

AUSTRALIAN PERSPECTIVE ON THE ROLE OF TARGETED THERAPIES IN GASTROESOPHAGEAL CANCER

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Abstract

Oesophagogastric cancers are the fourth highest cause of cancer related deaths worldwide and the sixth most common cause of cancer related deaths in the Australian population. Their incidence has been increasing in Australia, and unlike other tumour types such as breast, there has been no significant improvement in relapse-free or overall survival rates, with five year mortality rates for gastric cancer of 25% and less than 20% for oesophageal cancers. This is partly due to patients commonly presenting with late stage disease, but significantly also due to ineffective chemotherapeutic regimens for advanced oesophagogastric cancer. However, with the promise shown by targeted therapies in other previously poor prognostic tumour streams, there is a demand for a similar application in the treatment of these tumours.

Within the Australian population, the current annual incidence for gastric cancer and oesophageal cancer is projected to be 2000 and 1400 new cases per year,¹ representing 1.9 and 1.2 percent of all cancers respectively.¹ These tumours are associated with high mortality, with projections approximately 1300 and 1100 deaths from oesophageal cancer and gastric cancer respectively, disproportionately contributing to 5.8% of all cancer related deaths in total.¹ The majority of cancers are either squamous cell carcinomas (SCC), occurring predominantly in the proximal and mid-third of the oesophagus and stable in incidence,^{2,3} or adenocarcinomas,¹⁻³ which arise in the stomach and distal two thirds of the oesophagus, and which have shown a marked increase in incidence over time.

Current management of these tumours is generally poor due to a lack of well defined risk factors, non-specific symptoms and lack of biological markers, often resulting in patients presenting with regionally advanced or disseminated disease at diagnosis. Surgery is the mainstay of therapy for primary and locally advanced disease, but used alone demonstrates five-year survival rates of only 20-25%.^{4,5} With the addition of neo-adjuvant or adjuvant chemoradiotherapy, there is some benefit with slightly improved five-year survival rates of 30-35%.⁶⁻⁹ Treatment of metastatic disease is based on platinum based therapies with oral or intravenous fluoropyrimidine chemotherapeutic agents, with reported objective response rates of 20-50% and median overall survival universally less than 10 months.¹⁰⁻¹² However, the regimens are associated with significant toxicity, with patients reporting grade 3/4 neutropaenia and grade 3/4 diarrhoea in up to 82% and 20% of cases respectively when receiving combination of docetaxel, cisplatin, and 5-FU.^{11,12}

Additional targets of therapy have been identified following recent advances in the molecular understanding of oncogenic pathways. These advances promise a shift from 'one treatment for all' regimens to more effective cures with minimal toxicities. Early evidence of this success is seen

in HER-2 targeted treatment in breast cancer,¹³ epidermal growth factor receptor (EGFR) inhibitors in lung cancers,¹⁴ anti vascular endothelial growth factor (VEGF) therapy in renal cell carcinomas,^{2,15} and BRAF targeted therapy in melanoma.¹⁶ The potential for the use of targeted therapies in the future treatment of these cancers is significant, with the main developments in oesophagogastric cancer focused on the use of monoclonal antibodies and small molecule-based therapies targeting signal transduction pathways, in particular EGFR, HER-2/Neu and soluble VEGF or its receptor. Within Australia, trials are currently underway in the use of panitumumab in combination with first line chemotherapeutic agents in the treatment of advanced oesophago-gastric cancers (the ATTAX-3 trial, ACTRN12609000109202).

Targeted therapies

Epidermal growth factor receptor

EGFR, or ErbB1, is a member of the ErbB transmembrane growth factor receptor family, which also includes ErbB2 (Her2/neu), ErbB3 and ErbB4.¹⁷ Binding of the two known ligands, epidermal growth factor and transforming growth factor - α causes dimerisation of the receptor with any members of the ErbB family, leading to activation of the receptor's tyrosine kinase domain.¹⁸ This family of receptors is a key modulator of cell cycle regulation, proliferation, survival, avoidance of apoptosis, migration and differentiation.^{17,18}

Within oesophagogastric cancer, dysregulation of EGFR appears to be predominantly due to overexpression from gene amplification. It is detected either as increased cellular membrane staining of the protein by immunohistochemistry (IHC), or by in situ hybridisation (ISH), for the amplicon. EGFR overexpression is detected more so in SCC compared with adenocarcinoma and correlates with poorly differentiated histology, increased invasion and worse overall prognosis.^{19,20} While there is considerable variation between studies and techniques for EGFR expression, almost all international and local studies to date have

recruited patients without pretesting and following the dogma that EGFR overexpression is frequent in these tumours. Mutations of EGFR have also been identified but appear to be rare and are responsible for only a minority of the potentially treatable oesophagogastric cancer. These mutations are more commonly seen in oesophageal SCC (less than 14%)²¹ than in adenocarcinomas (<11%).²²⁻²⁴ The most common mutations included missense activating mutations (G719A, S768I, L858R),²¹⁻²⁴ truncation mutations (E872 GAA-TAA),²³ the tyrosine kinase inhibitors (TKI) drug resistance associated T790M,²² mutation and in-frame deletions (delE746-A750),²² all of which have been previously characterised in non-small cell lung cancer.²⁵⁻²⁷

The development of anti-EGFR targeted therapy is well underway in several tumour streams and includes several monoclonal antibodies (cetuximab, panitumumab and matuzumab) and oral TKI (erlotinib, gefitinib). The current anti-EGFR monoclonal antibodies are partially or completely humanised IgG1 and IgG2 antibodies designed to bind EGFR and block ligand-mediated tyrosine kinase activity.²⁸ They also promote EGFR internalisation by endocytosis and may activate tumour specific immune-mediated mechanisms.^{29,30} International trials have shown comparable response rates to other combined modality trials with relatively good toxicity.^{31,32} Locally, the Australasian GastroIntestinal Trials Group ATTAX-3 trial, which has recently commenced recruitment, is comparing the use of docetaxel, cisplatin, fluoropyrimidine with or without panitumumab over a 24 week course for the treatment of locally recurrent or metastatic oesophagogastric cancer (table 1). The study aims to recruit 100 patients and examine for tumour response rates, overall survival, progression free survival and treatment related toxicity. The study will provide further guidelines to the use of EGFR targeted therapy for oesophagogastric cancer in Australia.

The future incorporation of EGFR based targeted therapies for oesophagogastric cancer brings with it several challenges, one of the most of which will be the management of innate or selective tumour resistance. Lessons learnt from recent studies in colorectal carcinoma have shown that acquired and innate tumour resistance through mutational activation of the K-ras protein present

downstream of EGFR negates benefit from cetuximab or panitumumab.^{33,34} Similarly, the V600E mutation of B-raf protein downstream of EGFR is also associated with a lack of response to cetuximab in colorectal carcinoma.³⁵ Currently, limited studies estimate that up to 2% of oesophageal and 6% of stomach cancers harbour the K-ras mutations and up to 11% of oesophageal and 1% of stomach cancers harbour the B-raf mutation.³⁶ The impact of these and similar mutations on EGFR-based treatments is unknown and prospective management of oesophagogastric cancer may require mutational testing or identification of responsive cohorts either at the initiation of treatment or if resistance occurs, development of combination therapies and improved efficacy of second generation agents.

Her2/neu

The Her2/neu or ErbB2 tyrosine kinase is also a member of the ErbB growth factor receptor family, but unlike its other members, lacks an ectodomain for ligand binding.³⁷ Despite this, Her2 shares 64% homology with EGFR, with the most similar region located in its tyrosine kinase domain.³⁸ This allows heterodimeric partnering of Her2 with other family members, and within the potential combinations of homo and heterodimers that may be formed, it appears that each of the other receptors with their specific ligands prefer Her2 as their heterodimeric partner.^{39,40} Furthermore, Her2 containing heterodimers are characterised by extremely high signalling potency, as Her2 reduces the rate of dissociation considerably, allowing more potent and prolonged activation of downstream pathways,^{40,41} the most important appearing to involve mitogen activated protein kinase and phosphatidylinositol-3 kinase.⁴²⁻⁴⁴ As with EGFR, Her2 activation can induce mitogenic mechanisms and progression through the cell cycle and inhibition of apoptosis.^{42,44}

In oesophagogastric cancer, dysregulation of Her2 appears to be predominantly due to amplification and overexpression and is tested by IHC, ISH or a combination of both.^{45,46} Clinically, in oesophageal SCC, Her2 overexpression detected by IHC correlates with extramural invasion, poor response to neoadjuvant chemotherapy and amplification detected by ISH is related to poorer survival rates.^{46,47} In adenocarcinoma, study results have been mixed and less

Table 1: Summary of Australian trials of targeted therapy of oesophagogastric cancers.

Trial		Recruitment status	Agents	Disease stage	Targeted sample size	Measured outcomes
ATTAX3	Randomised, Phase II	Closed	1st line - wTCF/X + panitumumab	Metastatic/ Widespread	100	Tumour response (as per Response Evaluation Criteria in Solid Tumours - RECIST v1.1), overall survival, progression-free survival, treatment related toxicity.
wTCF, docetaxel/cisplatin or capecitabine/fluoropyrimidine.						

clear due to; a) differences in receptor testing based on IHC; b) fluorescent in situ hybridisation (FISH), silver in situ hybridisation (SISH) and chromogenic in situ hybridisation (CISH); c) use of different cut-offs to determine amplification and d) the distinction of true Her2 gene amplification and chromosome 17 polysomy. This has contributed to the wide range, between 9-34%, of reported Her2 positivity in adenocarcinomas.^{48,49} There is also variable clinical correlation with some studies demonstrating association between Her2 amplification (defined by FISH and using a lower threshold of 4 or more signals per nucleus) and increasing tumour depth of invasion, lymph node and visceral metastasis and overall poor survival,⁵⁰ while others using a cut-off incorporating higher copy numbers have shown no relationship.⁵¹

Currently, Her2 targeted therapies incorporated and evaluated in treatment of oesophago-gastric cancers are the monoclonal antibodies trastuzumab and the TKI lapatinib. Formally approved in Australia for the treatment of HER2-positive advanced adenocarcinoma of the stomach or gastro-oesophageal junction who have not received prior anti-cancer treatment for their metastatic disease, trastuzumab is a humanised IgG1 monoclonal antibody designed to bind to the extracellular segment of the Her2 receptor. Binding of the antibody prevents receptor dimerization and activation, increases receptor endocytosis and destruction, triggers tumour specific antibody dependent cytotoxicity and prevents proteolytic cleavage of Her2 from its extracellular domain, thus preventing anchor free activity.⁵²

The evidence of Her2 directed clinical efficacy in gastric adenocarcinoma and gastro-oesophageal junction tumours comes from the recently completed multinational phase III ToGA randomised control trial comparing chemotherapy alone or in combination with trastuzumab.⁵³ Participants with histologically confirmed inoperable locally advanced, recurrent and metastatic Her2 positive gastric adenocarcinoma or gastro-oesophageal junction tumours were recruited to the

study. As incorporated into current Australian Therapeutic Goods Administration guidelines, the ToGA study defined positive tumours if scoring 3+ on immunohistochemistry (defined as strong complete basolateral or lateral membranous staining in >10% of tumour cells in surgical specimens, or tumour cell clusters with a similar staining pattern in biopsy specimens irrespective of percentage) or if FISH demonstrated a Her2:CEP17 ratio > 2. The study showed an improvement in median overall survival (13.8 v 11.1 months; HR 0.74, 95% CI 0.060-0.91, p=0.0046) with no difference in rates of adverse cardiac or grades 3 or 4 adverse events between the two groups.

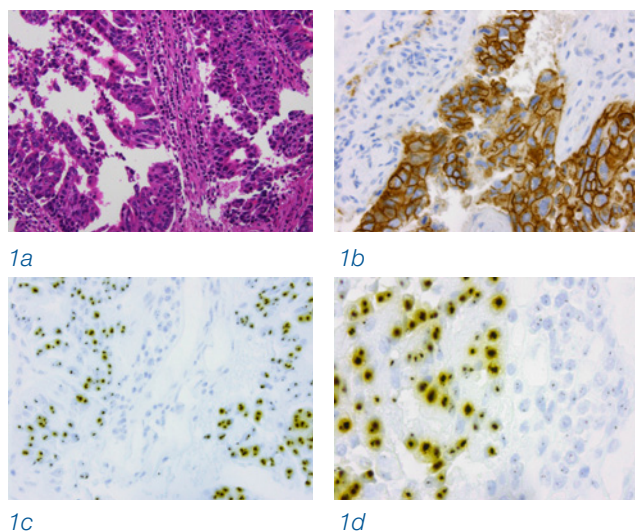
The Her2 TKI Lapatinib is also currently approved by the Australian Therapeutic Goods Administration for treatment of Her2 positive breast cancer, however to date it has only shown modest results as a first line treatment in patients with advanced gastric cancers with response rates <7%.⁵⁴ Further results from the phase III LOGiC trial, evaluating the combination of capecitabine/oxaliplatin +/- lapatinib as first line treatment of Her2 overexpressing advanced oesophago-gastric cancer therapy, are hoped to provide further direction in this area.

Current and future challenges in improving Her2 targeted therapy involve accurate identification of patients able to benefit from this treatment. While IHC and ISH based assays can theoretically identify tumours, (figure 1) accuracy may be limited by tumour heterogeneity and lack of experience in the technical and interpretive aspects of molecular testing. Recently published as an abstract at American Society of Clinical Oncology meeting, the GaTHER study was performed to evaluate Her2 testing accuracy of gastric cancers in Australia.⁵⁵ The results demonstrated that when IHC is interpreted by appropriately trained histopathologists experienced in reviewing gastric cancers, IHC3+ samples do not need further testing and that ISH is needed to confirm amplification only in IHC 2+ tumours using current reporting protocols for breast cancers. ISH for IHC 0 and 1+ tumours was not required. The study and recommendations are currently being formulated and the forthcoming publication is hoped to provide further guidelines and standardisation for Her2 testing and assessment within Australia. Simultaneously, the Royal College of Pathologists has also initiated a quality assurance programme for Her2 testing of gastric carcinomas, further acknowledging the importance of accurate testing. Additional studies are also required to evaluate the benefit of treatment in early stage primary disease and tumours with low level amplification of Her2, which have been untested to date for efficacy to current targeted therapies.

Vascular endothelial growth factor

VEGF is a potent vascular permeability and growth factor regulating normal and pathologic angiogenesis.^{56,57} VEGF binds with several high affinity transmembrane receptors, the most notable being VEGF receptors types 1 (flt-1), and 2 (KDR, flk-1) with the main biological effects including endothelial cell mitogenesis and migration, induction of proteinases, extracellular matrix remodelling, increased vascular permeability and survival of newly formed blood vessels.^{56,58}

Figure 1: Her2 staining. 1a) Gastric adenocarcinoma, H&E x 200, 1b) IHC 3+ staining of gastric adenocarcinoma (DAB x 200), 1c) and 1d) Silver in situ hybridization staining of gastric adenocarcinoma showing high level amplification of Her2 (x200,x 400).



Increased expression of VEGF has been measured in most tumours and patient serum, and biologically there is convincing evidence demonstrating the importance of angiogenesis with tumour growth and metastases.^{56,59} Approximately 30-60% of patients with oesophagogastric cancer have overexpression of VEGF with relatively similar data in both SCC and adenocarcinoma showing increasing serum levels and tumour VEGF expression associated with progression from dysplasia, invasive carcinoma to metastatic disease.⁶⁰⁻⁶² There is also positive correlation with vascular invasion, nodal involvement, metastatic disease and worse overall survival.⁶³⁻⁶⁶ While not examined in oesophago-gastric cancer, serum VEGF levels appear not to correlate with response to VEGF targeted therapies, possibly due to the limitation of current assays and further unknown complexities of angiogenesis and VEGF regulation.⁶⁷ Thus, trials to date have neither measured nor correlated VEGF with treatment decisions or outcome.

Currently, several targeted therapies have been developed for Australian Therapeutic Goods Administration approved use in other solid tumours. To date, most international phase II and III trials have evaluated the anti-VEGF humanised IgG1 monoclonal antibodies, bevacizumab, while the use of vascular endothelial growth factor receptor TKI sunitinib and sorafenib for oesophago-gastric cancer are early in development. Bevacizumab is thought to have dual anti-tumour activity providing both anti-angiogenic effects and also possibly improving chemotherapeutic drug delivery.^{68,69}

Due to expression of VEGF and vascular endothelial growth factor receptor in normal tissues, treatment is also associated with side-effects including hypertension, thromboembolism, proteinuria and haemorrhage.⁷⁰⁻⁷¹ Furthermore, due to reported fatal pulmonary haemorrhages in non-small-cell lung carcinoma with SCC histology, bevacizumab based trials have been limited to adenocarcinomas.⁷⁰ Trials to date have been encouraging and have shown improved overall survival and time to progression with similar toxicities to chemotherapy alone.⁷³ One encouraging phase II study has been led by Memorial Sloan-Kettering Cancer Centre, evaluating cisplatin/irinotecan with bevacizumab as a first-line treatment of advanced gastric and oesophagogastric junction adenocarcinomas in 47 patients.⁷³ The study reported significantly improved time to progression (8.3 months; 95% CI, 5.5 to 9.9 months) and overall survival (12.3 months; 95% CI, 11.3 to 17.2 months) compared with historic controls. Toxicity included a 6% incidence of gastric perforation or near perforation, 2% incidence of myocardial infarction, 2% rate of significant haemorrhage and 25.5% of grade 3/4 thromboembolic events, which were comparable to rates seen in patients receiving neoadjuvant cisplatin/irinotecan for advanced gastric cancer other studies.

A number of other phase 2 studies have shown benefit for the combination of chemotherapy with bevacizumab. These include trials using: oxaliplatin and docetaxel as the chemotherapy backbone (objective tumour response in 42% with median progression-free survival 6.6 months);⁷⁴ docetaxel, irinotecan and cisplatin (63% response rate);⁷⁵ and single agent docetaxel, which shows response in about one quarter of patients.⁷⁶⁻⁷⁷

Despite encouraging outcomes from these phase 2 studies, the phase 3 study AVAGAST did not meet its primary endpoint of improved overall survival.⁷⁸ This trial, a randomised, double blind, placebo controlled study of 774 patients, evaluated the use of cisplatin and capecitabine with either bevacizumab or placebo in the first line metastatic setting. Although there was no overall survival benefit, there was a significant improvement in progression free survival and overall response rate. The results from the AVAGAST trial also demonstrated a trend towards an overall survival benefit with the use of bevacizumab that was not statistically significant (overall survival 12.1 v 10.1 months, $p = 0.1002$). There was a 1.4 month increase in progression free survival with bevacizumab use (5.3 v 6.7 months, $p = 0.0037$) and statistically significant improvement in overall response rate (46 v 37%, $p=0.0315$). The study did find a higher rate of haemorrhage in the group receiving bevacizumab, although this was largely grade 1 bleeding not requiring intervention. Interestingly, the rate of arterial and venous thromboembolism was similar across both treatment arms.

Of note, subgroup analysis did show regional variation in overall survival both with and without the use of bevacizumab in addition to chemotherapy. The greatest benefit was seen in those patients in the Americas who demonstrated an overall survival hazard ratio of 0.63 with the use of bevacizumab combined with chemotherapy. In contrast, the Asian population in the study had a hazard ratio of 0.97 with the addition of an antiangiogenic agent, and essentially derived no benefit from this combination. There was also a similar trend noted for progression free survival. A number of reasons for this discrepancy have been postulated, including genetic variations in these populations, or practice differences in the approach to metastatic gastric cancer and patient selection in different geographical regions. Further evaluation of patient characteristics in the AVAGAST study compared to pooled data from patients in the United States receiving chemotherapy and bevacizumab for metastatic oesophagogastric cancer shows a significant difference in tumour location, histology and extent of disease.⁷⁹ Preplanned biomarker studies are ongoing to determine if there may be an identifiable subpopulation that might show benefit from the addition of bevacizumab to cytotoxic chemotherapy.

Although there would appear to be a sound biological rationale for the use of VEGF inhibitors in the setting of metastatic oesophagogastric cancer, there is currently no definitive evidence suggesting a benefit for the addition of bevacizumab to standard chemotherapy. Further research in this area may reveal a population for whom this is a beneficial addition, but the characteristics of this group are yet to be elucidated. Bevacizumab currently remains a prospect for further evaluation rather than an option for standard management of patients with metastatic gastric cancer.

Conclusion

Despite a marginal improvement in the treatment of oesophagogastric cancers, management options for locally advanced and metastatic disease are still limited with poor overall survival. Recent advances in the use of targeted

therapies have been promising, with an expanding body of both international and local experience, representing a significant shift from a 'one treatment for all' to a more tailored approach built on improving our molecular understanding of the oncogenic process.

While the survival benefits seen are significant, they are still modest and measured in months. Several challenges remain however, including the improved identification of patients most suitable for particular therapies, which is dependent on identifying and standardising the best tests for specific therapies. As with any other therapy, there will also be challenges pertaining to treatment toxicity and acquired resistance, which may be minimised with more specific biological targets identified and improved second and third generation therapies.

In the next few years, many of the current international and local trials are expected to provide further guidance in the treatment of oesophagogastric cancers. It is hoped that the initial promise will eventually lead to a wider armamentarium of target specific treatments that may be used in combination with conventional chemotherapy drugs and radiotherapy tailored specifically to the genetic profile of these tumours, with high efficacy and minimal toxicity.

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