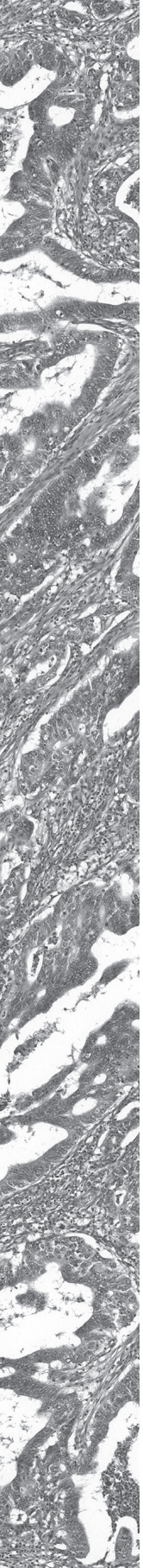
Prognostic and predictive biomarkers in oesophagogastric cancer



Valorisation

Oesophagogastric cancer (OGCa) remains a major public health issue with an estimated 1,407,000 new cases and 1,123,000 deaths worldwide in 2012 (1). This is despite the decline in newly diagnosed gastric cancer (GC) cases in recent years (1). OGCa is often asymptomatic and patients usually present with advanced stage disease. The standard of care treatment for locally advanced resectable disease is neoadjuvant/peri-operative chemo(radio)therapy and surgery. Survival remains poor, with 5-year overall survival up to 47% (2). Patients presenting with metastatic disease have a median life expectancy of less than 12 months if treated with cytotoxic chemotherapy (3). Thus, OGCa represents a substantial burden to patients in terms of morbidity and mortality.

Cancer-related health care costs have increased over the past decades (4), with OGCa having one of the largest expenditures in cancer care during the first 12 months after initial diagnosis (5). The estimated national cost of OGCa healthcare in the US was 3.15 billion USD in 2010 (5). In the Netherlands, €121 million was spent on OGCa patient health care in 2011 (6). With the use of emerging technologies such as advanced endoscopic imaging and deep-sequencing based technologies, and the high costs of new targeted therapies, including immune checkpoint targeting therapy, the already considerable economic burden related to OGCa is predicted to rise.

The prognosis prediction and treatment decisions for OGCa patients are currently based on TNM staging (7). As the cost of OGCa patient care increases with disease stage (8, 9), early detection is an important factor in reducing the economic burden. However, population screening by endoscopy is only cost effective in areas with high incidence (10, 11). New, potentially cheaper methods of screening, such as the cytosponge are currently under investigation (12). Furthermore, there are currently no biomarkers implemented in the clinic that could be measured in the blood, urine or tissue with sufficient sensitivity and specificity for early detection of OGCa (13). As patients with the same stage of disease can have very different outcomes, there remains a need to individualise and improve OGCa patient management to benefit the patient and improve efficiency in healthcare expenditure.

There are currently no prognostic or predictive biomarkers used in clinical practice for the management of OGCa patients. A prognostic biomarker provides information on clinical outcome. A predictive biomarker indicates the likely benefit of a treatment. Both likely prognosis and likely benefit from a particular treatment, together with patient's wishes, are used to guide patient management decisions (14). In this thesis, we investigated prognostic and predictive biomarkers in the epithelial tumour cells **(chapter 2, 3 and 4)** and tumour microenvironment **(chapter 5 and 6)** of patients with locally advanced resectable OGCa.

In **chapter 4**, we investigated the frequency of Epstein-Barr virus (EBV) and mismatch repair (MMR) deficiency in OGCa as they have been suggested as potential biomarkers for patient selection for immunotherapy or adjuvant cytotoxic therapy. We found the frequency of EBV and MMR is extremely low in OGCa, thus a large number of patients would need to be screened to identify the few patients with 'positive' tumours. Hence, we concluded that it

may not be economically feasible to screen patients for these tumour based molecular markers. We have recently used deep learning to predict MSI status (a surrogate marker for MMR deficiency) based on HE stained images (not part of this thesis) (15). Whilst this may offer a cost effective solution, the results from this study require validation in independent datasets which are ongoing. However, the challenge remains, that EBV and MSI/MMR deficiency status would only be able to influence the management in a minority of OGCa patients.

Similarly, the frequency of *KRAS* mutation **(chapter 2)** and *KRAS* amplification **(chapter 3)** is low in GC. Aside from the low frequency and economic feasibility of *KRAS* testing in OGCa, anti-epidermal growth factor receptor (EGFR) therapy in *KRAS* mutant OGCa does not appear to be effective (3). This is in contrast to colorectal cancer, where routine testing for *KRAS* mutation is implemented as a predictor of response to EGFR therapy (16). Thus, there is a clinical need to understand the biological differences in response to EGFR therapy between colorectal cancer and GC.

In subsequent chapters we expanded our work to the tumour microenvironment. In **chapter 5** we stratified oesophageal cancer (OeC) patients according to survival benefit from neoadjuvant chemotherapy based on the proportion of tumour/stroma heterogeneity between OeC biopsy pieces from the same patient. Patients with a low level of morphological heterogeneity had a survival benefit from cytotoxic chemotherapy. This was an exploratory, hypothesis generating image analysis based study which requires validation. If validated, future studies need to assess whether proportion of tumour/stroma heterogeneity can be assessed by a pathologist on routine haematoxylin and eosin (HE) stained slides.

In a separate study using tissue from 629 patients recruited into the Korean CLASSIC trial, we showed for the first time that tumour infiltrating lymphocyte (TIL) density measured on haematoxylin and eosin (HE) stained resection specimens may be used as a biomarker to predict survival benefit from adjuvant chemotherapy in GC patients **(chapter 6)** . Patients with high TIL density had little or no survival benefit from adjuvant cytotoxic chemotherapy and may therefore be potential candidates for treatment de-escalation. The results of this study also require validation but may have the potential to reduce patient morbidity due to (unnecessary) chemotherapy as well as reducing the healthcare costs normally related to the treatment of OGCa patients with adjuvant chemotherapy. From the patient perspective, the use of HE based TIL density as a predictive biomarker may offer more certainty about the potential success of chemotherapy. This may help to reduce the impact of unnecessary physical and psychological side effects of chemotherapy (either temporary or permanent), enabling patients to return to work earlier, thus reducing the financial impact of their illness on themselves and their families. Ultimately, predictive biomarkers may be able to improve outcomes and quality of life for OGCa patients. From the economic perspective, predictive biomarkers in OGCa to stratify patients for treatment with cytotoxic chemotherapy has the potential to improve the efficiency of the treatment and make it more cost-effective as only those patients who benefit and require chemotherapy would be treated. Importantly, for

prognostic and predictive biomarkers to reduce healthcare costs, the investment in technology should not offset the savings. As we performed TIL density using routine diagnostic HE stained slides produced at the time of pathological evaluation of the resection specimen and image analysis software, the costs are minimal and this test could be introduced relatively quickly into the routine setting, after appropriate validation. Studies in breast cancer have shown that TIL density on the HE can be assessed manually, thus reducing the cost even further. The results of this pilot work in the CLASSIC trial patients allowed us to obtain a Cancer Research UK project grant for validation and assessment of introduction of HE based TIL density into the routine clinic.

In conclusion, the knowledge generated in this thesis is not only of scientific importance, but will likely have societal and economic impact in the future. If validated, as described in the future perspectives **(chapter 7)**, HE based TIL density has the potential to improve the clinical management of GC patients while reducing expenditure on expensive chemotherapeutic drugs by ensuring only those patients benefiting from the drugs will be treated.

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