Prognostic and predictive biomarkers in oesophagogastric cancer

Globally, oesophagogastric cancer (OGCa) remains a major health problem with an estimated 1,407,000 new cases and 1,123,000 deaths each year. Patients with early stage OGCa are often asymptomatic. Due to the absence of an OGCa screening programme, patients in Western countries most commonly present with locally advanced disease at the time of diagnosis. Currently patient prognosis and treatment decisions in OGCa are based on TNM stage, patient's performance status and patient's preferences. However, OGCa patients with the same TNM stage can have very different outcomes. OGCa patients have a poor prognosis with a 5-year survival in Europe of 45-47% when diagnosed at a disease stage where the tumour is resectable and is treated with neoadjuvant/peri-operative chemo(radio)therapy and surgery. The survival benefit from neoadjuvant/peri-operative chemotherapy is modest at 6-14% improved 5-year survival compared to treatment by surgery alone, suggesting that only a subset of patients benefits from chemotherapy. Thus, there remains an urgent clinical need to identify biomarkers to individualise and improve OGCa patient management.

The **aim** of this thesis was to investigate prognostic and predictive biomarkers in locally advanced resectable OGCa. We first focussed on the molecular characterisation of the tumour cells and thereafter on the characterisation of the tumour microenvironment.

As *KRAS* and *BRAF* mutations in colorectal cancer are known predictors of poor response to EGFR targeting agents, in **chapter 2** we performed a literature review to analyze and summarize the current literature on *KRAS* and *BRAF* mutations, including *KRAS* amplifications in gastric cancer (GC). We included a total of 69 studies and found the current knowledge on *KRAS* and *BRAF* in GC to be limited due to small sample size of investigated tumours and the use of a variety of different methodologies, making any comparisons between studies difficult. The frequency of *KRAS* mutation and *KRAS* amplification is low (<10%) in GC. In particular, the frequency of *KRAS* mutations in GC is much lower than that in colorectal cancer. *KRAS* mutations and *KRAS* amplifications seem to be mutually exclusive, suggesting the potential need to screen GC patients for both genetic aberrations when searching for *KRAS* activation. *BRAF* V600E mutations are extremely rare in GC. So far, all clinical studies in unselected patients with metastatic GC have failed to show a significant benefit for EGFR targeting therapy. Post hoc analysis of the REAL3 trial showed no relationship between *KRAS* mutation status and EGFR treatment effect.

Studies in lung and ovarian cancer suggest a relationship between *KRAS* activation and histological phenotype. Therefore, we investigated whether *KRAS* mutation and/or *KRAS* amplification (collectively called *KRAS* activation) are also related to the histological phenotype in GC which could then potentially indicate whether KRAS activation is an early or late event in gastric cancer carcinogenesis **(chapter 3)**. Digitized Haematoxylin/Eosin stained slides from 1282 GC resection specimens were classified according to Japanese Gastric Cancer Association (JGCA) and the Lauren classification by at least two observers. *KRAS* mutation and *KRAS* amplification were found in 68 (5%) and 47 (7%) GCs, respectively. We confirmed a relationship between presence of *KRAS* mutation and mucinous phenotype in

GC as described in ovarian cancer and lung cancer. Interestingly, 724 GCs (57%) showed more than one histological phenotype. This relatively high level of intratumour morphological heterogeneity could reflect *KRAS* mutation heterogeneity, which may explain the failure of anti-EGFR therapy in GC.

Immune checkpoint targeting therapy has recently shown promise in several cancer types. Proposed biomarkers to predict potential response to immune checkpoint inhibitors include DNA mismatch repair (MMR) and/or Epstein-Barr virus (EBV) status. Therefore, in **chapter 4,** we determined the frequency of EBV and MMR in a large multicentre series of 988 oesophageal cancer (OeC) and 1213 GC using EBV-encoded RNA *in situ* hybridisation and MMR protein expression by immunohistochemistry (IHC), respectively. In a large subset of OeC, we tested microsatellite instability (MSI) in parallel with MMR IHC. The frequency of MMR deficiency and MSI was very low in OeC (0.8% and 0.6%, respectively) and much lower than in GC (10.3%). None of the OeCs were EBER positive in contrast to 4.8% EBER positive GC. This is the largest study to date demonstrating that in contrast to GC, EBV and MMR deficiency do not play a role in OeC carcinogenesis. Thus, the potential clinical usefulness of determining MMR deficiency/EBV status to screen patients for eligibility for immune checkpoint targeting therapy differs between OeC and GC patients.

Whilst many OGCa studies have focused on the characterisation of tumour epithelial cells, there is a growing interest in the role of the tumour microenvironment in cancer development and progression. Therefore, in **chapter 5,** we investigated whether the intratumour heterogeneity of the tumour/stroma content in the diagnostic biopsy of OeC patients is related to survival after neoadjuvant chemotherapy. Firstly, we established a new method using a statistical mixed effect model (MEM) to measure intratumour heterogeneity of the proportion of tumour (IHPoT). We used the newly developed method to estimate IHPoT (variation of the proportion of tumour in haematoxylin/eosin stained pre-treatment biopsy pieces from the same patient) in the pre-treatment biopsies from 218 OeC OE02 trial patients. We found that patients with a low IHPoT index (biopsies from the same tumour have a similar proportion of tumour) had a survival benefit from cytotoxic chemotherapy. This is the first study suggesting that IHPoT measured in the pre-treatment biopsy can predict chemotherapy survival benefit in OeC patients. IHPoT may represent a clinically useful biomarker for patient treatment stratification.

Based on these biopsy findings we were interested to know which components of the stroma (including fibroblasts, extracellular matrix, vessels and immune cells) are contributing to its relationship with chemotherapy response. Studies in GC suggested a clinical value of tumour infiltrating lymphocytes (TILs) with respect to patient prognosis (52, 53), thus we selected TILs as our initial focus of investigation of the stroma components **(chapter 6)**. We analysed the number of lymphocytes per area (so called TIL density) in patients with resectable, stage II-III GC from the Korean phase III CLASSIC trial. We used image analysis software (MIM from HeteroGenius, UK) to build a colour model for the identification of

lymphocytes. We calculated the TIL density using digital haematoxylin and eosin (HE) stained tissue microarrays constructed from GC resection specimens from 629 CLASSIC trial patients. TIL density proved to be an independent prognostic and predictive biomarker for survival benefit from adjuvant chemotherapy. Patients with high TIL density GC had a significantly improved survival and derived little or no benefit from adjuvant chemotherapy (Xelox) compared with patients with low TIL density GC. Patients with low TIL had a significant benefit from adjuvant chemotherapy. We concluded that TIL density measured on routine HE stained tissue sections may represent a new clinically useful biomarker identifying GC patients who may not require adjuvant chemotherapy and for whom treatment could be de-escalated. Validation of these results following the biomarker roadmap principle is ongoing.

In **Chapter 7,** we discuss the implications of our research in the context of the current literature. We also critically discuss the problems and shortcomings of current OGCa prognostic and predictive biomarker studies. To address one aspect of this, we outline plans for validation studies in the near future to fully assess the prognostic and predictive value of TILs. We also discuss the potential role of emerging technologies in the clinical management of OGCa patients in the future.