)	Post-diagnosis statin use	219 (14.3)	426.8	20.1 (16.3-24.9)	0.71 (0.57-0.89)	0.62 (0.44-0.86)[1]	0.817
OAC	No post-diagnosis statin use	498 (82.7)	728.6	41.5 (37.0-46.4)	1.00 (reference)	1.00 (reference)	
(n = 602)	Post-diagnosis statin use	104 (17.3)	237.4	19.8 (14.9-26.4)	0.70 (0.51-0.96)	0.61 (0.38-0.96)[1]	0.374
OGJA	No post-diagnosis statin use	186 (84.2)	303.1	42.3 (36.6-48.8)	1.00 (reference)	1.00 (reference)	
(n = 221)	Post-diagnosis statin use	35 (15.8)	66.9	34.6 (21.2-56.5)	0.63 (0.33-1.21)	0.58 (0.20-1.69)[1]	0.062
ESCC	No post-diagnosis statin use	310 (90.6)	440.0	30.7 (25.0-37.6)	1.00 (reference)	1.00 (reference)	
(n = 342)	Post-diagnosis statin use	31 (9.1)	46.2	15 (8.0-27.8)	1.08 (0.65-1.81)	0.65 (0.29-1.46)[1]	0.756
All-cause mortality							
Total OC	No post-diagnosis statin use	3615 (81.3)	4905.9	62.2 (60.0-64.5)	1.00 (reference)	1.00 (reference)	
(n=4445)	Post-diagnosis statin use	830 (18.7)	1379.5	43.7 (40.4-47.3)	0.82 (0.75-0.89)	0.67 (0.58-0.77)[2]	0.599
OAC	No post-diagnosis statin use	498 (82.7)	728.6	60.7 (55.3-66.6)	1.00 (reference)	1.00 (reference)	
(n = 602)	Post-diagnosis statin use	104 (17.3)	237.4	32.0 (25.6-40.1)	0.75 (0.59-0.96)	0.63 (0.43-0.92)[1]	0.290
OGJA	No post-diagnosis statin use	186 (84.2)	303.1	61.8 (54.9-69.6)	1.00 (reference)	1.00 (reference)	
(n = 221)	Post-diagnosis statin use	35 (15.8)	66.9	54.1 (36.5-80.0)	0.80 (0.51-1.24)	0.82 (0.38-1.73)[1]	0.418
ESCC	No post-diagnosis statin use	311 (90.9)	440.0	54.8 (47.0-63.8)	1.00 (reference)	1.00 (reference)	
(n = 342)	Post-diagnosis statin use	31 (9.1)	46.2	34.4 (22.9-51.8)	1.12 (0.74-1.68)	0.78 (0.41-1.50)[1]	0.751

Table 9: Oesophageal cancer-specific and all-cause mortality according to post-diagnostic use of statins

Abbreviations: ARB, angiotensin receptor blocker; ACEi, angiotensin conversing enzyme inhibitor; OC, oesophageal cancer; OAC, oesophageal adenocarcinoma; OGJA, oesophagogastric junctional adenocarcinoma; OSCC, oesophageal squamous cell carcinoma; NSAID, non-steroidal anti-inflammatory drugs.

[1]Adjusted for age, gender, body mass index, smoking status, cardiovascular diseases, diabetes, surgery, pre-diagnosis statin use, post-diagnosis use of aspirin, beta-blockers, NSAIDs, and ACEi/ARBs

[2]Adjusted for [1] except surgery

[3]p for interaction between pre and post-diagnosis statin use on survival

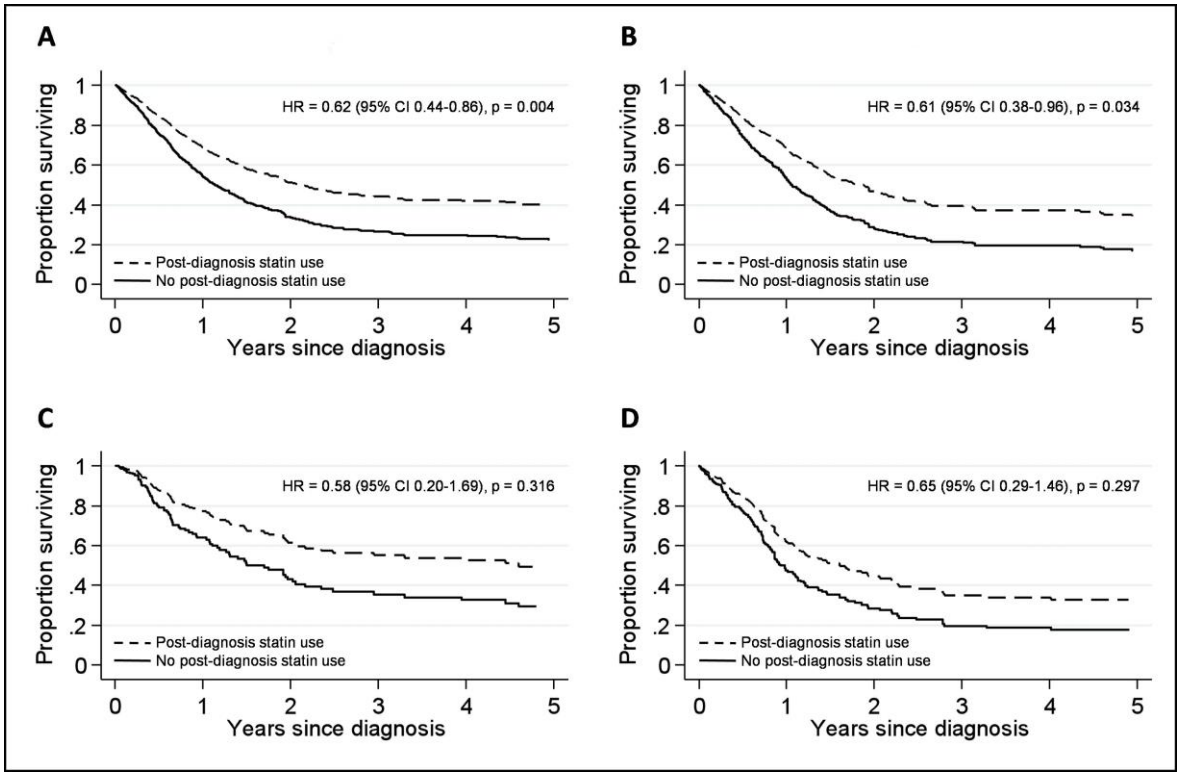

Figure 10: Adjusted time-dependent Cox proportional hazard regression survival curves with hazard ratios for oesophageal cancer-specific mortality stratified according to post-diagnosis statin use

Abbreviations: ACEi, angiotensin conversing enzyme inhibitor; ARB, angiotensin receptor blocker; CI, confidence interval; HR, hazard ratio; NCR, National Cancer Registry; NSAID, non-steroidal anti-inflammatory drugs

A – Total oesophageal carcinoma cases linked to NCR (n = 1222)

B – Oesophageal adenocarcinoma (n=470)

C – Oesophagogastric junctional adenocarcinoma (n=184)

D – Oesophageal squamous cell carcinoma (n = 267)

All adjusted for age, gender, body mass index, smoking status, cardiovascular diseases, diabetes, surgery, pre-diagnosis statin use, post-diagnosis use of aspirin, beta-blockers, ACEi/ARBs and NSAIDs

Only cases with complete body mass index and smoking data included

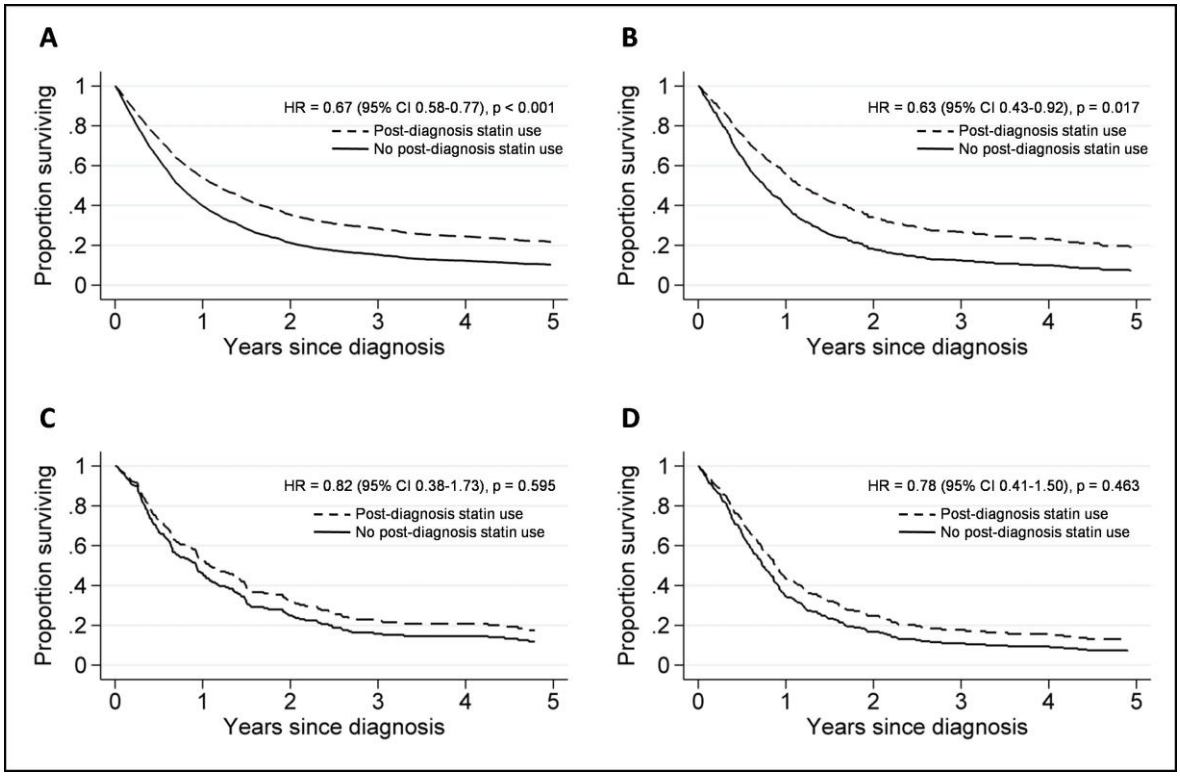

Figure 11: Adjusted time-dependent Cox proportional hazard regression survival curves with hazard ratios for all-cause mortality stratified according to post-diagnosis statin use

Abbreviations: ACEi, angiotensin conversing enzyme inhibitor; ARB, angiotensin receptor blocker; CI, confidence interval; HR, hazard ratio; NSAID, non-steroidal anti-inflammatory drugs

A – Total oesophageal carcinoma cases (n = 3595)

B – Oesophageal adenocarcinoma (n=470)

C – Oesophagogastric junctional adenocarcinoma (n=184)

D – Oesophageal squamous cell carcinoma (n = 267)

A adjusted for age, gender, body mass index, smoking status, cardiovascular diseases, diabetes, pre-diagnosis statin use, post-diagnosis use of aspirin, beta-blockers, ACEi/ARBs and NSAIDs

B, C, D adjusted for above including surgery

Only cases with complete body mass index and smoking data included

Statin type	Number of patients, n (%)	Person-Years	Mortality rate (95% CI) (per 100 person-years)	Unadjusted HR (95% CI)	Adjusted HR (95% CI)
OC-specific mortality					
No statin	1311 (85.69)	1703.91	42.2 (39.2-45.4)	1.00 (reference)	1.00 (reference)
Simvastatin	128 (8.37)	381.4	11.8 (8.8-15.8)	0.67 (0.50-0.91)	0.61 (0.41-0.89)[1]
Pravastatin	20 (1.31)	29.8	33.6 (18.1-62.5)	1.00 (0.54-1.87)	1.09 (0.56-2.12)[1]
Atorvastatin	63 (4.12)	137.1	21.9 (15.3-31.3)	0.81 (0.56-1.17)	0.56 (0.35-0.90)[1]
Rosuvastatin	3 (0.2)	8.7	0	NA	NA
Fluvastatin	5 (0.33)	14.3	7 (1-49.8)	0.23 (0.03-1.63)	0.24 (0.03-1.76)[1]
All-cause mortality					
No statin	3615 (81.33)	4592.4	66.5 (64.1-68.9)	1.00 (reference)	1.00 (reference)
Simvastatin	516 (11.61)	1066.3	34.4 (31.1-38.1)	0.83 (0.75-0.93)	0.68 (0.58-0.79)[2]
Pravastatin	57 (1.28)	132.6	30.9 (22.8-42)	0.69 (0.51-0.94)	0.58 (0.41-0.82)[2]
Atorvastatin	214 (4.81)	397.6	42.3 (36.3-49.2)	0.87 (0.74-1.02)	0.70 (0.58-0.85)[2]
Rosuvastatin	32 (0.72)	56.4	39 (25.7-59.2)	0.68 (0.45-1.04)	0.63 (0.40-0.99)[2]
Fluvastatin	11 (0.25)	40.0	12.5 (5.2-30)	0.36 (0.15-0.88)	0.33 (0.14-0.80)[2]

Table 10: Mortality according to first statin type used post-diagnosis of oesophageal carcinoma

[1]Adjusted for age, gender, body mass index, smoking status, cardiovascular diseases, surgery, diabetes, aspirin, beta-blockers, ACEi/ARB use and NSAIDs

[2]Adjusted for age, gender, body mass index, smoking status, cardiovascular diseases, diabetes, aspirin, beta-blockers, ACEi/ARB use and NSAIDs

Dose-response associations

No significant dose-response associations for either mean dose or cumulative dose in the 6-18 months prior to diagnosis were observed in the cohort for which OC-specific mortality data was available (n=1530) (p for trend 0.486 and 0.718 respectively) (see table 13). However, for all-cause mortality (n = 4445) there were significant dose-response associations for mean and cumulative dose categories (p for trend 0.003 and 0.002 respectively). For the dose response analyses defined by the first prescribed statin dose post diagnosis, while there were significant trends across dose categories, the point estimates did not consistently decrease from low to high dose use.

Statin exposure	Number of patients, n (%)	Person-Years	Mortality rate (95% CI) (per 100 person-years)	Unadjusted HR (95% CI)	Adjusted HR (95% CI)
OC-specific mortality					
Post-diagnosis statin use[3]					
No statin use	1311 (85.69)	1848.3	38.9 (36.2-41.9)	1.00 (reference)	1.00 (reference)
Low dose statin use[4]	118 (7.71)	245.1	18.8 (14.1-25.1)	0.70 (0.52-0.95)	0.56 (0.38-0.83)[1]
High dose statin use[4]	101 (6.6)	181.7	22 (16.1-30.0)	0.73 (0.53-1.00)	0.69 (0.46-1.03)[1]
P for trend				0.007	0.029
Pre-diagnosis statin use[5]					
No statin use	1301 (85.0)	1980.6	34.8 (32.3-37.5)	1.00 (reference)	1.00 (reference)
Low dose statin use[4]	149 (9.7)	232.8	42.4 (33.9-53.1)	1.08 (0.85-1.36)	0.90 (0.68-1.19)[1]
High dose statin use[4]	80 (5.2)	61.7	34.5 (25.2-47.2)	0.89 (0.65-1.23)	0.92 (0.64-1.33)[1]
P for trend				0.770	0.486
≥ 1 < 224 DDD[6]	146 (9.5)	191.2	36.6 (29.0-46.3)	0.97 (0.76-1.24)	0.89 (0.67-1.20)[1]
≥ 224 DDD[6]	83 (5.4)	103.2	44.6 (33.4-59.5)	1.07 (0.79-1.44)	0.99 (0.70-1.39)[1]
P for trend				0.820	0.718
All-cause mortality					
Post-diagnosis statin use[3]					
No statin use	3615 (81.3)	4905.9	62.2 (60.0-64.5)	1.00 (reference)	1.00 (reference)
Low dose statin use[4]	379 (8.5)	691.4	39.5 (35.1-44.5)	0.80 (0.70-0.90)	0.64 (0.54-0.75)[2]
High dose statin use[4]	451 (10.2)	688	48.0 (43.1-53.4)	0.83 (0.74-0.93)	0.70 (0.60-0.82)[2]
P for trend				<0.001	<0.001
Pre-diagnosis statin use[5]					
No statin use	3537 (79.6)	5128.2	56.8 (54.8-58.9)	1.00 (reference)	1.00 (reference)
Low dose statin use[4]	463 (10.4)	570.5	66.6 (60.2-73.7)	1.06 (0.95-1.18)	0.90 (0.80-1.02)[2]
High dose statin use[4]	445 (10.0)	586.6	61.7 (55.7-68.4)	0.95 (0.86-1.07)	0.83 (0.73-0.94)[2]
P for trend				0.706	0.003
≥ 1 < 224 DDD[6]	463 (10.4)	581.2	65.6 (59.3-72.5)	1.04 (0.94-1.16)	0.91 (0.80-1.02)[2]
≥ 224 DDD[6]	445 (10.0)	575.9	62.7 (56.5-69.5)	0.97 (0.87-1.08)	0.82 (0.72-0.93)[2]
P for trend				0.789	0.002

Table 11: Dose-response associations between statins use and risk of oesophageal cancer-specific and all-cause mortality

Abbreviations: ACEi, Angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; DDD, defined daily dose; OC, oesophageal cancer;

[1]Adjusted for age, gender, body mass index, smoking status, cardiovascular diseases, diabetes, surgery, aspirin, beta-blockers, ACEi/ARB use and NSAIDs

[2]Adjusted for 1 except surgery

[3]Dose categories determined using the first statin dose prescribed post-diagnosis

[4]Low dose equivalent to ≤ 20mg Simvastatin; high dose equivalent to > 20mg Simvastatin

[5]Measured between 6-18 months prior to diagnosis of OC

[6]Cut off of 224 DDDs selected as the median value in whole cohort

Sensitivity analyses

Pre-diagnosis statin use was associated with decreased all-cause mortality (HR 0.87, 95% CI 0.78-0.96) but not OC-specific mortality (HR 0.90, 95% CI 0.71-1.16) for the full cohort (table 14). No significant associations were observed between pre-diagnosis statin use and OC-specific and all-cause mortality for the histological subtypes. Including and excluding treatment modality (surgery, chemotherapy, radiotherapy) as individual covariates in models in analyses of post-diagnostic statin use did not materially alter the strength or precision of estimates (see table 15). Restricting the cohort to those who survived at least 3 months from diagnosis had a variable impact on associations between post-diagnostic statin use and survival: associations remained a similar magnitude in the full cohort for the assessment of all-cause mortality (main analysis: HR 0.67, 95% CI 0.58-0.77; sensitivity analysis: HR 0.70, 95% CI 0.60-0.82), however lost significance in the assessment of OC-specific mortality (main analysis: HR 0.62, 95% CI 0.44-0.86; sensitivity analysis HR 0.84, 95% CI 0.58-1.20). Lagging drug exposures weakened associations with OC-specific and all-cause mortality in the full cohort while they were strengthened for associations in patients with OAC. Ignoring new prescriptions in the final three months of follow-up did not materially alter associations for: OC-specific mortality for the full cohort (main analysis: HR 0.62, 95% CI 0.44-0.86; sensitivity analysis: HR 0.60, 95% CI 0.43-0.84); all-cause mortality for the full cohort (main analysis: HR 0.67, 95% CI 0.58-0.77; sensitivity analysis: HR 0.54, 95% CI 0.47-0.63); OC-specific mortality in patients with OAC (main analysis: HR 0.61, 95% CI 0.38-0.96; sensitivity analysis: HR 0.53, 95% CI 0.32-0.85); or for all-cause mortality in patients with OAC (main analysis: HR 0.63, 95% CI 0.43-0.92; sensitivity analysis: HR 0.49, 95% CI 0.33-0.73). For sensitivity analyses which stratified for pre-diagnosis cardiovascular disease status, risk of OC-specific mortality with post-diagnosis statin use was HR 0.66, 95% CI 0.44-1.00 (no cardiovascular disease) and HR 0.35, 95% CI 0.20-0.63 (with cardiovascular disease); and for all-cause mortality with post-diagnosis statin use HR 0.66, 95% CI 0.56-0.79 (no cardiovascular disease) and HR 0.66, 95% CI 0.52-0.83 (with cardiovascular disease).

Cohort	Statin exposure	Number of patients, n (%)	Person-Years	Mortality rate (95% CI) (per 100 person-years)	Unadjusted HR (95% CI)	Adjusted HR (95% CI)
EC-specific mortality						
Total OC	No pre-diagnosis statin use	1301 (85.0)	1980.6	34.8 (32.3-37.5)	1.00 (reference)	1.00 (reference)
(n=1530)	Pre-diagnosis statin use	229 (15.0)	294.5	39.4 (32.8-47.3)	1.01 (0.83-1.22)	0.91 (0.71-1.16)
OAC	No pre-diagnosis statin use	501 (83.2)	817.6	35.5 (31.6-39.8)	1.00 (reference)	1.00 (reference)
(n = 602)	Pre-diagnosis statin use	101 (16.8)	148.3	39.8 (30.8-51.3)	1.07 (0.81-1.41)	0.81 (0.55-1.20)
OGJA	No pre-diagnosis statin use	191 (86.4)	326.7	27.2 (22.1-33.5)	1.00 (reference)	1.00 (reference)
(n = 221)	Pre-diagnosis statin use	30 (13.6)	43.3	32.3 (19.2-54.6)	0.96 (0.55-1.69)	0.82 (0.40-1.69)
OSCC	No pre-diagnosis statin use	303 (88.6)	444.2	39.8 (34.4-46.2)	1.00 (reference)	1.00 (reference)
(n = 342)	Pre-diagnosis statin use	39 (11.4)	42.0	59.6 (40.3-88.2)	1.21 (0.79-1.84)	1.08 (0.65-1.79)
All-cause mortality						
Total OC	No pre-diagnosis statin use	3543 (79.6)	5128.2	56.8 (54.8-58.9)	1.00 (reference)	1.00 (reference)
(n=4445)	Pre-diagnosis statin use	908 (20.5)	1157.1	64.1 (59.7-68.9)	1.00 (0.93-1.09)	0.86 (0.78-0.95)
OAC	No pre-diagnosis statin use	501 (83.2)	817.6	52.6 (47.8-57.8)	1.00 (reference)	1.00 (reference)
(n = 602)	Pre-diagnosis statin use	101 (16.8)	148.3	59.3 (48.1-73.1)	1.08 (0.86-1.35)	0.77 (0.56-1.06)
OGJA	No pre-diagnosis statin use	191 (86.4)	326.7	50.2 (43.1-58.5)	1.00 (reference)	1.00 (reference)
(n = 221)	Pre-diagnosis statin use	30 (13.6)	43.3	57.8 (39.0-85.5)	0.96 (0.63-1.46)	0.72 (0.42-1.24)
OSCC	No pre-diagnosis statin use	303 (88.6)	444.2	59.0 (52.3-66.6)	1.00 (reference)	1.00 (reference)
(n = 342)	Pre-diagnosis statin use	39 (11.4)	42.0	83.4 (59.9-116.2)	1.18 (0.83-1.68)	0.95 (0.62-1.46)

Table 12: Oesophageal cancer-specific mortality according to pre-diagnostic use of statins

Abbreviations: ARB, angiotensin receptor blocker; ACEi, angiotensin conversing enzyme inhibitor; OC, oesophageal cancer; OAC, oesophageal adenocarcinoma; OGJA, oesophagogastric junctional adenocarcinoma; OSCC, oesophageal squamous cell carcinoma; NSAID, non-steroidal anti-inflammatory drugs

Adjusted for age, gender, body mass index, smoking status, cardiovascular diseases, diabetes, aspirin, beta-blockers, ACEi/ARB use and NSAIDs

Sesnitivity analyses (post-diagnostic statin use vs. non-use)	Statin users		Non users			
	Number of patients, n (%)	Mortality rate (95% CI) (per 100 person-years)	Number of patients, n (%)	Mortality rate (95% CI) (per 100 person-years)	Unadjusted HR (95% CI)	Adjusted HR (95% CI)
Total OC						
Oesophageal cancer-specific mortality						
Adjusted for surgery (main analysis)	219 (14.3)	15.1 (12.2-18.6)	1311 (85.7)	42.2 (39.2-45.4)	0.71 (0.57-0.89)	0.62 (0.44-0.86)[1]
Adjusted for surgery, chemotherapy and radiotherapy	219 (14.3)	15.1 (12.2-18.6)	1311 (85.7)	42.2 (39.2-45.4)	0.71 (0.57-0.89)	0.61 (0.44-0.85)[3]
Not adjusted for surgery	219 (14.3)	15.1 (12.2-18.6)	1311 (85.7)	42.2 (39.2-45.4)	0.71 (0.57-0.89)	0.65 (0.47-0.90)[2]
All patients surviving longer than three months from diagnosis	201 (16.7)	19.1 (15.3-23.7)	1001 (83.3)	30 (27.6-32.6)	0.71 (0.57-0.89)	0.84 (0.58-1.20)[1]
Exposure to precriptions lagged by three months	191 (12.5)	20.2 (16.2-25.3)	1339 (87.5)	38.4 (35.7-41.3)	0.84 (0.66-1.07)	0.66 (0.41-1.07)[1]
All prescriptions in the final three months of follow-up ignored	191 (12.5)	13.5 (10.8-16.9)	1339 (87.5)	42.6 (39.6-45.8)	0.64 (0.50-0.81)	0.60 (0.43-0.84)[1]
All-cause mortality						
Adjusted for surgery	830 (18.7)	43.7 (40.4-47.3)	3615 (81.3)	62.2 (60-64.5)	0.82 (0.75-0.89)	0.66 (0.58-0.76)[1]
Adjusted for surgery, chemotherapy and radiotherapy	830 (18.7)	43.7 (40.4-47.3)	3615 (81.3)	62.2 (60-64.5)	0.82 (0.75-0.89)	0.66 (0.58-0.76)[3]
Not adjusted for surgery (main analysis)	830 (18.7)	43.7 (40.4-47.3)	3615 (81.3)	62.2 (60-64.5)	0.82 (0.75-0.89)	0.67 (0.58-0.77)[2]
All patients surviving longer than three months from diagnosis	764 (21.8)	32 (29.4-34.8)	2749 (78.3)	49.4 (47.4-51.5)	0.88 (0.80-0.96)	0.70 (0.60-0.82)[2]
Exposure to precriptions lagged by three months	723 (16.3)	42.4 (38.9-46.3)	3722 (83.7)	61.8 (59.7-64.0)	0.92 (0.83-1.01)	0.85 (0.74-0.98)[2]
All prescriptions in the final three months of follow-up ignored	723 (16.3)	30.4 (27.9-33.2)	3722 (83.7)	68.1 (65.7-70.5)	0.67 (0.61-0.74)	0.54 (0.47-0.63)[2]
OAC						
Oesophageal cancer-specific mortality						
Adjusted for surgery (main analysis)	104 (17.3)	19.8 (14.9-26.4)	498 (82.7)	41.5 (37-46.4)	0.70 (0.51-0.96)	0.61 (0.38-0.96)[1]
Adjusted for surgery, chemotherapy and radiotherapy	104 (17.3)	19.8 (14.9-26.4)	498 (82.7)	41.5 (37-46.4)	0.70 (0.51-0.96)	0.61 (0.38-0.98)[3]
Not adjusted for surgery	104 (17.3)	15.5 (11.6-20.6)	498 (82.7)	41.5 (37-46.4)	0.70 (0.51-0.96)	0.60 (0.37-0.98)[2]
All patients surviving longer than three months from diagnosis	98 (20.3)	19.8 (14.9-26.4)	385 (79.7)	32.1 (28.2-36.5)	0.78 (0.56-1.08)	0.83 (0.50-1.36)[1]
Exposure to precriptions lagged by three months	93 (15.5)	19.7 (14.6-26.7)	509 (84.6)	40.8 (36.5-45.6)	0.81 (0.58-1.13)	0.47 (0.23-0.98)[1]
All prescriptions in the final three months of follow-up ignored	93 (15.5)	14.1 (10.4-19.0)	509 (84.6)	46 (41.1-51.4)	0.63 (0.45-0.87)	0.53 (0.32-0.85)[1]
All-cause mortality						
Adjusted for surgery (main analysis)	104 (17.3)	32 (25.6-40.1)	498 (82.7)	60.7 (55.3-66.6)	0.75 (0.59-0.96)	0.63 (0.43-0.92)[1]
Adjusted for surgery, chemotherapy and radiotherapy	104 (17.3)	32 (25.6-40.1)	498 (82.7)	60.7 (55.3-66.6)	0.75 (0.59-0.96)	0.65 (0.44-0.94)[3]
Not adjusted for surgery	104 (17.3)	32 (25.6-40.1)	498 (82.7)	60.7 (55.3-66.6)	0.75 (0.59-0.96)	0.63 (0.43-0.94)[2]
All patients surviving longer than three months from diagnosis	98 (20.3)	23.1 (18.3-29.2)	385 (79.7)	50.7 (45.6-56.5)	0.80 (0.62-1.04)	0.73 (0.48-1.09)[1]
Exposure to precriptions lagged by three months	93 (15.5)	30.5 (23.9-38.9)	509 (84.6)	60.2 (54.9-66)	0.82 (0.62-1.07)	0.59 (0.40-0.88)[1]
All prescriptions in the final three months of follow-up ignored	93 (15.5)	21.8 (17.1-27.8)	509 (84.6)	67.9 (61.9-74.4)	0.63 (0.49-0.82)	0.49 (0.33-0.73)[1]

Table 13: Sensitivity analyses for post-diagnostic statin use and esophageal cancer-specific and all-cause mortality

Abbreviations: ARB, angiotensin receptor blocker; ACEi, angiotensin conversing enzyme inhibitor; OC, oesophageal cancer; OAC, oesophageal adenocarcinoma; NSAID, non-steroidal anti-inflammatory drugs

[1]Adjusted for age, gender, body mass index, smoking status, cardiovascular diseases, diabetes, surgery, pre-diagnosis statin use, aspirin, beta-blockers, ACEi/ARB and NSAID use

[2]Adjusted for age, gender, body mass index, smoking status, cardiovascular diseases, diabetes, pre-diagnosis statin use, aspirin, beta-blockers, ACEi/ARB and NSAID use

[3]Adjusted for age, gender, body mass index, smoking status, cardiovascular diseases, diabetes, surgery, chemotherapy, radiotherapy, pre-diagnosis statin use, aspirin, beta-blockers, ACEi/ARB and NSAID use

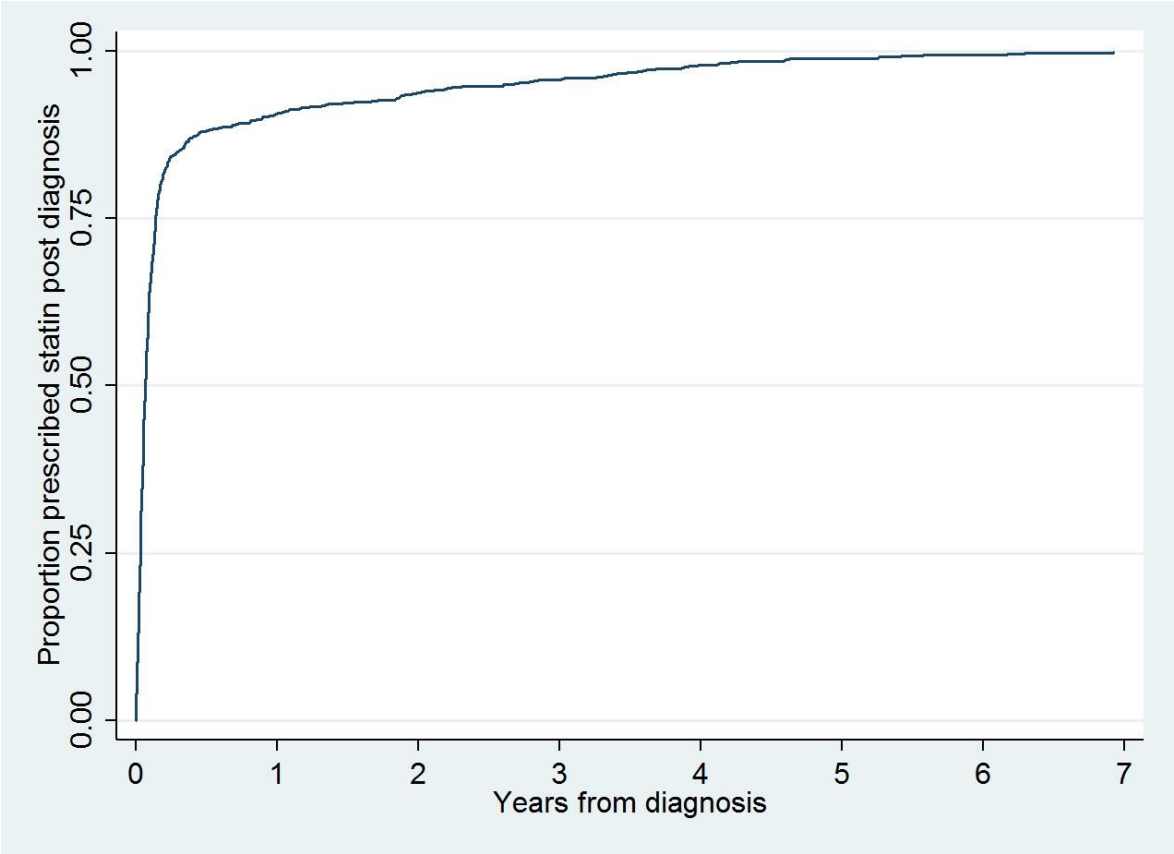

Figure 12: Kaplan-Meier plot of time to first statin prescription following diagnosis of oesophageal carcinoma among post-diagnosis statin users

2.5. Discussion

This large population-based cohort study of patients with incident OC found that post-diagnosis statin use was associated with a 39% reduction in OC-specific mortality and 33% reduction in all-cause mortality. In patients with OAC specifically, post-diagnosis statin use was associated with a 39% reduction in OC-specific mortality and 37% reduction in all-cause mortality. There were no significant improvements in survival associated with post-diagnosis statin use for OSCC or OGJA. Pre-diagnosis statin use did not significantly modify effects observed for post-diagnosis statin use on mortality. Significant dose and cumulative dose-response relationships were observed for pre-diagnosis statin use and all-cause mortality in the whole cohort. Estimates of the association between statin use and mortality for the histological subtypes, OGJA and OSCC, including the dose-response analyses with OC-specific mortality as the outcome, lacked precision. It therefore may not be possible to exclude a weak or moderate effect in these groups. While there were significant trends across dose categories defined by the first prescribed statin dose post-diagnosis, the estimated hazard ratios did not consistently decrease from low to high dose use suggesting that we should cautiously interpret this finding. It should be noted this approach would not take into account changes in dose or a cumulative exposure.

Biological mechanisms

Our findings are consistent with experimental studies which have demonstrated that statins promote apoptosis and limit proliferation and invasiveness in OAC cell lines[179, 180, 182, 235]. Inhibition of HMG-CoA reductase by statins decreases production of downstream intermediates of the mevalonate pathway, including farnesyl pyrophosphate, which are required for the prenylation and consequent membrane localisation of guanosine-triphosphate-bound proteins, including Ras[245]. Through limiting Ras farnesylation, statins reduce two protein kinases, extracellular signal-related protein kinase and protein kinase B/Akt, both of which are responsible for promoting cell survival and growth signal transduction in OAC cell lines[179]. Statins also reduce, in a dose-dependent manner, intracellular adhesion molecule-1[180], an adhesion molecule involved in trans-endothelial tumour cell migration and metastatic spread[185, 186]. Whether these mechanisms operate to explain the associations observed in this study is not clear.

Comparison with previous work

As far as we are aware, this is the first observational study to investigate the effect of statin use post-diagnosis on survival in patients with OC. However, one large observational study of 295, 925 patients diagnosed with cancer of any site within the entire Danish population examined the effect of statin use pre-diagnosis on cancer-specific mortality[189]. This study used a similar definition of pre-diagnosis statin exposure employed in our study, but also included all prescriptions between diagnosis and 18 months previously. In a sub-analysis of 4, 398 cases of OC, pre-diagnosis statin use was associated with reduced cancer-specific mortality (adjusted HR 0.81, 95% CI 0.69-0.95). This was similar to the effect size that we observed (HR 0.87, 95% CI 0.78-0.96) on all-cause mortality (n = 4445). However, while the effect size for pre-diagnosis statin use on OC-specific mortality in our study was similar, the estimate lacked precision, likely reflecting limited power to detect associations (HR 0.90, 95% CI 0.71-1.15) (n = 1530). Similarly to our study, significant amounts of data were missing for cancer stage, radiotherapy and chemotherapy. Use of concomitant medications that could plausibly confound associations were not included in multivariable analyses. The effect of dose-response on OC mortality specifically was not reported. This study did not determine associations according to the histological subtype.

Strengths and limitations

This study had several strengths. Read codes used to identify patients with OC in the GPRD have been shown to be valid (positive predictive value 0.97, sensitivity 0.92, and specificity 0.99)[232]. Overall five-year survival was 12.5%, consistent with UK data, suggesting the disease identified was clinically representative[58]**.** Participants with OC identified from the GPRD represent a large cohort with a median 9 (IQR 3.7 – 22.7) months follow-up post diagnosis to enable meaningful survival analyses. In a subset of patients, linkage with the NCR enabled associations between statin use and mortality for the histological subtypes of OC; and linkage with the ONS database enabled OC-specific mortality to be examined. Prospective prescription records within the GPRD avoid recall bias compared to self-reported medication use. Measurement error of drug exposures is likely to be minimal given the accuracy of prescription records in the GPRD[240]. While the GPRD does not record purchased over-the-counter medications, exposure misclassification for statin use is unlikely as such purchases account for only 0.7% of total statin use in the UK[246]. While the GPRD records prescribed medications, exposure misclassification could foreseeably arise where patients did not adhere to treatment: while prescriptions are accurately recorded by the GPRD, drug adherence is not directly captured. However, exposure misclassification through both sources would be expected to attenuate associations and underestimate the associations

observed in this study. For analyses of post-diagnosis statin use, the time-varying nature of drug exposures were accounted for and therefore avoided immortal time bias, which would have otherwise likely exaggerated associations. A form of selection bias, healthy survivor bias, could have influenced results for individuals who were prescribed their first statin after a substantial interval following diagnosis: this group would by definition have an improved prognosis as their risk of death due to the index cancer would be expected to diminish as the interval lengthened. However, this potential bias would seem unlikely to have influenced results overall as 90% of patients who were prescribed statins post-diagnosis, did so within 6 months (see figure 12). Analyses of OC-specific mortality censored for deaths due to other causes appropriately accounted for competing risk of death[244], an approach of particular relevance given the established efficacy of statins in reducing cardiovascular-related mortality[148-151].

Importantly time-dependent exposure to aspirin use was adjusted for as it is a plausible confounder in the association between post-diagnostic statin use and mortality outcomes in this population. Aspirin, through inhibition of cyclo-oxygenase to reduce inflammatory mediators and modulate platelet function, could improve survival in patients with OAC, a malignancy driven by inflammation[247, 248]. Indeed, in an individual patient meta-analysis of eight RCTs, allocation to aspirin (likely prior to the diagnosis of OAC) was associated with a significant reduction in death due to OAC[123]. While residual confounding by over-the-counter aspirin use is possible, any bias would be expected to have a negligible effect on study validity: nearly all long-term aspirin use is captured by the CPRD[249], the prevalence of aspirin use is low in this population, and any over-the-counter use would be expected to be non-differential between survivors and non-survivors prior to death[250].

This study has several limitations. There were substantial amounts of missing data for treatment modality and cancer stage. Completeness of treatment modality approached that expected for surgery and radiotherapy but not chemotherapy for patients linked to the cancer registry. For example, for OAC patients the proportion receiving surgery, chemotherapy, and radiotherapy, respectively, was 33%, 24% and 15% and national audit data indicate the approximate expected proportions to be 35%, 47% and 12%[251]. While treatment modality and stage are important predictors of outcomes, it is not clear as to whether they operate as confounders in the association between statin use and mortality. As clinical staging and treatment modality are closely related, treatment modality could be regarded as a proxy for staging: with surgery expected to be most discerning from the three captured modalities. Therefore, sensitivity

analyses were conducted (outlined above), to explore whether treatment modality, and by extension cancer staging, could operate as confounders in the association between statin use and mortality. Effect sizes and the precision of the estimates were similar for post-diagnosis statin use in analyses which did and did not adjust for surgery, chemotherapy and radiotherapy; suggesting that unmeasured confounding by treatment modality or clinical stage was not operating.

Reverse causation bias could theoretically operate in the association between post-diagnosis statin use and mortality. New users could represent a group with a more favorable prognosis, as determined by their GP, such that for these individuals prevention of cardiovascular disease (particularly primary prevention), a long-term outcome, is deemed a clinical priority, as opposed to adopting a more palliative approach. Although we cannot exclude this, our findings would suggest this mechanism of reverse causation bias is not a prominent explanation for the associations observed. First, of all statin users post-diagnosis, new statin users who were likely prescribed statins for primary prevention accounted for a minority (14%). Second, there was no significant interaction with statin use prior to diagnosis for the association between post-diagnosis statin use and OC-specific and all-cause mortality. Third, restricting the whole cohort to those surviving greater than three months from diagnosis did not materially alter associations in the whole cohort (associations examined with linked data were likely underpowered to assess this). Restricting the cohort to those surviving longer periods would have been underpowered: the prognosis from OC overall is poor and the remaining cohort size would be too small to permit meaningful analyses. Fourth, one would expect reverse causation bias to operate in the same manner as for other medications exposures used in the treatment of cardiovascular diseases (assuming they do not cause harm): indeed significant associations were not observed (all p values > 0.05). Fifth, pre-diagnosis statin use, a measure of statin exposure which would be expected to be free of reverse causation bias, was associated with reduced all-cause mortality. Deeming patients continuously exposed to statins once a prescription was issued until the end of follow-up prevented another guise of reverse causation bias, whereby treatment decisions made at the end-of-life, such as withdrawing regular medications, ensured patients were correctly classified as exposed. Similarly, sensitivity analyses which ignored all new prescriptions in the final three months' of follow-up, an exposure which may not plausibly influence outcomes but which could reflect a GP's assessment of prognosis, did not materially alter the strength or significance of associations.

The analyses may be susceptible to unmeasured confounding mediated by a healthy-user effect: statin users could represent a more health conscious group, whereby associated behaviors, either on the part of the patient or health-professional, may be associated with improved survival. For example, more health-conscious individuals may present and be diagnosed with an earlier stage of cancer; and GP have been reported to selectively under-prescribe lipid-lowering medications to obese patients or smokers[252], both of which are associated with increased cancer-related mortality[253, 254]. However, contrary to this, statin users appeared less healthy: they were more likely to be overweight or obese, smoke and have diabetes or cardiovascular disease than non-users - factors which independently predict mortality. Nevertheless, we attempted to minimize potential confounding from a healthy-user effect by including smoking, BMI, cardiovascular disease and concomitant medication use in multivariable analyses. Nevertheless, as with all observational studies, residual confounding is still possible.

Use of propensity scores as an alternative method to the multivariable outcome model used in the present study to account for confounding deserve consideration. A propensity score assigned to an individual is the probability of exposure status (in this case, post-diagnostic statin use) conditional all known confounders[255]. Propensity scores are most commonly estimated using multivariable logistic regression. The most common techniques for controlling for propensity scores are stratification, matching, weighting and adjustment. Adjustment is generally not advised as its validity is dependent on correctly specifying two models (used to derive the propensity score and the outcome model)[255]. Generally, the ability to control for confounding in traditional multivariable models and propensity score analyses is similar, with comparable effect sizes and precisions of estimates seen between the two when these methods are compared in the same dataset[256-258]. Nevertheless, propensity score analyses possess several advantages for treatment comparisons in epidemiological research. A major source of bias in pharmaco-epidemiology is confounding by indication/channeling bias/reverse causation bias: in the present study the probability of a GP initiating a statin after diagnosis of cancer is likely to be dependent on their assessment of a patients' prognosis and their anticipated long-term benefit. A propensity score is well placed to account for the indications and contraindications of use of the drug (as they focus on treatment indications and in addition are able to control for a large number of covariates), and enable comparison (by matching for or within strata of propensity scores) of patients with similar propensity to receive treatment, to generate valid treatment estimates[258]. This is in contrast to traditional multivariable models which are unable to specify this. Valid treatment comparisons can be further refined with the use of "trimming" of the study population: restriction to observations in comparison groups with overlapping propensity scores, through exclusion of

subjects in exposed and non-exposed groups with non-overlapping scores, before onward matching or stratification[255]. Therefore, individuals treated or not treated contrary to expectation (with low or high propensity scores, respectively) are excluded. Theoretically, unmeasured confounders should explain these non-overlapping populations. Indeed, this technique in simulation studies has been shown to reduce unmeasured confounding by frailty, an unobserved variable which is difficult to capture with known variables[259]. It should be noted that trimming the study population reduces the generalizability of causal inferences made to the restricted population only[255].

Propensity score analysis could be effectively implemented in the future to determine associations between post-diagnostic statin use and mortality in patients with OC. The propensity scores for post-diagnostic statin initiation would include variables which comprehensively account for the indications and contra-indications for statin therapy, cardiovascular disease (including severity), concomitant cardiovascular disease medications (including aspirin and statin therapy), and available contributing variables to clinically applicable cardiovascular disease risk calculators (such as QRISK®2[260]) to account for primary prevention measured prior to OC diagnosis. This approach would also account for confounding by medication use at baseline (such as aspirin) and in addition, would implement time-dependent exposures for their initiation post-diagnosis. Furthermore, trimming the population with non-overlapping propensity could limit unmeasured confounding, potentially by reverse causation or channeling bias (particularly the unobserved aspects of clinical decision making in determining treatment initiation), a key threat to the validity of such work. Subsequent matching or stratification for propensity scores with a sufficiently narrow caliper would further reduce residual confounding within strata or matched pairs[255]. Such an approach could improve the validity of such treatment estimates in population in future research.

An alternative, but equally valid approach to analysis of this cohort is the nested case-control study. In this setting, cases would be those participants with OC who had died during follow-up. Their date of death would be the assigned “index date” for their matched control(s) who would need to alive and undergoing follow-up at this time. Exposure data (including drug exposures) would be captured during fixed exposure windows measured prior to death (or index date), equally applied regardless of case/control status. Conditional logistic regression, with adjustment for the same factors listed in section 2.3, “covariates” (excluding matching factors) would generate odds ratios with 95% confidence intervals to estimate the association between post-

diagnostic statin use and mortality in patients with OC. These odds ratios would be expected to closely estimate the hazard ratio derived from the Cox proportional hazard regression model of the full cohort[290, 291]. Furthermore, this approach will account for the time-dependent nature of drug exposures and hence avoid immortal-time bias[261]. Such a nested case-control analysis could be included as a sensitivity analysis in similar future research.

Conclusions

In summary, post-diagnosis statin use was associated with large and significant reductions in OC-specific and all-cause mortality, specifically in those with OAC. There was evidence of significant dose and cumulative dose-response relationships with pre-diagnosis statin use on all-cause mortality in patients with OC. These results require replication in other large cohorts and provide further evidence in support of the conduct of randomized controlled trials of statins as adjuvant agents in patients with OC.

3. Chapter 3 – A Feasibility Study of Adjuvant Statin Therapy in the Prevention of Post-Operative Recurrence of Oesophageal Adenocarcinoma

3.1. Abstract

Background

Preclinical studies have demonstrated statins inhibit proliferation, promote apoptosis and limit invasiveness of oesophageal adenocarcinoma (OAC) cell lines. Observational research has demonstrated significant improvements in mortality associated with statin use after diagnosis of OAC. We aimed to determine the feasibility of assessing adjuvant statin therapy in patients with operable OAC in a phase III randomised controlled trial.

Methods

For this multi-centre, double-blind, parallel group, randomised, placebo-controlled trial, eligible patients were adults with OAC or Siewert type I/II adenocarcinoma due surgery. Participants were recruited from four UK centres and randomly assigned (1:1) to simvastatin 40mg or matching placebo by block randomisation, stratified by centre. Participants, clinicians and investigators were blinded to treatment allocation. Treatment started from the date of discharge following surgery and continued for up to one year. Feasibility assessments of recruitment, retention, drug absorption, adherence, safety, quality of life, generalisability, all-cause and disease-free survival were made. Trial registration: ISRCTN98060456.

Results

Between 23rd November 2014 and 22nd July 2016, 120 patients were assessed for eligibility, of which 32 (26.7%) were randomised. Of patients meeting eligibility criteria, 59.3% (32/54) were randomised. Patients allocated to simvastatin had significantly lower LDL cholesterol levels by three months (adjusted mean difference, -0.83 mmol/L, 95% CI -1.4 to -0.22, p=0.009). Median medication adherence for the preceding three months of follow-up at 3, 6, 9 and 12 months, respectively, was 83%, 94%, 99%, and 94%, with no significant differences in adherence between treatment groups. In total, 87.5% in the simvastatin group and 92.9% in the placebo group (p=0.626) experienced at least one adverse event. Completion of quality of life data was high (98.3% of questionnaire items) with no clinically significant differences observed between treatment groups. Cardiovascular disease (p=0.003), diabetes (p=0.003) and aspirin use (p=0.01)

were more prevalent in the non-randomised group compared with the randomised group. There were no significant differences between groups for overall (p=0.716) or disease-free survival (p=0.807).

Conclusions

This RCT supports the feasibility of assessing adjuvant statin therapy in a future phase III trial in patients with operable OAC. Feasibility estimates derived from this trial inform the design and conduct of a future study.

3.2.Introduction

3.2.1. Background

OAC is an important public health and clinical problem. The incidence has risen in the UK over recent decades by at least three fold[262] and the overall 5-year survival rates are less than 20%[263]. Patients with OAC commonly present at an advanced stage of disease and are therefore often only amenable to palliative treatments[264]. Even of those suitable for potentially curative treatment, the outcomes are still often poor, with 5-year survival estimated at 45%[114], and mortality predominantly attributed to recurrent disease[113]. Beyond current primary treatment modalities (oesophagectomy with or without peri-operative chemotherapy/chemoradiotherapy[111]), there are no established longer-term systemic therapies to reduce risk of recurrent disease. Consequently, there is an urgent need to assess potential novel therapies to improve current survival rates.

There is emerging experimental and epidemiological evidence that statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors), used in the primary and secondary prevention of cardiovascular disease, exert pleiotropic effects which are of relevance to cancer biology. Preclinical studies have demonstrated the effects of statin treatment on validated OAC cell lines, including inhibiting proliferation, promoting apoptosis and limiting invasiveness in a dose-dependent manner[179-182]. By inhibiting HMG CoA reductase, statins decrease production of intermediates of the mevalonate pathway, including farnesyl pyrophosphate, which are required for the prenylation and consequent membrane localisation of key members of the RAS superfamily of GTPases, including RAS, RAC and RHO[156, 245]. Through inhibiting RAS farnesylation, statins deplete extracellular signal-related protein kinase (ERK) and protein kinase B/Akt, both of which promote cell survival and growth signal transduction in OAC cell lines[179, 265]. Statins also reduce, in a dose-dependent manner, intracellular adhesion molecule-1 (ICAM-1)[180], a critical adhesion molecule involved in transendothelial tumour cell migration which promotes metastatic spread[185, 186].

A large observational study within the entire Danish population examined the effect of statin use pre-diagnosis on cancer specific survival in 295,925 patients diagnosed with cancer of any site[189]. In a sub-analysis of 4,398 cases of oesophageal cancer (including any histological subtype), statin use was associated with reduced cancer-related mortality (HR 0.81, 95% CI 0.69-0.95).

Furthermore, in a large pharmaco-epidemiological study, using the General Practice Research Database with linkage to the National Cancer Registry and Office of National Statistics Datasets, statin use after diagnosis of OAC (modelled as a time-dependent variable) was associated with significant reductions in cancer-specific mortality (HR 0.62, 95% CI 0.44-0.86)[207].

The emerging preclinical and epidemiological evidence justifies the conduct of randomised controlled trials to examine whether statins are efficacious adjuvant agents in patients with operable OAC. This proposed investigation is a feasibility study of adjuvant statin therapy in the prevention of post-operative recurrence of OAC, including adenocarcinoma of the gastro-oesophageal junction (Siewert I/II lesions). This group of post-surgical patients has been selected as they have minimal disease burden, yet substantial risk of recurrent disease. This implies the presence of undetectable residual micro-metastatic disease at oesophagectomy. We hypothesise that adjuvant and maintenance therapy could have a more pronounced clinical effect in this group, as opposed to those with macroscopic unresectable disease at presentation. Statins represent ideal agents to investigate as they are easily administered, inexpensive, well-tolerated and with an excellent safety profile at a population level[148-151].

3.2.2. Rationale

Before launching a definitive phase III RCT to determine efficacy of adjuvant and maintenance statin therapy in patients with OAC on a curative surgical pathway, important questions remain regarding study feasibility. Although statins are commonly prescribed for cardiovascular disease prevention, it is not known whether patients would be willing to consider entering a trial for the indication of investigating their anti-cancer potential. Valid and precise estimates of recruitment and retention would be required to determine feasibility and aid planning of a future trial. Prevalence of statin use has increased dramatically, however it is not clear to what extent this would impact recruitment to a future trial. It is important to determine whether patients are willing to adhere to treatment for this indication. Although a lesser concern, it is not known whether statins are adequately absorbed in this patient group: vagotomy performed during oesophagectomy may lead to reduced small bowel transit and could theoretically limit statin absorption. A future trial would be expected to capture patient-reported outcomes, particularly quality of life, and it would therefore be important to establish completion rates.

3.2.3. Objectives

The overarching aim of the STAT-ROC feasibility study was to determine the feasibility of assessing adjuvant statin therapy in patients with operable OAC in a future phase III randomised controlled trial. The following lists the objectives of the STAT-ROC feasibility study:

1. Recruitment and retention: to determine the recruitment and retention rates of eligible participants.

2. Absorption: to determine whether simvastatin is absorbed in patients following oesophagectomy.

3. Adherence: to determine whether participants adequately adhere to the allocated trial medication.

4. Safety: to determine a preliminary safety profile of simvastatin in this patient group.

5. Quality of life: to determine completion rates of questionnaires and conduct exploratory comparisons in quality of life reported between simvastatin and placebo treated groups.

6. Preliminary survival data: to estimate the effect of simvastatin 40mg following potentially curative surgery for OAC on disease-free and overall survival by one year post-randomisation.

7. Generalisability: to compare the demographic and clinical characteristics between randomised and non-randomised patients screened for this trial who meet inclusion criteria 2-4 (listed below).

3.3.Methods

3.3.1. Trial design

This study is a multi-centre, double-blind, parallel group, randomised, placebo-controlled trial to determine the feasibility of investigating adjuvant statin therapy in the prevention of post-operative recurrence of OAC in a future phase III RCT. Patients with OAC who underwent potentially curative surgery were randomised to receive either simvastatin 40mg nocte or placebo on discharge from hospital for up to one year. Participants were be assessed at screening, baseline and at three monthly intervals after discharge following surgery. Participants were followed-up for one year if recruited prior to 31/10/15. For patients recruited between 31/10/15 – 31/07/16, patients received at least 3 months' follow-up until 31/10/16 at the latest. Assessments include measurements of recruitment and retention, absorption, adherence, safety, quality of life, disease-free and overall survival and generalizability. A summary of the study design is shown in figure 13.

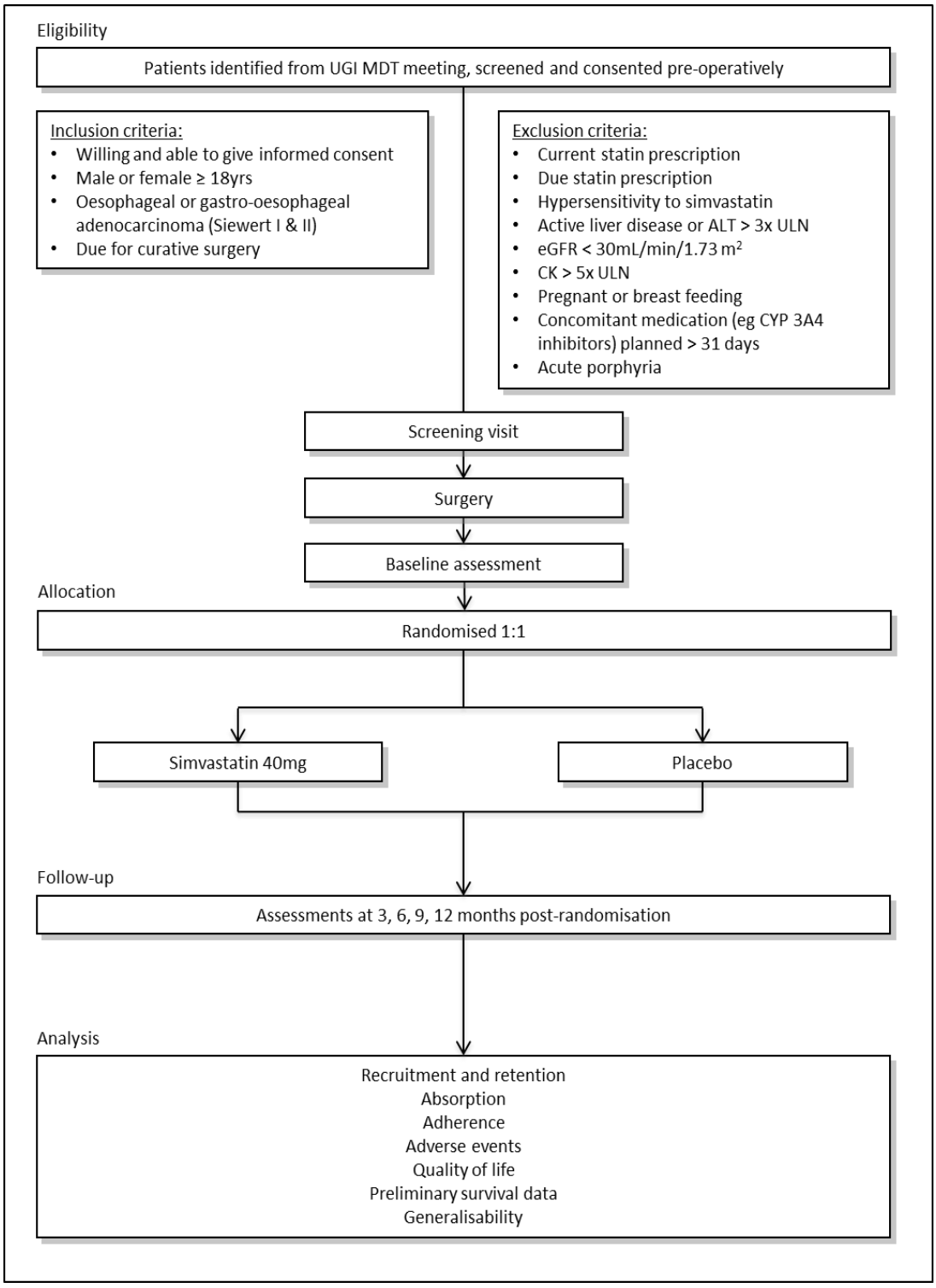

Figure 13: Summary of study design

3.3.2. Participants

Participants with OAC (including Siewert type I/II lesions) who underwent potentially curative surgery with either an oesophagectomy, oesophago-gastrectomy or extended total gastrectomy and whom survive to discharge from hospital following their operation.

Inclusion criteria

1. Participant was willing and able to give informed consent for participation in the trial.
2. Male or female, aged 18 years or above.
3. Diagnosed with OAC (including adenocarcinoma of the gastro-oesophageal junction [Siewert I/II lesions]) confirmed with both endoscopy and histology.
4. Due to undergo potentially curative surgery with either an oesophagectomy, oesophago-gastrectomy or extended total gastrectomy and survive to discharge from hospital following their operation.

Exclusion criteria

1. Currently prescribed a statin as part of their routine clinical care.
2. Were due to be prescribed a statin as part of their routine clinical care. Applicable to a participant who has agreed to statin therapy as recommended by their general practitioner (GP) for the primary or secondary prevention of cardiovascular disease. NB: patients who qualified for a statin but who choose not to be prescribed one for primary or secondary prevention of cardiovascular disease were still potentially eligible for this study.
3. Hypersensitivity to simvastatin.
4. Active liver disease or unexplained persistent elevations of serum transaminases (> 3x ULN).
5. Severe renal insufficiency (estimated glomerular filtration rate [eGFR] less than 30 mL/minute/1.73 m^2).
6. Creatine kinase (CK) > 5x ULN
7. Female participants who were pregnant, lactating or planning pregnancy during the course of the trial.
8. Concomitant drug prescription of potent CYP3A4 inhibitors planned for greater than one month during the study period (e.g. itraconazole, ketoconazole, fluconazole,

posaconazole, HIV protease inhibitors [e.g. nelfinavir], erythromycin, clarithromycin, telithromycin and nefazodone).

9. Concomitant drug prescription planned for greater than 1 month during the study period of amiodarone, verapamil, diltiazem, amlodipine, ciclosporin, danazol or gemfibrozil.
10. Acute porphyria.

Research Setting and delivery

This trial was conducted across four UK NHS sites: the Norfolk and Norwich University NHS Foundation Trust (NNUH), Nottingham University Hospitals NHS Trust (NUH), Mid Essex Hospital Services NHS Trust (MEHT), and James Cook University Hospital (JCUH) (South Tees Hospitals NHS Foundation Trust). Queen Elizabeth Hospital NHS Foundation Trust (QEH), Kings Lynn, Norfolk served as a single Patient Identification Centre (PIC) site for patients referred to NNUH. NNUH sponsored the study (ref: 2014GSURG01L[030114]). The research was hosted within respective departments of surgery at each NHS trust. The study was adopted by the UK Clinical Research Network Portfolio of studies and therefore received support from network-funded research delivery staff within division 1 (cancer). The Research Design Service East of England contributed to study design. Norwich Clinical Trials Unit (NCTU) supported all stages of delivery of the trial, including design, management, statistics, quality assurance and regulatory reporting.

3.3.3. Trial procedures

Trial procedures including recruitment, randomisation, baseline and follow-up assessments are summarised in Figure 14.

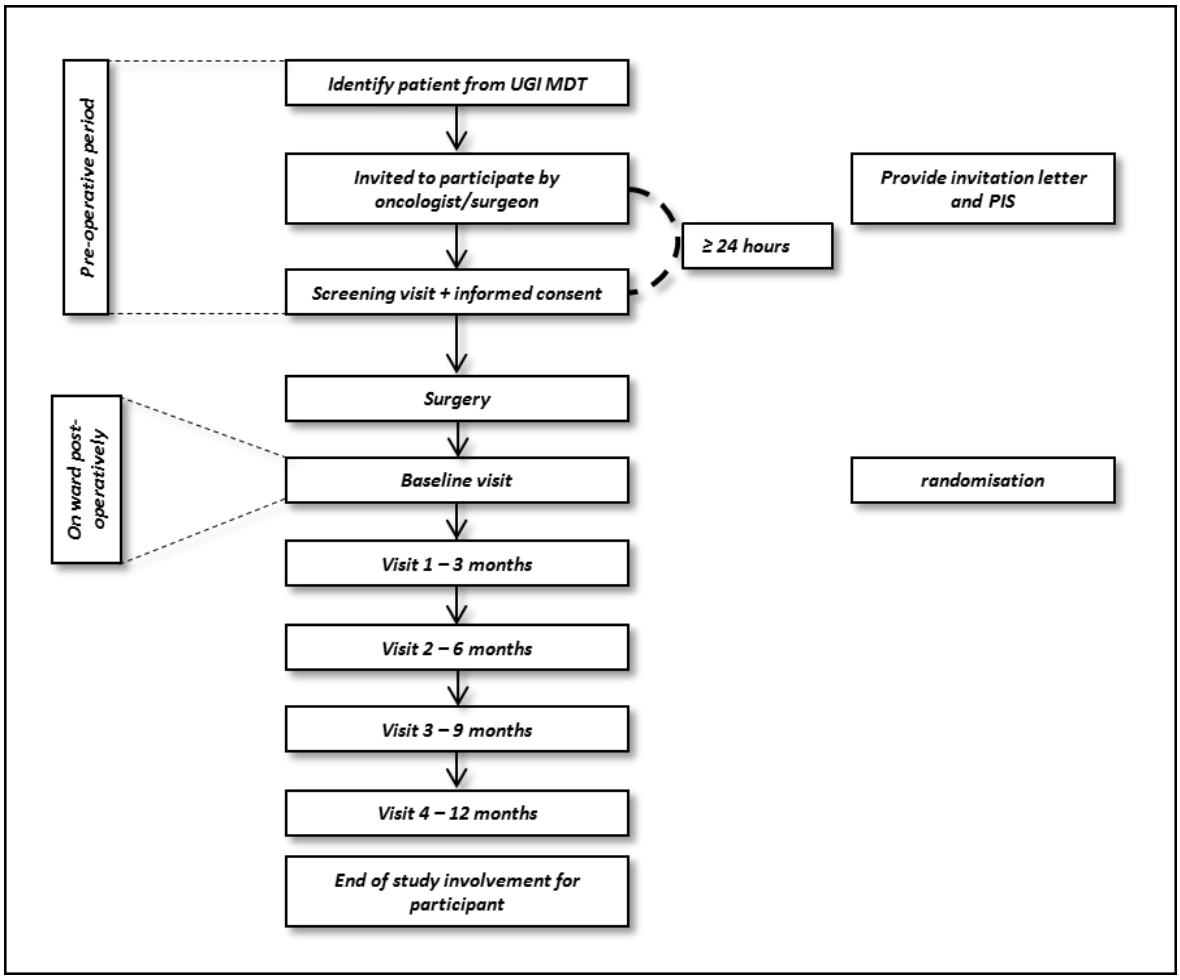

Figure 14: Flowchart of trial procedures

Abbreviations: MDT, multi-disciplinary team; PIS, participant information sheet.

Recruitment

Participants were identified at the local upper gastro-intestinal (UGI) cancer multi-disciplinary team (MDT) meetings at each NHS site. Each participant was asked in the pre-operative period by their surgeon or oncologist (or a member of their clinical team) if they would consider participating in this study. They were issued an invitation letter and a Participant Information Sheet (PIS). A member of the research team saw them at a screening visit prior to surgery, which was usually scheduled on the day of their pre-operative assessment. During this visit participants were screened to determine eligibility (see appendix F for the screening log) and informed consent was sought.

A retrospective case review of all patients meeting inclusion criteria numbered 2-4 at each site was conducted. The data were collected using an anonymised form. The data captured consisted of: patient demographics, clinical characteristics including tumour characteristics, performance status and adjuvant therapies. This generated a reference population against which generalisability was assessed for the randomised study population. Patients who met inclusion criteria numbered 2-4 who did not obviously meet any exclusion criteria at pre-screening were approached.

Informed consent

Recruited participants personally signed and dated the latest approved version of the Informed Consent Form (ICF) which was observed and countersigned by a member of the research team before any trial specific procedures were performed (see appendix G). Information presented to the participants detailed the trial rationale; the exact nature of the trial; what it would involve for the participant; participant responsibilities; the implications and constraints of the protocol; the known side effects of simvastatin; the safeguards in place; and how blood tests were due to be processed. The information clearly stated the participant was free to withdraw from the trial at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal. The participants were allowed at least 24 hours to consider the information, and the opportunity to question the Investigator, their GP or other independent parties to decide whether to participate in the trial. The member of the research team who took consent was familiar with the study and had the express authority to do so as detailed in the delegation log. Informed consent was sought during the screening visit. The original signed consent form was stored in the investigator site file. A copy of the signed informed consent was given to the participant, another was stored in the patient's medical notes and a copy was e-mailed to the NCTU.

Screening and eligibility assessments

Trial visit procedures are summarised in table 16. Participants were screened to ensure they fulfilled the inclusion criteria and did not meet any of the exclusion criteria. Some eligibility criteria required confirmation by laboratory tests, the results of which were available after patients had consented to the study. The research team wrote to the patient's GP to determine whether they were either currently prescribed a statin or due to be prescribed one for the primary or secondary prevention of cardiovascular disease. Members of the research team, including designated research nurses/practitioners and clinicians, performed screening and eligibility assessments. The maximum expected duration between screening and randomisation was two months. The assessments were:

1) Screening medical records to determine application of inclusion and exclusion criteria
2) Demographic information
3) Medical history
4) Drug history, including statin use
5) Quality of life questionnaires: EORTC QLQ-C30 and disease specific Oesophagogastric OG25 module
6) Height and weight
7) Blood tests for safety: thyroid function tests (TFTs), creatine kinase (CK), liver function tests (LFTs), creatinine. Blood test for research: non-fasting LDL cholesterol.
8) STAT-ROC feasibility study acceptance questionnaire or STAT-ROC feasibility study declined questionnaire (as appropriate)

Procedures	**Visits**					
	Screening	**Baseline**	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**
	Pre-op	Post-op	3 months	6 months	9 months	12 months
Informed consent	✓					
Demographics	✓					
Medical history	✓	✓				
Concomitant medications	✓	✓	✓	✓	✓	✓
Physical examination			✓	✓	✓	✓
Eligibility assessment	✓					
Randomisation		✓				
Dispensing of trial drugs		✓	✓	✓	✓	
Assessment of adherence			✓	✓	✓	✓
Blood tests for research	✓		✓	✓	✓	✓
Blood tests for safety	✓		✓	✓	✓	✓
EORTC QLQ-C30 and OG25	✓		✓	✓	✓	✓
STAT-ROC Acceptance or declined questionnaire	✓					
STAT-ROC withdrawal questionnaire (if applicable)		✓	✓	✓	✓	✓
Adverse event assessments			✓	✓	✓	✓
Clinical note review	✓	✓	✓	✓	✓	✓

Table 14: Schedule of procedures

Abbreviations: EORTC, European Organisation for Research and Treatment of Cancer; QLQ, quality of life questionnaire;

Randomisation, allocation concealment, blinding and code-breaking

Participants, clinicians, investigators and CTU staff were blinded to treatment allocation. Randomisation took place for consenting participants who satisfied the screening and eligibility assessments and whom were shortly to be discharged (within 1-5 days) from hospital following surgery for OAC. A computer generated randomisation code was produced by Ipswich Pharmacy Manufacturing Unit (PMU) and used to randomise participants in a 1:1 ratio to either simvastatin or placebo in blocks of six, stratified by NHS site. The final protocol deliberately did not stipulate the block size. The code stipulated the treatment allocation according to sequentially ordered four-digit subject number (starting from 0001). The unblinding code was sealed and stored at Ipswich PMU, NCTU and the NNUH pharmacy. Ipswich PMU produced identical sealed medication bottles which were individually labelled with corresponding subject numbers to preserve allocation concealment, and bottle numbers (from 1-12 corresponding to each month of treatment). Participants were sequentially allocated a subject number in the order they passed the baseline assessment. An Interactive Web Response System (IWRS) with password access limited to registered investigators, serially allocated the subject numbers to recruited patients. Confirmation e-mails were automatically sent to the user who performed allocation, the site principal investigator, site pharmacist and the trial co-ordinator to confirm the patient had been randomised. Prescription of the trial medication required the participant's name, date of birth, hospital number, subject number and the bottle numbers to be dispensed. Ipswich PMU produced identical active and placebo tablets to preserve blinding. Participants, their healthcare providers, data collectors and outcome adjudicators were all blinded to treatment allocation.

A mechanism was in place to facilitate treatment unblinding during the trial. Unblinding of the treating physician could only be justified in instances where knowing treatment allocation would alter the management of a severe adverse event. All suspected unexpected severe adverse reactions (SUSARs) would require unblinding and reporting as detailed below (under section x). Requests for unblinding should have been made via the trial co-ordinator or the CI to authorise this. In the case of out-of-hours emergency unblinding, the local PI or their delegate could log into the IWRS to reveal treatment allocation for a single participant. The IWRS which recorded the reason, date and time of the event, and identity of all recipients of the unblinding information to NCTU.

Baseline assessment

Baseline assessments were made while participants were an inpatient awaiting discharge following surgery. Provided there were no clinical contra-indications to receiving trial medication

and the participant was still willing to participate in the trial, they were randomised and hence allocated a subject number. To ensure sufficient medication supply, patients were prescribed four bottles each containing 31 tablets to ideally start on the day of discharge. Randomisation could be delayed by up to 31 days post discharge where a clinical contra-indication to starting the investigational medicinal product existed at the point of discharge but which may reasonably resolve to permit recruitment, such as renal impairment or awaiting the result of a barium swallow. Patients were asked to swallow the medication whole, in the evening. They were not permitted to crush the medication or open the capsules. Investigators were instructed to not inform participants of the reason for this: as it would risk unblinding.

Follow-up assessments

Participant follow-up began from the date of discharge from hospital following surgery with curative intent. Participants were invited to attend four follow-up assessments at 3, 6, 9 and 12 months post-discharge for those recruited prior to 31/10/15. A substantial amendment (described below) permitted extension of the recruitment period from 31/10/15 to 31/7/16; while the last possible date of patient follow-up remained 31/10/16. For participants recruited during this time, visits were still arranged at these three monthly intervals. Provided a three monthly visit was within 14 days (from 17/10/16) inclusive of the end of the study (31/10/16), this visit served at the final visit. If a three monthly visit was prior to this 14 day period the final visit was be made on 31/10/16 at the latest to maximise follow-up time. The IWRS calculated the target dates for each follow-up visit. Regardless of the period of recruitment (pre or post 31/10/15), the procedures for each visit were the same as detailed below:

1. Confirmation of any clinical contra-indication to patient receiving trial medication
2. Medical notes review to determine disease outcomes, including cancer recurrence
3. Record concomitant medications
4. Assessment of adverse events
5. Physical examination for evidence of recurrence if not already diagnosed
6. Quality of life: EORTC QLQ-C30 and disease specific Oesophagogastric OG25 module questionnaires
7. Perform pill counts
8. Specific drug safety assessments: LFTs at 3 and 12 months as routine, CK if muscle symptoms developed and the trial medication was felt likely to be causal. Blood tests for safety did not need to be repeated at study visits if they have already been determined within the prior 14 days of the patient visit as part of the patient's routine clinical care.
9. Assessment of simvastatin absorption: blood tests (non-fasting LDL-cholesterol [frozen]),

10. Dispensing of trial drugs at 3, 6, and 9 months

Follow-up assessments, where possible, were scheduled to coincide with hospital appointments as part of the participant's usual care. Symptoms of muscle toxicity were managed using an algorithm devised from the elected Summary of Medicinal Product Characteristics (SmPC) for Simvastatin 40mg and relevant clinical guidelines (appendix H)[266, 267]. Date and cause of death were verified using Death certificates or the Office for National Statistics. Provided the participant still met eligibility, trial medication were dispensed at each of these visits (3 bottles each containing 31 tablets at the 3 and 6 month visit, and 2 bottles at 9 months), excluding the final assessment which was the end of study involvement for that participant. The definition of the end of trial was the date of the last follow up visit of the last participant (31/10/16).

Discontinuation/withdrawal of participants from trial treatment

Participants could withdraw from the trial at any time. In addition, investigators could discontinue a participant from the trial at any time if they considered it necessary for the following reasons:

- Pregnancy, breast feeding or planning pregnancy during the course of the trial
- Ineligibility (either arising during the trial or retrospectively having been overlooked at screening)
- Significant protocol deviation
- An adverse event which requires discontinuation of the trial medication (anticipated for > 31 days) or results in inability to continue to comply with trial procedures
- Withdrawal of consent
- Loss to follow up

Participants could be withdrawn from trial treatment on a temporary (defined as ≤ 31 days) or permanent basis. When trial medication was withdrawn participants were still invited for the usual scheduled follow-up visits to permit intention-to-treat and safety analyses. The reason(s) for withdrawal were recorded. Patients who voluntarily withdrew from the study were asked to complete the STAT-ROC feasibility study patient withdrawal questionnaire, although they were under no obligation to do so nor were they required to provide their reason(s). If the participants were withdrawn due to an adverse event, the investigator arranged for follow-up visits or telephone calls until the adverse event has resolved or stabilised.

Adherence

Adherence was measured by pill counts recorded at each follow-up visit and corroborated at the end of the trial through LDL-cholesterol measurement (applicable only to the simvastatin treated group). In the MRC/BHF Heart Protection Study, simvastatin 40mg reduced LDL-cholesterol by 1.0 mmol/L on average in intention-to-treat analyses regardless of pre-treatment non-fasting LDL-cholesterol level[268].

For patients who had not already withdrawn treatment, adherence was encouraged at baseline and at each follow-up visit. Non-adherence was defined as intake of less than 80% of dispensed medications at each follow-up visit for the preceding three months. Reasons for non-adherence were sought which could be addressed in a future trial. Adherence was promoted through the following evidence-based approaches:

i. Patient focused: Patient education on adherence, the potential importance of the results[269] and to reduce perceptions of adverse events associated with statins[270]. Reinforcement by asking about adherence at each patient visit[271]. Acknowledge adherence verbally at follow-up visits[272].

ii. Health-professional focused: good communication skills with an empathic approach[273].

iii. Drug-focused: emphasis placed on the wide use and acceptable side-effect profile of simvastatin with a simple once daily dosing regimen[274, 275]. Medication will be supplied with clear information on dosing[276]. Encourage medication-taking with a daily event such as brushing teeth at night[270].

iv. System focused: Telephone call[277] to remind and encourage adherence at 2 and 4 weeks post-discharge. Minimise costs to the patient by reimbursing the financial costs of travel to follow-up visits[278].

Concomitant medication

The following medications were contra-indicated and necessitated temporary withdrawal of the trial medication if planned for up to 31 days, or permanent withdrawal if planned for longer than 31 days during follow-up:

- Potent CYP3A4 inhibitors (e.g. itraconazole, ketoconazole, fluconazole, posaconazole, HIV protease inhibitors [e.g. nelfinavir], erythromycin, clarithromycin, telithromycin and nefazodone).
- Ciclosporin, danazol, gemfibrozil, amiodarone, verapamil, diltiazem or amlodipine.

If treatment with a contra-indicated medication was planned for more than 31 days the study team liaised with their general practitioner or hospital consultant to prescribe a suitable alternative if possible.

Procedure for processing blood tests

Blood samples for research were centrifuged and serum was transferred to two cryovials at each site. Blood samples were frozen at -80°C and transferred on dry ice from participating centres to the NNUH Biochemistry department where they were be stored at -80°C. At the end of the trial one of the frozen samples from each time point from each participant was thawed at room temperature, mixed and centrifuged before being analysed for LDL cholesterol levels (a pharmacodynamic marker of statin absorption). The latest LDL cholesterol result was reported to the participant and their general practitioner. Blood tests for safety were processed in the usual way according to local hospital policy. Blood tests for research will be stored for a maximum of two years after the end of the trial. Samples were analysed at the end of study involvement for each participant (as opposed to during the study) to prevent unblinding. Participants were not expected to be disadvantaged by not knowing their non-fasting LDL cholesterol result until the end of the study as it would not be routinely measured as part of the standard clinical care of patients following potentially curative surgery for OAC. Furthermore, should participants have had their LDL cholesterol measured during the study for a non-trial indication and require treatment for high cholesterol they were aware to stop taking the trial medication in order to start treatment.

Trial medication preparation

Trial medication preparation was conducted by Ipswich PMU. Trial medications were simvastatin 40mg (the investigational medicinal product[IMP]) and placebo. To achieve blinding simvastatin 40mg capsules were manufactured by over-encapsulating simvastatin 40mg film coated tablets. These were identical in appearance to the placebo capsules. Opaque size 00 hard gelatine capsules were loaded into a capsule filling tray and the capsule tops were removed. For the active trial medication, one Simvastatin 40mg tablet (either PL 00289/1451 Teva UK Limited, Ridings Point, Whistler Drive, Castleford, WF10 5HX United Kingdom; PL 30306/0035 Actavis Group PTC ehf; and/or PL 17907/0127 Bristol Laboratories Ltd.) was placed into each empty capsule and subsequently Lactose monohydrate powder BP / Ph Eur (FrieslandCampina DMV BV, Veghel, The Netherlands) was added to the capsule until each lower shell was brim-full. For the inactive trial medication the capsule tops were removed and then filled to the brim with Lactose alone. The capsule tops were then refitted. For both active and inactive trial medication, the Lactose was sufficient to fully fill the capsule and in the case of the active medication, prevent movement of the enclosed tablet. Once processed, the bulk capsules were packaged into suitably labelled containers and quarantined until Quality Control checks had been performed. Thirty one capsules

were counted using a manual capsule counter and filled into each amber glass medicine bottle. One 0.5g Dilica Gel sachet was placed into each bottle and a child resistant cap tightly applied.

The smallest generic brands of Simvastatin (listed above) were selected such that the smallest possible capsule shell would not distort once prepared. The overall closed dimensions of each trial capsule was 23.3 x 8.53mm, with a capsule volume of 0.91 mls. The supplied certificate of analysis of the gelatin shell stipulated disintegration in less than 15 minutes, and in-house testing showed dissolution in water at 37°C of less than 5 minutes.

3.3.4. Safety reporting

Definitions

The definitions of harm of the EU Directive 2001/20/EC Article 2 based on the principles of ICH GCP applied to this trial (see table 17).

Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.
Adverse Reaction (AR)	An untoward and unintended response in a participant to an investigational medicinal product which is related to any dose administered to that participant. The phrase "response to an investigational medicinal product" means that a causal relationship between a trial medication and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out. All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the trial medication qualify as adverse reactions.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • results in death • is life-threatening • requires inpatient hospitalisation or prolongation of existing hospitalisation • results in persistent or significant disability/incapacity • consists of a congenital anomaly or birth defect. Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences. NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
Suspected Serious Adverse Reaction (SSAR)	An adverse event that is both serious and, in the opinion of the reporting Investigator, believed with reasonable probability to be due to one of the trial treatments, based on the information about the IMP provided in the Summary of Product Characteristics (SmPC).
Suspected Unexpected Serious Adverse Reaction (SUSAR)	A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out in the SmPC for that product

Table 15: Definitions of harm. Adapted from the EU Directive 2001/20/EC Article 2

Causality

The strength of the causal relationship between the study medication and each adverse event was assessed using the following definitions:

Unrelated: there is no evidence of any causal relationship

Unlikely: there is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the patient's clinical condition or other concomitant treatment).

Possible: There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition or other concomitant treatments).

Probable: There is evidence to suggest a causal relationship and the influence of other factors is unlikely.

Definitely: There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.

Capturing adverse event data

Adverse events (AEs) were captured from the date of first successful administration of the trial medication until 31 days following last administration. AEs occurring following consent but prior to first trial medication administration were not recorded as patients were exposed to normal care during this time. This approach was approved by the ethics committee and the MHRA. The date of discharge was the earliest date from which adverse events could be recorded. The following information was recorded on an adverse event form: description, defined using the Common Terminology Criteria for Adverse Events (CTCAE) V4.0 term; seriousness, using the definitions provided above; severity, defined using the CTCAE grade (1-5, where 1 indicates mild symptoms, and 5 indicates death); date of onset and end date; assessment of relatedness to trial medication; and action taken (none, IMP temporarily stopped, IMP permanently stopped). AEs were routinely recorded and reviewed at each follow-up visit and between visits when brought to the attention of the investigators.

Reporting adverse events

All SAEs that developed from first administration of the IMP to 31 days following last administration of the IMP (except those listed below) were recorded and reported to NCTU within 24 hours of the investigator becoming aware of the event. SAEs which did not require reporting include admissions or death secondary to known complication of adjuvant chemotherapy (eg. neutropenic sepsis, symptomatic anaemia, venous thromboembolism, cardiotoxicity and diarrhoea) or due to index cancer (eg. dysphagia or gastro-intestinal bleeding due to local tumour recurrence, ascites, metastatic disease, deep vein thrombosis or pulmonary embolism) or surgery (pneumonia, empyema, acute respiratory distress syndrome, pulmonary embolism, pleural effusion, surgical conduit dysfunction, anastomotic leak, wound infection, wound dehiscence or oesophageal stricture) but were be recorded in adverse event forms. This express list of exceptions to reporting SAEs was devised to limit the workload of contributing sites and NCTU as there would be no additional expected benefit to promoting pharmacovigilance through reporting these. For each reportable SAE, an SAE form was completed which included an assessment of causality and expectedness to determine whether the event was an SAE, SSAR or SUSAR.

Expected adverse events with Simvastatin

The Summary of Product Characteristics for simvastatin 40mg[279] lists the following adverse events which have been reported during clinical trials in adults and post-marketing experience. In the Heart Protection Study of 20,536 patients aged 50-80 years with cardiovascular disease or diabetes, 10,269 treated with simvastatin 40mg daily and 10, 267 treated with placebo, the safety profiles were comparable between groups over the five years of the study[268]. Individual adverse events, which were reasonably causally related to simvastatin 40mg, were categorised as “rare” (>1/10 000, <1, 000). Discontinuation rates due to side effects were comparable (4.8% treated with simvastatin 40mg and 5.1% treated with placebo). Furthermore, a meta-analysis of 14 RCTs in the primary prevention (46 262 participants) and 15 RCTs in the secondary prevention (37 618 participants) of cardiovascular diseases found a small minority of symptoms reported on statins were due to the drugs, and almost all would occur just as frequently on placebo[280]. Only new-onset diabetes was significantly higher on statins than placebo with an absolute risk of 0.5%, 95% CI 0.1-1.0%. Serious adverse events and treatment withdrawals were similar in both intervention and controls arms.

The sponsor elected one approved SmPC (Teva, Simvastatin 40mg, PL 00289/1453) as equivalent to the investigator’s brochure. Table 18 summarises the expected AEs as documented in the SmPC:

Frequency of adverse event	Adverse event
Very common (> 1/10)	None.
Common (> 1/100 to < 1/10)	None.
Uncommon (≥ 1/1000 to < 1/100)	Sleep disorders including insomnia, nightmares, depression, memory loss, sexual dysfunction, Diabetes Mellitus: Frequency will depend on the presence or absence of risk factors (fasting blood glucose ≥ 5.6mmol/L, BMI>30kg/m^2, raised triglycerides, history of hypertension).
Rare (> 1/10,000 to < 1/1000)	Anaemia, headache, paresthesia, dizziness, peripheral neuropathy, peripheral polyneuropathy, constipation, abdominal pain, flatulence, dyspepsia, diarrhoea, nausea, vomiting, pancreatitis, hepatitis, rash, pruritus, alopecia, asthenia, hypersensitivity syndrome (angioedema, lupus-like syndrome, polymyalgia rheumatica, dermatomyositis, vasculitis, thrombocytopenia, eosinophilia, ESR increased, arthritis and arthralgia, urticaria, photosensitivity, fever, flushing, dyspnoea and malaise), increases in serum transaminases, elevated alkaline phosphatase, increase in serum CK levels, rhabdomyolysis (CK more than 40 times the upper limit of normal plus evidence of end-organ damage).
Very Rare (< 1/10,000)	Insomnia, memory impairment, hepatic failure
Not known (cannot be estimated from the available data)	Depression, interstitial lung disease, tendonitis, tendinopathy sometimes complicated by tendon rupture, tendon rupture, erectile dysfunction.

Table 16: frequencies of adverse events expected with Simvastatin 40mg

Abbreviations: BMI, body mass index; ESR, erythrocyte sedimentation rate; CK, creatine kinase.

3.3.5. Statistical analysis

A statistical analysis plan was finalised and approved by the trial steering committee before masking was broken and analysis undertaken.

Flow of participants

A consort flow diagram was constructed to document the flow of participants through this trial: through enrolment, intervention allocation, follow-up and data analysis[281]. The number "assessed for eligibility" only applied to those participants meeting inclusion criteria numbered 2-4, regardless of the subsequent outcome of inclusion criteria 1 or any of the named exclusion criteria. This definition was provided to all contributing sites to ensure consistency of this population. The number who "received allocated intervention" applied only to those who successfully administered at least one dose of the trial medication; it did not apply to those who received dispensed medication bottles but did not swallow any trial medication.

Baseline characteristics

The baseline characteristics of all randomised participants were presented stratified according to treatment allocation. For each characteristic the summary measure used was appropriate to the nature of the variable and its distribution.

Outcomes and statistical analysis

As this was a feasibility study, the outcome measures were viewed with equal primacy and hence were not divided into primary and secondary outcomes overall. However, to reduce the probability of type 1 error, the primary outcome measure for drug absorption was prespecified (see below). Full analyses were conducted by intention to treat (ITT) and included all randomised participants. Per-protocol analyses (a non-randomised observational comparison[281]) comprised two definitions depending on the outcome assessed, for drug absorption and survival (see below).

1. **Recruitment.** Outcome defined as the randomisation of a trial participant. Three aspects of recruitment were calculated: i) the number of participants randomised per month per recruiting site; ii) the proportion of participants randomised from all those who met inclusion criteria 2-4 (regardless of exclusions) at pre-screening; and iii) the proportion of participants randomised from those not randomised who met all inclusion criteria except the first criterion (ie declined). These proportions were presented with 95% confidence intervals calculated using the binomial exact method.

2. **Retention.** Outcome defined as the last date of active participation in trial procedures including administration of trial medication. Withdrawal of participant included both complete withdrawal from the trial and withdrawal of treatment but still undergoing active follow-up, censored for recurrence and/or death (censored for recurrence first if later died during follow-up) as this is expected to be an outcome in the future phase three trial). This rate was presented with 95% confidence intervals calculated using the quadratic approximation to the Poisson log likelihood for the log-rate parameter. Kaplan-Meier survival curves were plotted (right censored for patients with truncated follow-up [until 31/10/16 at the latest] and death) for the randomised population and by treatment group. Differences in withdrawal between groups were tested using the log-rank test.
3. **Absorption.** The primary outcome was change in non-fasting LDL cholesterol at three months following discharge (visit 1), adjusted for LDL cholesterol measured at screening in the ITT population. The adjusted and unadjusted mean differences in LDL cholesterol, measured between treatment groups for visits 1-4 (3-12 months), were tabulated with 95% confidence intervals and p-values. Adjusted comparisons were conducted using ANCOVA. Significance testing for the primary outcome was assessed at the 5% level, while analyses at the other time points were exploratory at 1% significance. Plots were constructed with the mean unadjusted LDL cholesterol for each group plotted against serial visits over time (Screening, V1, V2, V3 and V4). Sensitivity analyses using the method above were repeated for the per-protocol population, defined for this outcome as participants adherent to least 80% of dispensed medications in the preceding three months. Individual participants could therefore leave and re-enter periods of adherence (and vice versa); and consequently could intermittently populate the per-protocol populations.
4. **Adherence.** Outcome defined as the proportion of medication consumed in the three months preceding the visit at which the pill count was performed. Median adherence (with interquartile range) were tabulated and presented using a range plot for all individuals included in the ITT population and according to treatment group, right censored for patients with truncated follow-up (until 31/10/16 at the latest), recurrence or death (whichever came first). Adequate adherence was defined as administration of at least 80% of trial medication in the three months preceding each follow-up visit. Estimates for adherence exceeding 105% for the preceding three months were considered implausible and were ignored. Estimates of adherence at all subsequent visits to implausible values were also ignored (as these were reliant on valid earlier estimates) for affected patients.

5. **Safety.** All reported adverse events were summarised according to treatment received and tabulated with frequencies (for the number of individuals with ≥ 1 adverse event) and percentages according to category of AE and worst grade experienced using CTCAE v4.0. The proportions of individuals with at least one adverse event were compared using the χ^2 test. Safety analyses were restricted to the trial population who successfully administered at least one dose of trial medication.
6. **Quality of life.** Compliance for completing quality of life questionnaire items were tabulated for each study visit (n, %). Items on both the EORTC QLQ-C30 and OG25 were scored and scaled, and missing values were imputed, in line with the EORTC manual[282]. Difference in mean scores adjusted for values observed at screening were tabulated (with 95% confidence intervals) for each follow-up visit stratified according to treatment allocation using ANCOVA. Plots were constructed with the mean scores for each group plotted against serial visits over time (Screening, V1, V2, V3 and V4).
7. **Exploratory survival comparisons.** Overall survival was defined as time elapsed from discharge from hospital to death from any cause. Disease-free survival was defined as the time elapsed from discharge to the first time point at which one of the following events occurred: local recurrence, distal recurrence or death. Kaplan-Meier survival curves and the log-rank test, with Cox proportional hazards modelling (which estimated hazard ratios and 95% confidence intervals) compared treatment groups. OS and DFS were compared between groups for the intention-to-treat population only: the number of events in the per-protocol populations (defined for this outcome as including participants adherent to least 80% of dispensed medications by the first follow-up visit at 3 months) were too low (no deaths and one recurrence).
8. **Generalisability.** Demographic and clinical characteristics were compared between randomised and non-randomised patients assessed for eligibility. Categorical data was compared using the χ^2 test, and continuous data were compared using the two sample t-test or Mann-Whitney U as dictated by the distribution.

Sample size calculation

As this is was a feasibility study, a formal sample size calculation was not required. Nevertheless, a sample size of 24 represents the inflection point between the number of participants randomised in a 1:1 ratio and the precision of mean difference between both groups[283] and would be expected to satisfy assessment of feasibility outcomes measured on a continuous scale. Therefore 24 was the minimum recruitment target for the trial. A sample of 22 participants (11 per arm) had 80% power at the 5% level to detect a difference of 1 mmol/l

in LDL cholesterol, assuming a standard deviation of 0.8[268]. An upper recruitment target limit of 36 participants was aimed for and felt to be potentially feasible given the inclusion criteria and study population available as this would have enabled improved precision in the assessment of feasibility outcomes. All analyses were performed with STATA version 13 (StataCorp LP, College Station, Texas, USA).

3.3.6. Trial oversight and quality assurance procedures

Trial monitoring

The aims of monitoring were to ensure that the trial was conducted and data generated, documented and reported in compliance with the protocol, good clinical practice (GCP) and the applicable regulatory requirements. Central monitoring was conducted by NCTU, with the option for on-site monitoring if required. There were three main aspects to trial monitoring as documented in a trial-specific working practice document (STAT-ROC quality management and monitoring plan): consent forms, patient safety and deliverability. Patients were consented to enable the NCTU to hold a copy of the consent form for the trial to facilitate central data monitoring. Consent forms were monitored for all randomised patients to ensure the correct version number was used; both the participant and investigator (as named on the delegation log) had signed the form; the date of consent preceded the date of randomisation; and that consent was recorded electronically on the IWRS. Patient safety was monitored to ensure that all inclusion and exclusion criteria (the latter were designed to preserve patient safety) were documented in the CRF and IWRS; blood tests for safety were taken as per the study schedule and that results were in the required ranges for participation in the trial; and serious adverse event forms were completed correctly and reported to NCTU within 24 hours of the investigator become aware of the event. Regarding trial delivery, the number of participants screened and recruited per month per site were monitored. On-site monitoring could be triggered following concerns raised by the chief investigator, trial steering committee or trial management group, if sites were to generate high volumes of data queries, or if sites did not respond to queries within three weeks.

Trial management group

The trial management group (TMG) met at least monthly to discuss the general progress and day-to-day running of the trial. The key members were the CI, trial co-ordinator, research practitioner, and all other STAT-ROC investigators. The TMG reviewed all AEs, CRF completion and data quality and dealt with all aspects of the quality control procedures.

Trial steering committee

A trial steering committee (TSC) was established to provide oversight for the trial, review the trial's progress, conduct and new relevant information. Specifically, their remit covered advising the Trial Management Group on all aspects of trial conduct; decision making for the continuation of the trial; approving the protocol, substantial amendments and the statistical analysis plan;

reviewing the recommendations of the safety committee; and assessing the impact and relevance of any accumulating new external evidence which may inform the conduct of the trial. Membership included independent and non-independent representation. Independent representation included a chair, consultant clinical oncologist, consultant gastroenterologist, NIHR representative, two patient representatives, and sponsor representative. Non-independent members were the team of investigators. TSC meetings were held twice per year for the duration of the trial (four were held in total).

Safety committee

A Safety committee (SC) was established in place of a full data monitoring and ethics committee (DMEC) given the relatively small size of the trial (compared with a large phase three RCT) and the relatively low risk of the intervention in terms of safety. Membership included two consultant physicians independent to the trial with clinical trial experience and routine clinical experience of prescribing statins. The main responsibilities of the safety committee were to review each serious and non-serious adverse event recorded during the trial; provide an independent opinion on whether the AEs were in line with that expected from this patient cohort (given the disease of interest and expected concomitant treatments including surgery and chemo/radiotherapy) or were related to simvastatin exposure; discuss and attempt to resolve any issues with the CI; and make recommendation to the TSC for either continuation, temporary or permanent stopping of the trial on grounds of the emerging safety data. The TSC reviewed blinded data, however, could at their disposal, request unblinded data. Safety committee meetings were held twice per year for the duration of the trial (four were held in total).

Ethical and regulatory considerations

Ethical approval was first granted in 1st July 2014 (appendix B), and subsequently for a substantial amendment on 29th June 2015 (NRES Committee South Central – Oxford B; reference: 14/SC/0247) (appendix C). Medicines and Healthcare products Regulatory Agency (MHRA) approval was first granted on 9th June 2014 (appendix D) and subsequently for a substantial amendment on 2nd July 2015 (reference: 13630/005/001-0002) (appendix E). The trial was registered with the European Clinical Trials Database (EudraCT Number: 2014-001318-24) and ISRCTN registry (ISRCTN98060456).

Role of the funding source

This research was funded by a Doctoral Research Fellowship (DRF-2013-06-115) awarded by the National Institute of Health Research (NIHR), the Norfolk and Norwich University Hospital Gastroenterology research fund (Dr Hugh Kennedy) and Surgical research funds (Mr Edward Cheong). The funding sources and sponsor had no input regarding the design, conduct or interpretation of this study. This research represents independent research funded by the National NIHR. The views expressed are those of the investigators and not necessarily those of the NHS, the NIHR or the Department of Health.

Substantial amendment

This feasibility study was originally planned to be conducted at a single site: the Norfolk and Norwich University Hospital NHS Foundation Trust. Within two months of this site being activated, it became clear that recruitment would be insufficient to meet the minimum target of 24 patients. NIHR, NCTU, sponsor, MHRA and ethical approval was sought to expand the study to three further NHS sites, extend recruitment from 31/10/15 to 31/7/16 with truncated follow-up for participants recruited after 1/11/15. The last date of follow-up was fixed at 31/10/16.

3.4. Results

Recruitment

Between 21st October 2014 and 22nd July 2016, 120 patients were assessed for eligibility (see figure 15). 88 were excluded in total, 54 for not meeting inclusion criteria (54 were current statin users and of these, two were also in receipt of contra-indicated medication [ciclosporin and amlodipine], and one had severe renal insufficiency); 22 declined to participate; and 12 patients assessed for eligibility during the recruitment period were excluded for other reasons (five patients originally on a curative surgical pathway progressed and were deemed unsuitable for curative resection; one patient died during neoadjuvant chemotherapy; two were pre-screened and provisionally met eligibility, however were not approached in time to gain consent prior to surgery; one patient consented to the trial however later required enteral feeding and would have been unable to take the trial medication; and three were pre-screened during recruitment but could not be approached as recruitment had completed at respective sites.

Between 23rd November 2014 and 22nd July 2016, 32 patients with oesophageal or oesophago-gastric junctional (Siewert type I or II) adenocarcinoma who underwent resection with curative intent were randomised to receive Simvastatin 40mg (n=16) or placebo (n=16) once daily from three UK NHS sites. Participants were followed-up until withdrawal from the trial, death, or one year from discharge from hospital following potentially curative surgical resection or 31st October 2016, whichever came first. Of the 16 patients randomised to placebo, two never received the study intervention (one withdrew consent prior to receipt of medication and one patient did not receive their trial medication prior to discharge, which was subsequently lost triggering a protocol violation). Of the 16 randomised to Simvastatin, all received the trial medication.

Participants recruited after 1st November 2015 underwent truncated follow-up for at least three months, affecting eight patients in the placebo arm and seven in the simvastatin arm. In total 12 patients in the placebo arm, and 14 patients in the simvastatin arm had both a screening and three month LDL cholesterol measurement. Reasons for missing values in the placebo group were: one death before the three months; one withdrawal before three months; one sample was insufficient volume; and for one hypertriglyceridaemia prevented calculation of LDL cholesterol. Missing values for two patients in the simvastatin group were also due to hypertriglyceridaemia.

The overall proportion of participants randomised from those assessed for eligibility (regardless of reasons for exclusion) was 26.7% (95% CI 19.0-35.5%) (32/120). The proportion of participants

randomised from those who met all inclusion criteria except the first one (ie. “participant is willing and able to give informed consent for participation in the trial”) was 59.3% (95% 45.0-72.4%) (32/54).

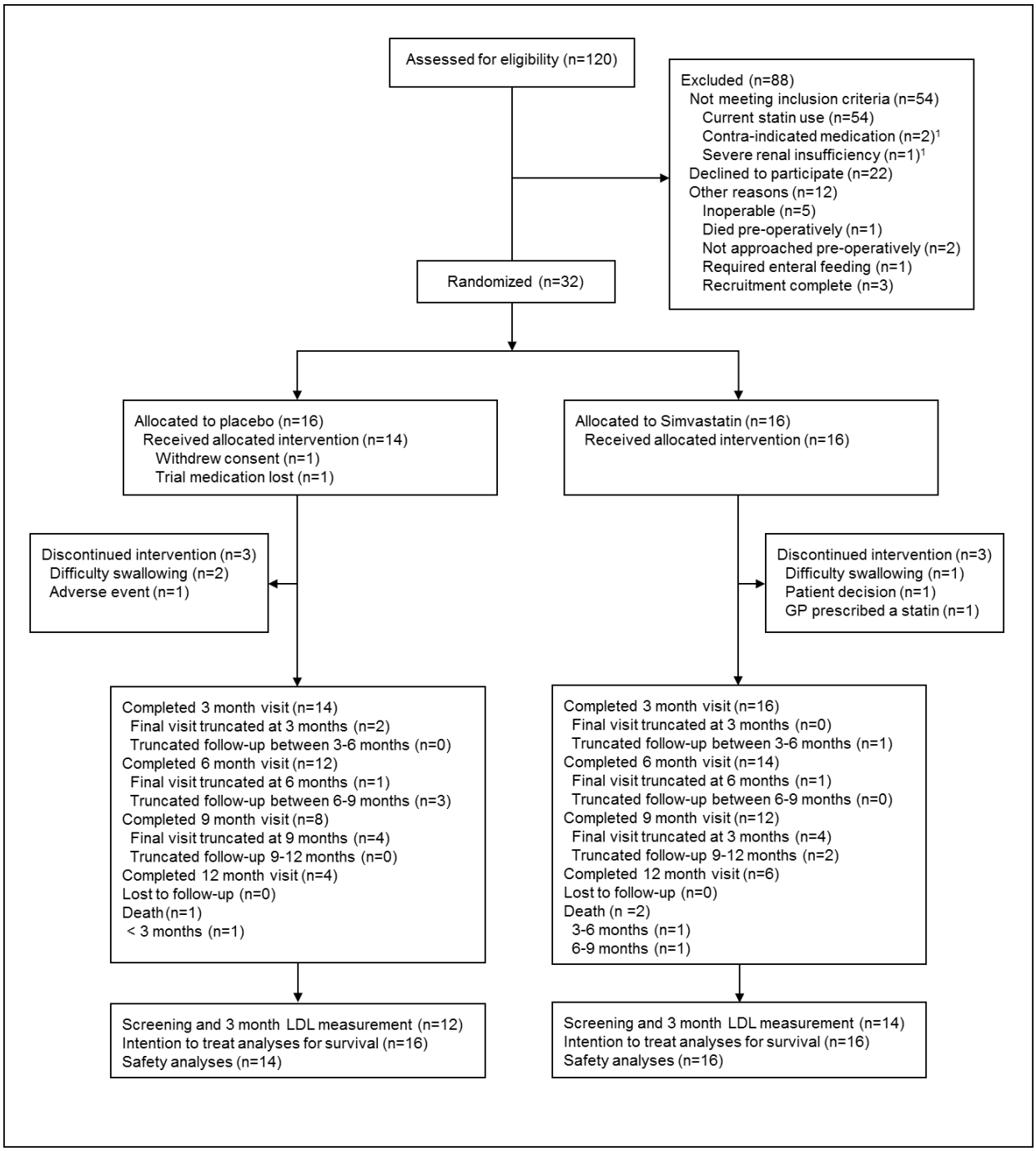

Figure 15: Trial profile

Table 19 shows the monthly recruitment rate for each NHS site. Of the four active sites, three recruited at least one patient. The overall cumulative monthly rate of recruitment was 3.01 (95% CI 2.59-3.48) participants per month. Variation in rate between centres was observed, the highest was seen at Mid Essex Hospital Services NHS Trust (recruitment completed of six allocated patients within 4.6 months), while no patients were randomised at South Tees Hospitals NHS Foundation Trust (of 16 assessed, six declined, eight were prevalent statin users, one progressed pre-op and one consented but was not randomised as their date of surgery was after recruitment to the study had closed).

Site	Recruitment time (months)	Number recruited	Rate (95% CI)
Norfolk and Norwich University Hospital	18	21	1.16 (0.73-1.76)
Mid Essex Hospital Services NHS Trust	4.6	6	1.31 (0.49-2.79)
Nottingham University Hospitals NHS trust	9.3	5	0.54 (0.18-1.24)
South Tees Hospitals NHS Foundation Trust	5.8	0	0
Cumulative total	37.7	32	3.01 (2.59-3.48)

Table 17: Monthly recruitment rate by contributing NHS site

Baseline characteristics

Baseline characteristics are presented in table 20. Baseline demographic and clinical characteristics, and quality of life function and symptoms scales were well-balanced overall between groups. The mean age at randomisation was 62.7 (SD 12.3) years in the placebo group and 66.6 (SD 8.7) years in the simvastatin group. As expected, most randomised patients were male, had a significant smoking history, where of white European descent, had minimal co-morbidity, were not aspirin users, and predominantly had a performance status score of zero. Mean non-fasting LDL cholesterol was similar between groups: 3.51 (SD 0.89) mmol/L in the placebo group, and 3.73 (SD 0.92) in the simvastatin group. The frequencies of individual tumour sites were similar between groups; for oesophageal, Siewert I and Siewert II tumours respectively there were seven, two and seven in the placebo group, and five four and seven in the simvastatin group. Most tumours were grade 2-3, based on biopsies taken at index. The majority of tumours were T3, (all were in the placebo group) with one T2, one T4 and two T4a tumours in the simvastatin group. Clinical N staging was broadly equivalent between groups. In both arms 15/16 (93.8%) received neoadjuvant chemotherapy and one patient in the simvastatin arm received pre-operative radiotherapy. VO_2 max was similar between groups, it was 20.7 ml/kg/min (SD 3) in the placebo group, and 21.8 ml/kg/min (SD 3.1) in the simvastatin group. Most oesophagectomies were hybrid procedures (9/16 in the placebo group and 10/16 in the simvastatin group). The lymph node yield and number of positive lymph nodes were similar between groups. Nine patients in the placebo group and five in the simvastatin group had a positive resection margin. Post-operative length of stay was similar between groups: median 10 days (IQR 6-12.5) in the placebo group, and 9 days (IQR 6-12) in the simvastatin group. The proportions of in-hospital complications were similar between groups, with seven (44%) in the placebo group, and six (38%) in the simvastatin group. Mean global quality of life, function scores (role, emotional, cognitive, social and physical) and symptom scales (dysphagia, eating difficulties and reflux) were similar between treatment groups.

Variable	Placebo (n=16)	Simvastatin (n=16)
Age at randomisation (years)	62.7 (12.3)	66.6 (8.7)
Time from diagnosis to randomisation (days)	153.4 (31.8)	155 (40.8)
Gender		
Male	13 (81.3)	12 (75)
Female	3 (18.8)	4 (25)
Smoking status		
Current	1 (6.3)	2 (12.5)
Past	10 (62.5)	11 (68.8)
Never	5 (31.3)	3 (18.3)
Body mass index (kg/m^2)	26.2 (4.1)	26.6 (4.7)
Ethnic origin (European)	16 (100)	16 (100)
Comorbid conditions		
Cardiovascular	0	1 (6.3)
Diabetes	0	0
Charleson co-morbidity index[1]		
0	15 (93.8)	14 (87.5)
1	1 (6.3)	2 (12.5)
Peri-operative aspirin use	0	0
Performance status		
0	16 (100)	13 (81.3)
1	0	2 (12.5)
2	0	1 (6.3)
LDL cholesterol (mmol/L)	3.51 (0.89)	3.73 (0.92)
Tumour site		
Oesophageal	7 (43.8)	5 (31.3)
Siewert I	2 (12.5)	4 (25)
Siewert II	7 (43.8)	7 (43.8)
Tumour grade		
Gx	2 (12.5)	1 (6.3)
G1	0	0
G2	5 (31.3)	8 (50)
G3	9 (56.3)	6 (37.5)
G4	0	1 (6.3)
Clinical T stage		
2	0	1 (6.3)
3	16 (100)	12 (75)
4	0	1 (6.3)
4a	0	2 (12.5)
Clinical N stage		
0	2 (12.5)	5 (31.3)
1	9 (56.3)	6 (37.5)
2	4 (25)	4 (25)
3	1 (6.3)	1 (6.3)
Neoadjuvant chemotherapy		
Yes	15 (93.8)	15 (93.8)
No	1 (6.3)	1 (6.3)
Pre-operative Radiotherapy		
Yes	0	1 (6.3)
No	16 (100)	15 (93.8)

Variable	Placebo (n=16)	Simvastatin (n=16)
Chemotherapy response		
Complete	2 (14.3)	2 (13.3)
Good	1 (7.1)	0
Moderate	4 (28.6)	3 (20)
Poor	4 (28.6)	0
No response	3 (21.4)	7 (46.7)
Unknown response[2]	2 (12.5)	1 (6.3)
VO_2 max (ml/kg/min)	20.7 (3)	21.8 (3.1)
Oesophagectomy		
Open	4 (25)	2 (12.5)
Hybrid	9 (56.3)	10 (62.5)
Minimally invasive	3 (18.8)	4 (25)
Lymph node yield, n (IQR)	26 (19-42)	21.5 (24.5-35)
Positive lymph nodes, n (IQR)	1.5 (0-4.5)	1 (0-3)
Vascular invasion		
Positive	9 (56.3)	5 (31.3)
Negative	7 (43.8)	11 (68.8)
Margin status		
R1	4 (25)	3 (18.8)
R0	12 (75)	13 (81.3)
Postoperative length of stay	10 (6-12.5)	9 (6-12)
Any postoperative in-hospital complication	7 (43.8)	6 (37.5)
Global Quality of life[3]	68 (20)	73 (10)
QLQ-C30 function scores		
Role[3]	85 (16)	82 (25)
Emotional[3]	82 (26)	79 (28)
Cognitive[3]	92 (17)	81 (24)
Social[3]	71 (31)	70 (31)
Physical[3]	95 (10)	92 (16)
OG25 symptom scales		
Dysphagia[4]	15 (26)	27 (36)
Eating restrictions[4]	20 (25)	34 (35)
Reflux[4]	13 (18)	24 (31)

Table 18: Baseline characteristics of randomised participants

Abbreviations: G, tumour grade; IQR, interquartile range; kg, kilograms; LDL, low-density lipoprotein; m, meters; SD, standard deviation; VO2 max, maximum volume of oxygen used

Values presented as frequencies (%) and means (SD) unless otherwise stated.

[1]Modified Charleson co-morbidity index (excludes solid tumours)

[2]Percentages presented for unknown categories reflect overall proportion of missing data for the relevant covariate; while percentages presented for known categories refer to complete data only.

[3]Global quality of life and functional scales: high score suggests a high level of functioning

[4]Symptom scales: high score suggest worse symptoms

Retention

The number and proportion of participants withdrawn from the trial (including withdrawing treatment with continued follow-up, and complete withdrawal from all trial related procedures) are presented in table 21. In total, for both treatment groups, one withdrew consent prior to receiving treatment, six discontinued the trial medication having received it: four reported difficulty swallowing the medication; one discontinued the medication and continued follow-up; one received a statin from their GP after randomisation, and one developed grade 3 transaminitis (discussed below, under safety) and treatment was discontinued by an investigator.

Number withdrawn	Proportion	Reason
3	9.4%	Difficulty swallowing trial medication
2	6.3%	Withdrew consent (1 from trial, 1 for treatment only)
1	3.1%	General practitioner prescribed statin during follow-up
1	3.1%	Adverse event (transaminitis)
Total: 7	21.9%	

Table 19: Reasons for withdrawal of treatment.

The overall annual rate of withdrawal from the study was 0.36 (95% CI 0.17-0.76) (see table 22). The rate was highest in the first three months: 0.74 (95% CI 0.31-1.77); before falling between three to six months to 0.36 (0.09-1.46); thereafter there were no further losses to follow-up. Retention overall and stratified by treatment allocation are further summarised in figure 16.

Follow-up	Person-years	Number withdrawn	Rate (95% CI)
0-3 months	6.8	5	0.74 (0.31-1.77)
3-6 months	5.5	2	0.36 (0.09-1.46)
6-9 months	4.2	0	0
9-12 months	2.8	0	0
Overall	19.3	7	0.36 (0.17-0.76)

Table 20: Withdrawal rates for recruited participants, stratified by follow-up period

Rates represent the annual rate of withdrawal

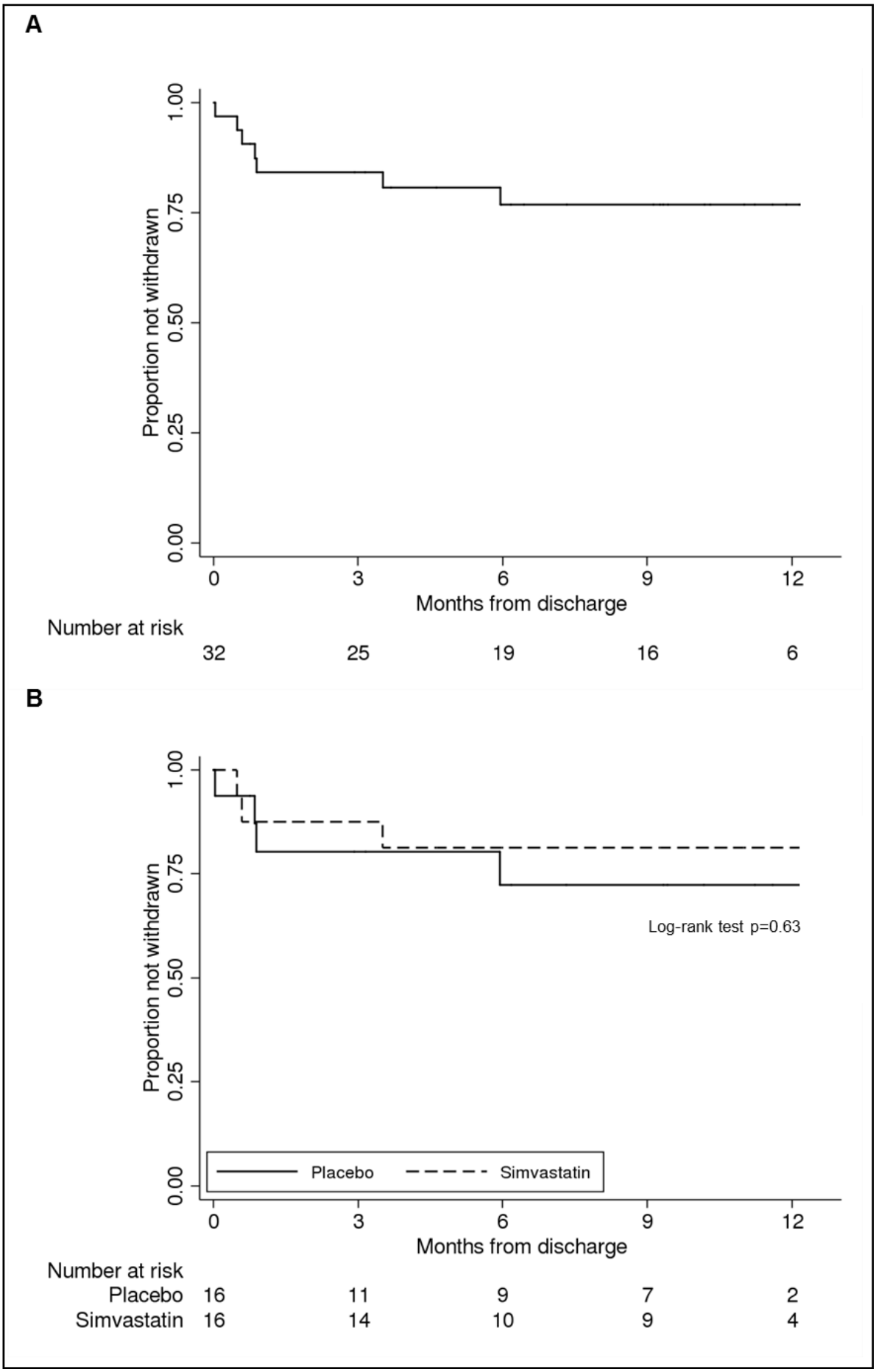

Figure 16: Kaplan-Meier plots of time to withdrawal (A) overall and (B) according to treatment allocation.

There were no significant differences in withdrawals between treatment groups (log-rank test = 0.63). Aside from two withdrawals between month three to six, all other withdrawals happened within 27 days of randomisation.

Absorption

Participants allocated to simvastatin, compared to placebo users, had a significant mean difference in LDL cholesterol by three months, adjusted for values at screening of -0.83 (95% CI -1.4 to -0.22), p = 0.009 (the primary outcome for this feasibility outcome) (see table 23). Exploratory analyses revealed significantly (at the 1% significance level) lower LDL levels at 6 and 12 months. None of the adjusted per-protocol comparisons reached significance at the 1% level.

	placebo		Simvastatin		Unadjusted mean		Adjusted mean	
Visit	n	LDL (mmol/L)	n	LDL (mmol/L)	difference (95% CI)	p value	difference (95% CI)	p value
Intention-to-treat								
3 months	13	3.00 (0.54)	15	2.20 (0.85)	-0.80 (-1.36 to -0.24)	0.007	-0.83 (-1.4 to -0.22)	0.009
6 months	10	3.09 (0.63)	14	2.14 (1.01)	-0.95 (-1.71 to -0.20)	0.016	-1.23 (-1.85 to -0.40)	0.004
9 months	8	2.89 (0.61)	12	2.17 (0.74)	-0.72 (-1.39 to -0.05)	0.036	-0.79 (-1.47 to -0.11)	0.025
12 months	4	3.00 (0.28)	6	2.07 (0.47)	-0.93 (-1.54 to -0.33)	0.008	-0.99 (-1.58 to -0.40)	0.007
Per-protocol								
3 months	7	3.00 (0.60)	9	2.46 (0.96)	-0.53 (-1.42 to 0.36)	0.224	-0.49 (-1.47 to 0.49)	0.300
6 months	8	3.09 (0.66)	12	2.09 (1.09)	-1.00 (-1.91 to -0.09)	0.034	-1.16 (-2.01 to -0.32)	0.010
9 months	7	2.86 (0.66)	12	2.17 (0.74)	-0.69 (-1.41 to 0.03)	0.058	-0.74 (-1.47 to -0.003)	0.049
12 months	3	3.07 (0.31)	5	2.02 (0.51)	-1.05 (-1.85 to -0.24)	0.019	-1.16 (-1.96 to -0.35)	0.016

Table 21: Comparison of non-fasting plasma LDL cholesterol by treatment group during follow-up according to intention-to-treat and per-protocol populations.

Abbreviations: CI, confidence interval; LDL, low density lipoprotein; n, number.

Mean scores adjusted for values at screening by ANCOVA. A negative difference implies that patients on Simvastatin have a lower LDL cholesterol than patients on placebo.

Mean plasma LDL cholesterol for participants over the duration of the study according to treatment allocation are shown in figure 17.

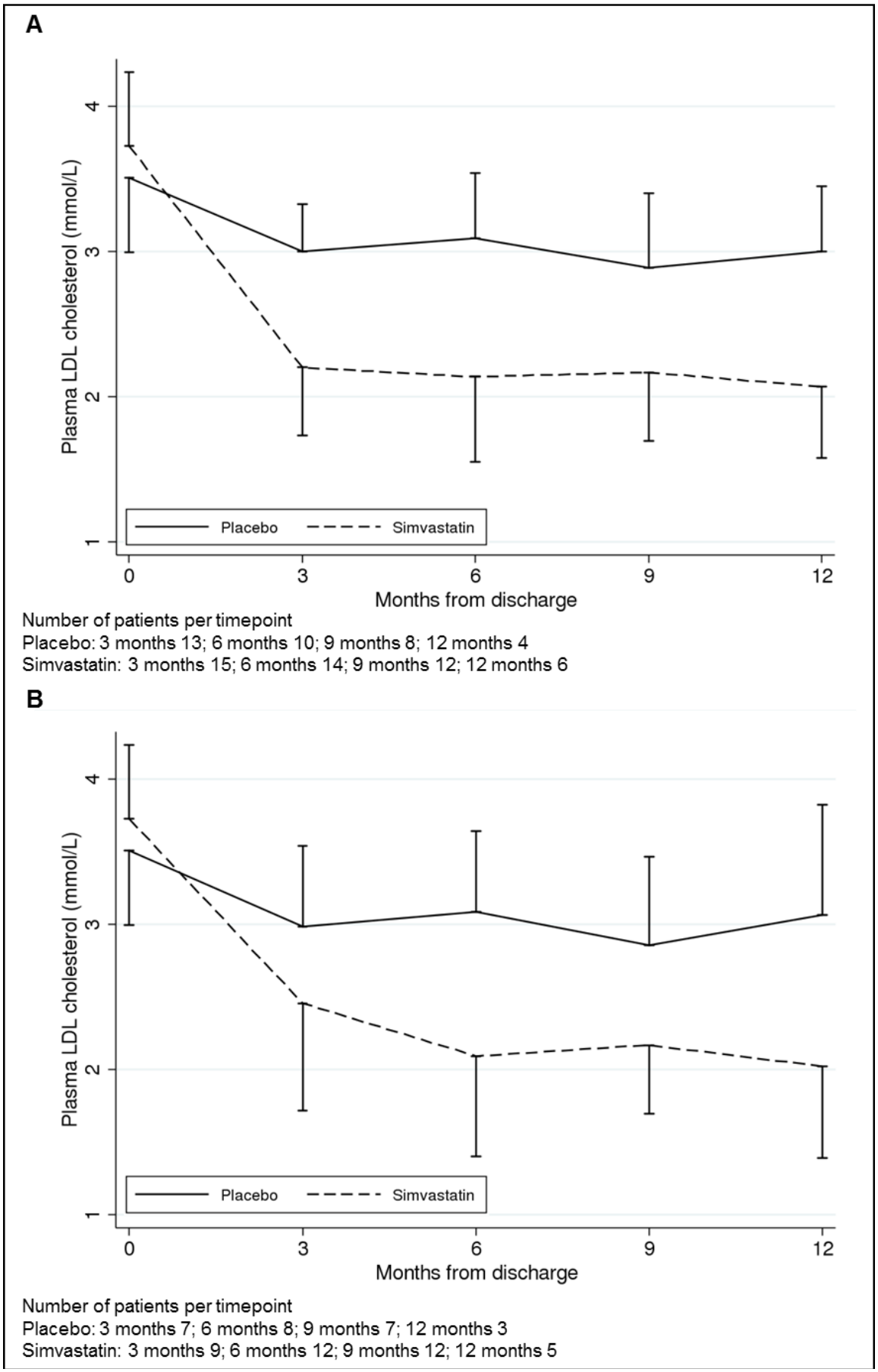

Figure 17: Plasma LDL cholesterol during follow-up according to treatment group for (A) the intention-to-treat and (B) per-protocol populations.

Half error bars span from the average to the upper limit or lower limit of the 95% confidence interval.

Adherence

Overall adherence was lowest in the first three months of treatment (52% adherence, median adherence 83%, IQR 45-98) before improving at subsequent visits at six months (78% adherence, median 94%, IQR 83-100), nine months (100% adherence, median 99%, IQR 96-100) and 12 months (75% adherence, median 89%, IQR 82-97) (see table 24 and figure 18). Adherence was similar in both statin and placebo treated groups.

Visit	3 months			6 months		
Allocation	n	median % (IQR)	≥ 80%	n	median % (IQR)	≥ 80%
Placebo	13	77 (38-98)	6 (46)	10	94 (90-100)	8 (80)
Simvastatin	14	85 (63-99)	8 (57)	8	92 (67-99)	6 (75)
Overall	27	83 (45-98)	14 (52)	18	94 (83-100)	14 (78)

Visit	9 months			12 months		
Allocation	n	median % (IQR)	≥ 80%	n	median % (IQR)	≥ 80%
Placebo	5	97 (96-99)	5 (100)	3	92 (78-98)	2 (67)
Simvastatin	5	100 (99-100)	5 (100)	5	96 (85-100)	4 (80)
Overall	10	99 (96-100)	10 (100)	8	94 (82-99)	6 (75)

Table 22: Adherence to trial medication during follow-up.

Abbreviations: IQR, interquartile range.

Median percentage (IQR) adherence and the proportion adherent (n, %) to ≥ 80% of trial medication in the preceding three months.

No implausible values for adherence (> 105%) were observed at three months. Values from three patients were ignored at six months (and thereafter), values from five patients were ignored at nine months (and thereafter) and none were ignored at 12 months.

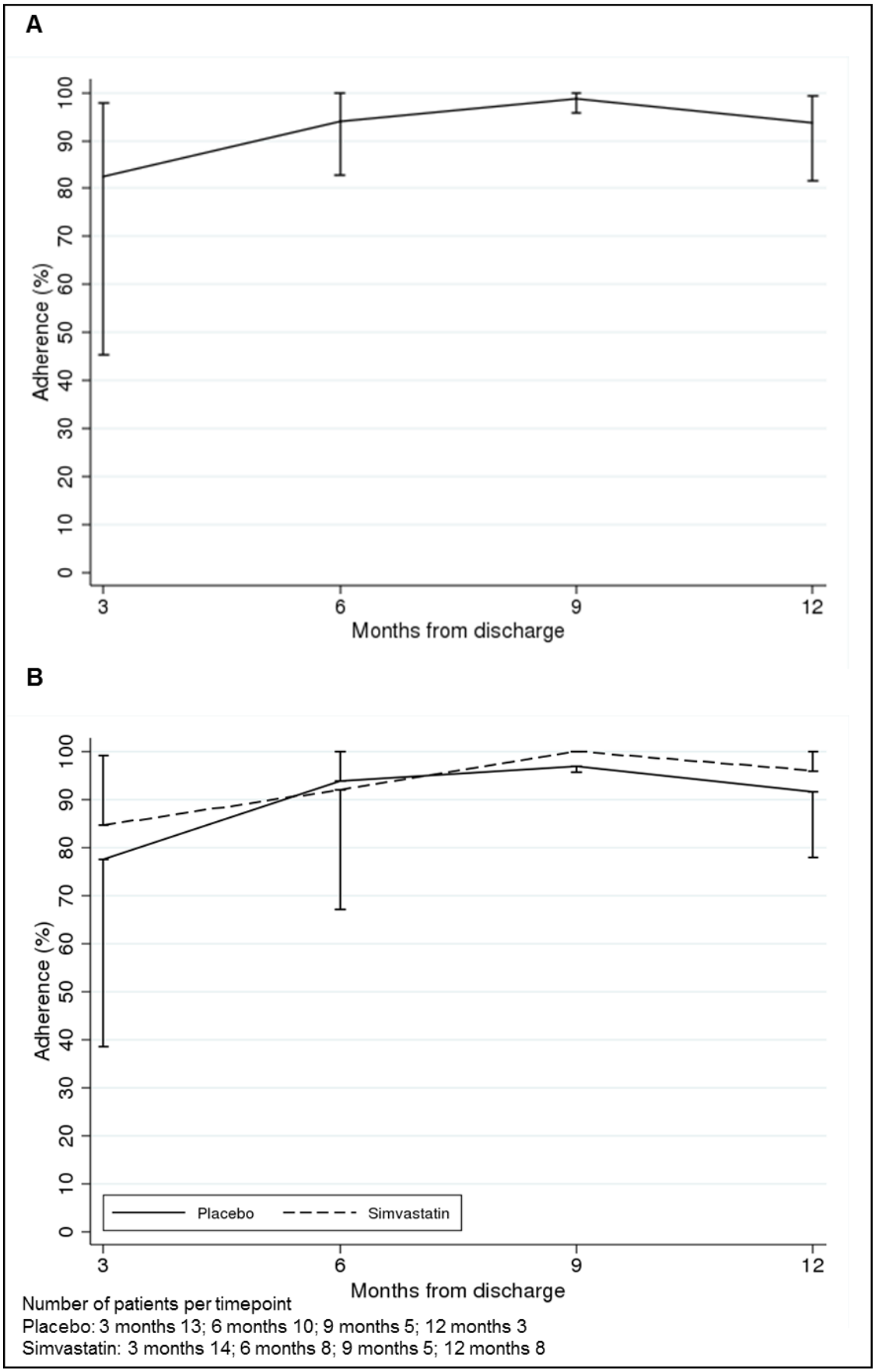

Figure 18: Median percentage adherence to trial medication (A) overall and (B) according to treatment allocation, calculated at each trial visit for the preceding three months.

Half error bars span from the median to the upper limit or lower limit of the interquartile range

Safety

Over the course of follow-up there were 108 individual adverse events affecting 27 participants. In total 20 (18.5%) were SAEs, with 13 in placebo and seven in the simvastatin arm. There were no suspected Serious Adverse Reactions (SSARs) or Suspected Unexpected Serious Adverse Reactions (SUSARs). Of all individual AEs, prior to unblinding, 94 were assessed as unrelated to trial medication, 12 were assessed as unlikely related, and two were assessed to be possibly related (grade 3 transaminitis [ALT > 5 ≤ 20 times upper limit of normal], which was subsequently downgraded to grade 2 [ALT > 3 ≤ 5 times upper limit of normal] in the same patient (who had been allocated placebo). As expected, the most frequent AEs were gastrointestinal (36 unique gastrointestinal AEs, excluding recurrent AEs in the same patient), of which dysphagia (8/36, 22.2%), abdominal pain (6/36, 16.7%), vomiting (6/36, 16.7%), diarrhoea (4/36, 11.1%), gastro-oesophageal reflux (4/36, 11.1%) and nausea (4/36, 11.1%) were the most common.

Table 25 shows the AEs according to CTCAE System Organ Class. There were no significant differences between treatment groups AEs categorised using this classification. Grade 1 (mild) myalgia was experienced by three patients, 1 receiving placebo and 2 receiving simvastatin, and was self-limiting and did not require discontinuation of trial medication. There were no cases of rhabdomyolysis. Severity of AEs (unique events for the worst grade experienced per patient) stratified by treatment (see table 26), showed no obvious differences between treatment groups (χ^2 test for distribution of severity according to treatment for any toxicity, p=0.639). The majority of grades reported were mild to moderate (34.0%, 29.8% and 31.9% respectively were grade 1-3 in the placebo group; and 43.8%, 31.3% and 18.8% respectively were grade 1-3 in the simvastatin group. There was a single grade 4 AE in the placebo group (dyspnoea) and in the simvastatin group (pleural effusion). Grade 5 AEs refer to death (one in placebo group and two in simvastatin group). All were due to metastatic disease and were not judged to be related to trial medication. No requests for unblinding on grounds of patient safety were made during the study. The safety committee held four meetings in total, during which there no safety concerns with the trial.

CTCAE System Organ Class	Placebo Number of individuals with ≥ 1 (%)	Simvastatin Number of individuals with ≥ 1 (%)	p value
Blood	1 (7.1)	0	0.277
Ear	1 (7.1)	1 (6.3)	0.922
Gastrointestinal	11 (78.6)	9 (56.3)	0.196
General disorders	3 (21.4)	5 (31.3)	0.544
Infections	3 (21.4)	4 (25)	0.818
Investigations	3 (21.4)	2 (12.5)	0.513
Transaminitis	1 (7.1)	0	0.277
Metabolism and nutrition	0	1 (6.3)	0.341
Musculoskeletal	1 (7.1)	5 (31.3)	0.100
Myalgia	1 (7.4)	2 (12.5)	0.626
Neoplasms	1 (7.1)	0	0.277
Nervous system	2 (14.3)	3 (18.8)	0.743
Psychiatric	2 (14.3)	2 (12.5)	0.886
Renal and urinary	1 (7.1)	1 (6.3)	0.922
Respiratory	4 (28.6)	2 (12.5)	0.272
Skin	1 (7.1)	1 (6.3)	0.922
Vascular	2 (14.3)	1 (6.3)	0.464
Any	13 (92.9)	14 (87.5)	0.626

Table 23: Adverse events by treatment allocation

Abbreviations: CTCAE, Common Terminology Criteria for Adverse Events.

Proportions of individuals with at least one adverse event were compared using the χ^2 test.

	Placebo (n=14)					Simvastatin (n=16)				
CTCAE System Organ Class	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Blood	0	0	1 (2.1)	0	0	0	0	0	0	0
Ear	1 (2.1)	0	0	0	0	1 (2.1)	0	0	0	0
Gastrointestinal	10 (21.3)	3 (6.4)	6 (12.8)	0	0	9 (18.8)	1 (2.1)	7 (14.6)	0	0
General disorders	0	1 (2.1)	1 (2.1)	0	1 (2.1)	1 (2.1)	1 (2.1)	2 (4.2)	0	2 (4.2)
Infections	0	3 (6.4)	0	0	0	1 (2.1)	3 (6.3)	0	0	0
Investigations	0	0	3 (6.4)	0	0	1 (2.1)	2 (2.1)	0	0	0
Metabolism and nutrition	0	0	0	0	0	0	1 (2.1)	0	0	0
Musculoskeletal	1 (2.1)	0	0	0	0	3 (6.3)	2 (4.2)	0	0	0
Neoplasms	0	1 (2.1)	0	0	0	0	0	0	0	0
Nervous system	1 (2.1)	2 (4.3)	0	0	0	1 (2.1)	2 (4.2)	0	0	0
Psychiatric	1 (2.1)	1 (2.1)	0	0	0	1 (2.1)	2 (4.2)	0	0	0
Renal and urinary	0	1 (2.1)	0	0	0	1 (2.1)	0	0	0	0
Respiratory	2 (4.3)	2 (4.3)	1 (2.1)	1 (2.1)	0	1 (2.1)	1 (2.1)	0	1 (2.1)	0
Skin	0	0	1 (2.1)	0	0	0	1 (2.1)	0	0	0
Vascular	0	0	2 (4.3)	0	0	1 (2.1)	0	0	0	0
Any toxicity	16 (34)	14 (29.8)	15 (31.9)	1 (2.1)	1 (2.1)	21 (43.8)	15 (31.3)	9 (18.8)	1 (2.1)	2 (4.2)

Table 24: Adverse events stratified by worst grade experienced and treatment group.

Data are n (%) for worst grade experienced for each adverse event recorded.

Quality of life

Completion of questionnaires was very high at each visit: 98.8% (1685/1705) at screening; 98.8% (1606/1625) at 3 months; 96.4% (1379/1430) at 6 months; 99.1% (1065/1075) at 9 months; 98.7% (543/550) at 12 months. Overall completion was 98.3% (6278/6385). Therefore 1.68% (107) values were imputed using EORTC guidelines. Table 27 and figures 20-21 shows the mean scores for global quality of life, QLQ-C30 function scores (role, emotional, cognitive, social and cognitive), and OG25 symptoms scales (dysphagia, eating restrictions and reflux) in placebo and simvastatin groups for each of the four follow-up visits and the mean difference between groups adjusted for scores at screening. Overall, adjusted differences between groups for each of these scores were small.

Quality of life measured at 3 months

	Placebo		Simvastatin		Difference at 3 months adjusted mean
	n	mean (SD)	n	mean (SD)	n (95% CI)
Global QOL	14	61.9 (20.3)	15	63.5 (16.9)	28 0.43 (-0.06 to 0.91)
QLQ-C30 function scores					
Role	14	65.5 (36.1)	15	70.8 (30.1)	29 0.53 (-0.09 to 1.14)
Emotional	14	76.8 (24.9)	15	78.0 (19.1)	28 0.51 (0.27 to 0.75)
Cognitive	14	76.2 (33.1)	15	80.2 (20.4)	28 0.70 (0.27 to 1.13)
Social	14	67.9 (33.0)	15	67.7 (22.3)	28 0.31 (-0.05 to 0.66)
Physical	14	76.2 (18.4)	15	79.6 (18.9)	29 0.37 (-0.15 to 0.90)
OG25 symptom scales					
Dysphagia	14	12.7 (14.4)	15	17.0 (25.8)	28 0.10 (-0.17 to 0.36)
Eating restrictions	14	34.5 (24.6)	15	37.4 (25.2)	28 0.13 (-0.19 to 0.44)
Reflux	14	29.8 (30.8)	15	24.4 (22.6)	28 0.45 (0.04 to 0.86)

Quality of life measured at 6 months

	Placebo		Simvastatin		Difference at 6 months adjusted mean
	n	mean (SD)	n	mean (SD)	n (95% CI)
Global QOL	12	75.0 (15.9)	13	71.8 (18.2)	23 0.30 (-0.18 to 0.77)
QLQ-C30 function scores					
Role	12	86.1 (15.6)	13	76.9 (28.5)	24 0.70 (0.12 to 1.28)
Emotional	12	88.2 (17.2)	13	78.8 (24.2)	23 0.57 (0.38 to 0.77)
Cognitive	12	84.7 (19.4)	13	74.4 (30.1)	23 0.97 (0.64 to 1.31)
Social	12	91.7 (15.1)	13	74.4 (33.1)	23 0.42 (0.11 to 0.74)
Physical	12	88.9 (15.7)	13	78.9 (22.2)	25 1.25 (0.55 to 1.95)
OG25 symptom scales					
Dysphagia	12	6.5 (11.1)	14	8.7 (12.5)	25 0.02 (-0.21 to 0.25)
Eating restrictions	12	24.3 (22.0)	14	28.6 (20.6)	25 0.28 (-0.06 to 0.62)
Reflux	12	23.6 (25.1)	14	13.1 (23.7)	25 0.44 (0.02 to 0.87)

Quality of life measured at 9 months

	Placebo		Simvastatin		Difference at 9 months adjusted mean
	n	mean (SD)	n	mean (SD)	n (95% CI)
Global QOL	8	75.0 (10.9)	12	75.7 (12.5)	18 0.12 (-0.31 to 0.56)
QLQ-C30 function scores					
Role	8	87.5 (19.4)	12	81.9 (25.1)	19 0.77 (0.15 to 1.39)
Emotional	8	84.4 (22.5)	12	78.5 (22.0)	18 0.50 (0.31 to 0.69)
Cognitive	8	87.5 (19.4)	12	81.9 (21.9)	18 0.41 (-0.02 to 0.84)
Social	8	93.8 (17.7)	12	81.9 (26.1)	18 0.05 (-0.38 to 0.47)
Physical	8	93.3 (6.2)	12	84.4 (15.4)	19 0.67 (0.15 to 1.20)
OG25 symptom scales					
Dysphagia	7	7.9 (12.4)	12	7.4 (10.9)	18 0.07 (-0.17 to 0.32)
Eating restrictions	7	16.7 (17.3)	12	25.7 (19.6)	18 0.21 (-0.15 to 0.55)
Reflux	7	16.7 (16.7)	12	23.6 (21.9)	18 0.53 (0.22 to 0.83)

Quality of life measured at 12 months

	Placebo		Simvastatin		Difference at 12 months adjusted mean (95% CI)	
	n	mean (SD)	n	mean (SD)	n	
Global QOL	4	83.3 (0)	6	75 (13.9)	9	0.20 (-0.81 to 1.22)
QLQ-C30 function scores						
Role	4	87.5 (8.3)	6	77.8 (25.1)	9	1.64 (0.72 to 2.57)
Emotional	4	97.9 (4.2)	6	75.0 (19.0)	9	0.45 (0.14 to 0.75)
Cognitive	4	95.8 (8.3)	6	77.8 (31.0)	9	1.40 (0.81 to 1.99)
Social	4	100 (0)	6	83.3 (18.3)	9	0.26 (-0.09 to 0.62)
Physical	4	95 (6.3)	6	81.1 (22.9)	9	6.17 (2.53 to 9.80)
OG25 symptom scales						
Dysphagia	4	2.8 (5.6)	6	1.9 (4.5)	9	0.28 (-0.30 to 0.86)
Eating restrictions	4	6.3 (8.0)	6	22.2 (16.4)	9	0.08 (-0.77 to 0.94)
Reflux	4	12.5 (16.0)	6	19.4 (19.5)	9	-0.71 (-2.33 to 0.90)

Table 25: Global quality of life, function and symptoms scores by treatment group measured during follow-up.

Global quality of life and functional scales: high score suggests a high level of functioning. Symptom scales: high score suggest worse symptoms. Difference in mean scores adjusted for values at screening by ANCOVA. For global quality of life and functional scales a positive difference implies that patients on Simvastatin have less deterioration than patients on placebo. For symptom scales a positive difference implies that patients on Simvastatin have more deterioration than patients on placebo. For adjusted difference, n is the number of observations included in the model.

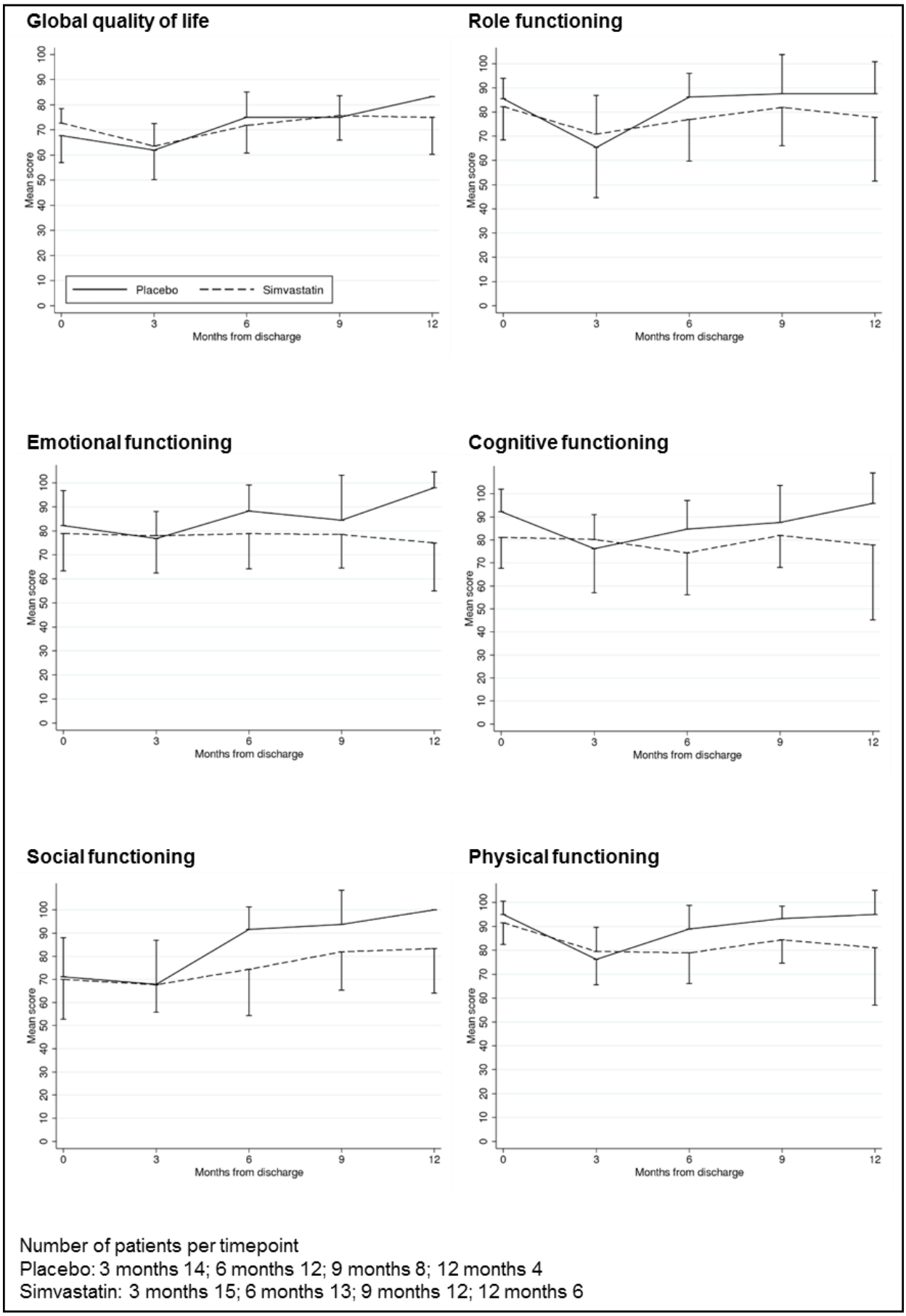

Figure 20: Mean unadjusted scores for global quality of life and function during following up by treatment group.

Higher scores suggest better quality of life and functioning. Half error bars span from the mean to the upper limit or lower limit of the 95% confidence interval.

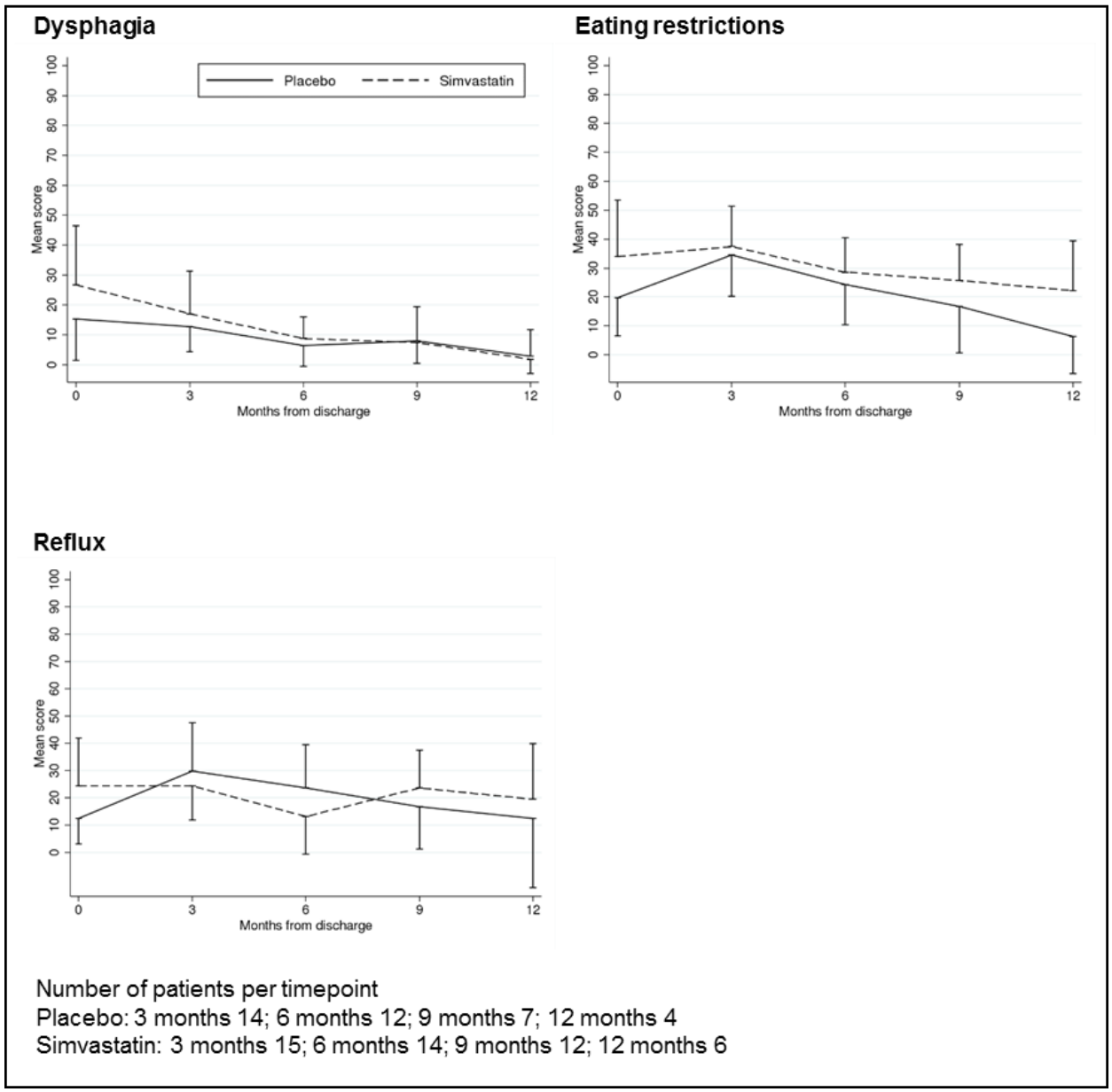

Figure 21: Mean unadjusted scores for symptom scales during following up by treatment group.

Higher scores suggest worse symptoms. Half error bars span from the mean to the upper limit or lower limit of the 95% confidence interval.

Survival

During follow-up four participants developed distal recurrent disease (two in both placebo and simvastatin groups) and of these, three died (one in the placebo and two in the simvastatin group). There were no local recurrences in either treatment group. Median overall and disease-free survival was not reached. There was no significant difference between groups for overall survival (HR 1.56, 95% CI 0.14-17.3, p=0.716) or disease-free survival (HR 0.79, 95% CI 0.11-5.61, p=0.807) (see figure 22). Clinical examination (as part of research visits) did not detect recurrence in advance of diagnosis by the clinical team.

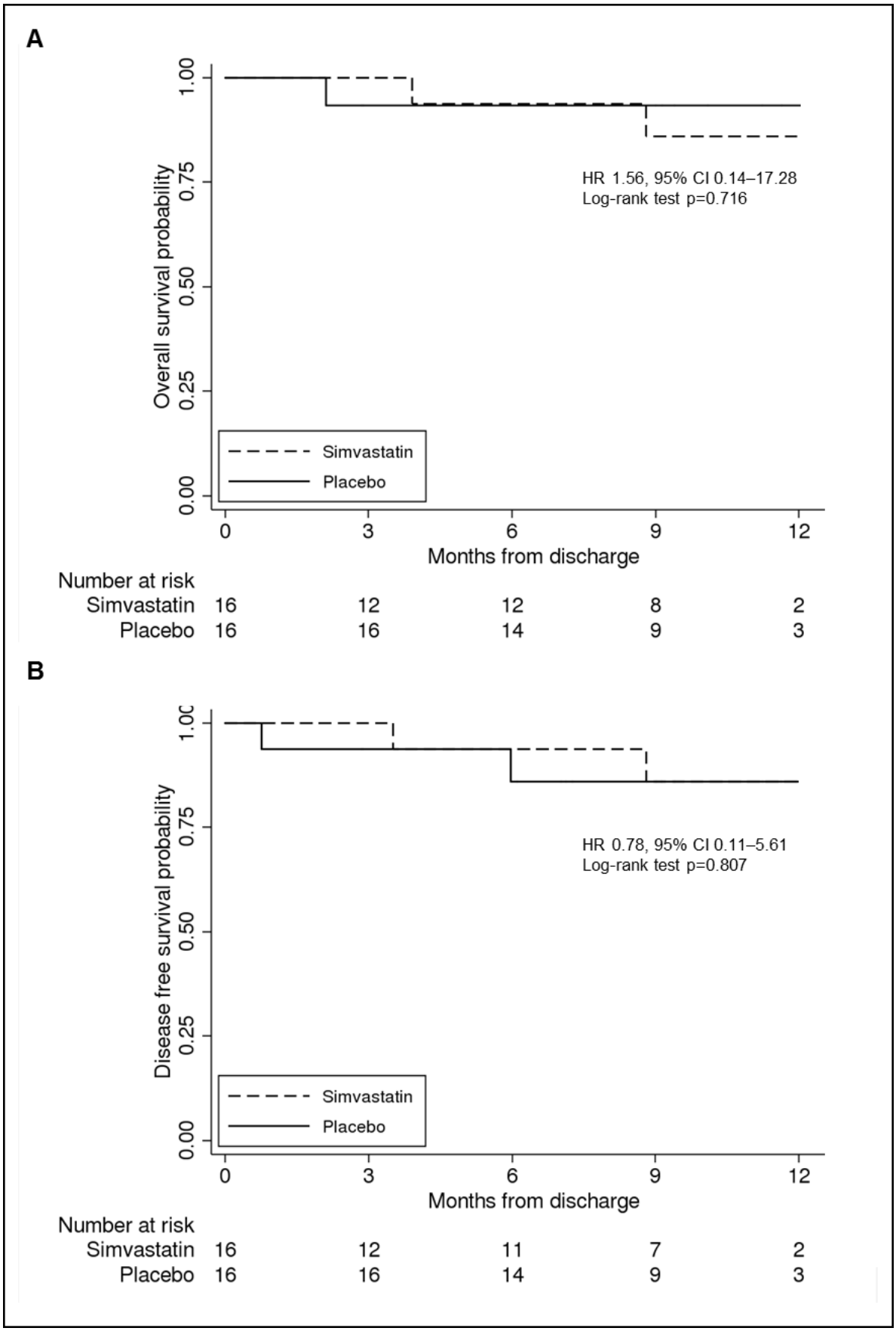

Figure 22: Kaplain-Meier estimates of overall survival (A) and disease-free survival (B) by treatment allocation in the intention-to-treat population.

Hazard ratios and 95% confidence intervals estimated with cox proportional hazards regression.

Generalisability

No significant differences were found between non-randomised and randomised patient populations for age at diagnosis, gender, smoking status, BMI, tumour site, tumour grade, clinical staging or pre-operative radiotherapy. As expected there were significant differences between treatment groups for cardiovascular diseases (28.4% in non-randomised and 3.1% in the randomised group, p=0.003), diabetes (22.5% in the non-randomised population, and no patients with diabetes in the group, p=0.003) and aspirin use (17.9% in the non-randomised group, and no patients in the randomised group, p=0.010). There were also significantly more patients (p=0.037) with a lower ECOG performance status (indicating better performance status) in the randomised group compared to the non-randomised group (87.5% in the randomised group and 63% in non-randomised had a score of 0). Significantly more (p=0.035) patients underwent neoadjuvant chemotherapy in the randomised group (93.8%) than in the non-randomised group (76.7%).

Variable	non-randomised (n=88)	randomised (n=32)	p-value
Age at diagnosis, years	67.8 (8.9)	64.0 (10.8)	0.059
Gender			
Male	77 (88.5)	25 (78.1)	0.151
Female	10 (11.5)	7 (21.9)	
Smoking status			
Current	13 (16.5)	4 (12.5)	0.485
Past	42 (53.2)	21 (65.6)	
Never	24 (30.4)	7 (21.9)	
Body mass index (kg/m^2)	27.6 (5.2)	26.4 (4.4)	0.245
Comorbid conditions			
Cardiovascular	25 (28.4)	1 (3.1)	0.003
Diabetes	18 (22.5)	0	0.003
Peri-operative aspirin use	15 (17.9)	0	0.010
Performance status			
0	53 (63.9)	28 (87.5)	0.037
1	27 (32.5)	3 (9.4)	
2	3 (3.6)	1 (3.1)	
Unknown[1]	5 (5.7)	0	
Tumour site			
Oesophageal	42 (50.0)	12 (37.5)	0.458
Siewert I	11 (13.1)	6 (18.8)	
Siewert II	31 (36.9)	14 (43.8)	
Unknown	4 (4.5)	0	
Tumour grade			
G1	1 (1.3)	0	0.342
G2	41 (51.9)	13 (46.4)	
G3	37 (46.8)	14 (50)	
G4	0	1 (3.6)	
Unknown[1]	9 (10.2)	4 (12.5)	
Clinical T stage			
1	3 (3.5)	0	0.080
2	15 (17.9)	1 (3.1)	
3	63 (75.0)	28 (87.5)	
4	3 (3.6)	3 (9.4)	
Unknown[1]	4 (4.5)	0	
Clinical N stage			
0	38 (45.8)	7 (21.9)	0.077
1	22 (26.5)	15 (46.9)	
2	16 (19.3)	8 (25.0)	
3	7 (8.4)	2 (6.3)	
Unknown[1]	5 (5.7)	0	
Neoadjuvant chemotherapy			
Yes	66 (76.7)	30 (93.8)	0.035
No	20 (23.3)	2 (6.3)	
Unknown[1]	2 (2.3)	0	
Pre-operative radiotherapy			
Yes	5 (6.0)	1 (3.1)	0.531
No	78 (94)	31 (97.9)	
Unknown[1]	5 (5.7)	0	

Table 27: Comparison of demographic and clinical variables between the non-randomised and randomised patient populations.

Abbreviations: G, tumour grade; kg, kilograms; m, meters; SD, standard deviation; maximum volume of oxygen used.

Values are frequencies (%) or means (SD) unless otherwise specified.

[1]Percentages presented for unknown categories reflect overall proportion of missing data for the relevant covariate; while percentages presented for known categories refer to complete data only.

3.5. Discussion

This multi-centre, double-blind, parallel group, randomised, placebo-controlled trial reported outcomes for 32 participants randomised to simvastatin 40mg or placebo to determine the feasibility of adjuvant statin therapy in patients with operable OAC in a future phase III trial. Overall, 26.7% of patients assessed for eligibility were randomised. Of patients assessed for eligibility who did not meet any exclusions 59.3% were randomised. Across four sites, overall three participants were recruited per month (3.01, 95% CI 2.59-3.48). In total, seven participants withdrew during the course of the trial: three had difficulty swallowing the trial medication, two withdrew consent, one was started on a non-trial statin by their general practitioner, and one patient was withdrawn following an adverse event (transaminitis). The overall annual rate of withdrawal was 0.36 (95% CI 0.17-1.77). The rate of withdrawal was highest in the first three months, followed by the next three months, with no withdrawals thereafter. Patients allocation to simvastatin had significantly lower LDL cholesterol levels than the placebo group by three months (adjusted mean difference -0.83 mmol/L, 95% CI -1.4 to -0.22, p=0.009) in the intention-to-treat population, indicating drug absorption, a significant pharmacodynamic effect and suggesting drug adherence. Drug adherence was poorest in the first three months of follow-up (52% took at least 80% of administered medications), and improved to acceptable levels thereafter. The majority of adverse events recorded during follow-up were expected given the patient cohort, and most were attributable to the underlying malignancy, surgery or oncological treatments. There were no statistically significant or clinical significant differences in the adverse event profile between treatment groups. Completion rates of quality of life questionnaires (QLQ-C30 and OG25) were very high over the course of the study (98.3% of individual items were completed overall). Although there were some statistically significant differences in quality of life scores between groups for certain domains at certain time-points, absolute differences were small and there were no clinically significant differences between groups. The absolute number of deaths (three) and recurrences (four) for the cohort were low. As expected, there were no significant differences between groups for overall survival or disease free survival. No systematic differences between the randomised and non-randomised groups were found for certain demographic characteristics (age, gender, smoking, BMI) or tumour characteristics (tumour stage or grade).

Trial interpretation

Recruitment and retention. This trial demonstrated that patients were willing to enter the trial and be randomised to simvastatin or placebo. The trial also demonstrated that patients'

consultants (all oesophago-gastric surgeons) were willing to approach and enrol their patients in the trial. The proportion of patients randomised from those assessed for eligibility and from those meeting inclusion criteria was favourable. The recruitment rates across all sites should be seen as a valid and relatively precise estimate of anticipated recruitment rates for a future trial. The main caveat is that initiation rates of statins varies by geographic location[284], and adjustment for projected statin prevalence across regions would be required for planning anticipated recruitment nationally. Comparison of recruitment rates between centres should be interpreted in light of the precision of the estimates, a function of duration of follow-up and absolute numbers of participants recruited. The rates calculated for the Norfolk and Norwich University hospital, with the longest period of follow-up and highest number recruited were predictably the most precise, while estimates rates at other centres lacked precision and should be interpreted with less certainty. Rates of withdrawal were highest in the first three months of follow-up, mainly contributed to by difficulties in swallowing trial medication. Participants who continued to participate between three-six months, and more so thereafter were more likely to be retained. There is uncertainty about the precise rate of withdrawal overall and particularly stratified by period of follow-up (and diminishes further over time) as person-time at risk was limited. This data provides strong impetus to manufacture bespoke, more easily swallowed (and ideally suitable for crushing) trial medication for a future trial. As a result, there may be difficulties in generalising these rates of withdrawal to a future phase III RCT for which, production of smaller trial medications could be justified. Despite the inherent uncertainty in estimates of retention, the current data are reassuring and would support trial feasibility.

Baseline characteristics. The baseline characteristics were generally well balanced overall. Caution needs to be made in interpreting proportions for individual characteristics within treatment groups as the denominator for each group was 16. Therefore discrepancies of small numbers of patients between categories, expected by chance, dramatically alter percentages. It is important that such discrepancies are not over-interpreted. Well balanced characteristics (both observed and non-observed) are essential for a future phase III trial, though are of lesser importance for interpreting the feasibility of a future trial.

Absorption. A significant reduction in non-fasting LDL cholesterol between randomised treatment groups at three months in the ITT population provides good evidence to infer that simvastatin 40mg is absorbed sufficiently to produce a pharmacodynamic effect. Reductions in LDL cholesterol were consistent with those observed in the MRC/BHF Heart production study (mean

difference overall -1.0 mmol/L, standard error 0.02)[285]. Exploratory analyses conducted at remaining time points in the ITT population at the 6 and 12 months were significant at the pre-specified 1% level, indicating longer term absorption over the course of the trial. Highly significant differences in LDL cholesterol between groups would be expected to be a function of drug absorption, drug potency (sufficient to exert a pharmacodynamics effect) and overall drug adherence. While it is possible to infer that adherence was sufficient to permit an observable difference in LDL cholesterol between groups, it is not possible to quantify adherence further based on these results. Per-protocol analyses lacked precision and did not meet significance 3 months. Per-protocol analyses were underpowered given the assumptions set out in the power calculation to detect significant differences were they to exist. The per-protocol analysis should therefore not be seen as evidence that statins do not lower LDL cholesterol, instead that results were inconclusive for this population.

Adherence. Adherence as determined using pill counts demonstrated poorest adherence in the first three months, followed by greatly improved adherence thereafter. At least 75% of the cohort adhered to at least 80% of the trial medication thereafter. Adherence data is only applicable to the first year of treatment, it is not possible to draw further inferences on longer term follow-up, such as that which would be expected from a full trial. Patterns of adherence were very similar between treatment groups. Furthermore, interpretation should also consider the known limitations of pill counts, as they can overestimate adherence (discussed below)[286]. Nevertheless, the data from pill counts taken together with the comparison of LDL cholesterol between groups would suggest adherence is sufficient to not obviate a future trial.

Safety. There was no evidence to suggest an adverse safety profile in this patient population with statin use, either in terms of the absolute numbers of AEs nor in terms of their severity. Particular AEs of interest with statins are rhabdomyolysis, deranged live function tests, rash and depression. Comparisons involved small numbers of AEs and this analysis would unlikely be adequately powered to detect even modest differences between groups. Although there is no plausible reason to suggest the adverse event profile should be different in this cohort, this has not previously been determined and favourable safety profile would support the feasibility of a future trial. There are no known interactions with current chemotherapy regimens and statins.

Quality of life. Completion of both the QLQ-C30 and OG25 questionnaires was very high overall (98.3%) and at each follow-up visit. Importantly this demonstrates the feasibility of assessing quality of life in a future phase III RCT. This study was not intended (nor originally powered) to detect significant differences between groups. Statistically significant differences were observed (as demonstrated in the table by non-overlapping 95% CIs, p values not shown) for emotional and cognitive functional domains, and reflux, during follow-up. This is most plausibly the result of multiple testing and the product of calculating quality of life scales following linear transformation of raw scores; scores can in practice only occupy limited values and not each potential value from 0-100. It is reassuring the absolute differences between scores were not clinically significant: most differences which reached statistical significance were less than two, and a value of eight points difference has previously been deemed to be of clinical importance in a landmark oesophageal cancer trial[287]. The mean and standard deviations for function scores and symptom scales can be used to inform sample size calculations for future trials where patient reported outcomes are used the primary outcome.

Preliminary survival data. Estimates of overall and disease free survival were very imprecise. This is attributable to the limited numbers of events and short durations of follow-up for participants (particularly with truncated follow-up). This would be consistent with previous trials and observational data, few events would be expected with minimal follow-up[25, 39, 288].

Generalisability. Comparisons between randomised and non-randomised groups provide strong evidence for systematic differences between groups for cardiovascular disease (p=0.003), diabetes (p=0.003) and aspirin use (p=0.01). This is expected as the trial eligibility precludes statin users, which are indicated in patients with these conditions, similarly with shared indications for aspirin use. Statin use was associated with greater co-morbidity (presence of any cardiovascular comorbidity and/or diabetes, 84.6% for statin users vs 15.4% for non-statin users, p<0.001) and more advanced age (mean 63.1 [SD 10.0] for statin users vs. 71.5 [SD 6.8] for non-statin users, p<0.001) and may explain poorer ECOG performance status scores, and lower use of neoadjuvant chemotherapy and radiotherapy in the non-randomised population. It should also be noted that not all patients in the non-randomised group proceeded to surgery (five progressed and were deemed unsuitable for curative resection and one patient died during neoadjuvant chemotherapy), and of statin users and patients who declined involvement, the proportions who ultimately underwent resection is not known.

Comparison with previous trials

This is the first RCT to determine the feasibility of assessing post-operative statin therapy in patients with OAC in a future phase III. The effect of simvastatin 80mg has been assessed in patients undergoing oesophagectomy in a single-centre RCT (conducted in Belfast, Northern Ireland) previously[289]. The aim of this study was to determine effect of simvastatin 80mg (versus placebo) when administered four days preoperatively and seven days post-operatively on pulmonary dead space (primary endpoint determined using volumetric capnography) to determine the potential of high dose statin therapy in preventing acute lung injury. No significant differences were found between groups. Similarly to STAT-ROC, prevalence of statin use was high (31/63 excluded were prevalent statin users). The Add-Aspirin trial, a study most readily comparable to STAT-ROC in design, is currently underway[290]. This is a phase III RCT assessing the effects of aspirin on disease recurrence and survival after primary therapy in four cohorts of non-metastatic solid tumours (breast, colorectal, gastro-oesophageal and prostate). Aside from the intervention (a routinely prescribed medication for cardiovascular disease prevention for which there is compelling evidence of cancer chemo-preventive effects[123]), key distinctions are the inclusion of squamous cell carcinomas and gastric tumours (in addition to OAC) in the gastro-oesophageal cohort; the exclusion of R1 resections; exclusion of patients with risk factors for gastrointestinal toxicity; and the use of a run-in period of eight weeks to assess toxicity and adherence prior to randomisation and dose escalation from 100mg to 300mg if under 75 years of age. Published trials have assessed the effect of allocation to statins on cancer-related outcomes in solid tumours previously[197, 211-216]; including gastric cancer[211, 212], colorectal cancer[197], pancreatic cancer[213], breast cancer[215] and lung cancer[216]. It is difficult to draw direct comparison with these studies which included predominantly patients with advanced disease and duration of statin therapy was short. Of relevance, there was no evidence to suggest a clinically significant increase in toxicity with statin allocation.

Strengths and limitations

This study has a number of strengths. First, we were able to assess the “real world” feasibility of a future phase RCT study in the setting of a multi-centre trial, to provide valid estimates of feasibility parameters. Feasibility estimates from multiple sites are more likely to be applicable to a future multi-centre RCT than from a single centre alone. Second, this trial has established the prevalence of statin use in the target trial population, a notable risk to study feasibility. This data is informative for assessing trial feasibility and to enable planning of expected recruitment. Third, recruitment exceeded the minimum target (of 24 patients) with 32 patients in total. This has

enabled assessment of the feasibility outcomes with greater precision. This is important for planning a future trial, where precise estimates of recruitment can be used to plan the number and size of recruiting oesophago-gastric centres required. Fourth, this trial has provided valuable information to devise strategy to improve retention in a future trial, particularly a strong impetus to manufacture smaller trial medication which can be easily swallowed and potentially crushed. Data from this feasibility study can be used to justify the cost of manufacturing a bespoke placebo (for a generic statin brand) to funding bodies, which would be expected to exceed the cost of over-encapsulation. Fifth, we were able to establish that trial procedures were acceptable at different sites to clinicians, research staff and patients. Sixth, we established effective central trial-specific procedures at NCTU which would be expected to form the basis of procedures for a definitive trial: a high-level risk assessment (to consider the trial risks and risk reduction strategies relating to the safety of trial participants; study design; and project management and governance); a safety management plan (detailing safety oversight in the trial, trial specific procedures to preserve the safety of participants and serious adverse event reporting procedures); and a quality management and monitoring plan (a bespoke approach to quality assurance and a detailed central monitoring plan).

This study has a number of limitations. First, patient follow-up was limited for patients recruited after 1/11/15 due to truncated follow-up. Assessment of outcomes of interest beyond 6 months are therefore limited. This particularly applies to the endpoints of retention, overall survival, disease-free survival, measures of adherence and measurement of quality of life. While assessments of these to 12 months were possible, estimates were imprecise as a result. This will have contributed to the relatively few recurrences and deaths captured. Longer follow-up with more events could have enabled more informative estimates of overall survival and disease-free survival which could have been used to inform a sample size calculation for a future trial. Second, despite use of the smallest available simvastatin tablets and smallest possible gelatin capsules to preserve blinding, the trial medication were deemed relatively large (measuring 23.3 x 8.53mm). To aid comparison, the largest available gelatin capsules, size 000, measure 26.1 x 9.91mm. Of the patients who withdrew trial medication, difficulty swallowing the tablets was the most commonly cited reason. Although this trial estimated retention, this is unlikely to be applicable to a future trial where manufacture of an easily-swallowed bespoke placebo would be justified and viable. This makes estimates of retention less certain, and hence require further assumptions be made for a future trial. Third, this study estimated feasibility for up to one year's follow-up only. Feasibility beyond this time point have not been established. This does not preclude planning a

future trial, but necessitates sound assumptions on longer-term adherence to trial-related procedures.

An application for funding a phase 3 RCT has been made to the NIHR Efficacy and Mechanism Evaluation programme based on the results of the STAT-ROC feasibility study.

Conclusions

This multi-centre, double-blind, parallel group, randomised, placebo-controlled trial has demonstrated the feasibility of assessing adjuvant statin therapy in patients with operable OAC in future phase III trial. Estimated recruitment and retention rates, adherence to medication, drug absorption, adverse events, and patient completion of trial-related procedures support the conduct and inform the design considerations for a future trial.

4. Chapter 4 – Statins in the prevention of oesophageal adenocarcinoma: nested case-control analysis

4.1. Abstract

Background

There are no current non-endoscopic evidence-based approaches to reduce the risk of malignant progression in patients with non-dysplastic Barrett's oesophagus (BO), the only known precursor to oesophageal adenocarcinoma (OAC). Statins exert plausible anti-carcinogenic mechanisms and are attractive potential chemoprotective agents. There is uncertainty in current estimates for associations between statin use and malignant progression in BO populations. This study aimed to investigate whether statin use is inversely associated with either high-grade dysplasia (HGD) or OAC in a BO population.

Methods

Participants diagnosed with BO with follow-up from 1st January 2000 to 13th June 2013 were identified from two contributing centres of the UK National Barrett's Oesophagus Registry (UKBOR). Patients with incident or prevalent OAC were matched with up two controls with non-dysplastic BO and no evidence of progression, for gender, centre and date of birth. Duration of follow-up was matched within each case-control set. Data on relevant exposures were extracted from patient records. Statin use was measured between 6 months to 5 years prior to the date of diagnosis of each case and the equivalent index date in matched controls.

Results

In total, 79 cases with HGD/OAC were matched to 138 controls with non-dysplastic BO. Statin use was equally prevalent (17.7%) in cases and matched controls. Statin use was not significantly associated with malignant progression in either unadjusted (OR 1.13, 95% CI 0.53-2.41) or adjusted analyses (OR 0.69, 95% CI 0.23-2.02). Dose and duration response relationships, defined with categories of mean statin dose (p for trend=0.758), cumulative dose (p for trend=0.289) and cumulative duration (p for trend=0.216) were all non-significant. Prevalence of statin use and the number of included participants were lower than required to meet the assumptions of the sample size calculation.

Conclusions

No significant associations were demonstrated between statin use and risk of malignant progression in a BO population registered with UKBOR. This study was underpowered and therefore at risk of type 2 error.

4.2.Introduction

4.2.1. Background

Barrett's oesophagus (BO) is the only known premalignant lesion to oesophageal adenocarcinoma (OAC). This is the most common histological subtype of oesophageal malignancy in the west, an aggressive malignancy with a poor prognosis[4, 5]. Endoscopic surveillance is practiced to identify and treat dysplastic and early cancerous lesions, to improve long-terms outcomes of patients at risk of progression. There has been considerable interest in the potential for chemoprevention as a future strategy. The results of the AspECT trial (Study of Aspirin and Esomeprazole Chemoprevention in Barrett's Metaplasia) are eagerly awaited and could change practice in the future[291]. There is considerable interest in the potential for statins, currently used in the primary and secondary prevention of cardiovascular diseases, to reduce the risk of malignant progression in BO populations[3].

In vitro studies have demonstrated the effects of statins in validated OAC-cell lines, with resultant inhibition of proliferation and promotion of apoptosis, in a dose-dependent manner[179-182]. The functional relevance of inhibiting the mevalonate pathway, has been demonstrated as likely causal, mediated through depletion of downstream isoprenoid intermediates which permit propagation of growth-signalling pathways, which are of relevance to Barrett's carcinogenesis[179].

Seven previous observational investigations have determined associations between statin use and risk of malignant progression in BO populations[188, 221-226]. Unfortunately, most studies were at substantial risk of bias, likely mediated by immortal-time bias[222, 224], time-window bias (discussed below)[188, 221, 222], and confounding[222-224, 226]. Furthermore, the definition of statin exposure in some studies inadequately considered temporal associations[188, 221, 223-226]. It is therefore difficult to draw strong conclusions from the existing epidemiological literature.

4.2.2. Aims and Objectives

The overarching aim of this study was to examine associations between statin use and risk of malignant progression to HGD/OAC in a nested case-control study conducted within two of the largest contributing centres to the United Kingdom Barrett's Oesophagus Registry. Specific objectives were:

1. To determine associations between statin use and risk of HGD/OAC with adjustment for plausible confounders, in particular BMI and relevant common drug exposures (acid suppressive medications, NSAIDs, and aspirin).
2. To determine whether dose and duration-response relationships exist.

4.3. Methods

4.3.1. Study population and data source

This study was conducted using the United Kingdom National Barrett's Oesophagus Registry (UKBOR), the world's largest BO registry[292]. This resource was established in 1996 as joint initiative of the Oesophageal Section of the British Society of Gastroenterology and the European Cancer Prevention Organisation. It is currently administered by the University Department of Surgery, Royal Free Hospital, London. The aims of the registry are to determine the descriptive epidemiology of BO and identify risk factors for malignant progression. In total 12, 500 patients with BO have been registered by gastroenterologists from 46 UK centres. Of these, a core dataset of approximately 3000 patients with detailed demographic, endoscopic and clinical data, has been constructed; and is suited to analytical epidemiology, including pharmacoepidemiology[293]. In this subset, routinely available medical data have been extracted from medical records, including endoscopic reports (including date of endoscopy and endoscopic findings), linked histology records, demographic information (age, gender, ethnicity) and other clinical covariates (smoking, alcohol history and co-morbidity). This study was conducted using patients identified from Rotherham General Hospital and Wexham Park NHS foundation trusts, research-active sites which contribute to this detailed subset. For cases and controls selected for this study further collection of pseudo-anonymous data from hospital records was required to capture detailed time-dependent medication exposures and other covariates required. Data sources for medication exposures within medical notes were inpatient prescription charts, general practitioner referral letters (including copied repeat prescription scripts where available), any correspondence, or hand written inpatient or outpatient records. Ethical approval for existing consented UKBOR participants was provided by the registry's existing ethics approval (The London Multi-centre research Ethics Committee, MREC/02/2/5). For additional cases not previously registered with UKBOR from Rotherham General Hospital (having not previously declined participation with UKBOR), separate study-specific approval was granted to extract pseudo-anonymous data from their medical records (Brent Research Ethics Committee, 16/LO/1741) (appendix I).

4.3.2. Case-control definitions

We used a nested case-control analysis of a cohort dataset to investigate the association between statin use and risk of HGD/OAC in patients with non-dysplastic BO. Men and women who developed HGD or OAC, diagnosed between 1st January 2000 and 13th June 2013 (cases), were

identified centrally from UKBOR. Pseudo-anonymous data were provided by Rotherham General Hospital for additional cases not previously registered with UKBOR. Both incident (HGD or OAC diagnosed greater than one year since diagnosis of non-dysplastic BO) and prevalent (HGD or OAC diagnosed within one year of BO or without a previous BO diagnosis) cases were included. Prevalent cases were included as the expected number of incident cases was expected to preclude sample size considerations. The date of diagnosis of HGD or OAC first recorded from correlation of endoscopic and histology reports was the index date. Patients low-grade dysplasia at baseline or follow-up were excluded. Each case was matched with up to two patients with non-dyplastic BO (controls) according to gender, date of birth (+/- 2 years), and centre. The same index date for each case was assigned to each of the matched controls, who were required to have no evidence of malignant progression at this time (with non-dysplastic BO on histology following a subsequent surveillance endoscopy).

Compared with a full cohort analysis, a nested case-control analysis is an efficient study design which permits time-consuming and expensive data extraction of covariate information to be limited to only the selected cases and controls[294]. The calculated odds ratios from a nested case-control analysis should closely estimate unbiased rate ratios derived from a proportional hazards model of a full cohort with minimal or no loss of precision[295, 296]. Furthermore the matching procedure is a recognized method to account for the time-varying nature of drug exposures with fixed exposure definitions applied equally prior to the index date for both cases and controls for each risk set, therefore avoiding immortal time bias[261].

4.3.3. Statin prescription and categorisation

“Ever use” of statins was defined as at least one entry of statin use made in the hospital notes between 6 months to 5 years prior to the index date. “Regular use” was defined as at least two records, separated in time by at least 30 days, in the same time window defined above. Mean daily dose and cumulative statin dose were calculated for individuals where dosage was recorded during this time window using the defined-daily dose (DDD) categories. Median DDDs determined the category thresholds for both mean and cumulative statin dose analyses. Cumulative statin durations during this time period were determined.

4.3.4. Covariates

Potential confounders were extracted for time periods preceding the index date, including: smoking status (ever or never smoked); body mass index (BMI) (kg/m^2) recorded closest to 5 years

prior to the index date, and other medications (aspirin, non-aspirin NSAIDs, proton pump inhibitors [PPIs], histamine receptor antagonists [H2As] were extracted using the same definitions as for statin use (separately ever and regular use).

4.3.5. Statistical analysis

Baseline characteristics, excluding matching demographics (age, gender and centre), were compared between cases and their matched controls. For controls, means and percentages were weighted by the inverse number of controls (with complete data for the variable of interest) matched to each case. This ensured proportions were comparable between sets. To ensure unbiased comparisons between prior exposures, follow-up was restricted to the shortest duration of the present case and matched control(s) for each set. Follow-up therefore varied between sets but was controlled for within sets. This approached was used as it was not possible to match for follow-up as part of the matching procedure, which in turn determined which medical notes were to be interrogated. While maintaining adherence to the restrictions defined above, all medication exposures were measured between 6 months to a maximum of 5 years prior to the date of diagnosis of the cases and the index date for controls. Equal follow-up between cases and controls (in this case within sets) is important to avoid "time-window bias"[297]. In the context of case-control studies this bias can arise if differential follow-up periods between cases and controls occur are not adequately accounted for. Differential follow-up would naturally be expected as follow-up is function of disease course, where progressors would be expected to have shorter prior follow-up than non-progressors. This bias has been responsible for producing spurious protective associations between statin use and lung cancer[297, 298].

Nested case-control analyses were performed using conditional logistic regression, to investigate the association between statin use and malignant progression according to each definition of statin use (ever, regular use, and according to mean dose, cumulative dose and cumulative duration of use). Participants not prescribed statins in the 6 months to 5 years preceding the index date were used as the reference group, to calculate odds ratios (ORs) with 95% confidence intervals (95% CI). Age, gender and calendar period were controlled for using the matching procedure. Analyses were adjusted for smoking, BMI, aspirin, NSAIDs, PPIs and H2As. A test for trend was applied across dose and duration categories. All analyses were performed with STATA version 13 (StataCorp LP, College Station, Texas, USA).

4.4. Results

Participants

In total, 2, 543 patients were registered with UKBOR from two of the largest contributing centres: Rotherham General Hospital NHS foundation trust (n = 1, 396) and Wexham Park NHS Foundation Trust (1, 147) (figure 23). From this cohort, 967 were excluded (671 with BO had no endoscopic follow-up after 1/1/2000; 139 with HGD/OAC were diagnosed prior to this date; and 157 were excluded with LGD at baseline or follow-up). The nested cases-control study was drawn from 123 patients with HGD/OAC and 1, 453 patients with non-dysplastic BO. It was not possible to match controls to 13 of the cases, and in total 1243 controls were not selected by the matching procedure. The medical records were unobtainable for particular cases and/or their matched controls leading to the exclusion of 103 participants: matched sets were retained provided medical notes were obtainable for at least one case and one matched control. The final study population included 79 cases and 138 controls. The 79 cases comprised 24 with HGD and 55 OAC. In total, 19 (24.1%) cases were incident (known diagnosis of BO at least one year prior to diagnosis). The majority of study participants were selected from Rotherham General Hospital NHS foundation trust (n=211, 76 cases and 135 controls), a more contemporaneous cohort; and the minority were from Wexham Park NHS Foundation Trust (n=6, 3 cases and 3 controls).

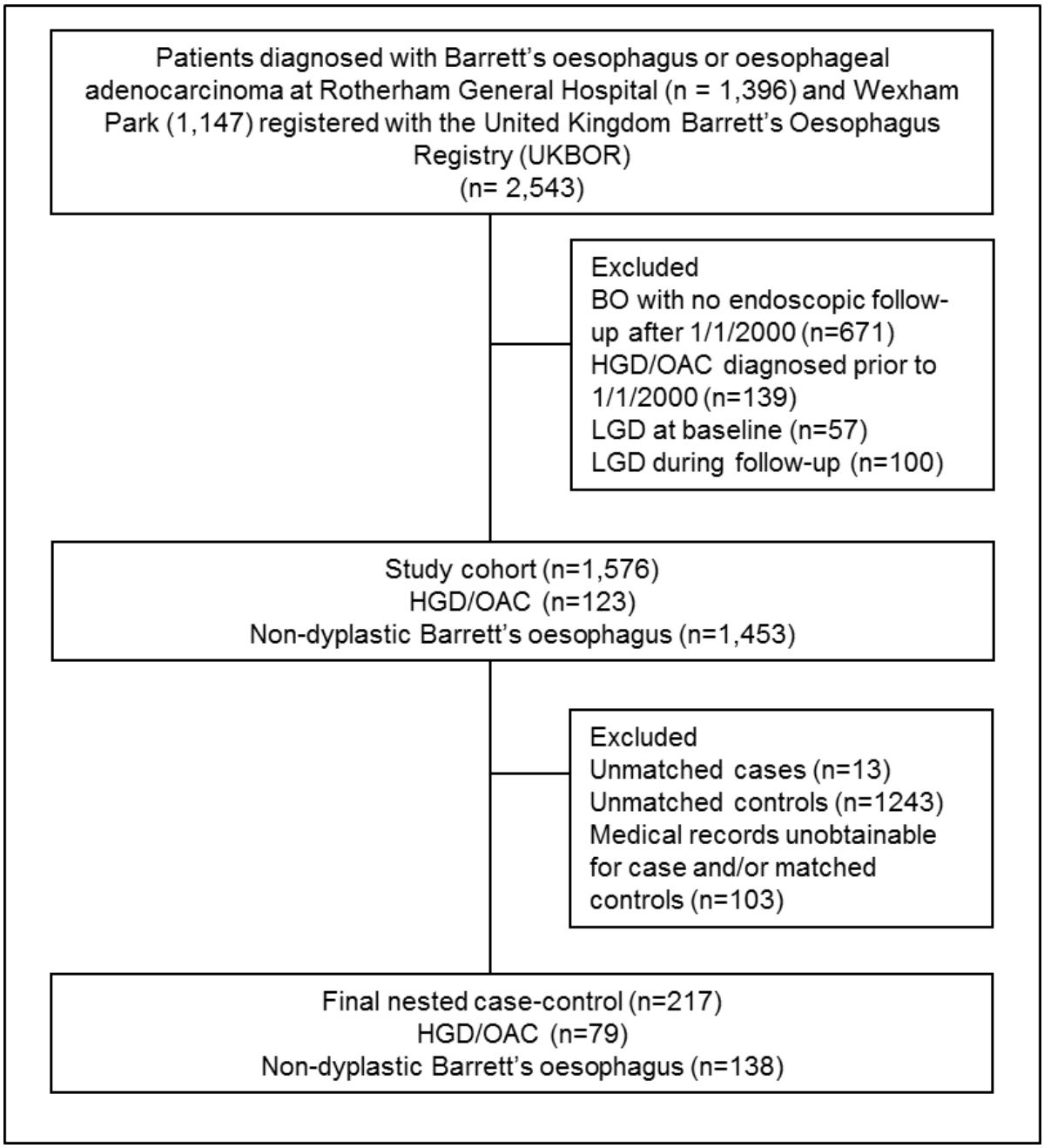

Figure 23: Flow chart of study participants

Abbreviations: BO, Barrett's oesophagus; HGD, high-grade dysplasia; LGD, low grade dysplasia; OAC, oesophageal adenocarcinoma.

Baseline characteristics

Baseline characteristics of study participants are summarised in table 30. Median follow-up prior to the index date was 3.5 years (IQR 0.4-6) for cases and was 3.9 years (IQR 2-5.6) for the controls. Age and gender were controlled for using the matching procedure. The mean age of cases was 67.9 years (SD 10.7) and 68 (86.1%) were male. In total, 66.2% of cases and 55.8% of controls were smokers (percentages and means weighted by the inverse number of controls per case). For BMI, 32.3% and 33.9% of cases and 26.7% and 35% of controls respectively were in the overweight (BMI ≥ 25 to < 30 kg/m^2) or obese category (BMI ≥ 30 kg/m^2). 38 (48.1%) cases were known to have a diagnosis of BO. The median (maximal) length of BO in cases with known BO was 4.5cm (IQR 3-7), and in controls was 4cm (IQR 2.5-5). Intestinal metaplasia was observed in 39.2% of cases and 37.7% of controls. Statin use was observed in 25.3% of cases and 22.8% of controls. Aspirin use was observed in 21.8% of cases and 24.7% of controls. NSAID use was observed in 5.1% of cases and 13.3% of controls. H2A use was observed in 7.6% of cases and 6.3% of controls. PPI use was observed in 39.2% of cases and 67.1% of controls. Crude unadjusted odd ratios for risk of HGD/OAC, calculated prior to restricting follow-up within sets, demonstrated no significant associations for smoking status (OR 1.57, 95% CI 0.85-2.88, p=0.148), BMI (OR 0.65, 95% CI 0.30-1.43, p=0.286 for BMI ≥ 25 to < 30 kg/m^2; OR 1.00, 95% CI 0.45-2.24, p=0.994 for BMI ≥ 30 kg/m^2), intestinal metaplasia (OR 1.18, 95% CI 0.66-2.11, p=0.587), statin use (OR 1.28, 95% CI 0.66-2.48, p=0.462); aspirin use (OR 0.89, 95% CI 0.45-1.75, p=0.734) or H2A use (1.21, 95% CI 0.43-3.43, p=0.718). Known associations with HGD/OAC were demonstrated in unadjusted analyses for length of the Barrett's segment (OR 1.22, 95% CI 1.02-1.47, p=0.031, per 1cm increase), NSAID use (OR 0.27, 95% CI 0.08-0.99, p=0.049) and PPI use (OR 0.32, 95% CI 0.17-0.60, p<0.001).

Characteristics	Cases (n=79)	Controls (n=138)	Crude odds ratio (95% CI)
Median (IQR) duration of follow-up (years)	3.5 (0.4-6.0)	3.9 (2.0-5.6)	-
Age (years) mean (SD)[1]	67.9 (10.7)	67.9 (10.3)	-
Male sex, n (%)[1]	68 (86.1)	119 (86.1)	-
Smoking status			
Never	25 (33.8)	57 (44.2)	1.00 (reference)
Ever	49 (66.2)	73 (55.8)	1.57 (0.85-2.88)
Unknown[2]	5 (6.3)	8 (5.8)	1.35 (0.32-5.77)
Body mass index (kg/m^2)			
< 25	21 (33.9)	34 (26.7)	1.00 (reference)
≥ 25 to < 30	20 (32.3)	53 (43.3)	0.65 (0.30-1.43)
≥ 30	21 (33.9)	35 (30.0)	1.00 (0.45-2.24)
Unknown[2]	17 (21.5)	16 (11.6)	2.03 (0.80-5.14)
Known Barrett's, n (%)	38 (48.1)	138 (100)	-
Median (IQR) length of Barrett's (cm)	4.5 (3-7)	4 (2.5-5)	1.22 (1.02-1.47)[3]
Intestinal metaplasia, n (%)	31 (39.2)	52 (37.7)	1.18 (0.66-2.11)
Statin use, n (%)	20 (25.3)	30 (22.8)	1.28 (0.66-2.48)
Aspirin use, n (%)	17 (21.5)	32 (24.7)	0.89 (0.45-1.75)
NSAID use, n (%)	4 (5.1)	18 (13.3)	0.27 (0.08-0.99)
PPI use, n (%)	31 (39.2)	92 (67.1)	0.32 (0.17-0.60)
H2A use, n (%)	6 (7.6)	9 (6.3)	1.21 (0.43-3.43)

Table 28: Baseline characteristics of HGD/OAC cases and matched controls

Abbreviations: H2A, histamine receptor blocker; IQR, interquartile range; NSAID, non-steroidal anti-inflammatory drugs; PPI, proton pump inhibitor.

[1]Matching variables (along with centre).

For controls, means and percentages were weighted by the inverse number of controls (with complete data for the variable of interest) matched to each case.

[2]Percentages presented for unknown categories reflect overall proportion of missing data for the relevant covariate; while percentages presented for known categories refer to complete data only.

[3]per cm.

Medication use defined as any use between 6 months - 5 years prior to index date.

Statin use and risk of high-grade dysplasia/adenocarcinoma

The association between statin use and HGD/OAC is shown in table 31. Statin use measured between 6 months to 5 years prior to diagnosis or (index for the matched cases) was observed in 17.7% of cases and 17.7% (percentage weighted by the inverse number of controls per case) of controls. These proportions are discrepant to those presented in table 30 and is a product of the restricted follow-up windows within sets. Any documented exposure to statins (ever use) was not significantly associated with HGD/OAC in either unadjusted (OR 1.13, 95% CI 0.53-2.41, p=0.747) or adjusted (0.69, 95% CI 0.23-2.02, p=0.492) analyses. Regular statin use (defined as a record of at least two records of statin use between 6 months to 5 years prior to date of diagnosis or index) was not significantly associated with HGD/OAC in either unadjusted (OR 1.11, 95% CI 0.42-2.75,p=0.816) or adjusted (OR 0.68, 95% CI 0.17-2.65, p=0.576) analyses.

Statin exposure prior to index	cases (n=79)	controls (n=138)	Unadjusted odds ratio (95% CI)	Adjusted odds ratio (95% CI)[1]
No statin use	65 (82.3)	116 (82.3)	1.00 (reference)	1.00 (reference)
Ever use between 0.5-5 years	14 (17.7)	22 (17.7)	1.13 (0.53-2.41)	0.69 (0.23-2.02)
Regular use between 0.5-5 years[2]	10 (13.3)	14 (12.0)	1.11 (0.45-2.75)	0.68 (0.17-2.65)

Table 29: Statin use and risk of high-grade dysplasia/oesophageal adenocarcinoma

Follow-up prior to index date matched within sets

For controls percentages were weighted by the inverse number of controls matched to each case.

[1]Adjusted for body mass index, smoking status, use of aspirin, NSAIDs, proton pump inhibitors and histamine receptor antagonists

[2]At least two records documented at least 30 days apart

Dose and duration-response analyses

No significant associations were observed for dose-response analyses (see table 32) in which categories of statin use were defined according to the mean DDD documented between 6 months to 5 years prior diagnosis of the cases or index date of the controls (adjusted analyses, for mean DDD < 0.82: OR 0.36, 95% CI 0.08-1.62; for DDD ≥ 0.82: OR 1.05, 95% CI 0.24-4.53; P for trend=0.758). ORs decreased with increasing cumulative dose categories of statins use, however were individually non-significant (adjusted analyses, for cumulative DDD < 749: OR 0.85, 95% CI 0.20-3.65; for DDD ≥ 749: OR 0.38, 95% CI 0.07-2.07) and there was no significant trend across categories (P for trend=0.289). Similarly, ORs decreased with increasing duration of statin use, though were individually non-significant (adjusted analyses, for durations < 3 years: OR 1.38, 95% CI 0.36-5.29; for durations ≥ 3 years: OR 0.23, 95% CI 0.05-1.38) and there was no significant trend across categories (P=0.216).

Statin exposure prior to index	No (%) of cases (n=79)	No (%) of controls (n=138)	Unadjusted odds ratio (95% CI)	Adjusted odds ratio (95% CI)[1]
No statin use	65 (82.3)	116 (82.3)	1.00 (reference)	1.00 (reference)
Mean daily dose of statin				
< 0.82 DDD[2]	5 (7.1)	11 (12.1)	0.74 (0.25-2.19)	0.36 (0.08-1.62)
≥ 0.82 DDD[2]	8 (10.3)	8 (5.5)	1.83 (0.63-5.29)	1.05 (0.24-4.53)
P for trend			0.494	0.758
Cumulative dose of statin				
< 749 DDD[3]	7 (9.0)	9 (10.4)	1.21 (0.43-3.43)	0.85 (0.20-3.65)
≥ 749 DDD[3]	6 (7.7)	10 (7.0)	1.07 (0.34-3.37)	0.38 (0.07-2.07)
P for trend			0.791	0.289
Cumulative duration				
< 3 years	9 (11.4)	11 (10.1)	1.53 (0.56-4.17)	1.38 (0.36-5.29)
≥ 3 years	5 (6.3)	11 (9.3)	0.80 (0.27-2.41)	0.26 (0.05-1.38)
P for trend			1.000	0.216

Table 30: Mean dose, cumulative dose and cumulative duration of statin use and risk of high-grade dysplasia/oesophageal adenocarcinoma

Abbreviations: DDD, defined daily dose.

Follow-up prior to index date matched within sets

For controls percentages were weighted by the inverse number of controls matched to each case.

Missing dosage data affected one case and three controls. Percentages presented for known dosage categories refer to complete data only.

Cumulative dose and duration excludes the 6 month period preceding the index date

[1]Adjusted for body mass index, smoking status, use of aspirin, NSAIDs, proton pump inhibitors and histamine receptor antagonists

[2]0.82 DDD cut off selected as the median daily dosage value in whole cohort. 0.82 DDD is equivalent to 25mg simvastatin or 16mg of atorvastatin (these do not correlate with dispensed doses).

[3]749 DDD cut off selected as the median cumulative dosage value in whole cohort. 749 DDD is equivalent to 22, 470mg simvastatin or 14, 980mg of atorvastatin

4.5. Discussion

This nested-case control analysis, using data from two contributing centres of the UK National Barrett's Oesophagus Registry, demonstrated non-significant inverse associations between statin use and risk of malignant progression to HGD/OAC in patients with non-dysplastic Barrett's. Effect sizes, and their precision, were not materially altered when the exposure definition considered associations for regular use instead. There was no significant evidence to support dose-response relationships (with exposures categorised according to mean statin dose, or cumulative dose) or duration-response relationships.

Comparison with previous epidemiological studies

There are seven previously published epidemiological investigations which examine associations between statin use and risk of HGD and/or OAC in populations with BO[188, 221-226], each with distinctive population and design characteristics. In total, four used population-based healthcare datasets[188, 222, 225, 226], and three were hospital-based[221, 223, 224]. The present study is hospital-based, both in terms of ascertainment of case/control status and exposure variables. In six studies, cases were known to have a prior diagnosis of BO[188, 222-226], while in one, 94% of cases presented de novo (prevalent cases)[221]. This is a key feature which distinguishes our study from previous work (discussed below). Of the previous hospital-based studies, two were conducted at a single centre[221, 223]. For the outcome of interest, two studies considered HGD/OAC[224, 226], three considered OAC[188, 221, 223] and two considered a composite diagnosis of OC (with no distinction according to histological subtype)[222, 225]. Choice of outcome is very likely a function of ascertainment from available data sources. Our study used HGD/OAC as a composite outcome given the availability of histology data. Five considered both relevant patient characteristics (such as gender and age) and potential drug exposures as relevant confounders in either the matching procedure or in adjusted analyses[188, 221, 223-225], while two did not (both population-based cohorts)[222, 226], and would expected to be at greater risk of confounding. Five adjusted for BMI[188, 221-223, 225], and two did not[223, 226]. Our study adjusted for body mass index, smoking status, use of aspirin, NSAIDs, proton pump inhibitors and histamine receptor antagonists. One study adjusted for number of gastroscopies prior to index, to account for confounding by healthy user status (and in doing so significant inverse associations persisted)[188]. Our study did not make the same adjustment as only 19 cases (24.1%) had been diagnosed with BO more than one year previously. Two studies were at risk of immortal-time bias[222, 224], which was subsequently adequately addressed in one[230], while five adequately addressed time-dependent exposures through analysis or study design[188, 221, 223, 225, 226]. Six studies considered in the exposure definition of statin use up until the point of diagnosis of the case or index date of the control (for case-control studies)[188, 221,

[223-226], while one excluded measurement during the year preceding cancer diagnosis (however did not apply the same to controls)[222]. Unlike our study, three studies were at risk of time-window bias[188, 221, 222]. The prevalence of statin use varied between studies: in case-control studies, between 26-40% in cases and 25.5-54% in controls[188, 221, 222, 226]; and in cohorts overall between 13.6-36.7% [223-225]. In our study, the prevalence was 17.7% in cases and controls.

In the context of BO literature in which estimates of exposure and their associations with malignant progression are made, it is reasonable to draw direct comparisons between studies which use different measures of effect size (ORs, RR, HRs): incidence rates of progression in non-dysplastic BO populations are low[36], and therefore estimates can be seen to approximate one another. Absolute effect sizes from studies which reported significant associations were consistent with one another: OR 0.65 (95% CI 0.49-0.91)[188]; 0.57 (95% CI 0.28-0.94)[221]; HR 0.61 (95% CI 0.45-0.83)[225]; as were those from studies which did not reach statistical significance: HR 0.55 (95% CI 0.23-1.29)[224]; HR 0.68 (95% CI 0.30-1.54)[223]; HR 0.82 (95% CI 0.43-4.56)[222]; OR 0.5 (95% CI 0.1-1.7)[226]. The absolute adjusted effect size from our study for the primary is consistent with these previous studies analysis (OR 0.69, 95% CI 0.23-2.02), and there is a considerable area of overlap of the confidence intervals with all estimates from previous studies. There is, however, considerable uncertainty in our estimates: the 95% confidence interval spans one, and is potentially consistent with up to a 77% reduction and 202% increase in odds of HGD/OAC. Therefore the results from our study do not conclusively establish either a harmful, absent or protective association.

Plausible explanations for some studies demonstrating significant and while others demonstrated non-significant inverse associations are potentially explained by differences in study design, sample size, prevalence of statin use in the studied population, periods of study conduct (earlier periods are associated with lower prevalence of statin use), ascertainment and definition of statin exposure, and choice of covariates adjusted for. To date there have been no RCTs in populations of BO to examine whether statin use affects subsequent risk of malignant progression.

Biological mechanisms

Previous preclinical studies have demonstrated the effects of statin treatment on validated OAC cell lines, including inhibiting proliferation, promoting apoptosis and limiting invasiveness in a

dose-dependent manner[179-182]. Inhibition of the mevalonate cascade and subsequent depletion of its downstream products, have been shown to be of functional relevance to these observations[179]. Depletion of intermediates, particularly farsenyl pyrophosphate, an isoprenoid responsible for farnesylation (lipidation) and hence membrane tethering of the RAS superfamily of GTPases, reduces activity of downstream growth signalling cascades, extracellular signal regulated kinase (ERK) and protein kinase B (Akt) pathways, which have been shown to be active in BO and OAC[179, 299]. Statins also exhibit pro-apoptotic effects in non-malignant BO cells[183]. Mutant p53 defines the boundary between non-dysplastic BO and HGD, and is the most commonly recurrently mutated gene in OAC[77]; in other settings has demonstrated gain-of-function properties to upregulate transcription of mevalonate pathway enzymes to sustain malignant proliferation[77], a viable target which can readily be inhibited by statins. However, whether these observations translate into clinically relevant chemopreventive actions in BO populations is not clear. While it is difficult to draw direct comparison from experimental data in predominantly malignant cell lines to premalignant contexts; and although the results of our study are inconclusive, the weight of previous observational evidence (its inherent limitations notwithstanding) does suggests inverse associations between statin use and risk of progression, which would be consistent with the emerging preclinical data.

Strengths and limitations

This study has a number of strengths. Patient demographic and clinical characteristics were consistent with other BO populations, and unadjusted associations between length of BO segment, NSAID and PPI use, would suggest the disease is clinically representative[225, 300, 301]. Unlike previous observational work[222, 224, 226], we were able to adjust for BMI and other medication exposures which could plausibly confound associations. The nested case-control dataset was constructed from the cohort-study dataset by sampling controls at random from the risk sets who were being followed-up and had not progressed by the date of diagnosis of the case. Therefore, calculated odds ratios were precisely controlled for calendar time. This is important as calendar time could otherwise plausibly confound associations: statin use has become increasingly prevalent in the UK population during the study period (simvastatin prescriptions increased 300%, from 12.7 to 37.8 million annual prescriptions from 2004 to 2014[302]) and patients with non-progressing BO would be expected to live longer than progressors, and hence more likely survive to time periods with increasing prevalence of statin use. Extraction of medication exposure data from medical records, as opposed to interviewing participants, prevented recall bias and enabled exposures to be captured during a defined time window, enabling their time-dependent nature to

be accounted for. While it was not possible to blind investigators conducting extraction from notes, algorithms for dataset construction uniformly defined drug exposure status measured prior to the index date, independent to participant case/control status. To ensure maximum follow-up data, ideally duration of follow-up should have been controlled for by the matching procedure. However, the duration of follow-up of each participant prior to matching was not known (it was not feasible to establish). Nevertheless, this study controlled for follow-up by ensuring exposure windows were matched exactly within sets. This was likely at the expense of measurement error: documented exposures captured prior to restricting exposure windows were ignored. It is therefore likely that some statin users (recorded more than five years prior to diagnosis for the cases or index date for the controls) were considered non users. Such measurement error would be expected to non-differential and apply equally to both cases and controls. The consequence of not measuring prior exposures equally could have resulted in time-window bias, and in other aetiological studies has been shown to produce "illusory" protective effects between statin use and other malignancies[297]. Importantly, aspirin use was adjusted for as it is likely to be an important confounder: aspirin users are more likely to be statin users (given the shared treatment indications of these medications), and aspirin use is independently associated with a reduced risk of malignant progression in patients with BO[303]. Inhibition of cyclo-oxygenase-2 enzyme is proposed as the predominant mechanism through which aspirin exerts its chemopreventive effects in this population[304]. This mechanism is largely distinct (and hence independent) to that exerted by statins from the existing experimental evidence summarized above. While, there is some evidence in OAC cell lines that statins inhibit COX-2 expression[182], the functional relevance of this observation has not been assessed. The effects of combined statin and COX-2 inhibition (with a selective COX-2 inhibitor) are additive, in OAC cell lines, which again underscores an independent mechanisms of action[179].

This study has a number of limitations. The main limitation is the final sample is likely underpowered to optimally examine associations. Assumptions from the power calculation were not met, both in terms of the included numbers (actual: 79 cases and 138 controls, required: 200 cases and 400 controls), but also in terms of the prevalence of statin use (actual 17.7% in both cases and controls, required: 20% in cases and 35% in the controls). Despite original counts of 262 patients with HGD/OAC and 2281 registered with UKBOR from the included centres, after excluding all cases diagnosed prior to 1/1/2000 (n=139) and not being able to obtain medical records for selected cases/controls and/or their matched counterparts, it was not possible to meet the study's sample size requirements. Data from the remaining 44 NHS sites which have previously collaborated with UKBOR, were largely historical and did not cover the time period required by the study.

It is unclear whether use of prevalent OAC cases could violate a key premise of the case-control study design: it is assumed, but not known, whether controls are truly randomly selected from the same well-defined at-risk base population as all cases. While we are confident that incident cases of OAC (diagnosed at least 1 year after diagnosis of BO) are drawn from the same baseline population, it is assumed, but not known whether the same applies to prevalent cases (either diagnosed within 1 year of diagnosis of Barrett's oesophagus or without prior history of Barrett's oesophagus). While it is widely accepted that OAC arises from BO (either previously diagnosed or undiagnosed), it is obviously not possible to corroborate that OAC our prevalent cases were truly preceded by undiagnosed BO. While our approach of using both incident and prevalent OAC cases is well-established and consistent with prominent published case-control studies in the aetiological BO literature[221, 305, 306], emerging evidence indicates caution should be applied in the interpretation of such studies[307]. A recent case-case comparison study using the Surveillance, Epidemiology and End Results (SEER) Medicare database demonstrated large and significant differences for patients with OAC known and not known to have prior BO. Patients with known prior BO compared to patients without were older, attained higher educational level, higher burden of co-morbidity, more physician visits in the two years prior to diagnosis, favourable cancer staging, lower cancer grade, smaller tumour size, more likely to undergo surgery, and longer survival. Regardless of whether these observations are due to differences in underlying biology or selection bias, this raises the possibility of the premise of the case-control study detailed above as potentially being violated, and therefore risking selection bias. However, it is not clear whether this would affect associations between exposure and outcome.

The use of routinely collected data on medication exposures from hospital notes presents several potential limitations. Unlike automated records for filled prescriptions electronically captured in large primary care datasets, such the Clinical Practice Research Datalink, this study relied upon the use of routinely collected medication data documented in medical notes. Therefore entries in the notes typically require a hospital visit and for the health professional to document the full list of patient medications. It is therefore almost certain that capture of medication use over time in medical notes is an incomplete record of all dispensed and over-the-counter medications and will be a function of the setting of interaction (out-patient or emergency attendance) and will be expected to be dependent on the specialty. The extent of medication history documentation is likely to be context specific: for example, at a cardiology clinic appointment or full medical admission the physician would be expected to detail a more thorough medication history than for example an ophthalmology appointment or accident and emergency attendance where generally such information may be of lesser importance. Information bias may be introduced where the frequency and/or level of detail in record keeping for medication histories is differential between cases and controls[307]. This may be expected for patients with prevalent OAC who may be more

likely to seek less frequent medical attention, compared with patients with BO undergoing regular endoscopic surveillance (with or without outpatient follow-up) and frequent interaction with healthcare professionals[307].

It is likely that data for BMI is missing not at random (MNAR): completeness of BMI is plausibly dependent on the absolute value, with extreme values (either low or high) assumed to be more likely to be captured than values within the normal range[308]. It is therefore possible that complete case analysis (as used in this study) may bias associations. Sensitivity analyses represent a pragmatic method of assessing the impact of such bias, if it exists. However, given associations for statin use were non-significant, and participant numbers are small, sensitivity analyses were expected to be uninformative and were therefore not conducted.

Conclusions

This nested case-control study, conducted using data from hospital records from two of the largest contributing centres to the United Kingdom Barrett's Oesophagus Registry, demonstrated non-significant inverse associations between statin use and malignant progression. No significant cumulative dose or duration-response relationships were demonstrated. The results were inconclusive and could not establish beneficial, null or harmful effects with certainty. Further large, well-conducted observational studies are required which adequately consider exposure windows, latency, immortal person-time and adjust for plausible candidate confounders.

5. Chapter 5 - Overall discussion

Statins as chemopreventive agents in Barrett's oesophagus

Current approaches to reducing the burden of HGD/OAC in patients with known BO are primarily endoscopic. The purpose of endoscopic surveillance is to identify dysplastic or overtly malignant lesions at an early stage suitable for curative intervention (ideally endoscopic rather than surgical). A key challenge is the low absolute rate of malignant progression[36, 309], therefore exposing the majority of patients with BO (of whom individually will unlikely benefit) to an invasive procedure, which in itself is not free of risk[27]. Results from the Barrett's Oesophagus Surveillance Study (BOSS) are awaited with anticipation to establish the efficacy and cost-effectiveness of surveillance versus an "at need only" approach[36]. Considerable global efforts underway are focussing on identifying those with BO at highest risk of progression to inform optimum surveillance strategies[291, 310]. There are, however, no current trial data to inform non-endoscopic strategies to reduce malignant progression in BO populations. Such approaches would be welcome as they could be administered at a wider level in primary care. Results of the AspECT trial (Study of Aspirin and Esomeprazole Chemoprevention in Barrett's Metaplasia) could have substantial implications for future prevention strategy[291]. A role for statins as a novel future chemopreventive strategy would be welcome; if empirically demonstrated in the setting of a trial, these agents are inexpensive and easily administered and have been demonstrated as safe and well-tolerated at a population level[148-151].

There is a growing and prominent perception that statins are causally protective against the development of OAC[3]. While the sum of available epidemiological evidence would suggest significant inverse associations between statin use and malignant progression in BO populations in meta-analyses[311, 312]; a wider appreciation of the strengths and limitations of the current observational literature is warranted. Such significant inverse associations, if causal, would have important implications, and as such, alternative explanations deserve consideration. While the available results are encouraging, most published studies suffer such significant methodological limitations, such as immortal-time bias[222, 224], time-window bias[188, 221, 222], with little consideration of temporality, let alone latency[188, 221, 223-226], and confounding[222, 226]; that drawing dichotomous conclusions are difficult. To summarise understanding of the current observational literature (including the present study), the evidence base is best considered inconclusive. Furthermore, because of these inherent limitations, a more contemporaneous meta-estimate of all currently available studies in their current form, would not be expected to further this position.

On the background of this uncertainty, future epidemiological investigation would be recommended. An attractive research strategy would be in an individual patient data (IPD) meta-analysis. Such an approach would yield particular advantages over a traditional meta-analysis: cohorts may have been followed up for longer periods of time since original publications (to therefore better capture long term outcomes which are of particular relevance to BO populations); results of unpublished studies can be included (reducing publication bias); model assumptions for complex time-dependent associations can be checked across populations; and meta-analysis of subgroups effects can be assessed across individuals[313]. This would need to be an international collaboration to combine datasets of individual participants from a number of BO populations. A strict, pre-specified assessment of the suitability of included populations would be required in advance, together with strict inclusion and exclusion criteria for individual participants included. Particular essential criteria would be a standardised agreement for the definition of ascertainment of the BO population; exposures and outcomes. A critical issue will be the quality of available prescription information. Electronic prescription data, such as those used in population-based datasets would be the most desirable: they are suited to capturing particularly longer term prescriptions dispensed in primary care, for statins and other medications which may confound associations. Although information on adherence is usually not available, the benefits of accurately capturing the timing and posology of dispensed routine medication would represent a substantial advantage. Capture of routine over-the-counter medications is a consideration, although potentially of lesser significance, where cumulative dosage and duration of prescription medication would likely have primary influence. Datasets would need to be relatively contemporaneous, during periods where prevalent statin use is sufficient to estimate significant associations where they exist. In addition to an approved protocol, a detailed pre-specified data management and statistical analysis plan would be required, with the aim of introducing by design and analysis, standard processes of data extraction to ensure comparability of studies, and valid means of addressing relevant time-related biases, consideration of temporality and latency, and confounding.

Should the results of such research suggest persistent significant (statistically and clinically) inverse associations, with evidence of dose-response relationships, clinical practice would not necessarily change. As with all observational research, the effects of bias (particularly unmeasured confounding and selection bias) cannot be excluded. Such research may, however, more reliably inform the decision to conduct a trial of the efficacy of statins as a chemopreventive agents in patient with non-dysplastic BO. A tentative and well justified approach to this decision is

necessary given the substantial considerations required for the design and conduct of such a trial. Assuming an absolute cumulative risk of HGD/OAC (as a composite outcome) of 2% at 10 years[28, 314] in the unexposed group, with 1:1 allocation of a statin and placebo, 10% drop-out rate (including withdrawals and contamination of the exposure), a HR of 0.60[225, 312] for the effect of allocation to statins on progression, with alpha set at 5% (two-sided) and power set at 80%, the required estimated sample size for such a trial would be 8, 838. While, this is not outside the realms of possibility, it would be a considerable undertaking, require international collaboration and be dependent on prioritisation for funding. Further challenges to such a trials' conduct would be the high prevalence of statin use in at-risk populations (potentially excluding approximately half of eligible patients immediately) and the long durations required to observe the events needed to meet assumptions of the sample size calculation. Further threats to the interpretation of such a trial would be use of current endoscopic techniques (particularly radiofrequency ablation for LGD) which alter the natural history of the disease and would lower event rates further[41]. Should high dose esomeprazole and/or aspirin be regarded as a new standard of non-endoscopic management in the future (if demonstrated efficacious), further reductions in event rates would be expected and sample size considerations would need to adequately reflect this. A body of well-conducted observational research, such as an IPD meta-analysis could provide further detail which would be of considerable importance for the design of such a trial: a more reliable estimate of effect size could better inform the sample size considerations of a future trial; and a contemporaneous estimate of the cumulative risk of progression over time among non-statin users. Should high-dose statin use be associated with further increments in effect size, with consequent reductions in sample size, the feasibility of a future trial would be further supported. For example, holding all other assumptions constant, with an effect size of 0.31 for high dose statin use[221], the required sample size would be 2400. Empirical RCT evidence that statins exert causative chemopreventive effects to prevent the malignant progression would have profound implications for reducing disease burden in populations with BO.

Statins as adjuvant and maintenance therapy in patients with operable oesophageal adenocarcinoma

OAC is an aggressive malignancy with a dismal prognosis overall[5]. Even patients selected for treatment with curative intent for invasive disease, at best 5 year survival is 45%, with mortality largely attributable to recurrent disease[113, 114]. Aside from current primary surgical and oncological treatment modalities there are no other evidence-based interventions to reduce the risk of

recurrence and improve related-prognosis. For particular solid organ tumours, longer-term maintenance modalities are advocated to reduce the risk of recurrent disease and death; for example, androgen deprivation therapy is recommended for 2-3 years in men with prostate cancer at high risk of prostate-cancer mortality receiving neoadjuvant hormonal therapy and radical radiotherapy[315]; and aromatase inhibitors (or tamoxifen if these are contra-indicated) are recommended for the adjuvant treatment of early oestrogen receptor-positive invasive breast cancer in postmenopausal women for 2-3 years[316]. Given risk of death from recurrent disease is substantial in patients with OAC selected for treatment with curative intent, consideration is required for novel, safe and tolerable adjuvant therapies, of which statins are a potentially logical candidate.

Existing *in vitro* evidence demonstrates the anti-proliferative, pro-apoptotic, and anti-metastatic effects of statins in validated OAC cell lines[179-182]. These studies establish a role for mevalonate pathway products in mediating and propagating the activity of relevant downstream signalling cascades, which are potently and dose-dependently inhibited by statins. However, it is not clear whether these effects would be manifest and hence relevant in patients with OAC. A significant, clinically evident cytotoxic effect of statins (to the same degree as current chemotherapeutic regimens in OAC) in patients, lacks face validity and would seem highly improbable. However, a predominant cytostatic role may seem more plausible. Statin use measured prior to diagnosis of OC in large population-based cohorts have demonstrated significant reductions in risk of cancer-related mortality[189]. Furthermore, we have for the first time demonstrated significant reductions in cancer-specific and all-cause mortality with post-diagnostic statin use in patients with OC and OAC specifically[207]. In both studies, censoring for deaths due to other causes appropriately accounted for competing risk of death[244], of particular relevance given the established efficacy of statins in reducing cardiovascular-related mortality[148-151]. Although we demonstrate evidence to suggest reverse causation bias and confounding by staging is unlikely a prominent explanation for these associations, we are unable to completely exclude this. As with all observational research, the role of unknown confounders cannot be discounted.

Although there are biologically plausible and highly supportive experimental and epidemiological evidence to suggest a potential role for statins in the treatment of OAC, there exists clinical equipoise: an RCT is required to definitively determine whether statins are efficacious adjuvant agents in this setting, and therefore whether they should be used for this indication in clinical practice. We hypothesise that if statins are a viable treatment strategy, they would be most likely

to be shown to be effective in a patient cohort treated with curative intent to prevent recurrence, a group with minimal disease burden (following surgery +/- chemotherapy), in contrast to cohorts with advanced metastatic disease at diagnosis. We have estimated "real world", feasibility parameters in the setting of a multi-centre feasibility RCT across four UK NHS sites. In doing so we have demonstrated the feasibility of assessing statin therapy in patients with invasive OAC selected for oesophagectomy in a future phase III RCT. We know that clinicians support the trial and their patients are willing to be recruited. Estimates of recruitment and retention are favourable. Adherence to trial medication improves after the first three months of treatment. Simvastatin allocation was sufficient to cause significant reductions in LDL cholesterol comparable to estimates in patients with cardiovascular disease[285], confirming drug absorption and a pharmacodynamic effect. There was no evidence to suggest an unfavourable adverse event profile in this patient population. Feasibility of trial-related procedures, such as patient-completion of quality of life questionnaires was demonstrated. These estimates, together with the effect size derived from observational data have informed the design of a potential future trial.

A future trial would require essentially the same eligibility criteria as the feasibility work to ensure feasibility estimates remain valid. Survival estimates for all-cause mortality for patients with OAC (including Siewert I and II lesions) at three (46%)[317] and five years (45%)[118] are similar in large UK patient cohorts following oesophagectomy, a finding also reflected in relevant clinical trials, where absolute differences are small between these time points[113, 318]. This would suggest most recurrences occur within the first three years, with few occurring between years three to five, as demonstrated in observational cohorts[288]. For this reason, we propose a treatment duration of three years, during the period of highest risk of recurrence. This has practical advantages, as it would help ensure more efficient delivery of the trial overall, it would restrict the burden of trial involvement for participants to the minimum duration possible and would likely be more acceptable to clinical investigators who may not necessarily follow-up their cohort for longer periods. To ensure the greatest chance of demonstrating a significant difference between groups and a successful trial outcome, it follows the most potent statin at the highest tolerated dose be used for the intervention. Simvastatin 40mg was originally selected as the intervention for the feasibility study as it is the most widely used statin for which the adverse event profile has been best characterised[285]. However, doses as high as 80mg are associated with higher risk of myopathy (0.9%)[319], precluding their routine use at this dose. Atorvastatin 80mg may be a more logical candidate statin to select as the intervention for a future trial: it potently inhibits of the mevalonate pathway without the risk of myopathy[151], has a higher bioavailability (12% vs 5% for

simvastatin) and longer half-life (15-30 hours vs 2-3 hours for simvastatin)[170]. The feasibility estimates derived from the STAT-ROC feasibility study would be expected to remain valid even if an alternative statin was selected. The primary outcome would be overall survival by three years, with secondary outcomes including disease-free survival, health-related quality of life and cost-effectiveness. Assuming a HR for all-cause mortality of 0.63 based on our observational estimates[207], baseline survival at three years of 45% in the unexposed group[118, 317], 15% drop out overall (including treatment withdrawals, with and without contamination of the exposure, and complete withdrawal from the trial), with 90% power, at the 5% significance level (two-sided), a sample size of 508 (254 per arm) would be required. This would detect an absolute difference of 15.5% in all-cause mortality between groups. Even with 80% power, for this number of participants a HR for all-cause mortality of 0.68 could be detected, with an absolute difference in mortality between groups of 13.3%. Assuming recruitment rates from our feasibility work are applicable across 25 prospective NHS sites (with the highest oesophago-gastric surgical case-loads nationally[264]), weighted for case load, an estimated 657 participants due surgery for OAC could be recruited over a three year period. The STAT-ROC phase III trial would need to involve not only the major oesophago-gastric surgical centres, but also the peripheral NHS trusts in order to facilitate recruitment and follow-up, and in doing so operate a hub and spoke system. Current recruitment estimates for the feasibility work include the impact of competing trials, however, this landscape during a future recruitment period would be expected to change, particularly with recruitment to Add-Aspirin[290] and Neo-AEGIS[320]. For future trial success, a collaborative approach will be required in which co-enrolment between trials is permitted. Co-enrolment should still enable valid treatment estimates to be made (a product of randomisation), and ensures that estimates of effect remain contemporaneous given that interventions in current trials may become established as the future standard of care. If statins are empirically demonstrated to be efficacious agents in patients with operable OAC in improving mortality, this would represent a major advance in treatment strategy to benefit patients and the wider NHS.

6. Reference list

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chemoradiation (CROSS protocol) in adenocarcinoma of the esophagus and esophagogastric junction. Journal of Clinical Oncology 2014;32.

Appendix A

Description	Code
Cancinoma of the oesophagus or gastro-oesophageal junction	(Read/OXMIS code) B110z00, B103.00, B10z.11, B110000, B801200, B102.00, B101.00, B110111, B10y.00, B10z.00, B801.00, B801000, 150 A, B10..00, B104.00, B801z00, B100.00, B110100, B801100, 150 C
Oesophagectomy, oesophago-gastrectomy, gastrectomy	(OPCS code v4.6) G01.1, G01.2, G01.3, G27.1, G27.2, G27.3, G27.4, G27.5, G02.1, G02.2, G02.3, G02.4, G02.5 (Read/OXMIS code) 7600z00, 7610.12, 7600013, 7601.11, 7600.11, 7600012, 7601000, 7601, 7600000, 7602y00, 7602, 7602000, 7600, 7420300, 7610400, 7601z00, 7600100, 7601y00, 7601200, 7601400, 7601111, 7600200
Adenocarcinoma	(Morphology code, national cancer data repository) 81403, 81443, 81453, 82603, 82113, 84803, 84903, 81402, 85743, 84813
Squamous cell carcinoma	(Morphology code, national cancer data repository) 80703
Cerebrovascular disease	(Read/OXMIS code) G65z.00, G63..12, Gyu6600, G65zz00, G671.00, G65y.00, G677400, G63y.00, G65..00, G671z00, G641000, 4350, G633.00, G660.00, G661.00, G666.00, G665.00, G662.00, G64..13, G613.00, G6...00, G67..00, G61X100, G63z.00, 4380, G63..00, G61z.00, 4389, Gyu6.00, 1477, G61X000, G61X.00, Gyu6F00, G617.00, 4319CR, G61..12, G61..00, G66..11, G66..00, G667.00, G66..13, G663.00, G664.00, G668.00, G66..12, 14A7.00, 14A7.12, 4369B
Ischaemic heart disease	(Read/OXMIS code) G301.00, G30z.00, G30..14, G30..15, G305.00, 4109TC, G307.00, 14AH.00, G30y.00, G303.00, 4109NC, G30..00, G300.00, G32..12, G30X000, G30..13, G307100, G307000, G301100, G32..00, G30..17, G32..11, G30..12, 429 AH, G30yz00, G302.00
Peripheral vascular disease	(Read/OXMIS code) 4439A, G732100, G732200, G73z000, G73z011, G73..11, 7A13411, R054.00, G715.00, 14AE.00, G73..00, Gyu7100, G710.00, G73y.00, G731100, G713000, G715000, g71..00, R054200, G732000, G732.00, G716000, G732400, 4459TE, 7A13.11, 7A11311, G73yz00, C107.12, 4459FT, R054300, G714100, R054z00, G716.00, 4410N, 7A11211, G712.00, 4459CR, G711.00, G73z.00, G73zz00, G71z.00, G713.00, 7A14.11, 4419, 14NB.00, G71..00, G713.11, Gyu7200, G732300, R054000, G714.11, G714.00, Gyu7400, G718.00, 7A14411, 4439GD, 4459N, 4430G, G711.11
Beta blockers	(Product code) 5, 24, 26, 197, 220, 297, 472, 581, 594, 599, 707, 739, 751, 753, 769, 786, 817, 822, 940, 1006, 1048, 1050, 1124, 1288, 1290, 1295, 1333, 1334, 1448, 1572, 1597,

	1684, 1788, 2361, 2414, 2432, 2499, 2587, 2590, 2629, 2775, 2780, 3005, 3087, 3167, 3344, 3474, 3516, 3526, 3588, 3691, 3748, 3827, 4004, 4025, 4265, 4410, 4429, 4542, 4588, 4605, 4725, 4771, 4796, 4983, 5284, 5330, 5478, 5713, 5721, 5858, 5968, 6066, 6751, 7049, 7066, 7091, 7429, 7474, 7528, 7543, 7553, 7620, 7852, 7853, 7974, 8023, 8061, 8068, 8071, 8113, 8147, 8172, 8189, 8262, 8290, 8331, 8369, 8555, 8623, 8642, 8673, 8707, 8807, 8935, 8978, 8987, 9016, 9143, 9178, 9185, 9273, 9292, 9783, 10191, 10294, 10429, 10627, 10716, 10777, 10892, 11338, 11380, 11711, 11793, 12037, 12054, 12141, 12296, 12456, 12495, 12517, 12519, 12651, 13051, 13394, 13415, 13487, 13499, 13526, 13871, 14030, 14057, 14058, 14117, 14126, 14146, 14438, 14502, 14552, 14673, 14808, 15042, 15117, 15176, 15488, 15619, 15730, 16645, 16776, 16786, 17082, 17149, 17322, 17462, 17615, 17679, 17783, 18185, 18287, 18414, 18743, 18950, 19055, 19068, 19142, 19172, 19178, 19182, 19191, 19200, 19202, 19437, 19853, 19858, 19998, 20012, 20082, 20093, 20169, 20468, 20502, 20728, 21025, 21133, 21182, 21838, 21839, 21866, 21873, 21885, 21905, 21966, 22208, 22793, 22912, 23131, 23134, 23326, 23587, 24083, 24094, 24191, 24195, 24218, 24280, 24461, 24635, 24832, 25359, 25363, 25367, 25462, 25644, 25730, 26211, 26228, 26229, 26248, 26255, 26529, 26741, 26895, 26922, 27357, 27486, 27700, 27719, 27727, 27946, 27964, 28048, 28128, 28177, 28700, 28788, 28996, 29180, 29230, 29368, 29398, 29427, 29610, 29762, 29763, 29827, 29998, 30400, 30519, 30541, 30636, 30770, 31214, 31470, 31536, 31708, 31776, 31833, 31934, 32094, 32114, 32135, 32162, 32552, 32630, 32787, 32836, 33079, 33085, 33092, 33184, 33374, 33376, 33569, 33578, 33602, 33644, 33650, 33657, 33659, 33836, 33839, 33850, 33909, 34012, 34034, 34092, 34094, 34125, 34171, 34177, 34185, 34188, 34208, 34214, 34265, 34365, 34371, 34378, 34407, 34430, 34443, 34449, 34492, 34501, 34509, 34520, 34575, 34584, 34585, 34600, 34640, 34690, 34695, 34740, 34741, 34754, 34783, 34804, 34821, 34825, 34854, 34867, 34868, 34882, 34884, 34890, 34899, 34925, 34945, 34949, 34963, 34976, 35054, 35062, 35695, 35710, 35778, 35938, 35940, 36261, 36576, 36603, 37118, 37725, 37837, 38370, 38433, 38498, 38991, 39233, 39423, 39646, 39819, 39846, 40167, 40240, 40241, 40761
Angiotensin converting enzyme inhibitors	(Product code) 65, 69, 78, 80, 82, 97, 147, 196, 277, 448, 593, 633, 654, 709, 756, 761, 1021, 1121, 1143, 1144, 1299, 1520, 1807, 1904, 2982, 3069, 3203, 3310, 3720, 3839, 3929, 4103, 4571, 5047, 5159, 5189, 5275, 5612, 5735, 5800, 5861, 6078, 6200, 6261, 6288, 6314, 6359, 6362, 6364, 6408, 6468, 6765, 6786, 6794, 6806, 6807, 7314, 7419, 8025, 8026, 8105, 8106, 8268, 8800, 8830, 9646, 9693,

	9731, 9764, 9915, 9948, 10882, 10902, 11133, 11197, 11351, 11561, 11567, 11641, 11937, 11965, 11983, 11987, 12313, 12411, 12412, 12574, 12815, 12858, 13026, 13589, 13755, 14228, 14387, 14477, 14478, 14960, 15031, 15085, 15096, 15108, 15121, 15135, 15605, 15958, 16196, 16197, 16212, 16701, 16708, 16710, 16924, 17006, 17120, 17474, 17624, 17633, 17655, 18219, 18223, 18263, 18269, 18325, 19198, 19204, 19208, 19223, 19690, 20188, 20579, 20849, 20975, 21053, 21162, 21231, 21943, 22439, 22708, 23252, 23478, 23642, 24041, 24482, 25998, 26995, 27871, 28127, 28438, 28486, 28586, 28724, 28725, 28820, 28902, 29130, 29530, 29627, 30039, 30921, 31307, 31587, 31716, 31810, 32048, 32166, 32241, 32514, 32560, 32597, 32857, 32934, 33057, 33078, 33095, 33336, 33353, 33646, 33811, 33894, 33977, 34357, 34382, 34390, 34400, 34412, 34429, 34431, 34432, 34453, 34471, 34490, 34505, 34528, 34539, 34540, 34544, 34562, 34567, 34583, 34589, 34651, 34652, 34657, 34696, 34698, 34710, 34712, 34719, 34732, 34768, 34798, 34799, 34877, 34893, 34936, 34937, 34943, 34952, 34953, 35007, 35302, 35731, 35794, 36742, 36753, 37080, 37087, 37655, 37710, 37778, 37908, 37930, 37964, 37965, 37971, 37978, 38026, 38034, 38285, 38308, 38510, 38854, 38899, 38995, 39137, 39147, 39227, 39242, 39355, 39421, 39512, 40355, 40384
Angiotensin receptor blocker	(Product code) 520, 529, 531, 575, 624, 764, 828, 1293, 1780, 2971, 3222, 4155, 4226, 4540, 4645, 4685, 4741, 4818, 5013, 5117, 5723, 5988, 6217, 6243, 6285, 6351, 6437, 6518, 6877, 6939, 7043, 7338, 9196, 9745, 10316, 10323, 11251, 11252, 11348, 11448, 11469, 11526, 11864, 12836, 12874, 13123, 13821, 14283, 14738, 14870, 14943, 14965, 14983, 16060, 16161, 16285, 16371, 17545, 17686, 17689, 18200, 18202, 18903, 18910, 20117, 21423, 23456, 24268, 24359, 24484, 24632, 25382, 27520, 29634, 31072, 35096, 35173, 35174, 35189, 35196, 35304, 35317, 35329, 35343, 35380, 35481, 35697, 36939, 37573, 37650, 37747, 38367, 38395, 38459, 38889, 39021, 39199, 39786, 39944, 39984, 40316, 40571, 40639, 40668, 40711
Statins	(Product code) 25, 28, 42, 51, 75, 379, 420, 490, 713, 730, 745, 802, 818, 1219, 1221, 1223, 2137, 2718, 2955, 3411, 3690, 4961, 5009, 5148, 5251, 5278, 5775, 5985, 6168, 6213, 7196, 7347, 7374, 7552, 7554, 8380, 9153, 9315, 9316, 9897, 9920, 9930, 10172, 10183, 10206, 11627, 11815, 13041, 14219, 15252, 16186, 17059, 17683, 17688, 18442, 21020, 22579, 31658, 31930, 32909, 32921, 33082, 34312, 34316, 34353, 34366, 34376, 34381, 34476, 34481, 34502, 34535, 34545, 34560, 34746, 34814, 34820, 34879, 34891, 34907, 34955, 34969, 36377, 37434, 39060, 39652, 39675, 39870, 40340,

	40382, 40601
Aspirin	(Product code) 3, 16, 34, 254, 306, 377, 381, 393, 395, 430, 434, 484, 645, 657, 685, 1049, 1137, 1902, 2047, 2105, 2237, 2607, 2628, 2986, 3155, 3275, 3309, 3386, 3726, 4319, 4679, 5288, 6006, 6007, 6226, 6666, 6696, 7516, 7518, 7520, 7539, 8185, 8186, 8271, 8645, 9044, 9129, 9144, 9301, 9432, 9939, 10031, 10298, 10305, 10310, 11326, 11951, 11961, 11977, 12047, 12964, 12976, 12992, 13882, 14517, 15364, 15367, 15779, 16184, 16611, 17180, 17456, 17704, 17920, 17926, 18030, 18217, 18261, 18329, 19189, 19255, 20127, 20650, 20840, 21067, 21380, 21382, 21770, 21921, 22138, 22232, 22305, 22618, 22776, 23142, 23488, 23593, 23841, 23878, 23932, 24025, 24309, 24622, 24828, 24960, 25211, 25335, 25718, 26967, 27435, 28784, 28810, 29054, 29759, 29848, 30022, 30920, 31001, 31210, 31211, 31499, 31858, 31870, 31894, 31938, 31953, 31954, 31956, 32036, 32154, 32178, 32210, 32314, 32728, 32992, 33139, 33293, 33317, 33320, 33656, 33662, 33668, 33676, 34233, 34309, 34385, 34386, 34434, 34485, 34611, 34666, 34762, 34796, 34797, 34942, 35967, 36521, 36543, 37541, 39738, 40144, 40381
Non-steroidal anti-inflammatory drugs	(Product code) 15, 40, 120, 126, 129, 140, 141, 157, 162, 167, 177, 259, 296, 341, 344, 360, 387, 389, 392, 402, 407, 416, 417, 447, 474, 497, 499, 518, 526, 538, 560, 580, 586, 589, 597, 612, 613, 628, 637, 640, 647, 649, 650, 661, 666, 676, 706, 723, 736, 754, 784, 807, 838, 849, 850, 917, 919, 920, 928, 1030, 1043, 1051, 1073, 1075, 1086, 1096, 1115, 1116, 1139, 1210, 1231, 1233, 1246, 1392, 1446, 1468, 1469, 1470, 1496, 1571, 1621, 1688, 1692, 1739, 1755, 1757, 1766, 1778, 1866, 1983, 1984, 2129, 2197, 2200, 2234, 2235, 2243, 2257, 2258, 2288, 2293, 2363, 2366, 2382, 2386, 2387, 2463, 2622, 2671, 2827, 2863, 2904, 2938, 3043, 3053, 3168, 3170, 3182, 3216, 3262, 3266, 3311, 3326, 3409, 3416, 3421, 3431, 3432, 3492, 3496, 3597, 3599, 3710, 3739, 3817, 3852, 3897, 3899, 3901, 3935, 3939, 3958, 3972, 3974, 4043, 4045, 4049, 4095, 4216, 4298, 4320, 4368, 4469, 4506, 4564, 4565, 4625, 4631, 4692, 4710, 4713, 4731, 4806, 4880, 4911, 4965, 4984, 5080, 5085, 5173, 5175, 5200, 5254, 5266, 5268, 5401, 5407, 5455, 5482, 5648, 5695, 5739, 5812, 5841, 5938, 6249, 6460, 6464, 6498, 6663, 7058, 7118, 7424, 7426, 7432, 7434, 7481, 7483, 7490, 7522, 7524, 7535, 7667, 7688, 7840, 7913, 8062, 8145, 8385, 8401, 8451, 8544, 8600, 8663, 8672, 8789, 8969, 9222, 9439, 9465, 9474, 9500, 9637, 9688, 9736, 9822, 9886, 9899, 9912, 9978, 10033, 10149, 10169, 10209, 10212, 10295, 10325, 10336, 10481, 10558, 10589, 10625, 10678, 10711, 10785, 10792, 10917, 10978, 11168, 11215, 11322, 11466, 11495, 11550, 11907, 11952, 11970, 11980, 11995, 11999, 12000, 12075, 12122,

	12188, 12766, 13347, 13380, 13459, 13606, 13627, 13639, 13818, 14084, 14085, 14333, 14380, 14385, 14422, 14476, 14541, 14672, 14678, 14707, 14776, 15005, 15023, 15068, 15104, 15180, 15201, 15286, 15501, 15732, 16001, 16170, 16176, 16192, 16193, 16194, 16221, 16222, 16225, 16272, 16286, 16473, 16474, 17029, 17030, 17124, 17126, 17128, 17131, 17165, 17201, 17491, 17525, 17532, 17572, 17680, 17733, 17750, 17754, 17818, 18066, 18196, 18234, 18364, 18371, 18448, 18527, 18640, 18647, 18662, 18798, 18812, 18820, 18921, 19007, 19036, 19046, 19320, 19322, 19382, 19575, 20016, 20059, 20105, 20230, 20384, 20385, 20386, 20395, 20621, 20805, 20978, 21045, 21050, 21123, 21150, 21387, 21419, 21421, 21444, 21610, 21807, 21811, 21813, 21815, 21816, 21821, 21824, 21831, 21840, 21843, 21846, 21864, 21955, 22206, 22230, 23026, 23121, 23204, 23323, 23795, 24007, 24020, 24121, 24122, 24128, 24137, 24193, 24212, 24236, 24305, 24308, 24320, 24356, 24469, 24531, 24682, 25205, 25257, 25283, 25329, 25341, 25342, 25358, 25361, 25362, 25619, 25643, 25701, 25750, 25790, 25794, 25800, 26083, 26165, 26205, 26214, 26216, 26231, 26234, 26242, 26247, 26351, 26404, 26522, 26575, 26631, 26888, 26970, 26994, 27013, 27055, 27082, 27200, 27362, 27366, 27484, 27490, 27677, 27723, 27782, 27783, 27968, 28168, 28171, 28255, 28256, 28332, 28348, 28383, 28390, 28479, 28553, 28695, 28764, 28816, 28888, 28900, 29010, 29037, 29068, 29110, 29181, 29316, 29330, 29332, 29345, 29352, 29455, 29465, 29524, 29587, 29674, 29704, 29749, 29772, 30122, 30168, 30243, 30282, 30297, 30327, 30382, 30389, 30391, 30724, 30790, 30806, 30811, 30849, 30892, 30923, 30942, 30982, 31064, 31383, 31429, 31469, 31482, 31589, 31777, 31787, 31916, 31944, 31945, 31950, 31959, 31962, 32090, 32097, 32100, 32105, 32108, 32136, 32227, 32234, 32242, 32365, 32366, 32509, 32536, 32601, 32641, 32854, 32862, 32875, 32916, 33111, 33113, 33180, 33308, 33318, 33321, 33357, 33457, 33559, 33568, 33589, 33645, 33669, 33704, 33785, 33801, 33994, 34091, 34143, 34190, 34199, 34212, 34218, 34271, 34289, 34290, 34354, 34359, 34362, 34425, 34438, 34447, 34487, 34527, 34536, 34550, 34595, 34610, 34621, 34663, 34670, 34725, 34729, 34738, 34743, 34744, 34757, 34769, 34793, 34850, 34889, 34898, 34910, 34911, 34922, 34923, 34924, 34931, 34961, 34977, 34980, 35265, 35292, 35653, 35711, 35890, 35893, 35935, 36260, 36486, 36577, 36597, 36606, 36650, 37002, 37053, 37094, 37253, 37553, 37562, 37587, 37648, 37750, 37763, 38332, 38493, 38511, 38770, 38817, 38881, 38944, 38948, 38992, 39019, 39085, 39109, 39264, 39317, 39354, 39502, 39693, 39722, 39758, 39823, 39873, 40083, 40086, 40141, 40185, 40215, 40253,

	40336, 40394, 40401, 40484, 40516, 40664, 40756

Table 32: comprehensive list of all Read/OXMIS, OPCS, morphology and product codes used to generate the study dataset for chapter 2.

Appendix B

NHS
Health Research Authority

NRES Committee South Central - Oxford B
Whitefriars
Level 3, Block B
Lewin's Mead
Bristol
BS1 2NT

Telephone: 0117 342 1333
Fax: 0117 342 0445

01 July 2014
Amended and Reissued 10 July 2014

Dr Andrew Hart
University of East Anglia
Chancellor's Drive
Norwich
NR4 7TJ

Dear Dr Hart

Study title:	**A feasibility study of adjuvant STATin therapy in the prevention of post-operative Recurrence of Oesophageal adenoCarcinoma (The STAT-ROC feasibility study)**
REC reference:	**14/SC/0247**
Protocol number:	**2014GSURG01L(03-01-14)**
EudraCT number:	**2014-001318-24**
IRAS project ID:	**135174**

Thank you for your letter of 26th June 2014, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to make a request to postpone publication, please contact the REC Manager, Mr Thomas Fairman, nrescommittee.southcentral-oxfordb@nhs.net.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the

study.

You should notify the REC in writing once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. The REC will acknowledge receipt and provide a final list of the approved documentation for the study, which can be made available to host organisations to facilitate their permission for the study. Failure to provide the final versions to the REC may cause delay in obtaining permissions.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett (catherineblewett@nhs.net), the HRA does not, however, expect exceptions to be made. Guidance on where to register is provided within IRAS.

Clinical trial authorisation must be obtained from the Medicines and Healthcare products Regulatory Agency (MHRA).

The sponsor is asked to provide the Committee with a copy of the notice from the MHRA, either confirming clinical trial authorisation or giving grounds for non-acceptance, as soon as this is available.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites listed in the application, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Non-NHS sites

The Committee has not yet completed any site-specific assessment (SSA) for non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. We will write to you again as soon as an SSA application(s) has been reviewed. In the meantime no study procedures should be initiated at non-NHS sites.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Covering letter on headed paper		17 April 2014
Covering letter on headed paper		19 June 2014
GP/consultant information sheets or letters	1.1	10 June 2014
Letter from sponsor		15 April 2014
Letter from statistician		14 April 2014
Letters of invitation to participant	1.0	14 April 2014
Non-validated questionnaire [EORTC QLQ-C30]		
Other [CV - Dr L Alexandre]		
Other [Letter for Oncologist]	1.0	14 April 2014
Other [GP statin prescription intention]		
Other [SmPC]	4	10 June 2012
Other [CV - Dr A Hart]		
Participant consent form	1.1	10 June 2014
Participant information sheet (PIS)	1.1	04 June 2014
REC Application Form		16 April 2014
Research protocol or project proposal	1.2	10 June 2014
Validated questionnaire [Disease Specific Oesophagogastric]		

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website:
http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

14/SC/0247	Please quote this number on all correspondence

With the Committee's best wishes for the success of this project.

Yours sincerely

p.p. T. Fairman

Mr Chris Foy
Chair

Email: nrescommittee.southcentral-oxfordb@nhs.net

Enclosures: "After ethical review – guidance for researchers"

Copy to: *Mr Michael Sheridan, Norfolk and Norwich University Hospitals NHS Foundation Trust*

Appendix C

NHS
Health Research Authority

NRES Committee South Central - Oxford B

Whitefriars
Level 3, Block B
Lewin's Mead
Bristol
BS1 2NT

Tel: 0117 342 1333

29 June 2015

Dr Andrew Hart
University of East Anglia
Chancellor's Drive
Norwich
NR4 7TJ

Dear Dr Hart

Study title:	**A feasibility study of adjuvant STATin therapy in the prevention of post-operative Recurrence of Oesophageal adenoCarcinoma (The STAT-ROC feasibility study)**
REC reference:	**14/SC/0247**
Protocol number:	**2014GSURG01L(03-01-14)**
EudraCT number:	**2014-001318-24**
Amendment number:	**Substantial Amendment: AM01 15/05/2015**
Amendment date:	**15 May 2015**
IRAS project ID:	**135174**

The above amendment was reviewed by the Sub-Committee in correspondence.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

The Sub-Committee reviewed the amendment and found there to be no items of ethical concern.

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Covering letter on headed paper		09 June 2015
GP/consultant information sheets or letters [Letter to GP V1.2]	1.2	14 May 2015
GP/consultant information sheets or letters [Letter to GP V1.2]	1.2 (Tracked changes)	14 May 2015

A Research Ethics Committee established by the Health Research Authority

Letters of invitation to participant [Invitation letter V1.1]	1.1	22 May 2015
Letters of invitation to participant [Invitation letter V1.1]	1.1 (Tracked changes)	22 May 2015
Non-validated questionnaire [STATROC acceptance questionnaire V1.0]	1.0	14 May 2015
Non-validated questionnaire [STATROC declined questionnaire V1.0]	1.0	14 May 2015
Non-validated questionnaire [STATROC Patient withdrawal questionnaire V1.0]	1.0	14 May 2015
Notice of Substantial Amendment (CTIMP) [Substantial Amendment: AM01 15/05/2015]	Substantial Amendment: AM01 15/05/2015	15 May 2015
Other [Letter to the oncologist V1.1]	1.1	14 May 2015
Other [Letter to the oncologist V1.1]	1.1 (Tracked changes)	14 May 2015
Participant consent form [Consent form V1.3]	1.3	04 May 2015
Participant consent form [Consent form V1.3]	1.3 (Tracked changes)	04 May 2015
Participant information sheet (PIS) [STATROC PIS]	1.3	15 May 2015
Participant information sheet (PIS) [STATROC PIS v1.3]	1.3 (Tracked Changes)	15 May 2015
Research protocol or project proposal [STAT-ROC trial protocol]	1.4 (Tracked Changes)	14 May 2015
Research protocol or project proposal [STAT-ROC trial protocol]	1.4	14 May 2015
Sample diary card/patient card [Patient card V1.0]	1.0	14 May 2015

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

This Committee is recognised by the United Kingdom Ethics Committee Authority under the Medicines for Human Use (Clinical Trials) Regulations 2004, and is authorised to carry out the ethical review of clinical trials of investigational medicinal products.

The Committee is fully compliant with the Regulations as they relate to ethics committees and the conditions and principles of good clinical practice.

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

14/SC/0247:	Please quote this number on all correspondence

Yours sincerely

Dr Liesl Osman
Vice-Chair

E-mail: nrescommittee.southcentral-oxfordb@nhs.net

Enclosures: List of names and professions of members who took part in the review

Copy to: Mr Michael Sheridan

NRES Committee South Central - Oxford B

Attendance at Sub-Committee of the REC meeting on 26 June 2015

Committee Members:

Name	*Profession*	*Present*	*Notes*
Dr Liesl Osman (Chair)	Retired Research Advisor	Yes	
Mrs Kate Thompson	Retired In patient and day hospice manager	Yes	

Also in attendance:

Name	*Position (or reason for attending)*
Mrs Siobhan Bawn	REC Manager

Appendix D

MHRA
Regulating Medicines and Medical Devices

MHRA
151 Buckingham Palace Road
London SW1W 9SZ
United Kingdom

mhra.gov.uk

Dr A R Hart
UNIVERSITY OF EAST ANGLIA
NORWICH MEDICAL SCHOOL
CHANCELLOR'S DRIVE
NORWICH
NR4 7TJ
UNITED KINGDOM

09/06/2014

Dear Dr A R Hart

THE MEDICINES FOR HUMAN USE (CLINICAL TRIALS) REGULATIONS 2004 S.I. 2004/1031

Our Reference:	13630/0005/001-0001
Eudract Number:	2014-001318-24
Product:	Simvastatin
Protocol number:	2014GSURG01L(03-01-14)

NOTICE OF ACCEPTANCE OF AMENDED REQUEST

I am writing to inform you that the Licensing Authority accepts your amended request for a clinical trial authorisation (CTA), received on 04/06/2014.

The authorisation is effective from the date of this letter although your trial may be suspended or terminated at any time by the Licensing Authority in accordance with regulation 31. You must notify the Licensing Authority within 90 days of the trial ending.

Finally, you are reminded that a favourable opinion from the Ethics Committee is also required before this trial can proceed; changes made as part of your amended request may need to be notified to the Ethics Committee.

Yours sincerely,

Clinical Trials Unit
MHRA

Medicines and Healthcare Products Regulatory Agency

Appendix E

MHRA
Regulating Medicines and Medical Devices

MHRA
151 Buckingham Palace Road
London SW1W 9SZ
United Kingdom

mhra.gov.uk

Dr A R Hart
UNIVERSITY OF EAST ANGLIA
NORWICH MEDICAL SCHOOL
CHANCELLOR'S DRIVE
NORWICH
NR4 7TJ
UNITED KINGDOM

02/07/2015

Dear Dr A R Hart

THE MEDICINES FOR HUMAN USE (CLINICAL TRIALS) REGULATIONS 2004 S.I. 2004/1031

Our Reference:	13630/0005/001-0002
Eudract Number:	2014-001318-24
Product:	Simvastatin
Protocol number:	2014GSURG01L(03-01-14)
Substantial Amendment Code Number:	Code Number: 2014GSUR01L/030114)
Version: 1.4	
Date: 2015/05/15	

NOTICE OF ACCEPTANCE OF AMENDMENT

I am writing to inform you that the Licensing Authority accepts the proposed amendment to your clinical trial authorisation (CTA), received on 28/05/2015.

This amendment may therefore be made.

You are reminded that where it is appropriate, the Ethics Committee should also be notified of amendments.

Yours sincerely,

Clinical Trials Unit
MHRA

Medicines and Healthcare Products Regulatory Agency

Norfolk and Norwich University Hospitals NHS Foundation Trust

University of East Anglia

STAT-ROC Screening Log

Study: The STAT-ROC Feasibility Study

Sponsor ref: 2014GSURG01L(030114)

Please use this form to record details of ALL patients assessed for eligibility at pre-screening (upper GI MDT) and screening.

Visit	Initials	Date of Birth	Hospital number	Date of P/S	Trial number (if S)	List inclusion criteria which apply (e.g. 1-4)	List exclusion criteria which apply (e.g. 1-10)	Patient status[1]	If P give detailed reason if not approached for S If S give detailed reason why not consented[2]
P									
S									
P									
S									
P									
S									
P									
S									

Abbreviations: P, pre-screening; S, Screening.

[1] (1) signed consent, (2) Declined, (3) Ineligible, (4) not approached, (5) awaiting patient decision

[2] complete only if justification not already clear.

STAT-ROC Screening Log, V1.0, 16/09/14

Appendix G

Our Vision
To provide every patient with the care we want for those we love the most

Norfolk and Norwich University Hospitals NHS
NHS Foundation Trust

Professor Andrew Hart
Norwich Medical School
Floor 2, Bob Champion Research and Education Building
James Watson Road
University of East Anglia
Norwich Research Park
Norwich NR4 7UQ
Tel: 01603 593611
Email: a.hart@uea.ac.uk

Protocol reference: 2014GSURG01L(030114)
Screening ID:
Patient Hospital Identification Number:

Consent form

Trial Title: A feasibility study of adjuvant statin therapy in the prevention of recurrence of operable oesophageal adenocarcinoma

Short title: The STAT-ROC Feasibility Study

Name of Researcher: Professor Andrew Hart

Initial each box

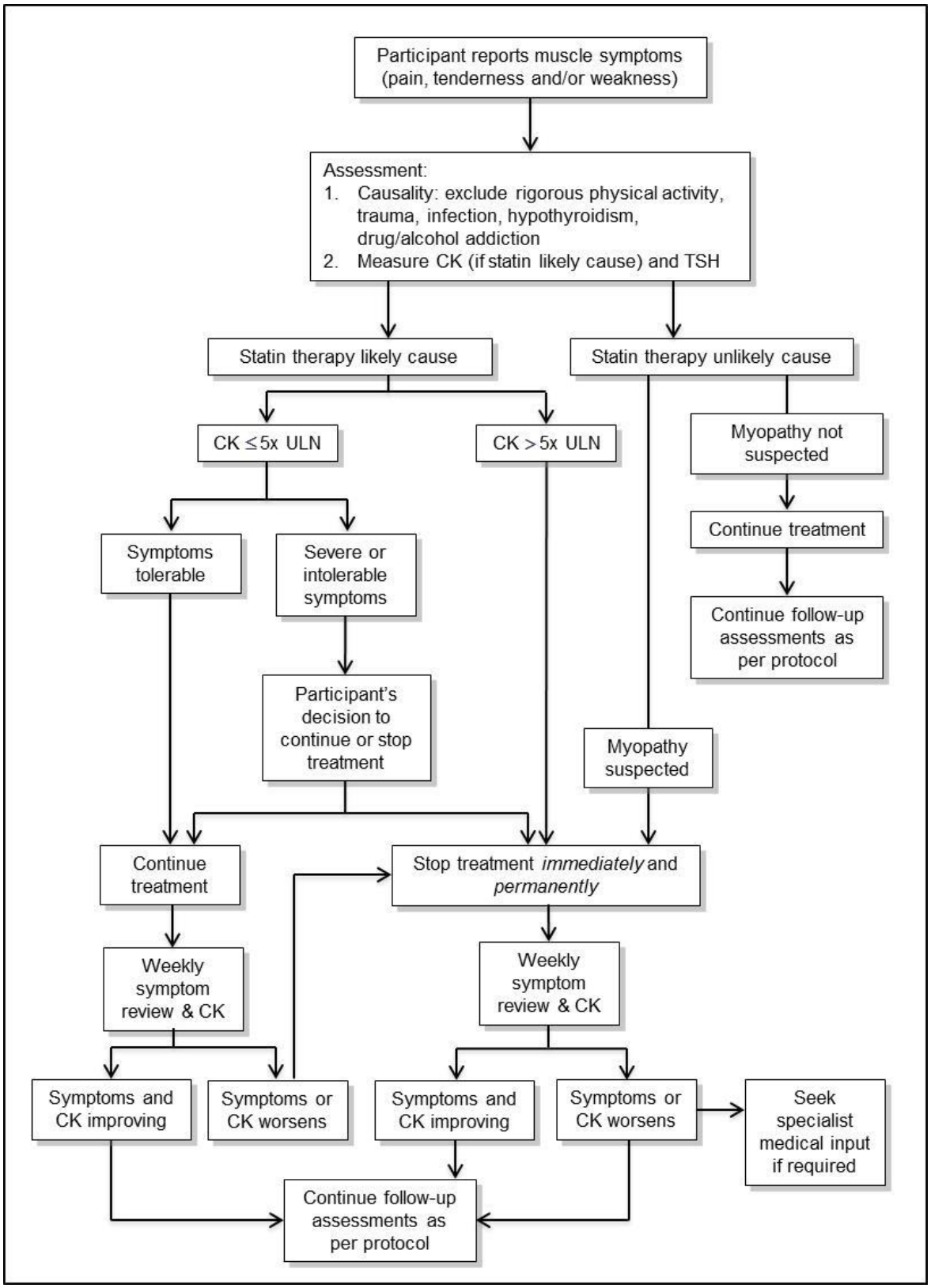
1. I confirm that I have read and understand the information sheet dated 14/05/15 v1.3 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from the NHS Trust where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
4. I understand that blood tests for safety and research purposes will be taken during this study as per the schedule outlined in the information sheet dated 14/05/15 v1.3. Blood tests for research will be stored for a maximum of two years after the end of the study at the Norfolk and Norwich University Hospital. ☐
5. I understand that Office for National Statistics data may be collected to determine health outcomes. ☐
6. I agree to my GP and my oncology consultant (if applicable) being informed of my participation in the study. ☐
7. I give permission for the Norwich Clinical Trial's Unit (NCTU) to hold a copy of this form. ☐
8. I agree to take part in the above study. ☐

Print name of Participant in full	Date in full	Signature
Print name of person taking consent in full, as per study delegation log	Date in full	Signature

Original: Investigator site file. Copies: 1 for patient; 1 faxed to NCTU; 1 to Patient Medical Notes

Consent form date of issue: 26/08/15
Consent form version number: 1.4

Appendix H



Abbreviations: CK, creatine kinase; ULN, upper limit of normal

Appendix I

NHS
Health Research Authority

London - Brent Research Ethics Committee
80 London Road
Skipton House
London
SE1 6LH

Telephone: 020 7972 2554

Please note: This is the favourable opinion of the REC only and does not allow you to start your study at NHS sites in England until you receive HRA Approval

14 September 2016

Dr Leo Alexandre
NIHR Doctoral Research Fellow
University of East Anglia
Bob Champion Research and Education Building
James Watson Road
University of East Anglia, Norwich
NR4 7UQ

Dear Dr Alexandre

Study title:	**A nested case-control study to investigate if statin use is inversely associated with the risk of high-grade dysplasia or oesophageal adenocarcinoma in patients with non-dysplastic Barrett's oesophagus.**
REC reference:	**16/LO/1741**
Protocol number:	**1.4**
IRAS project ID:	**207008**

The Proportionate Review Sub-committee of the London - Brent Research Ethics Committee reviewed the above application on 16 September 2016.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact the REC Manager Ms Julie Kidd, nrescommittee.london-brent@nhs.net . Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

Ethical opinion

On behalf of the Committee, the sub-committee gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

You should notify the REC once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. Revised documents should be submitted to the REC electronically from IRAS. The REC will acknowledge receipt and provide a final list of the approved documentation for the study, which you can make available to host organisations to facilitate their permission for the study. Failure to provide the final versions to the REC may cause delay in obtaining permissions.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for HRA Approval (England)/ NHS permission for research is available in the Integrated Research Application System, www.hra.nhs.uk or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host

organisations.

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database. This should be before the first participant is recruited but no later than 6 weeks after recruitment of the first participant.

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be permissible with prior agreement from the HRA. Guidance on where to register is provided on the HRA website.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion").

Approved documents

The documents reviewed and approved were:

Document	*Version*	*Date*
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [I&I letter]	N/A	05 September 2016
IRAS Application Form [IRAS_Form_07092016]		07 September 2016
IRAS Application Form XML file [IRAS_Form_07092016]		07 September 2016
IRAS Checklist XML [Checklist_07092016]		07 September 2016
Letter from sponsor [I&I letter]	N/A	05 September 2016
Letter from statistician [Statistician's letter]	NA	27 April 2016
Other [No material ethics issues tool]	N/A	07 September 2016
Research protocol or project proposal [Study protocol]	1.4	27 April 2016
Summary CV for Chief Investigator (CI) [LA IRAS CV 2016]	N/A	04 January 2016
Summary CV for supervisor (student research) [ARH CV]	N/A	18 April 2016

Membership of the Proportionate Review Sub-Committee

The members of the Sub-Committee who took part in the review are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website:
http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

16/LO/1741	**Please quote this number on all correspondence**

Yours sincerely
PP

Mrs Sunder Chita

Vice Chair

Email: nrescommittee.london-brent@nhs.net

Enclosures: *List of names and professions of members who took part in the review*

"After ethical review – guidance for researchers"

Copy to: *Ms Yvonne Kirkham*
Dr Philippa Collins, Rotherham General Hospital