

**Methods:** HUVEC cells tubule formation was measured in the presence of amidated gastrin-17 (G17) and glycine-extended gastrin-17 (GlyG17) peptides. HB-EGF gene and protein expressions were measured by qRT-PCR, immunocytochemistry, and Western blotting, and HB-EGF shedding by ELISA. Matrix metalloproteinases were assessed by Western blotting. Microvessel density (MVD) was assessed by immunohistochemistry and serum-amidated gastrin levels by RIA.

**Results:** HUVEC cells showed increased tubule and node formation in response to G17 which was blocked by the cholecystokinin-2 receptor (CCK-2R) antagonist, JB95008 and by antiserum to gastrin and HB-EGF. Gastrin peptides increased HB-EGF gene expression/protein secretion in HUVEC and micro-vessel-derived endothelial cells and the levels of MMP-2, MMP-3, and MMP-9. G17 promoted angiogenesis in a chorioallantoic membrane assay, and MVD was significantly elevated in pre-malignant large intestinal tissue from hyper-gastrinaemic APC<sup>Min/+</sup> mice. MVD in the normal mucosa surrounding colorectal adenocarcinomas correlated with patient serum gastrin levels and HB-EGF expression.

The extent to which *H. pylori* to up-regulation of HB-EGF can be attributed to its effect on gastrin was examined. Gastric cells, transfected with either gastrin small interfering RNA or antisense plasmid or the CCK-2R, were cultured for 24 hours with *H. pylori* (+/-), a CCK-2R antagonist.

*H. pylori*-induced significantly higher levels of HB-EGF gene expression and ectodomain shedding in the CCK-2R-transfected cells than the vector control which was reversed by the CCK-2R inhibitor. Gastrin down-regulation reduced the effect of the bacteria on HB-EGF gene and protein expression levels. Endogenous gastrin and CCK-2R expression were also found to be significantly up-regulated in all cell lines as a result of exposure to *H. pylori*. Finally gastrin has been shown along-with VEGF to be a target gene of HIF1a and be up-regulated under hypoxic conditions in vitro.

**Conclusions:** The above studies confirm the multi-factorial role of gastrin in gastro-intestinal malignancy confirming it as a target for both chemoprophylaxis and established cancers.

## Symposium (Tue, 25 Sep, 14:45–16:45) FECS/ASCO symposium – what have we learnt from pre-surgical medical therapy in breast cancer?

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INVITED

### The role of neoadjuvant therapy

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Experiments on preclinical models have suggested that preoperative introduction of chemotherapy (CT) of endocrine therapy can improve survival by abrogating a post surgical growth spurt and by addressing micrometastatic disease at an earlier stage than when given postoperatively. Clinical trials have not confirmed this hypothesis, although they have demonstrated that neoadjuvant systemic therapy (NST) reduced overall tumor burden, expanded the indications for breast-conserving surgery to patients (pts) with more advanced disease, and provided an in vivo assessment of response that facilitated the safe and effective administration of systemic treatment. NST also provides an improved investigational model, since sequential monitoring with the primary tumor in place offers the opportunity for multiple biopsies to monitor the biological effects of treatment. Large randomized trials have shown preoperative CT to be at least equivalent in disease-free and overall survival to the same CT administered postoperatively. Emerging data suggest, however, that the effects of systemic therapy in general, and NST in particular, vary with different subclasses of breast cancer (BC). The first such observations were based on estrogen receptor (ER). The magnitude of benefit from CT is proportionately more modest for pts with ER+ tumors than for ER- tumors. This is dramatically expressed in preoperative trials, where the pathologic complete remission (pCR) rate is four to six-fold higher for ER- than for ER+ BC. Similar variation in pCR rate is observed by grade and histologic type (invasive lobular cancers vs. invasive ductal cancers). Paradoxically, although pCR identifies a group of patients with improved survival compared with patients who do not achieve pCR, patient groups with lower pCR rates (those with ER+ tumors, those with low grade or lobular cancer) have better overall survival than those that tend to have higher pCR rates. This observation emphasizes the importance of understanding the biological heterogeneity of BC. Studies based on gene expression profiling in BC have confirmed the existence of at least three distinct forms of BC: ER+/HER2-, HER2+, and "triple-negative". These three groups differ by much more than the individual gene (ER or HER2) expression, and their clinical course and responsiveness to different treatments is quite different too. Thus, HER2+ and triple-negative tumors achieve a high pCR rate (40-50%) with standard combinations,

while ER+/HER2- tumors do not (pCR rate <10%). Gene profiling also leads to the identification of potential new therapeutic targets. Validation of such novel targets and development of specific therapeutics might be the best legacy of NST.

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INVITED

### Biological lessons from adjuvant therapy

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Neoadjuvant therapy offers a convenient setting to test for predictive factors to drug response. Primary tumours in general are suitable for tissue sampling and tumour response evaluation. In addition, such studies allow us to draw important conclusion from a limited number of patients. However, while a complete response on primary chemotherapy has been found associated with long term outcome, patients achieving a complete response may still develop distant metastasis. Thus, micro-metastasis may harbour resistant cells not easily detectable in the primary tumour. Further, not every patients progressing locally on therapy are subject to subsequent relapse even in case they are treated with local salvage therapy only. These findings illustrate the complex issue of primary response versus risk of metastases and long-term outcome.

Despite these limitations, neoadjuvant therapy offers an important setting to test for chemoresistance. While success so far has been limited with respect to identify predictive factors validated for clinical use, this is likely to change. Following development of improved methodologies and, in particular, more knowledge about biological parameters to look for, it is likely that such studies may offer unique results in the not-to-far future. With more protocols becoming available and longer patient follow-up, the potential exist to obtain tissue from distant metastasis in patients who subsequently relapse following participation in neoadjuvant trials. Thus, comparing biological parameters of such metastatic deposits to primary tumour biopsies may offer a unique opportunity to identify key biological events involved in a transformation of cells towards resistance.

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INVITED

### Neoadjuvant antiHER2 therapy

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With the success of trastuzumab treatment of HER2 positive metastatic disease, a number of phase II trials were launched evaluating various trastuzumab-chemotherapy combinations in patients with HER2 positive stage II/III breast cancer. These combinations, which utilized trastuzumab in conjunction with agents including paclitaxel, docetaxel, carboplatin, cisplatin, and vinorelbine, demonstrated high response rates and relatively high pathologic complete response rates. A randomized phase II trial from MD Anderson Cancer Center demonstrated a pathologic complete response rate in excess of 50% with a combination of trastuzumab and paclitaxel followed by trastuzumab with an epirubicin-based regimen. While there are multiple possible explanations for the extraordinarily high pathologic complete response rate in this study, the most intriguing explanation relates to the concurrent use of trastuzumab and an anthracycline. A phase III trial has been designed to evaluate this regimen further.

From a research perspective, preoperative studies represent an ideal opportunity to pose translational questions related to HER2 positive disease. To date, preoperative studies have suggested that neither ER status nor HER2 status change frequently in response to trastuzumab-based therapy. Furthermore, it appears that a brief exposure to trastuzumab does not change proliferation rate, but does induce apoptosis. Preliminary work also suggests that there may be a gene signature associated with trastuzumab resistance.

Three randomized trials will soon be open in BIG, the US Intergroup, and the NSABP to compare trastuzumab and lapatinib combinations in the preoperative setting. Not only will these studies compare pathologic complete responses rates, but more importantly, they will collect and interrogate tissue. In this way, it is hoped that the studies will lead to a more complete understanding of factors that predict and/or mediate sensitivity and resistance to HER2-directed therapy.

Recent results and planned trials will be reviewed.

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INVITED

### Biological lessons from pre-surgical endocrine therapy

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Pre-surgical medical studies allow a unique opportunity to integrate clinical and biological observations as a result of the taking of sequential biopsies

from the tumour. Proliferation (as measured by the nuclear antigen, Ki67) is substantially reduced in ER+ primary tumours by SERMs such as tamoxifen and aromatase inhibitors. With aromatase inhibitors at least 90% of patients show a reduction in Ki67 indicating that the large majority of ER+ breast carcinomas have some dependence on oestrogen although the withdrawal of this may lead to an insufficient change to elicit response. No increase in apoptosis has been measured, indeed significant decreases occur with aromatase inhibitors probably as a result of the close association between proliferation and cell death. Changes in Ki67 are only modestly predictive of clinical response but in the IMPACT trial they were predictive of the improved RFS seen with anastrozole over tamoxifen and the combination of tamoxifen and anastrozole. In keeping with this, Ki67 levels after 2 weeks treatment with endocrine therapy is more closely associated with recurrence free survival than pretreatment levels suggesting that the treatment induced change contributes to prediction of long-term outcome. The potential for this to be used for improved prediction of outcome in individual patients will be tested in the PeriOperative Endocrine Therapy for Individualising Care (POETIC) trial. Expression array analysis of biopsies before and after treatment with aromatase inhibitors reveals the profound changes in transcription that result from estrogen deprivation. The changes can be summarised as a Global Index of Dependence on Estrogen (GIDE) that may provide an additional index of benefit from the therapy. It is higher in patients with high ER+ tumours and lower in patients with HER2 positive disease. The study of the expression of other such markers or activity of biological pathways with Ki67 and/or the GIDE as indices of response should shed further light on the importance of the respective markers/pathways. Detailed analysis of the changes in gene expression after estrogen deprivation may identify pathways other than proliferation that determine the progression of estrogen independent disease and its response to endocrine therapy.

## Symposium (Tue, 25 Sep, 14:45–16:45)

### Treatment of advanced colorectal cancer in the era of biologics

**89** INVITED  
**Molecular markers and patient selection in colorectal cancer**

P. Johnston. UK

Abstract not received.

**90** INVITED  
**Signal transduction pathways in colorectal cancer (CRC)**

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An emerging understanding of the molecular pathways that characterize cell growth, cell cycle, apoptosis, angiogenesis and invasion has provided novel targets in cancer therapy. Numerous proteins have been implicated as having a crucial role in CRC. There are different targets according to their cellular localization like: (a) membrane receptor targets (epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGFR), insulin-like growth factor receptor (IGFR), platelet-derived growth factor receptor (PDGFR), tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)); (b) intracellular signaling targets (Ras/Raf/MEK/MAPK pathway, phosphoinositol-3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR), src kinase and the hdm2/p53 complex, among others); and (c) other protein kinases that regulate cell division (Aurora and Polo kinases and cyclin dependent kinases (CDK)). In this session we aim to review the current knowledge of some of these signaling pathways as well as the potential targets for innovative drugging in CRC.

The PI3K/Akt/mTOR pathway controls many cellular processes that are important for the formation and progression of cancer, including apoptosis, transcription, translation, metabolism, angiogenesis and cell cycle progression. The PI3K signaling pathway is upregulated in many CRC, and this upregulation positively correlates with increased tumorigenic potential of colon adenocarcinoma cell lines. Mutations in PI3CA (which encodes the P110 $\alpha$  catalytic subunit) have been identified in up to 40% of CRC. A correlation between PI3K mutations and advanced stage of tumorigenesis, just before cell invasion, has been observed. Increased expression and activation of Akt has been noted in CRC. mTOR downstream and upstream effectors have been shown to be activated in around 1/3 of CRC.

C-src is a non-receptor tyrosine kinase protein overexpressed and activated in many human cancers, including CRC and is associated with advanced-stage and distant metastases. C-src is also of particular interest in colon

cancer because it is overexpressed and/or activated in a wide range of tumors that also overexpress several receptor tyrosine-kinases, indicating the potential role for cross-talk interactions in promoting tumorigenesis. Emerging data from the clinical development of new drugs directed to these targets is providing novel opportunities in the treatment of patients with CRC that will probably translate in efficacy advantage in the next years.

**91** INVITED  
**Optimal strategy for integration of biologics in treatment of metastatic colorectal cancers**

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The management of patients with metastatic colorectal cancer (CRC) has changed dramatically over the last years, with increasing chances of prolonged survival. The development of new cytotoxic and targeted agents as well as the multidisciplinary management of patients with resectable and initially non-resectable metastases contribute to the progress. The development of the cytotoxic agents irinotecan, oxaliplatin and capecitabine and of the biological agents bevacizumab, cetuximab and panitumumab has clearly increased the therapeutic options for patients with metastatic CRC.

It has been shown in randomized phase III trials that bevacizumab, when combined with irinotecan plus bolus 5-FU/LV (IFL) in the first-line treatment of metastatic CRC and with FOLFOX in second-line treatment leads to an increased median survival, progression-free survival (PFS) and response rate (RR) compared to the cytotoxic chemotherapy alone. Moreover, it has been demonstrated in a few randomized phase II studies and in a combined analysis of these phase II studies that bevacizumab increases the activity of 5-FU/LV in the first-line setting. The recent randomized phase III study of FOLFOX compared to capecitabine plus oxaliplatin  $\pm$  bevacizumab in the first-line treatment shows that capecitabine is as effective as IV 5-FU/LV when combined with oxaliplatin and that bevacizumab increases the PFS of the fluoropyrimidine/oxaliplatin combination. The data from phase 2 studies with irinotecan and capecitabine (without bevacizumab) show also a high activity, although more uncertainty remains on the optimal dose of this combination in view of some reports of higher toxicity of the combination capecitabine plus irinotecan.

Cetuximab is active in epidermal growth factor receptor (EGFR)-expressing irinotecan refractory metastatic CRC. The combination of cetuximab with irinotecan is more active in this setting than cetuximab alone. The combination of cetuximab plus irinotecan leads to an increased RR and TTP compared to cetuximab alone in irinotecan-refractory CRC. It has been shown also that panitumumab, a human monoclonal antibody against the EGFR is active in irinotecan- and oxaliplatin-refractory metastatic CRC. The RR of the anti-EGFR antibodies cetuximab and panitumumab as single agent in EGFR expressing chemorefractory CRC is consistently around 10%. In a large phase III trial it was shown that panitumumab increased significantly the PFS compared to best supportive care in EGFR expressing metastatic CRC refractory to oxaliplatin and irinotecan. In another large randomized trial of cetuximab versus best supportive care, cetuximab prolonged the PFS as well as the survival. The PFS of the combination FOLFIRI/cetuximab was significant longer than that of FOLFIRI alone in a phase 3 trial in the first line treatment of CRC.

With this information in mind, bevacizumab is often used in clinical practice in combination with an active cytotoxic regimen in the first-line treatment of metastatic CRC (FOLFIRI or FOLFOX) and cetuximab plus irinotecan in chemorefractory CRC, at least if patients are fit and if there are no contraindications for these therapeutic options.

Many open questions and challenges remain in relation to the use of the anti-VEGF and anti-EGFR antibodies in metastatic CRC. Answers are needed to optimize the outcome for patients and the more optimal use of the resources. A crucial challenge is to demonstrate which patients are more likely to respond to bevacizumab-containing regimens and to the anti-EGFR antibodies cetuximab and panitumumab. Predictive molecular markers for a benefit on angiogenesis inhibitors are not yet available. Despite intensive research, large studies validating predictive molecular markers for response to anti-EGFR antibodies are not yet available in metastatic CRC. The clinical studies evaluating the activity of cetuximab and panitumumab have been carried out in EGFR-expressing tumors, as determined by immunohistochemistry (IHC). The intensity of EGFR immunostaining is not related to antitumor activity, and a clinical benefit has also been noted in patients whose tumors had no EGFR immunostaining. EGFR gene mutations have not been demonstrated to play a role in the response prediction in CRC. Although it has been reported in a small study that EGFR gene copy number, as assessed by fluorescence in-situ hybridization (FISH), correlates with the propensity of CRC to respond to EGFR-directed antibodies, this finding is at the moment very controversial. In a few other retrospective studies K-ras mutations were associated with low activity to cetuximab.