Breast tumor heterogeneity: causes and consequences

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Recently, novel agents, the PARP inhibitors, have been shown to be effective in the phase II trials in women with BRCA1 or BRCA2 mutations and metastatic ovarian or breast cancer. These drugs target DNA repair pathways that are particularly vulnerable in women with BRCA1/2 mutations. The agents may also be effective in women with sporadic breast cancer, and are currently in trials in Europe and the United States alone and in combination with cytotoxic agents.

S1
Identifying and assessing women at high risk for breast cancer
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There has been progress in the identification of factors that confer important risk for the development of breast cancer. The factors include: heritable mutations in susceptibility genes; exposure to therapeutic radiation during breast development (as for Hodgkin’s disease survivors who received therapeutic radiation to the chest); and histologic lesions, including LCIS and atypical hyperplasias.

Testing for mutations in the BRCA1 and BRCA2 breast ovarian cancer susceptibility genes has become part of the established care of breast cancer patients. Genetic information from BRCA1/2 testing is used to help healthy at-risk women to avoid breast and/or ovarian cancer, and ultimately to avoid death from those cancers. Data accumulated over the past decade have provided evidence that breast cancer surveillance can be improved with the addition of breast MRI, that prophylactic oophorectomy substantially reduces the risk of ovarian cancer and, when performed before menopause, can reduce the risk of breast cancer as well, and that prophylactic mastectomy reduces the risk of breast cancer by more than 90%.

It has been observed that approximately 80% of BRCA1-associated breast cancers are negative for ER, PR and HER2 (so-called triple negative) and cluster with basal-like breast cancers by DNA microarray, while 80% of BRCA2-associated breast cancers are ER+ and PR+, but HER2 negative, and luminal. These data are surprising given the close relationships between these genes in their DNA repair activities, and raise some concern that hormonal interventions will not successfully reduce the risk of BRCA1-associated breast cancers. Other strategies may be necessary to reduce breast cancer risk for this group.

Genetic information has been shown to have important implications for women with breast cancer as well. Women with strong family histories of breast and/or ovarian cancer, and women diagnosed before age 40 may consider testing at the time of breast cancer diagnosis if they would use the information to make treatment decisions. Some women choose bilateral mastectomies over breast-conserving treatment if they learn that their risk of second primary breast cancer exceeds 50%, and if their prognosis from the original breast cancer is good. Some women opt for oophorectomy as part of the management of their ER+ breast cancer if they are premenopausal mutation carriers (and could participate in TEXT).

S2
Magnetic resonance imaging for diagnosis, staging, and follow-up
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There is considerable debate regarding the role of magnetic resonance imaging (MRI) in the management of the breast cancer patient. MRI should not be used as a diagnostic test to exclude the presence of carcinoma. In one multi-institutional study of 1,004 women, the positive predictive value of MRI was 72%, and the overall sensitivity 88% [1]. Recognition that MRI identifies additional areas of cancer not detected by other imaging modalities in an average of 16% of breast cancer cases [2] has led to great interest in its use to select women for breast-conserving surgery (BCS). Suggested benefits of MRI include a reduction in margin positivity and conversion from BCS to mastectomy, and a decrease in local recurrence rates. Several retrospective studies and one prospective randomized [3] trial have addressed the impact of MRI on the short-term surgical outcomes. These studies have uniformly failed to demonstrate a benefit for MRI. In the prospective randomized Comparative Effectiveness of Magnetic Resonance Imaging in Breast Cancer (COMICE) trial, re-excision was required in 10% of the MRI group and 11% of the non-MRI group, with conversion to mastectomy in 6% and 8%, respectively. Most studies show that MRI approximately doubles the overall likelihood of undergoing mastectomy without decreasing unplanned mastectomy. Solin and colleagues examined the effect of MRI on local recurrence after BCS with radiation therapy (RT) and on contralateral cancer [4]. At 8 years the incidence of contralateral cancer was 6% in both the MRI and non-MRI groups, and local recurrence was seen in 3% of those with an MRI at diagnosis and in 4% of those without. The repeated observation that MRI finds two to four times as much disease as becomes evident as local recurrence indicates that the majority of this disease is controlled with RT. In addition, the existence of local recurrence after mastectomy indicates that some local recurrence is a manifestation of biologically aggressive disease (first site of metastases), which is unlikely to be influenced by the use of MRI. Current indications for the use of MRI in patients with breast cancer include: known or suspected BRCA1&2 mutation carriers who choose not to undergo bilateral mastectomy; patients presenting with metastases to the axillary nodes and no evident breast tumor; patients with Paget’s disease of the nipple and no evident breast tumor; or the uncommon patient with a major discrepancy between clinical findings and the results of mammography and ultrasound.
A benefit for MRI in the follow-up of the breast cancer patient is also
unproven. Local recurrence after BCS and RT is uncommon, occurring
in fewer than 8% of patients at 10 years. The size of the local
recurrence is not a prognostic factor, and the idea that early detection
will improve prognosis does not reflect the biology of local recurrence.
The treatment of local recurrence regardless of size is mastectomy, and
salvage mastectomy results in local control in 85 to 95% of patients.
These good outcomes must be weighed against the cost of MRI, the
high rate of short-interval follow-up and biopsies generated, and the
lack of a clear rationale for using the examination for follow-up.

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early-stage invasive breast carcinoma or ductal carcinoma in

S3
Genetic profile sets: differences and preferences
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High-throughput genetic analysis of breast cancer results in improved
classification of breast cancer and can be used to identify prognostic
and predictive factors. These prognostic and predictive factors are
increasingly useful to guide therapy decisions in individual breast
cancer patients.

There is a rapidly increasing body of literature on gene expression
signatures associated with prognosis; and on gene expression
signatures associated with response to specific systemic treatment,
including chemotherapy, hormonal therapy and HER2 targeted therapy.
The main prognostic profiles published to date are a 70-gene signature
identified by our group in the Netherlands Cancer Institute (Amster-
dam, the Netherlands); a 76-gene prognosis profile identified by investi-
gators from Erasmus Medical Centre (Rotterdam, the Netherlands; a
21-gene recurrence score developed by the company Genomic Health
(Redwood City, USA); and a genomic-grade signature developed by
investigators at the Institut Bordet (Brussels, Belgium).

Breast cancer is a very heterogeneous disease, and prognostic and
predictive profiles may differ in breast cancer subtypes. We are there-
fore currently performing gene expression profiling studies, assessment
of DNA copy number changes and miRNA expression profiles in triple-
negative breast cancers (n = 96) and HER2-positive breast cancers
(n = 140).

Prognostic classifiers can be used to identify patients that will benefit
most from adjuvant systemic therapy, but other classifiers will be
needed to decide which treatment should be given.

To guide the choice of chemotherapy, hormonal therapy and targeted
therapy, neoadjuvant studies are well suited to identify predictive
factors for therapy response. For this purpose, we have analysed gene
expression profiles in pretreatment biopsies of 191 patients treated
with neoadjuvant chemotherapy; and patients with HER2-positive
breast cancer treated with the combination of chemotherapy and
trastuzumab. Our results and studies from various other groups show
that basal-type/triple-negative tumors show a pathological complete
remission in 30 to 40% of cases; as compared with <5% in luminal-
type tumors. It has been more difficult to identify gene expression
profiles associated with response to chemotherapy and response to
trastuzumab using supervised classification techniques. Research
aimed at the identification of genetic classifiers for responsiveness to
specific systemic therapies is expanding rapidly and should lead to
clinically useful tests in the coming years.

At present, there are several ongoing randomised clinical trials investi-
gating genetic profiling in guiding adjuvant systemic therapy; and in
neoadjuvant systemic therapy. These studies will enable us to better
understand differences between genetic sets; and will allow us to develop
our preferences based on results obtained in large well-controlled trials.

S4
Molecular heterogeneity of breast cancer: implications for treatment and clinical trial design
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Comprehensive molecular analysis of breast cancer indicates that
breast carcinoma is not a single disease with homogeneous
morphology and variable hormone receptor expression, but a collection
of molecularly distinct neoplastic diseases of the breast. The magnitude of
doctoral differences that are seen between estrogen receptor (ER)-positive
and ER-negative cancers at the mRNA expression
or the DNA copy number levels are similar to the extent of
molecular differences that distinguish acute myeloid leukemias from
lymphoid leukemias. The clinical differences between ER-negative and
ER-positive cancers have long been recognised and the recent
 genomic data provided further evidence that these cancers represent
different diseases. Furthermore, two major groups within the ER-
positive cancers can also be easily recognised at the molecular level:
that one corresponds to high-grade, highly proliferative tumors; and
the other to lower-grade cancers with low proliferation rate. These two
molecular subgroups have very different prognosis: the former group is
less sensitive to endocrine therapy and has poor prognosis but is more
sensitive to cytotoxic drugs, and therefore adjuvant chemotherapy may
improve outcome. The latter group has excellent prognosis with
endocrine therapy alone, and does not appear to benefit much from
adjuvant chemotherapy. Several different molecular assays can assist
in distinguishing between these different prognostic subsets of ER-
positive cancers; the most commonly used commercial test in the
United States is Oncotype DX; however, the MammaPrint and
GenomicGrade molecular assays are also able to identify ER-positive
patients with excellent prognosis with endocrine therapy. In the ab-
scence of the statistically more accurate multivariate molecular predic-
tion models, the histologic grade, HER2 status and Ki67 may be used as
a poor man’s alternative to estimate prognosis and chemotherapy
sensitivity of ER-positive cancers. The recognition that breast cancer is
not a single disease has important consequences for clinical trialists
and academic investigators. Different diseases require separate clinical
trials, and different biomarkers may be needed to predict response or
prognosis accurately in the different disease subsets. Different clinical
issues are pressing for the different types of breast cancers, better
systemic therapies are needed for triple receptor-negative cancers,
proper sequencing and length of adjuvant endocrine therapy is an
issue yet to be clarified for endocrine-sensitive ER-positive cancers,
and optimal use of chemotherapy and better drugs are needed for the
proliferation ER-positive cancers. It is also apparent that different
biomarkers are needed for the different cancer subsets. For example,
proliferation-related markers are strongly prognostic and also predictive
of chemotherapy sensitivity among ER-positive cancers but these same
markers are not predictive in ER-negative cancers.

Traditional breast cancer studies where all patients with cancer of the
breast are eligible for the same therapy will soon be regarded as naïve.
as a clinical trial proposal to treat all acute and chronic leukemias with
the same drug in a single trial and perform subset analysis for the
various cytologic types at the end.

S5
Clinical utility and future of genetic profiles for breast cancer
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The era of breast cancer as a single disease, and one-size-fits-all
treatment, has passed. Hormone receptor status has allowed us to
identify two phenotypic subtypes of breast cancer superimposed on
the existing light microscopic histologic classifications, and in recent
years HER2 has added a third axis of categorization. These three
markers (ER, PR, and HER2) share the fact that they are linked
inextricably to treatment decisions as the functional targets of specific
agents and therefore to a degree the treatment has defined the
disease. With the availability of molecular subtyping relying on mRNA
in paraffin-embedded tissue or fresh-frozen material, precise assess-
ments of gene loci for amplification, deletion, or mutation, and the
development of high-throughput techniques, we now are at the begin-
ing of an era when it may be possible and appropriate to generate a
genetic profile for each patient’s tumor such that we will subset breast
cancer further and will tailor therapy for these subsets. Already two
commercial tests are available to clinicians (MammaPrint and OncotypeDx),
each offering prognostic information based on a collection
of genes – and the latter also providing predictive information with
regard to the value of chemotherapy. Each is currently being tested in
prospective studies to provide additional information about how best to
integrate them into routine care. On the horizon are newer and potentially
more informative techniques, such as representational oligonucleotide microarray, a version of comparative genomic
hybridization, which can provide more detail regarding gene amplifica-
tions and deletions. This presentation will review the available
technologies and discuss their potential clinical utility.

SYMPOSIUM II
New surgical approaches

S6
Axillary staging: new approaches and treatment of
minimal disease
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Axillary staging is necessary since 30 to 40% of patients with curable
breast cancer will have metastases in the axilla. Years ago, the most
radical method of staging was performed: an axillary clearance.
However, in patients with a negative axilla, who make up 60 to 70% of
all patients, this lymph node dissection has no additional value. Today,
with the sentinel node procedure it is possible to offer patients
accurate staging of the axilla without the morbidity of axillary clearance.
Discussion has now started about the fact that there are subgroups of
patients in which the risk of axillary metastases is so low that axillary
staging can be omitted. Furthermore, it is standard practice to perform
axillary clearance in patients with positive sentinel lymph nodes.
However, studies show that, of patients with a positive sentinel lymph
node, 40 to 60% have no additional involved lymph nodes.
The following dilemmas, which are all related to minimal disease, are
therefore still open for debate and will be discussed in the
presentation: Is a sentinel node procedure necessary when only
minimal disease is present in the breast? Is axillary treatment necessary
when only minimal disease is present in the node? What is the role of
the PET/CT scan in axillary staging in minimal disease? How can we
stage the axilla after primary systemic treatment when potentially only
still minimal disease is present?

S7
Surgical management of the breast and axilla after
neoadjuvant treatment: the role of sentinel node
biopsy
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Several unique surgical issues arise in the management of patients
who are selected to receive neoadjuvant systemic therapy. These
involve the original diagnostic assessment of the extent of disease in
the breast and axilla, the preoperative planning, and ultimately the
surgical management of the primary breast tumor and that of axillary
lymph nodes. Careful consideration of these issues is critical in order to
maximize local control of the disease, while minimizing the extent of the
required surgical resection and the ensuing surgical morbidity.
Adequate diagnostic assessment with core needle biopsy before
initiation of neoadjuvant systemic therapy ensures the presence of
invasive carcinoma and provides adequate material for routine
biomarker evaluation (such as ER, PR and HER-2 neu), while minimally
disturbing the primary breast tumor. Consideration should also be
given to assessing the status of axillary nodes by minimally invasive
techniques such as ultrasound of the axilla with fine needle aspiration
of suspicious nodes. Optimal preoperative planning aims at accurately
determining the patterns of primary tumor shrinkage and the amount
and location of any residual disease in the breast.
Surgical treatment after neoadjuvant systemic therapy focuses on the
management of the primary breast tumor and that of axillary lymph
nodes. Regarding the primary breast tumor, several studies have
shown that neoadjuvant systemic therapy converts a proportion of
mastectomy candidates to candidates for breast-conserving surgery.
Neoadjuvant systemic therapy can also decrease the amount of breast
tissue that needs to be removed at lumpectomy even in patients who
are lumpectomy candidates at presentation.
Neoadjuvant systemic therapy (primarily neoadjuvant chemotherapy)
downstages axillary lymph nodes in up to 30 to 40% of the patients.
Although this observation was of little clinical significance when axillary
node dissection was the sole method for staging the axilla, the
development and validation of sentinel lymph node biopsy (SNB) has
provided an additional potential advantage for neoadjuvant chemother-
apy; that is, the possibility of decreasing the extent and morbidity
of axillary surgery. This approach is, naturally, predicated on the premise
that SNB is feasible and accurate following neoadjuvant chemother-
apy. Initially, small, single-institution studies examined the efficacy
of lymphatic mapping and the accuracy of SNB after neoadjuvant
chemotherapy with significant variability in the rate of SN identification
and in the rate of false negative SN [1]. However, when these studies are
examined collectively [1,2] or when larger, multicenter datasets are
analyzed [3], SNB after neoadjuvant chemotherapy appears to have
similar performance characteristics to those of SNB before systemic
therapy [4-6].
Some have proposed that candidates for neoadjuvant systemic therapy
should have SNB before, rather than after, neoadjuvant systemic
therapy so that information on the status of the axillary nodes be
obtained without the potential confounding effects of systemic
treatment, and sentinel node-negative patients can avoid axillary
dissection [7-9]. Although this approach may be useful in patients who
will not need systemic therapy (that is, chemotherapy) if the SN is
negative, it is not generally useful for the majority of candidates for
neoadjuvant systemic therapy, for whom little – if any – is to be gained
by knowing the pathologic nodal status upfront. In addition, this
approach commits patients to two surgical procedures and does not
take advantage of the downstaging effect of neoadjuvant chemotherapy on the axillary nodes.

References

S8 Immediate versus delayed repair of partial mastectomy defects in breast conservation

S9 Prophylactic contralateral surgery: current recommendations and techniques

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The use of contralateral prophylactic mastectomy (CPM) from both invasive cancer and ductal carcinoma in situ is increasing in the United States [1], in spite of the fact that only 3% of breast cancer patients in the Surveillance, Epidemiology, and End Results database developed a contralateral cancer at 5 years [2]. Endocrine therapy and trastuzumab, both widely used as adjuvant therapy, reduce the incidence of contralateral cancer by about 50%, suggesting that the risk of contralateral cancer in women treated today is quite low.

Most guidelines for prophylactic mastectomy address women at high risk, not women with unilateral cancer. Known or suspected BRCA mutation carriers who develop unilateral cancer have a 40 to 60% risk of developing a contralateral cancer, and CPM is accepted as appropriate therapy in this circumstance.

To address the issue of what kind of women undergo CPM, we identified 477 patients who had the procedure at Memorial Sloan-Kettering Cancer Center within 1 year of a unilateral cancer diagnosis and compared them with 2,500 women undergoing unilateral mastectomy in the same period (1997 to 2005). CPM patients were younger (median 46 vs 53 years; P<0.0001), had smaller tumors (1.2 vs 1.8 cm; P<0.0001), and were less likely to have positive nodes (47 vs 57%; P<0.0001). Hormone receptor and HER2 status did not differ between cases and controls. Although 68% of CPM patients reported a family history of breast cancer, only 2% underwent genetic testing. Of these, 34 (7%) were found to have a mutation. The pathology specimens of 6% of patients undergoing CPM were found to contain malignancy (eight invasive, 20 ductal carcinoma in situ). When CPM is performed, it should be done to the same anatomical limit as a therapeutic mastectomy. Skin sparing to facilitate reconstruction is appropriate, but flaps should be the same thickness as is used in a therapeutic mastectomy. The use of nipple sparing is controversial [3].

In order to maintain a blood supply to the nipple areolar complex, some breast tissue must be left behind. Most studies of local recurrence after nipple preservation have been limited to patients with breast cancer, and little is known about its use in the prophylactic setting, particularly regarding risks in BRCA carriers. However, local recurrence is uncommon after nipple sparing for cancer treatment, and improved body image and psychological adjustment after nipple sparing has been reported [3], making this option worthy of consideration in patients undergoing CPM.

References
**SYMPOSIUM III**

**Advances in radiotherapy**

**S10**

**Other new radiotherapy techniques**

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**Objective** To report the NYU research on novel radiation therapy of breast cancer. Radiation therapy has enabled effective breast preservation in the majority of newly diagnosed breast cancer patients. This milestone in the history of breast cancer management is currently revisited to identify the optimal selection of target and fractionation, while assuring minimal radiation exposure of normal tissues adjacent to the breast.

**Methods** Five consecutive prospective trials explored hypofractionated, accelerated regimens of breast radiotherapy that also aim at optimal normal tissue sparing.

**Results** After pilot-testing a 3-week prone regimen of IMRT to the breast with a concomitant boost to the tumor bed, we have prospectively studied in a cohort of 400 women whether a prone versus a supine setup for treatment was superior at sparing lung and heart tissue, while assuring target (index breast) coverage. The results of this trial indicate that the prone setup is superior in >90% of patients.

The prone setup also characterizes our two studies of partial breast irradiation delivered over five fractions of 6 Gy each. At NYU this approach is only offered to the subset of patients at the lowest risk of irradiation delivered over five fractions of 6 Gy each. At NYU this patients.

**Conclusions** The IMPACT trial, in which over 300 ER+ patients were treated with tamoxifen or anastrozole or the combination, revealed that change in the proliferation marker Ki67 was greater with anastrozole than with either of the other arms, a result parallel to the drug’s greater effectiveness in the far larger and longer ATAC adjuvant trial. In addition, Ki67 after 2 weeks was more predictive of recurrence-free survival than pretreatment Ki67 [1]. The value of on-treatment Ki67 as an index of long-term outcome is being studied in detail in the 4,000-patient Perioperative Endocrine Treatment for Individualised Care (POETIC) trial. The combination of on-treatment Ki67 with standard clinical features has allowed the derivation of a Preoperative Endocrine Therapy Index, which identified a group of patients with a very low likelihood of relapse on endocrine treatment alone [2]. Most recently we have created expression array data from over 100 patients treated with anastrozole. The quantitative expression of ER at a transcript level correlated strongly with the decrease in Ki67 and a Global Index of Dependence on Estrogen such that tumours with low ER showed little reaction to oestrogen deprivation. However, not all tumours with high ER expression showed high oestrogen dependence. Some of the less dependent tumours were HER2-positive but other mechanisms must account for the discordance in others. The POETIC trial should help to identify molecular factors associated with resistance to aromatase inhibitors.

**References**


**SYMPOSIUM IV**

**Changes in preoperative treatment**

**S11**

**Biomarkers and predictive factors of response to neoadjuvant treatment**

M Dowsett1, A Dunbier1, H Anderson1, J Salter1, S Detre1, R Jones1, A Skene2, M Dixon3, IE Smith1

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**Introduction** Neoadjuvant therapy provides a unique and powerful opportunity to derive biopsy material before, during and subsequent to the treatment of otherwise untreated breast cancers and, by measuring the expression of biomarkers in these, to study the biology of the disease in vivo. We have conducted such studies involving endocrine therapy or chemotherapy and have focused on the concept that measurement of change in expression of critical biomarkers shortly after starting therapy may be more closely associated with clinical outcome than before therapy.

**Endocrine therapy** The IMPACT trial, in which over 300 ER+ patients were treated with tamoxifen or anastrozole or the combination, revealed that change in the proliferation marker Ki67 was greater with anastrozole than with either of the other arms, a result parallel to the

**S12**

**Adjuvant therapy after preoperative treatment for breast cancer**

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The increase in the use of preoperative chemotherapy has raised new questions regarding how to optimize locoregional and systemic adjuvant treatment. When patients are given preoperative systemic therapy, the preferred therapeutic regimens are the same as those established as safe and active in the adjuvant setting. At present, no data suggest that systemic treatment should be tailored, in one direction or another, based on initial tumor response or lack thereof (except for frank disease progression while on treatment), or based on the extent of residual disease.

Adjuvant locoregional and systemic treatment after preoperative chemotherapy for breast cancer is still controversial. No high-quality data from prospective trials are available; nevertheless, locoregional therapy decisions should be based on both pretreatment and the clinical extent of disease. Sentinel node biopsy can be performed before and after preoperative therapy in patients with clinical N0...
disease at diagnosis. It is not clear whether resection margins should be differently evaluated after preoperative therapy than in the standard setting. The success of breast-conserving surgery depends on careful patient selection and on an adequate surgical technique that achieves negative margins. Adjuvant breast irradiation is indicated for all patients who undergo breast conservation; for patients treated with mastectomy, chest-wall and regional nodal radiation must be done in those who present with clinical stage III disease or who have histologically positive lymph nodes at diagnosis.

Additional studies are needed to determine the value of postmastectomy irradiation in clinical stage II breast cancer, as well as to determine the convenience of adjuvant systemic therapy in patients who do not achieve a good pathological response with preoperative treatment. Multidisciplinary treatment teams are critical in order to improve therapeutic management of these patients.

SYMPOSIUM V

Advances in adjuvant therapy

S13 Adjuvant therapy of HER2+ breast cancer
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Adjuvant therapy of HER2+ breast cancer has evolved dramatically over the past 4 years. It is now widely appreciated that treatment with trastuzumab, when administered concurrently or sequentially with chemotherapy, will decrease the risk of disease recurrence by approximately 50%. Clinical trials have also demonstrated a modest improvement in overall survival that is anticipated that this survival advantage will increase with further follow-up. Nevertheless, there are patients who will experience disease recurrence in spite of optimal trastuzumab-based therapy, and new treatment approaches are needed for these individuals. The results of the initial randomized trials have also given rise to a number of difficult questions that clinicians and patients face on a daily basis. The presentation will focus on controversial topics in the adjuvant treatment of HER2+ disease, and patients face on a daily basis. The presentation will focus on the adjuvant setting will also be discussed.

S14 Controversies in hormonal adjuvant therapy for premenopausal patients
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The major controversy concerning adjuvant hormonal therapy for premenopausal patients is the absence of appropriate clinical trial data to indicate the appropriate way to treat patients presenting with oestrogen receptor (ER)-positive operable breast cancer. This is despite the fact that the first randomized trial in breast cancer, begun in 1947, was a comparison of ovarian irradiation versus no further treatment after primary surgery for breast cancer [1] and the availability of recent overviews [2,3]. The Oxford overview indicates that tamoxifen is as effective in premenopausal women as in postmenopausal women. It also indicates that ovarian ablation is effective alone but not in addition to chemotherapy [3]. Subsequent studies indicate that ovarian suppression is effective in addition to chemotherapy in young women who do not develop chemotherapy-induced amenorrhoea. Multiple randomised studies indicate in patients with ER-positive tumours that ovarian suppression with or without tamoxifen is as effective as chemotherapy. All of these studies have been of poor design since none has had a third arm where both endocrine therapy and chemotherapy are given. It is likely that in the chemotherapy-alone arm there would be additional patients who would have responded to endocrine therapy and vice versa so that, although no differences between endocrine and chemotherapy were seen in these trials, it is not logical to conclude that either treatment alone is optimal without appropriate trial data. Because trials comparing methods of ovarian suppression have relatively few patients, we do not know the most effective method. LHRH agonists are associated with temporary ovarian suppression. The ZEBRA trial demonstrated that 2 years of goserelin was as effective as chemotherapy, but in this and subsequent trials of the use of LHRH agonists we do not have a clear indication of the duration of treatment required. Finally, no adequate trials indicate whether an LHRH agonist adds to the effectiveness of tamoxifen as adjuvant therapy. Given the uncertainties of treatment of premenopausal ER-positive early breast cancer, it is vital that patients are entered into appropriate trials such as SOFT.

References
more potent endoxifen, indicate a poorer efficacy for tamoxifen and therefore greater relative benefit from an AI. The decision to deliver adjuvant chemotherapy in addition to endocrine treatment is judged largely on the basis of prognosis after considering the impact of the endocrine therapy. While the RS is a validated instrument for this purpose, the prognostic effect of markers such as PgR, HER2 and Ki67 is substantial and may allow the development of widely applicable immunohistochemical approaches to risk evaluation.

References

SYMPOSIUM VII
Bone-targeted therapies

S16
Targeting the tumour microenvironment: denosumab, a new RANKL inhibitor
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Bone is the most common site for metastasis and is of particular clinical importance in breast cancer, which is common and associated with a relatively long clinical course. Metastatic bone disease results from the interactions between cancer cells in the bone marrow microenvironment and normal bone cells rather than direct destruction by cancer cells. These growth factor and cytokine-mediated interactions typically lead to stimulation of both osteoclast and osteoblast function with uncoupling and imbalance in bone remodelling. This provides the rationale for bone-targeted therapies to reduce the risk of skeletal complications such as fracture, and to relieve bone pain. Additionally, bone-derived growth factors released from bone promote a fertile environment for the survival and proliferation of cancer cells, creating a vicious cycle of bone destruction. Receptor activator of NF-κB ligand (RANKL) is a key mediator in this process. Within the bone microenvironment, factors secreted by tumour cells stimulate stromal cells and osteoblasts to secrete RANKL, which binds to its cognate receptor RANK on the surface of precursor and mature osteoclasts. RANKL is a critical mediator of osteoclast differentiation, function, and survival.

Denosumab is a fully humanised monoclonal antibody that inhibits RANKL. A dose of 120 mg, 4-weekly, administered by subcutaneous injection has been defined in a large dose-finding randomised phase II study for the treatment of advanced malignancy [1]. To prevent treatment-induced bone loss, an osteoporosis dose and schedule of 60 mg every 6 months has been evaluated, and was shown to prevent aromatase inhibitor-induced bone loss [2]. The large phase III trials in metastatic bone disease comparing denosumab with zoledronic acid have completed accrual and will report in the next year. Preclinical data suggest that denosumab is a more complete inhibitor of osteoclast function than the bisphosphonates (BPs). Additionally, a randomised phase II study in patients with increased bone resorption despite ongoing BPs has compared changing to denosumab, an antibody to RANK ligand, with continuation of the BP. This showed rapid and sustained biochemical response in >60% of patients with denosumab compared with <30% for those on standard BP treatment. Additionally, the number of skeletal events appeared to be less in the denosumab-treated patients [3]. The Austrian Breast Cancer Study Group are conducting a large randomised trial in postmenopausal women receiving endocrine treatment with the 60 mg every 6 months schedule. This is primarily to evaluate effects on bone mineral density and fractures, but with disease-free survival as a secondary endpoint. Other metastasis prevention trials are planned.

References

S17
Bisphosphonates in the adjuvant treatment of early breast cancer
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Bisphosphonates are the standard of care for preventing skeletal morbidity and treating hypercalcemia of malignancy in patients with bone metastases. Zoledronic acid (intravenous; 4 mg monthly) is approved to prevent skeletal-related events in patients with bone metastases from several tumor types, and can improve survival in some subsets of patients with skeletal metastases and high baseline bone turnover. In the adjuvant setting, bisphosphonates have shown clinical efficacy for preventing cancer treatment-induced bone loss and promise for reducing disease recurrence. For example, early studies of clodronate showed the potential for bisphosphonates to prevent bone metastases and prolong survival, but results with clodronate have been inconsistent. Recently, the more active bisphosphonate zoledronic acid (4 mg every 6 months) prevented bone loss and significantly reduced the risk of disease-free survival events by 36% (P = 0.01) compared with adjuvant endocrine therapy alone in a large phase III trial (n = 1,803) in premenopausal women with early breast cancer [1]. Notably, these benefits were not limited to bone because the addition of zoledronic acid reduced disease recurrence at all sites. This fuels the See-and-Soil hypothesis about dormant tumor (stem) cells in early disease, and hints towards a potential impact of bisphosphonate treatment on the bone marrow microenvironment. These results of twice-yearly zoledronic acid have been confirmed indirectly in bone-protection trials in postmenopausal patients [2]. In addition, several ongoing trials (involving more than 20,000 patients altogether) are evaluating the efficacy of bisphosphonates for the prevention of metastases in breast, prostate, and lung cancers, and multiple myeloma. Results from these studies are likely to expand the role of bisphosphonates, particularly zoledronic acid, in the adjuvant therapy setting, and help us in elucidating the underlying biology as well as resolving open clinical questions.

Acknowledgements The authors acknowledge the invaluable contribution of our patients who contributed to ABCSG-12 and other ABCSG trials as well as the work of all ABCSG investigators, study nurses, and data-management associates, both in the individual trial centers and in the ABCSG center. ABCSG-12 is an academic trial that received support from AstraZeneca and Novartis.

References
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S18
Breast tumor heterogeneity: causes and consequences
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Introduction
In breast and other cancer types, a high degree of diversity exists among and within tumors. Breast tumors are composed of a variety of cancer cells with distinct phenotypes and genotypes. The molecular mechanisms responsible for this intratumoral heterogeneity are not well defined. Two currently popular hypotheses that attempt to explain intratumoral heterogeneity are the cancer stem cell and the clonal evolution models. Each of these concepts has been investigated for some time, leading to the accumulation of findings that support one or the other. Although the two views share some similarities, they are fundamentally different notions with very different implications for clinical practice.

Methods
To characterize cells with stem-like characteristics, we determined the gene expression, DNA methylation, and genetic profiles of distinct cell populations purified from breast carcinomas and normal breast tissue using cell surface markers CD24 and CD44 that have been associated with stem cell-like properties. Gene expression profiles were analyzed using serial analysis of gene expression, DNA methylation profiles by methylation-specific digital karyotyping, whereas genetic alterations were investigated using SNP arrays and fluorescence in situ hybridization.

Results
SNP array and methylation-specific digital karyotyping analyses suggested that CD24+/CD44− and CD24−/CD44+ cells from the same tumor are clonally related, but can be both genetically and epigenetically distinct. CD44+ cells have an activated TGFβ signaling pathway, whereas it is decreased in CD24+ cells due to TGFβR2 promoter hypermethylation. As a consequence of this, CD44+ cells specifically respond to an inhibitor of TGFβR, and acquire more differentiated epithelial cellular morphology and membrane localization of E-cadherin and β-catenin. Furthermore, gene expression profiling revealed that breast cancer patients with lymph node-negative breast tumors that have a higher fraction of CD44+ cells had shorter overall survival as well as shorter distant metastasis-free survival. In contrast, CD24+ cells appeared to be more prevalent in distant metastases even when the primary breast tumor was enriched for CD44+ cells. This suggests that the tumor cells may be altered during the metastatic process, or that CD24+ breast cancer cells are intrinsically more competent for metastasis.

Conclusions
The results of our recent studies investigating breast cancer cells with stem cell characteristics and the genetic diversity of cellular populations within tumors imply multiple layers of heterogeneity and a combination of different molecular mechanisms underlying breast tumor heterogeneity. Understanding these molecular mechanisms will facilitate the development of more effective ways to treat and prevent breast cancer.

References

SYMPOSIUM VIII

New drugs beyond herceptin

S19
Neratinib, an irreversible pan ErB receptor tyrosine kinase inhibitor active for advanced HER2+ breast cancer
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Neratinib (HKI-272) is a dual inhibitor of the tyrosine kinase receptors, erbB1 (EGFR) and erbB2 (HER2). In a phase I study, neratinib was tolerable and demonstrated antitumor activity in patients with solid tumors, including 8 of 26 evaluable patients with erbB2-positive advanced breast cancer. In an open-label, two-arm phase II study, patients with stage IIIB, IIIC or IV erbB2-positive advanced breast cancer were evaluated to further characterize the safety and efficacy of neratinib. The maximum tolerated dose of once-daily oral neratinib is 320 mg. The most common neratinib-related toxicity was diarrhea. Antitumor activity was observed in patients with breast cancer who had previous treatment with trastuzumab, anthracyclines, and taxanes, and tumors with a baseline ErbB-2 IHC staining intensity of 2+ or 3+.

Neratinib has demonstrated a potent activity against trastuzumab-resistant and trastuzumab-naive HER2-positive breast cancer in a phase II study that evaluated the safety and efficacy of a daily 240 mg oral dose on 136 women with locally advanced or metastatic breast cancer (stage IIIB, IIIC or IV). The 16-week progression-free survival (PFS) rate was the primary end point. Secondary end points included safety, objective response rate and clinical benefit rate. Patients were assigned to one of two study arms based on prior treatment with trastuzumab. The efficacy analysis included 127 evaluable patients, 61 in arm A and 66 in arm B. In patients who were previously treated with trastuzumab (arm A), the 16-week PFS rate was 60%, and the median PFS was 23 weeks. The objective response rate was 26%, and the clinical benefit rate was 36%.

At the present time, several phase II studies of neratinib in combination with chemotherapy (capcitabine, vinorelbine, paclitaxel and doxorubicin) are ongoing, some of them already finished. The tolerance and efficacy will be presented. A large worldwide randomized phase III study of the combination of trastuzumab versus neratinib plus paclitaxel in chemonaive advanced HER2-positive breast cancer patients will start very soon in order to determine the efficacy of neratinib compared with standard treatment.

References
SYMPOSIUM IX

New targets and new drugs

S20

Future role of bevacizumab in breast cancer

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In the past years, anti-angiogenic therapies have rapidly developed for many solid tumors, including breast cancer.

The first phase III trial of bevacizumab in combination with capecitabine versus capecitabine alone was conducted in 462 patients with MBC previously treated with an anthracycline or taxane: although the addition of bevacizumab did not improve PFS, there was an absolute increase of approximately 11% in ORR (20 vs 9%; P = 0.001) [1]. In the E2100 trial the addition of bevacizumab to first-line weekly paclitaxel resulted in doubling of both the objective RR (38.9 vs 21.2%; P < 0.0001) and median PFS (11.8 vs 5.9 months; P < 0.0001), compared with paclitaxel alone in more than 700 HER2-negative MBC patients, although there was no impact on OS [2]. These findings were corroborated by more complete analyses for regulatory purpose and confirmed by IRF. The AVADO phase III trial compared bevacizumab with placebo in combination with docetaxel as first-line chemotherapy for patients with HER2-negative locally recurrent breast cancer or MBC, showing a significant improvement in PFS (8.8 months for bevacizumab 15 mg/kg vs 8 months for docetaxel; P = 0.0001) [3]. These results have led to the development of multiple phase III trials of bevacizumab in combination with chemotherapy agents in first-line MBC, along with trials investigating the addition of bevacizumab to other anticancer therapies such as trastuzumab and endocrine agents.

To date no predictive biomarkers for benefit from bevacizumab have been identified; several ongoing trials incorporate molecular studies with the aim of targeting the correct subset of patients (such as triple-negative patients) for bevacizumab therapy. Clinical trials are underway to evaluate the use of bevacizumab in the adjuvant and neoadjuvant setting. The BEATRICE phase III trial will assess the benefit of adding bevacizumab to standard adjuvant chemotherapy (anthracycline ± taxane or taxane only) in triple-negative early breast cancer. The addition of bevacizumab to adjuvant chemo-therapy (docetaxel/carboplatin or docetaxel+–FEC) and trastuzumab will be studied in a further phase III trial (BETH) in patients with HER2-positive breast cancer. Other phase III adjuvant trials are evaluating the addition of bevacizumab to standard chemotherapy in HER2-negative and in node-positive or high-risk breast cancer. The NSABP B-40 ongoing phase III trial will evaluate the addition of bevacizumab to either neoadjuvant docetaxel, docetaxel plus capecita-bine or docetaxel plus gemcitabine followed by doxorubicin plus cyclophosphamide in patients with operable breast cancer. The phase III GeparQuinto trial will evaluate the integration of bevacizumab, everolimus and lapatinib into current neoadjuvant regimens [4]. Bevacizumab will probably have an established role in the treatment of some subgroups of MBC and as adjuvant therapy in early breast cancer; we need to identify which subgroups of patients may specifically benefit from bevacizumab therapy.

References


S21

nab-Paclitaxel (Abraxane®): an albumin-bound cytotoxic exploiting natural delivery mechanisms into tumors

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Taxanes (paclitaxel and docetaxel) are highly active chemotherapeutic agents in the treatment of breast cancer. Being hydrophobic, taxanes require solvents (Cremophor EL or polysorbate) to enable parenteral administration. These solvents contribute to the main toxicities seen with taxanes (hypersensitivity, peripheral neuropathy, and myelo-suppression). Cremophor EL can also leach plasticizers from polyvinyl chloride tubing, which can result in severe, sometimes fatal, infections. Currently, paclitaxel is administered by intravenous infusion over 3–6 hours. nab-Paclitaxel is a solvent-free, albumin-bound nanoparticle formulation of paclitaxel that takes advantage of the increased delivery of albumin to tumors through receptor-mediated transport called transcytosis. nab-Paclitaxel binds to gp60, the albumin receptor on endothelial cells, which in turn activates caveolin-1 and the formation of caveolae. Caveolae transport the albumin–paclitaxel conjugate to the extracellular space, including the tumor interstitium. In the tumoral interstitium, SPARC (secreted protein, acidic and rich in cysteine) is selectively secreted by the tumors and binds to albumin-bound paclitaxel with the resultant release of paclitaxel in the vicinity of tumor cells. Together the absence of solvents and the receptor-mediated delivery result in decreased toxicity and increased antitumor activity of nab-paclitaxel compared with solvent-based paclitaxel [2].

nab-Paclitaxel (Abraxane®) has been approved for the treatment of metastatic breast cancer (MBC), based on a phase III trial in 460 patients comparing 260 mg/m² nab-paclitaxel administered over 30 minutes every 3 weeks (Q3W) without premedication with 175 mg/m² solvent-based paclitaxel given over 3 hours Q3W with premedication. Overall response rates were 33% and 19% and the median time to progression was 23.0 weeks and 19.6 weeks, respectively. A significant difference was reported in overall survival in patients receiving nab-paclitaxel versus solvent-based paclitaxel in ≥2nd lines of treatment (56.4 vs 46.7 weeks, respectively) [3]. Weekly schedules of nab-paclitaxel have proven more effective than Q3W schedules in a randomized phase II study in MBC. Furthermore, outcomes were more favorable for weekly nab-paclitaxel than for 100 mg/m² docetaxel administered Q3W, with significantly less toxicity [4]. Development of weekly nab-paclitaxel continues in MBC, NSCLC, melanoma, and pancreatic cancer.

References


Molecular mechanisms of resistance to HER2-targeted therapy

S22

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The human epidermal growth factor receptor 2 (HER2, also known as ErbB-2) is amplified in 20 to 25% of invasive breast cancers. HER2 overexpression has been associated with poor survival rates [1]. Trastuzumab (Herceptin) is a monoclonal antibody directed against domain IV of the extracellular domain of HER2. Lapatinib (Tykerb) is a small-molecule tyrosine kinase inhibitor targeted against the intracellular domain of HER2 (ATP binding site). Both agents produce objective response rates in 20 to 30% of patients with HER2-positive metastatic breast cancer. Efficacy is improved when these targeted therapies are combined with chemotheraphy or endocrine therapy. However, the majority of patients that respond to trastuzumab or lapatinib develop progressive disease within 1 year of treatment initiation. Trastuzumab has been shown to improve survival rates in women with early-stage breast cancer. Approximately 15% of patients develop metastatic breast cancer despite adjuvant trastuzumab, chemotherapy and endocrine therapy [2]. Potential molecular mechanisms of HER2-targeted therapy include increased signaling via the phosphatidylinositol 3-kinase/protein kinase B (PI3K) pathway through overexpression or cross-talk between HER2 and the insulin-like growth factor-I receptor or PTEN loss. Decreased interaction between trastuzumab and HER2 may be caused by steric hindrance of the HER2 receptor by MUC-4, or by the presence of a truncated HER2 protein, which may block inhibitory actions of trastuzumab [3]. One of the mechanisms of action of trastuzumab is the induction of antibody-dependent cellular cytotoxicity, which may play an important role in the adjuvant setting. In patients with metastatic breast cancer, continuation of trastuzumab beyond progression results in prolongation of the time to progression [4]. The mechanism of action of trastuzumab in this setting is not known. Novel therapies targeted against these aberrant molecular pathways are being studied in laboratory and clinical settings, and offer hope that the efficacy and duration of response to trastuzumab can be greatly improved. These include pertuzumab, a monoclonal antibody that targets domain II of the HER2 extracellular domain and prevents heterodimer formation between HER2 and HER3 or EGFR. Trastuzumab-Dm1, a potent antibody-drug conjugate that is effective in the setting of resistance to multiple lines of HER2-directed therapy; Hsp90 inhibitors that degrade the HER-2 protein (for example, 17-AAG); irreversible small molecule tyrosine kinase inhibitors (for example, HKI-272); and IGF-IR inhibitors. Indirect approaches include immunotherapy and anti-angiogenic therapy. Understanding mechanisms of resistance in vivo will help us identify what is the optimal treatment for individual patients.

References
Results Two hundred and fifty women have been enrolled, stratified and studied, as described previously, and we are evaluating whether they are affected by metabolic syndrome and how it impacts on the onset of breast cancer.

Conclusions Metabolic syndrome can be considered an important risk factor in developing breast cancer. Weight control, reduction of insulin serum levels and a correct lifestyle should be praised as efficient instruments to prevent breast carcinoma.

P3 HER2 assessment using fluorescence in situ hybridization compared with Oncotype DX and association with risk of breast cancer death
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Objective Guidelines mandate demonstration of 95% concordance to another laboratory or method before reporting patient HER2 results. In this analysis, HER2 results obtained with Oncotype DX, a validated 21-gene recurrence score assay utilizing RT-PCR, were compared with standard fluorescence in situ hybridization (FISH) results. The Oncotype DX HER2 results were then associated with relative risk of breast cancer death.

Methods Breast cancer specimens from the Kaiser Oncotype DX study were evaluated for HER2 by FISH with positive >2.2, equivocal 1.8 to 2.2, and negative <1.8. HER2 was also assessed with Oncotype DX (RT-PCR) with positive ≥11.5 units, equivocal >10.7 to <11.5 units, and negative ≤10.7 units (each unit = twofold change in expression). Concordance analyses were conducted following ASCO/CAP guidelines that mandate 95% concordance. Logistic regression was used to estimate association between Oncotype DX HER2 results and risk of breast cancer death.

Results Of 568 patients, 12% (67 patients) were HER2+ by Oncotype DX and 11% (60) by FISH. Fifty-five patients were HER2+ by both methods. Of the 12 patients Oncotype DX HER2+ but FISH negative, using FISH 11 patients were HER2+ and one patient was HER2 equivocal. The positive and negative HER2 concordance by FISH and Oncotype DX was 97% (95% CI = 96% to 99%). Risk of breast cancer death was significantly greater in patients with HER2 ≥11.5 (OR = 1.84, 95% CI = 1.13 to 2.99) compared with patients with HER2 ≤10.7. However, in patients with HER2 >10.7 to <11.5 compared with HER2 ≤10.7, there was no greater risk (OR = 0.75, 95% CI = 0.46 to 1.20). There were 71 (12.5%) polyosy cases.

Conclusions There is a high degree of concordance between Oncotype DX and standard IHC methods for ER, PR and HR status, indicating Oncotype DX may be a valid alternative to IHC. The notable incidence of IHC HR-negative patients who were Oncotype DX HR-positive, which could affect treatment decisions, deserves further study.

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Disclosure FLB is an employee and shareholder of Genomic Health, Inc.

P5 Breast cancer presenting as axillary abscess and unilateral lymphedema
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Introduction This is a case report illustrating the initial presentation of breast cancer as an axillary abscess and ipsilateral arm lymphedema.

Case report A 62-year-old Indian lady presented with the main complaints of left arm swelling and pain of 6 months duration. Clinical examination revealed gross right upper limb lymphedema; associated with tender axillary abscess with possibility of infected lymphadenopathy. Right breast examination revealed multifocal hard breast lumps with a retracted nipple. Ultrasound and mammography of the breasts showed an edematous right breast with several suspicious nodules. As the patient was septic and symptomatic from the axillary abscess, we performed an axillary drainage and axillary lymph node biopsy, which proved positive for adenocarcinoma. Ultrasound-guided core biopsy of the breast lesion was invasive ductal carcinoma. The estrogen and progesterone receptors were negative, c-erb2 was positive. Metastatic work-up showed that the patient had bilateral pulmonary metastases with bony metastases as well.

Conclusions The present case highlights the fact that, despite a breast screen programme established in Singapore, there are patients presenting with locally advanced cancers. This case is particularly interesting as she presented as a surgical emergency for an axillary abscess.
study were to determine the epidemiological features of premenopausal patients with primary breast carcinoma diagnosed in our unit for the past 20 years, and to evaluate any significant changes observed during this period.

Methods Between January 1988 and December 2008, 162 premenopausal patients with primary breast carcinoma were evaluated at our center. A total of 85 patients came prior to January 2000, and 77 patients between January 2000 and December 2008. We used statistical parametrical tests (Student t test and chi-square test) to compare mean age at the time of diagnosis, maternal age at first pregnancy, parity, lapse time between last delivery and diagnosis of cancer, staging, histological tumor grade, and whether the diagnosis was made based on on-demand screening mammography or based on clinical findings.

Results No significant differences were found in female age at the time of diagnosis (43.1 ± 5.2 years vs 43.6 ± 4.6 years). There were significant differences in maternal age at first pregnancy (27.2 ± 4.6 years vs 31.5 ± 4.4 years), lapse time between last delivery and cancer (11.9 ± 6.3 years vs 9.3 ± 6.1 years), parity (54% vs 45% in multiparous patients), staging (47% vs 56% stage 0 or I), histological grade (26% vs 42% grade III tumors) and diagnosis (43% vs 57% by screening).

Conclusions The results of this study indicate that female age at the time of diagnosis has remained constant for the past two decades while maternal age for first delivery has increased. The lapse time until cancer diagnosis decreased from the last delivery. In the past decade, on-demand screening programs have helped us diagnose more cases that were at lower stages even if they were of higher histological grade.

P7 Breast carcinoma: molecular markers and subtypes to predict patients at risk of developing metastatic disease

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Objective To identify molecular markers and molecular subtypes in breast carcinoma that predict patients at higher risk of developing bone, brain, and visceral metastasis.

Methods Immunohistochemical analysis using a panel of antibodies against ER, PR, Her2, EGFR, CK5/6, CK14, Ki67, E-cadherin, Bcl2 and p53 was performed on tissue microarray sections of breast carcinoma with bone, brain and visceral metastasis (33, 13 and 30 cases) and 453 cases of breast carcinoma without metastasis. Using standard methods of scoring, the expression levels of receptors and subtype distribution (luminal A, luminal B, Her2, basal, triple negative) were compared within these groups.

Results When compared with breast tumours without metastases, bone metastases were significantly associated with ER (P = 0.01) and E-cadherin (P = 0.014) positive breast tumours, brain metastases were significantly associated with ER (P = 0.01) and PR (P = 0.001) positive breast tumours, and visceral metastases were significantly associated with ER (P < 0.001), PR (P = 0.013), Ki67 (P = 0.009), EGFR (P = 0.01) and p53 (P = 0.002) positive breast tumours. The triple-negative subtype was significantly associated with breast tumours with bone (P = 0.001) and brain (P < 0.001) metastases, while the basal subtype was significantly associated with breast tumours with visceral metastases (P = 0.005), when compared with breast tumours without metastatic disease. There was a significant association with tumour size >2 cm in breast tumours with bone (P = 0.021) and visceral metastases (P < 0.001), compared with breast tumours without metastatic disease.

Conclusions We have shown that, using immunohistochemistry, a standard panel of molecular markers of breast carcinoma can be of significant value in predicting sites of metastases.

P8 How many sentinel lymph nodes should we excise?

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Objective Radioactivity may be detected over several nodes during sentinel node biopsy (SNB), thus indicating that they all should be excised for examination. Biopsy of an insufficient number of lymph nodes (LN) may produce a false negative result, while excision of too many contradicts the SNB idea itself, turning it into an incomplete lymphadenectomy. We aim at establishing the minimal number of LNs that must be removed without compromising the reliability.

Materials and methods During years 2004 to 2008, 1,478 SNBs were performed in our department. Invasive cancer was diagnosed in 1,202 cases and these were included in our present study. LNs were removed one by one, according to the expressed radioactivity, until the last node showed radioactivity below 10% of the previous one. All LNs were marked in order of their expressed radioactivity and subsequent removal.

Results Metastases were found in 228/1,202 (19%) patients with invasive breast cancer. The postoperative full pathologic report showed that 143/228 patients (62.7%) had no LN metastases other than to sentinel LNs; they were, however, found, in the remaining 37.3%. The number of excised sentinel nodes varied from one to nine, the average was 2.04. Table 1 presents groups of patients with particular numbers of sentinel LNs excised and percentages of metastases found. In our experience, almost full (97%) information about the presence of metastases could be based on the biopsy of three first sentinel nodes, and in full (100%) of five first sentinel nodes.

Table 1 (abstract P8)

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Conclusions In most cases, full information about the presence of metastases to sentinel nodes could be obtained from the biopsy of the first three LNs, and 100% information of the first five. Biopsy of more than five LNs proved to be of no additional value.

P9 Prognostic value HER-2/neu expression in T1 to T3 breast cancer with axillary lymph node metastasis

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Objective Studies on the association of HER-2/neu with the axillary lymph node metastasis are controversial. Amplification of the protein
product of the HER-2/neu oncogene in primary breast cancer specimens is associated with an adverse prognosis.

Methods From January 2000 to December 2008, 504 breast cancer patients were operated on at General Hospital 'Sveti Vracevi' in Bijeljina. We selected 253 (50.2%) patients with breast cancer who had metastases to axillary lymph nodes.

Results Extracapsular extension (ECM) was found in 103 (40.7%) patients. The patients were identified and divided into two groups: the HER-2-positive group (38 patients) and the HER-2-negative group (65 patients). In the HER-2-positive group ECM was seen in 62.5% of patients, compared with 37.4% in the HER-2-negative group ($P = 0.059$). The total number of lymph nodes showing ECM were ECM also significantly more in the HER-2-positive group (48 out of 81, 59.25%), versus 13 out of 60 (21.66%) in the HER-2-negative group ($P < 0.001$). With a median follow-up of 96 months, factors with independent prognostic value for disease-free survival by multivariate analysis included HER-2/neu overexpression with extracapsular extension ($P < 0.05$), pN category ($P < 0.01$), presence of lymphovascular invasion ($P < 0.005$), and ECM ($P < 0.001$). An independent negative prognostic effect on overall survival was observed for HER-2/neu overexpression with extracapsular extension ($P < 0.05$), pN category ($P < 0.05$), and presence of lymphovascular invasion ($P < 0.005$) and ECM ($P < 0.001$).

Conclusions In patients whose tumors expressed HER-2/neu who had positive lymph nodes and extracapsular extension, prognosis was significantly worse compared with those who were HER-2/neu-negative and lymph node-positive with extracapsular extension. These findings have led to the conclusion that HER-2/neu overexpression is associated with a more aggressive subtype of cancer.

P10 Role of p27 in tamoxifen response in breast cancer cell lines

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Objective The aim of the study was to experimentally point out the clinical results of p27 as a predictive factor for tamoxifen response in estrogen receptor-positive breast cancer cell lines. Normal human mammary epithelium expresses the CDK inhibitor p27, whereas p27 is downregulated in a fraction of breast cancer, associated with poor prognosis and aggressive features. Besides prognostic information, p27 has also been linked to prediction of treatment effects. In a randomized study, where patients were either treated with adjuvant tamoxifen or no treatment, we observed that low p27 was not associated with prognostic information but instead linked to poor treatment effect of the selective estrogen receptor modulator tamoxifen. In this study we evaluated the clinical data with an experimental approach using breast cancer cell lines.

Methods Proliferation and cyclin D1 protein levels were monitored after treatment with estrogen alone or together with tamoxifen, and p27 levels were modulated with siRNA transfection.

Results Surprisingly, p27-downregulated MCF-7 cells responded to tamoxifen treatment but showed a decreased sensitivity to estrogen stimulation. For Cama-1 and T-47D cells there was no difference in estrogen or tamoxifen response in relation to p27. Cyclin D1 protein levels corresponded to p27 in the siRNA experiments, validating the function of p27 in the model system.

Conclusions Our results indicate that p27 was not required for mediating a tamoxifen effect in the tested cell lines but could function as an assembly factor essential for estrogen-induced proliferation. This potential assembly factor function for p27 might explain the observation in the primary breast cancer of a tamoxifen treatment predictive function for p27 in breast cancer.

P11 Inactivation of the retinoblastoma tumour suppressor pathway in premenopausal breast cancer is associated with resistance to tamoxifen

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Objective shRNA mediated knockdown of the retinoblastoma tumour suppressor (pRb) in oestrogen receptor (ER*) cell lines leads to resistance to tamoxifen, and pRb inactivation has further been associated with more aggressive disease. By studying the tamoxifen response in premenopausal patients randomised to either control or tamoxifen treatment, we aim to determine the importance of pRb inactivation in relation to tamoxifen response.

Methods Breast cancer samples were assembled in tissue microarrays, immunohistochemically stained for phos-pRb and evaluated as the fraction of positive nuclei divided into four groups: 0%; 1 to 10%; 11 to 25%; and 26 to 100%. pRb-inactivated tumours were defined using the phos-Rb parameter in combination with the proliferation marker Ki67. Tumours with none or low expression of phos-pRb displaying a high proliferation rate were defined as pRb inactivated ($n = 57$), whereas the remaining tumours were considered to have a functional pRb pathway ($n = 273$).

Results Inactivation of pRb was significantly correlated to larger tumours ($P = 0.001$), lymph node-negative disease ($P = 0.91$) and a higher histological grade ($P < 0.001$). There was a positive correlation to cyclin E levels ($P < 0.01$) but a negative correlation to cyclin D1 ($P < 0.001$) and ER as well as progesterone receptor levels (both $P < 0.001$). A significant difference was noted in recurrence-free survival when comparing no treatment with tamoxifen treatment in the patient group with functional pRb ($P = 0.003$); however, the beneficial effect of tamoxifen was lost in the pRb-inactivated group ($P = 0.619$). A multivariate analysis confirmed that inactivation of pRb was significantly associated with impaired tamoxifen response.

Conclusions The functional inactivation of pRb seems to be part of the explanation to why a subgroup of ER* tumours does not respond to tamoxifen. An evaluation of pRb status in ER* tumours could therefore possibly contribute to a more effective treatment.

P12 Risk of breast cancer amongst women who start smoking as teenagers

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Introduction We examined the effect of smoking on breast cancer risk in women who started smoking as teenagers. The women, 30 to 50 years of age who had smoked for at least 20 years, were surveyed through a mailed questionnaire at recruitment. Altogether, 1.34% of the women were diagnosed with incident, invasive breast cancer. In contrast, women who had smoked for at least 20 years, but started after their first child’s birth, did not experience an increased breast cancer risk. Our results support the notion that women who start smoking as teenagers and continue to smoke for at least 20 years may increase their breast cancer risk. Recent studies have shown breast cancer risk among women who start to smoke as teenagers, especially those who began before their first child’s birth. Ninety percent of all smokers began to smoke before the age of 19 – being the target of the cigarette manufacturers at this tender and immature age. Because of this early start and the addiction from nicotine, it is almost impossible to quit, thereby leaving enough time for the carcinogens present in tobacco smoke to damage the body.
Results among nondrinkers, and separately for each country.

Subjects

The number of women used for this study was a stunning 102,098 who completed a mailed questionnaire at recruitment through a period of 9 years (1996 to 2004). All Kenyan–Nigerian women were aged from 30 to 50.

Methods

We estimated the relative risk (RR) of breast cancer associated with different measures of smoking initiation: duration – the period in which the smoker had smoked, which was a strong determinant of the risk; and intensity – the number of cigarettes that were smoked during this period. Cox proportional hazard regression models were used to estimate these risks so as to adjust for confounding variables. We conducted analyses on the entire study population, among women who had smoked for at least 20 years, among nondrinkers, and separately for each country.

Results

Altogether, 1,240 women were diagnosed with incident, invasive breast cancer. Compared with women who had never smoked, women who smoked for at least 20 years and who smoked 10 cigarettes or more daily had a RR of 90%. In contrast, women who had smoked for at least 20 years, but started after their first birth, did not experience an increased breast cancer risk (Figure 1).

Discussion

The risk of breast cancer is almost double if young women start smoking within 5 years of their first menstrual cycle. Cigarette smoke contains over 40 potent cancer-causing chemicals. Scientists have been able to show that these chemicals can cause breast cancer cells in laboratory cultures to become cancerous. Women who started smoking in their teens developed breast cancer before menopause. One reason for this is that teenage breast tissue is still developing, which makes it more susceptible to the cancer-causing effects of the chemicals in smoke. Another factor that turned out to be important from this study was the amount and duration of smoking. More cigarettes per day and more years of smoking led to a higher chance of breast cancer (Figure 2).

Recommendations

From this study we recommend that smoking prevention should be reinforced among adolescents, especially in high schools worldwide. Irma H Russo, MD, from Fox Chase Cancer Center in Philadelphia points out that tobacco already has a devastating effect on women by causing lung cancer. Lung cancer and breast cancer are the major causes of cancer deaths in women. The results of this study mean women have even more reasons to avoid tobacco.

Conclusions

Our results support the notion that women who start smoking as teenagers and continue to smoke for at least 20 years may increase their breast cancer risk.

P13

VEGF-A, VEGF-C, VEGF-R2, EGFR and HER2 in serum plus EGFR in tissue of patients with triple-negative breast cancer

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Objective

Triple-negative breast cancer (ER–PR–HER2-negative) is not very common, has very poor prognosis and its therapeutic approach is a problem. This cancer type overexpresses EGFR in tissue. Elevated VEGF-C levels may be a predictor of lymph node metastases. VEGF-R2 plays an important role in tumor angiogenesis. We measured VEGF-C, VEGF-R2, EGFR, VEGF-A and HER2 in serum and EGFR in tissue. We compared all these parameters to find correlations between them.

Patients and methods

Seventy-three patients with triple-negative breast cancer were enrolled in this study. All patients had chemotherapy and radiotherapy after the surgical treatment. All of the parameters were measured in serum by ELISA.

Results

VEGF-C, VEGF-A, VEGF-R2 and HER2 in serum were measured in 73 patients. From our results, serum VEGF-C was overexpressed in 77% (11,393 ± 2,160 pg/ml, normal values: 2,459 to 6,651). Serum VEGF-R2 was overexpressed in the same patients (8,948 ± 1,234 pg/ml, normal values: 2,000 to 6,000). Serum VEGF-A was overexpressed in four patients but we found that they had extremely elevated levels of all of the VEGF agents. EGFR was measured in 54 patients who do not have any recurrence of the disease. It was overexpressed in 21/54 (>0.13 fmol/ml). In those patients, EGFR was also overexpressed in tissue (57%). We found also that in patients with overexpression of both serum and tissue EGFR, VEGF-C was not overexpressed but VEGF-R2 was overexpressed. Serum HER2 was overexpressed in eight patients. In those patients, serum and tissue EGFR (HER1) was also overexpressed.

Conclusions

We found interesting correlations between these factors. VEGF-C and VEGF-R2 have significant correlation. On the contrary, patients with overexpression of serum and tissue EGFR and VEGF-R2 do not overexpress VEGF-C. We need more patients to evaluate these results as they can help in the search for anti-angiogenic therapies.

P14

A supernumerary muscle complicated axillary lymphadenectomy: case report

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During lymphadenectomy in the left axilla of a 38-year-old woman with a 1.4 cm invasive ductal breast carcinoma, when the surgeon attempted to prepare the lateral margin of the pectoralis major muscle, an aberrant muscular slip was observed slightly lateral and deep to the pectoralis major muscle. The two muscles were separated by a narrow band of connective tissue that was completely dissected. Following this, the supernumerary muscle located in the center of the surgical field was pulled towards the latissimus dorsi muscle. As a result, the...
muscle formed an arcuate course and the lymphadenectomy was carried out troublesomey through a limited field. Nevertheless, the amount of the lymph node dissected was satisfactory (N: 0/25). Based on the anatomical characteristics of the muscle, it was recognized as a pectoralis quartus muscle. To our knowledge this is the first report of a pectoralis quartus muscle as a surgical finding. The surgeon should be aware of the possible presence of this supernumerary muscle as well as its anatomical characteristics in order to avoid any complications.

P15
Differential expression of cytoplasmic and stromal β-arrestin-1 is associated with separate aspects of tumor behavior in breast cancer
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Objective The main focus of our study was to investigate the importance of β-arrestin-1 in breast cancer and to elucidate a possible link between β-arrestin-1 protein overexpression and CCND1 amplification, since both genes map to chromosome locus 11q13, a region often amplified in breast cancer.

Methods The β-arrestin-1 protein expression was assessed in two different patient cohorts. The first included 179 premenopausal and postmenopausal breast cancer patients and the second included 500 breast cancer cases from premenopausal patients randomized to either 2 years of tamoxifen or no adjuvant treatment.

Results High cytoplasmic expression of β-arrestin-1 turned out to be associated with estrogen receptor negativity and HER2 amplification, whereas high β-arrestin-1 expression in stromal cells was associated with a more aggressive tumor phenotype. In the randomized cohort, cytoplasmic β-arrestin-1 expression was inversely correlated to amplification of CCND1 and positively correlated to expression of Chk1, previously described as a marker for distal 11q deletion, implying that the ARRB1 gene, instead of being co-amplified, might be deleted in the 11q13 amplification event.

Conclusions Interestingly, β-arrestin-1 may play different roles for tumor cells, depending on whether it is expressed by the tumor cell itself or by the surrounding stroma. In addition, amplification of the CCND1 gene might be associated with a loss of the ARRB1 gene.

P16
Value of diffusion-weighted imaging in differentiating benign from malignant breast lesions
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Introduction MRI has a proven high sensitivity for the detection of breast cancer. Besides contrast enhancement which is related to the vascularity of breast tumours, tumour high cellularity can be characterized or better measured via diffusion-weighted imaging (DWI), which is an important characteristic of tumours. DWI, combined with morphology and kinetics of contrast enhancement, may increase the specificity of MRI and may help in differentiating between normal and malignant breast lesions.

Materials and methods So far, 11 patients with biopsy-proven breast cancer and eight patients with benign breast lesions underwent DWI of the breast with an echo-planar imaging (EPI) sequence at 1.5 T. The apparent diffusion coefficient (ADC) was measured using four different b-values: 0, 300, 500, and 800 seconds/mm².

Results The ADC values of the malignant lesions ranged from 0.82 x 10⁻³ mm²/seconds to 1.65 x 10⁻³ mm²/seconds, with a mean ADC value of 1.14 ± 0.22 x 10⁻³ mm²/seconds. In benign lesions, the mean ADC was 1.62 ± 0.24 x 10⁻³ mm²/seconds, varying from 1.17 x 10⁻³ mm²/seconds to 2.00 x 10⁻³ mm²/seconds.

Conclusions The preliminary results show that contrast-enhancement patterns are mostly aspecific, while there is a better concordance between tumour malignancy and ADC values. Inclusion of more patients in the future might result in a threshold value of ADC allowing malignant lesions to be distinguished from benign lesions.

P17
Phase I study of combination therapy with weekly paclitaxel and cyclophosphamide for advanced or recurrent breast cancer
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Objective Although anthracycline is a key agent in breast cancer treatment, there is a concern that it may cause cardiotoxicity. Recently, the usefulness of combined therapy with docetaxel and cyclophosphamide (C) was reported. Because paclitaxel (P) has different features, such as induction of apoptosis and anti-angiogenic activity on weekly administration, establishment of combination therapy of P/C is required. We initiated a phase I study to determine the maximum tolerated dose and the recommended dose (RD) of the combination therapy of P/C for advanced or recurrent breast cancer.

Methods P was given intravenously on days 1, 8 and 15 and C on day 1, every 3 weeks. P was given at 80 mg/m² for level 1 and 100 mg/m² for level 2, and C at 600 mg/m² for both. Onset of dose-limiting toxicity was evaluated during course 1, and tolerability through course 4.

Results Four patients each were enrolled in levels 1 and 2 from October 2006 to November 2007. Main adverse events were four cases of grade 3 neutropenia (50%) and one case of peripheral nerve disorder (12.5%). During the first course of levels 1 and 2, hematologic toxicity of grade 4 and nonhematologic toxicity of grade 3 or higher were not observed, and a MTD was not attained. The response rate among assessable cases (one in level 1, two in level 2) was 66.7%.

Conclusions Safety was confirmed during four courses at level 2, and this was regarded as the RD. This is the first report on the phase I study of the combination therapy of weekly P and C, and its safety and efficacy should be evaluated in the phase II trial.
expression is frequently decreased in sporadic cancers. Women with hereditary mammary gland and ovarian cancer due to BRCA1 mutations are born with a mutation in one BRCA1 allele, but only develop cancer after mutation or allelic loss of the other BRCA1 allele. BRCA1 plays a critical role in the differentiation of ER-negative stem/progenitor cells from ER-positive luminal cells. Defects of the BRCA1 gene may result in the accumulation of genetically unstable mammary gland stem cells, providing prime targets for further carcinogenic events, because BRCA1 also plays a role in DNA repair. Our study indicates that restoration of normal wild-type BRCA1 expression levels in many mammary gland cancers may inhibit tumours by a genetic correction strategy, wherein the loss of BRCA1 expression contributes to tumorigenesis.

P19 Advanced topics in array comparative genomic hybridization for diagnosis of hereditary breast cancer
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Array comparative genomic hybridization (aCGH) is an efficient approach for scanning entire genomes to seek variations in genomic copy number variations, genotyping and medical genetics. It is changing from being only a research tool to being a tool for clinical diagnostics in patients with cancer, global developmental delay, mental retardation, autism, multiple congenital anomalies and dysmorphism, and is becoming a powerful tool in disease gene discovery and prenatal diagnostics. This tool is also showing promising data in cancer research and in the diagnosis, classification and prognosis of hereditary breast cancer. This presentation discusses the techniques available for aCGH analysis and possible approaches for using aCGH as a tool for the identification of hereditary breast cancer.

P20 Advanced topics in BRCA1/BRCA2 analysis by genetic analyzer and real-time PCR
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The most common gene changes in breast cancer are those of the BRCA1/BRCA2 genes. As both are large multi-exon genes, mutation screening of BRCA1/BRCA2 is technically challenging, because each gene harbors more than 1,000 different disease-associated mutations, the vast majority of which are individually rare. However, methods widely used in research laboratories such as SSCP, DGCG and HPLC miss nearly one-third of the BRCA mutations that are detected by genetic analyzer. These methods for scanning mutation fail to detect all BRCA1 germline defects. In the meantime, an alternative approach is to use a mutation scanning technique to highlight variations in genomic sequences that are then characterized by genetic analyzer. On the other hand, large genomic rearrangements are not detectable by current PCR-based methods, perhaps explaining the discrepancy between linkage analysis and genetic testing. Quantitative real-time PCR can determine large genomic rearrangements, gene duplications or deletions in BRCA1/BRCA2 genes. Furthermore, melting curve analysis immediately after PCR can identify small mutations, down to single base changes. These techniques are becoming easier and faster and can be multiplexed. In summary, sequencing in combination with real-time PCR methods is a favorable option for the analysis of BRCA genes.

P21 Breast cancer survivors and lymphoedema: the relevance of education
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Introduction Secondary arm lymphoedema is a chronic and distressing condition, relatively common after axillary lymph node dissection (AND) for breast cancer. It may be associated with functional, esthetic, and psychological problems that actually could affect the quality-of-life (QOL) of breast cancer survivors. The present study describes the prevalence and characteristics of arm and hand swelling in patients undergoing breast cancer surgery in our institution.
Methods We studied patients who underwent AND for breast cancer who had been diagnosed 4 years ago. We analyzed the prevalence of lymphoedema in these patients and reported data about pathologic and surgical characteristics.
Results Seventy-five women, median age 61 years, were included. Nine women (12%) reported arm or hand swelling since their surgery, eight of them with mild swelling. Twenty-one (28%) reported current swelling and one-half of them constant swelling, mainly in the hand. Swelling was considered mild when it affected just the hand. Women who reported severe swelling had significantly worse physical functioning and depressive symptoms as well. Since the swelling has reduced the possibility of dressing appropriately and modified perceptions about general appearance, 92% of these women began treatment for swelling with a high grade of compliance (86%). We detected that just five women had high body mass index; all of them had more than 10 axillary lymph nodes evaluated and one-half of them were affected by the tumor.
Conclusions Arm lymphoedema is a chronic problem for a subgroup of long-term survivors of breast cancer that negatively affects physical functioning. Educational efforts are needed for being aware of this problem so that survivors could identify this complication early and begin treatment to improve their physical functioning.

P22 Sentinel lymph node biopsy for breast cancer: how many nodes to stop at? Experience at a tertiary centre in Singapore
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Objective Sentinel lymph node biopsy (SLN) using blue dye and radioisotope is a widely used tool in the staging of breast cancer. However, multiple SLNs are frequently found. We sought to determine whether there is safe number of SLNs at which to stop the procedure without affecting accuracy.
Methods We reviewed the records of 182 patients who underwent successful SLN biopsy using radioisotope and/or blue dye in Singapore General Hospital. SLNs with counts more than 10% of the background axilla count, with retention of blue dye or SLNs that were enlarged were removed and the order of removal recorded. All SLN sites removed were categorized as a dye success, an isotope success or both. The count for each SLN site (if applicable) was recorded.
Results Seventy-nine percent of the patients who underwent SLN biopsy (144) had multiple SLNs. The mean number of SLNs removed per patient was 2.4. Twenty-six percent of the patients (48) were node-positive. Eighty-five percent of node-positive patients (41) had multiple...
SLNs. Eighty-eight percent of the node-positive patients were identified within the first two sites, while 97.5% and 100% were identified by the third and fourth sites, respectively.

Conclusions Seventy-nine percent of patients undergoing SLN biopsy for breast cancer have multiple SLNs. Positive nodes were detected within the first three sites removed in 97.5% of node-positive patients. Terminating the procedure at the third SLN site may help in lowering morbidity and costs without compromising accuracy.

P24
Sentinel node in breast cancer: retrospective analysis of 212 patients and factors associated with lymphatic involvement
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Objective To perform a retrospective study/review of the sentinel lymph node biopsy (SLNB) technique at our institution, analysing (from the results obtained) the relationship with the patients' age, tumour characteristics, injection technique, lymph node involvement and locoregional recurrence.

Materials and methods A total of 212 patients were included; 209 patients underwent subdermal periareolar injection (of 99mTc nano-colloid) and peritumoral injection was performed in three patients. Lymphoscintigraphy was performed after 15 to 30 minutes post-injection until the sentinel lymph nodes (SLN) were identified, and anterior and lateral views were registered. In the case of a next-day surgery protocol, a delayed 24-hour post-injection view was registered preoperatively. Skin marking of the SLN location was performed preoperatively. SLN were defined as the ones presenting with the highest count rate as measured by the intraoperative probe, as well as those with count rate of at least >10% of the former. The remaining resected LN were regarded as non-SLN (NSLN). A descriptive (and association) analysis of the following variables was performed: age, size and tumour location, histology type and grade, injection technique, number of removed SLN and NSLN, malignant involvement of SLN and NSLN, surgical axillary clearance (AC), malignant lymphadenopathy in AC and axillary recurrence.

Results The mean patient age was 59 (range 32 to 85) years; mean of removed SLN 2.94; mean of removed NSLN 0.82. The primary tumour was palpable in 115 patients (54.25%). The most frequent location was the supero-external quadrant (45.75%). The size of most tumours was between 1 and 2 cm (34.43%). The most common histological group was ductal carcinoma (60.38%) and the most common histology grade was 2 (37.74%). In 91.08% of cases, the number of removed SLN was ≤5. SLN were positive in 49 patients (23.11%). In 92.45%, ≥3 NSLN were removed. In six patients (2.83%) the NSLN were positive on histology, and in three of them SLN were negative. There was a statistically significant relationship between tumour size and malignant involvement of the SLN and NSLN, the indication of AC and the presence of malignant lymphadenopathy in AC. Moreover, there was a statistically significant relationship between histology grade and the number of removed SLN.

Conclusions The local and regional lymph node involvement of disease only showed a statistically significant relationship with tumour size. The number of removed SLN showed a statistically significant relationship with histology grade. It would be advisable to perform a histological analysis of all removed SLN and NSLN.

P24
Efficacy and safety of the administration of bevacizumab in combination with first-line chemotherapy for the treatment of advanced breast carcinoma: ATHENA MO19391 study results in Spanish patients
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Introduction Bevacizumab (BVZ) is a humanized monoclonal antibody that targets vascular endothelial growth factor, that leads to the inhibition of growth and proliferation of new blood vessels that are next to the tumor. The administration of BVZ plus first-line chemotherapy (paclitaxel, docetaxel) in the treatment of advanced breast carcinoma has lead to better outcomes in terms of response rate and time to progression in previous published studies.

Methods The ATHENA MO19391 study is a nonrandomized, open-labeled, international trial that tests the addition of BVZ (10 mg/kg/2 weeks or 15 mg/kg/3 week) to first-line chemotherapy (nonanthracycline based) in the treatment of advanced breast cancer. Results of the ATHENA trial have been previously reported. In the global study, 2,027 patients were included in 37 participating countries, between September 2006 and June 2008. This report shows the current efficacy and safety data of ATHENA participating patients included in Spanish centers.

Results A total of 119 patients in 20 Spanish centers were included in the trial, with the following basal characteristics: median age 51 years (27 to 79); postmenopausal status, 83 patients (69.7%); estrogen receptor-positive, 64 patients (66.7%); HER2-negative, HER2-positive, unknown, 92 patients (95.8%), two patients (2.1%), two patients (2.1%), respectively; prior adjuvant therapy, 92 patients (95.8%) – anthracycline-based, 63 patients (72.4%) and taxane-based, 38 patients (43.6%). The majority of patients (68 patients, 57.1%) who received previous adjuvant treatment had a disease-free interval >24 months.

The most common chemotherapy regimens that were combined with BVZ were paclitaxel (48 patients, 40.3%) and docetaxel (41 patients, 34.5%). Other used regimens were paclitaxel + gemcitabine (six patients, 5%), capecitabine (three patients, 2.5%), and others. The median number of administered cycles per patient was 8 (1 to 27). With a median follow-up of 10.9 months (0.9 to 24.9), 46% of patients had disease progression. The median time to progression was 11.5 months (10 to 13.6), and median overall survival was 22.5 months (19.2 to NR). One hundred and ten patients were evaluable for efficacy. Fourteen patients (12.7%) achieved a complete response, and 60 patients (54.5%) a partial response, for an overall response rate of 67.2%. Twenty-seven patients (24.5%) had stable disease, and nine (8.2%) progressed. The clinical benefit rate was 91.7%. Thirty-one patients (26.1%) had at least one adverse event grade ≥3. The most relevant SAEs were: febrile neutropenia, five patients (4.2%); grade 1 to 2 hypertension, 41 patients (34.4%); grade 3 to 4 hypertension, eight patients (6.7%); grade 1 to 2 epistaxis, 60 patients (50.4%); grade 3 DVT, one patient (0.8%); grade 1 to 3 proteinuria, 32 patients (26.9%); grade 4 nephrotic syndrome, two patients (1.7%); grade 3 GI perforation, one patient (0.8%); and grade 1 to 2 impaired healing, four patients (3.4%). Thirty-two patients (26.9%) had died at the time of the analysis, the majority (31 patients) due to breast cancer progression.

Conclusions Chemotherapy (nonanthracycline based) plus BVZ as first-line treatment for advanced breast cancer has a high antitumor
activity in terms of response rate and clinical benefit and of time to progression. The toxicity profile is manageable and due to toxic effects of both treatments separately. Administration of BVZ plus chemotherapy must be considered first-line therapy for the treatment of advanced breast cancer.

**P25**

**Circulating epithelial tumor cells in patients with metastatic breast cancer treated with bevacizumab**

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**Introduction**

Circulating epithelial tumor cells (CTCs) in peripheral blood are an ideal source for the detection of disseminated tumor cells of an easy sampling procedure. Their prognostic significance has been demonstrated in metastatic breast carcinoma and has the potential to influence the clinical management of patients with breast cancer. The aims of the present study are the evaluation of the prevalence and kinetics of CTS before and after anti-angiogenic treatment with bevacizumab (Avastin) in patients with metastatic breast cancer.

**Methods**

We analyzed 7.5 ml peripheral blood from 40 metastatic breast cancer patients treated with bevacizumab before (baseline) and after the first cycle of treatment. The presence of CTCs was assessed with the CellSearch System (Veridex, USA). Samples were subjected to immunomagnetic enrichment with an anti-EpCAM antibody and were fluorescence labeled. CTCs were defined as nucleated cells (DAPI+) expressing cytokeratin 8, 18 and 19 but CD45-negative phenotype. A sample was considered positive when one or more cells were detected.

**Results**

Data are available for 40 patients. We found \( \geq 1 \) CTCs before the first cycle of treatment with bevacizumab in 60% of the patients (n = 24). After the first treatment, reduction of one or more CTCs was found in 35% of the patients (n = 14). The median number of CTCs was 15 cells/7.5 ml blood in the first determination and 8 cells/7.5 ml in the second determination. In 30% of the patients (n = 12) we found an increase of baseline CTCs before and after treatment. In 26 patients we did not find any variation of baseline CTCs before and after treatment. In eight patients with CTCs positive at baseline (20%) the second determination after treatment was 0 cells/7.5 ml. Persistence of at least one CTC after the first cycle of treatment was found in 35% of the patients.

**Conclusions**

The results of this explorative study are preliminary and a large number of patients and follow-up are required. The study is ongoing to explore the prognostic significance of reduction or persistence of CTCs during treatment with anti-angiogenic agents (bevacizumab) and the relationship with response rate to treatment. Complete data will be presented.
P28
ZD6474 in combination with paclitaxel and UVB irradiation enhances the antiproliferative effect and apoptosis on breast carcinoma
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Breast cancer is the leader of cancer deaths among women. Despite developments in surgery, chemotherapy and radiotherapy, the long-term survival of patients with metastatic and higher staging breast cancer has remained very low. Breast cancers and their aggressive nature are characterized by overexpression of epidermal growth factor receptor (EGFR) and vascular endothelial growth factor receptor (VEGFR). There is a common downstream signaling molecule involved in both EGFR and VEGFR mediated pathways. There are reports that cancer cells acquired resistance to anti-EGF/EGFR therapy by increased tumor-induced angiogenesis due to the constitutive overexpression of VEGF. Hence, the inhibition of both EGFR and VEGFR signaling pathways simultaneously may provide greater benefit than blockade of either pathway alone. There are also reports that breast cancer acquired resistance to conventional chemotherapy and radiotherapy by increased phosphorylation of EGFR and VEGFR. Moreover, patients suffering from breast cancer have a poor prognosis because of a lack of effective treatment strategies. We hypothesized that inhibiting the phosphorylation of the EGFR and VEGFR by ZD6474, a dual tyrosine kinase inhibitor of EGFR and VEGFR, in combination with paclitaxel or radiation would inhibit breast cancer cell growth. We tested this hypothesis using the human breast cancer cell lines MCF-7 and MDA-MB-231. In culture, ZD6474 in combination with paclitaxel or UVB had a synergistic effect in inhibition of cell proliferation, and lowered the IC₅₀ by 10-fold and fivefold, respectively, compared with paclitaxel/UVB alone. Flow cytometry data suggest that combination therapy of ZD6474 with paclitaxel increases apoptosis from 20% to 45% in MDA-MB-231 and from 16% to 38% in MCF-7 compared with paclitaxel alone. This is further supported by a decrease in cell cycle regulatory protein cyclin D1, and an increase in apoptosis marker poly(ADP-ribose) polymerase in combination therapy compared with paclitaxel and UVB irradiation alone. Moreover, combination therapy sensitizes the paclitaxel resistance MCF-7/PTX and MDA-MB-231/PTX. ZD6474 along with paclitaxel or radiation can therefore be used for treatment in drug-insensitive advanced breast cancers.

P29
Developing a prediction model for benefit from fulvestrant in heavily pretreated metastatic breast cancer patients
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Introduction Fulvestrant use in heavily pretreated patients with metastatic breast cancer is associated with highly variable responses. The present study aimed to characterize the benefit of fulvestrant therapy and to develop a prediction model for clinical benefit in this setting.
Methods A nationwide, retrospective chart review of patients enrolled in a Canadian compassionate use program was performed. This program mandated prior therapy with tamoxifen and both steroidal and nonsteroidal aromatase inhibitors. Charts from the seven highest accruing centers were reviewed. The sample size was based on the derivation of a model to predict the probability of a patient remaining on fulvestrant and free from chemotherapy for at least 3 months.
Results A total of 305 women received at least one dose of fulvestrant; 207 went on to receive chemotherapy (68%). Of these, 48 (23%) required chemotherapy at 3 months, 113 (55%) at 6 months, and 170 (82%) by 12 months. The median duration of fulvestrant treatment was 126 days (range 23 to 1,920). The median overall survival from start of fulvestrant was 698 days (25th percentile, 316 days to 75th percentile, 1,359 days). The preliminary prediction model showed that older age (OR = 0.96, 95% CI = 0.93 to 0.99) and having received no adjuvant hormonal therapy (OR = 0.5, 95% CI = 0.2 to 1.25) predicted a greater chance of remaining chemotherapy-free at 3 months. Presence of lung (OR = 2.55, 95% CI = 1.1 to 5.9) or brain metastases (OR = 12.8, 95% CI = 4.1 to 55.4) predicted a lower chance of remaining chemotherapy-free at 3 months.
Conclusions Older age and having received no prior adjuvant hormonal therapy predicted a greater chance of remaining chemotherapy-free at 3 months, while lung and brain metastases predicted a lower chance. These factors may be considered when prescribing fulvestrant.

P30
Employing multiplex immunofluorescence to quantify Her2 and phosphorylated Her2 in formalin-fixed, paraffin-embedded breast tumor specimens
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Objective Her2 overexpression is a predictor for response to trastuzumab; however, only one-third of patients initially respond and the majority progress within 1 year. Recently, levels of the phosphorylated form of Her2 (pHer2) in hormone receptor-positive, primary tumors was determined to be an independent predictor for poor disease-free and overall survival, suggesting that additional variables may be important for determining outcome. We sought to develop a multiplex immunofluorescent (IF) quantitative assay for Her2 that included pHer2 and to compare the initial results with traditional methods of evaluating Her2 levels in formalin-fixed, paraffin-embedded (FFPE) breast tissue specimens.
Methods We developed a Her2 multiplex IF assay (Her2multiplex) composed of AE1/AE3 (cytokeratin), TAB250 (Her2-EC), c-erb B2 (Her2), and Her2 pY1248 (pHer2) utilizing breast tumor cell lines (BTC: MCF7, T47D and SKBR3) that differentially express Her2 and pHer2. The assay was applied to a 37-patient tissue microarray (TMA) with known Her2 status (munochemical (IHC) HercepTest). Image analysis generated quantitative IF intensity features that were evaluated univariately, and then multivariately with feature selection to discriminate Her2 (2+) from Her2 (3+). The AUC was used to estimate performance.
Results We developed baseline intensity thresholds from the component biomarkers in the Her2multiplex (TAB250 Her2, c-erb B2 and phosphorylated Her2) utilizing a series of BTC: MCF7, T47D and SKBR3. IF images were acquired from 72 TMA cores, and 52 cores (26 patients) had appropriate tumour content (>50%) and quality for analysis. Both c-erb B2 and pHer2 were significantly associated with IHC data and were selected (AUC = 0.96, sensitivity = 0.91, specificity = 1) to discriminate Her2 (2+) from Her2 (3+).
Conclusions Quantitation of Her2 and pHer2 by IF is feasible in FFPE breast tumor specimens. The results correlate with IHC protein data and also provide a quantitative strategy for the development of a standardized assay useful for tumor phenotyping, patient stratification and therapeutic indication.
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Biopsy confirmation of metastatic breast cancer: interim results of a prospective biopsy study

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Objective Decisions about systemic treatment of women with metastatic breast cancer are often based on oestrogen receptor (ER), progesterone receptor (PgR), and HER2/neu status of the primary tumour. The present study prospectively investigated concordance in receptor status between primary tumour and distant metastases at various stages of progression and assessed the impact of any discordance on patient management.

Methods Biopsies of suspected metastatic lesions were obtained from patients and were analysed for ER/PgR and HER2/neu. The receptor status of metastases was compared with the primary tumour. The treating physician completed questionnaires before and after biopsy to determine whether the biopsy resulted in a change to the treatment plan.

Results Eighty women were enrolled and 70 of them underwent biopsy. Of these, 43 (61%) were newly diagnosed with metastatic disease, 10 (14%) had received one line of treatment in the metastatic setting and 17 (24%) had received two or more lines of treatment in the metastatic setting. Fifty-six samples were sufficient for analysis; of these, 4/56 biopsies (7%) showed benign disease and one biopsy (2%) confirmed a different malignancy (idolent lymphoma). Changes in hormone receptor status were observed in 34% and in HER2/neu status in 5% of women. Loss of PgR was the most common change in hormone receptor status (29%). All changes in HER2/neu involved gain of receptor. Biopsy results led to a change of management in 9/70 patients (13%). Patients with newly diagnosed metastatic disease were more likely to show discordance than those previously treated in the metastatic setting. Triple-negative tumours were least likely to show discordance.

Conclusions This prospective study demonstrates the presence of substantial discordance in receptor status between primary tumour and metastases. The number of patients needed to biopsy to alter management is 7.8. Tissue confirmation should be considered in patients with clinical or radiological suspicion of metastatic recurrence.

P32

Sentinel lymph node biopsy in breast cancer: day surgery and local anaesthetic approach

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Introduction Sentinel node biopsy (SNB) is widely used in the management of breast cancer patients without axillary metastases.

Methods From 1 January 2006 through 31 December 2008 we performed 113 SNBs at St M. Goretti Hospital. Mammary carcinoma was diagnosed as malignant by aspiration cytology and/or biopsy. In all cases with positive cytology, we performed at the same time quadrantectomy and SNB. The patients underwent preoperative lymphoscintigraphy. We never used vital blue dye. All patients underwent surgical treatment 3 to 4 hours later. We performed SNB and quadrantectomy in day surgery (DS) and local anaesthesia (LA). Axillary incision was 2 to 3 cm. This approach was discussed with all patients and informed consent was obtained. The objective of the present study is to investigate the validity of this procedure.

Results Four patients underwent preoperative lymphoscintigraphy, the radiotracer did not show any sentinel lymph node (SLN), and we performed axillary dissection. One case showed a double SLN in the axilla and internal mammary chain. Only the internal mammary node was positive. After surgery we distributed a questionnaire to the patients about the acceptability of this approach. We identified the SLN in all patients (100%).

Conclusions The results achieved are extremely accurate. This procedure is safe and well accepted by doctors and patients (98%). The oncological results are absolutely reliable. As regards hospital logistics, operations in DS and LA can be easily managed, leading to a significant cost reduction – 42.15% less expensive than the same operation performed under general anaesthesia.

P33

A randomized, phase III trial exploring the effects of neoadjuvant sequential treatment with steroidal ( exemestane) and nonsteroidal (anastrozole) aromatase inhibitors on biomarkers in postmenopausal women with hormone receptor-positive locally advanced breast cancer

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Introduction Despite many large randomised trials assessing adjuvant endocrine treatment for postmenopausal breast cancer patients, the optimal endocrine strategy remains unknown. Neoadjuvant endocrine studies provide the opportunity to model an appropriate study design in a more expeditious manner. Several large adjuvant trials are exploring sequential aromatase inhibitor (AI) strategies. The present study compared the effect of two sequences of AI use – steroidal ( exemestane (E)) and nonsteroidal (anastrozole (A)) – on serological and pathological biomarkers, when given in the neoadjuvant setting to patients with locally advanced breast cancer.

Methods Thirty postmenopausal women with estrogen and/or progesterone receptor-positive disease were randomised to receive either E followed by A (E→A group) or A followed by E (A→E group). Each agent was given for 8 weeks. Serum estrone sulfate, and estradiol levels, as well as intratumoral Ki67 were evaluated at baseline, 8 weeks, and 16 weeks. Clinical response, patient preference, and quality of life were also assessed.

Results Despite rapid falls in sex steroid levels with AI use, there was no difference in estradiol, estrone sulfate or Ki67 levels between groups. There was no significant difference in toxicities, or in quality of life scores. Overall clinical response rate was 68% and clinical benefit was 93%. There was a trend towards improved clinical response in the A→E group. The majority of patients expressed a preference for treatment.

Conclusions Neither sequence of steroidal or nonsteroidal AI appears to offer a significant advantage over the other. A trend towards improved clinical response in patients treated with A→E is hypothesis-generating and needs confirmation in larger trials.