PhD Thesis in Medicine
(Sub-specialization Biomedicine)

Clinical decisions in breast cancer, a heterogenous disease

Student: Sofia Braga,
Faculdade de Ciências Médicas
Instituto Gulbenkian de Ciência
José de Mello Saúde

Supervisor: José Pereira Leal, Instituto Gulbenkian de Ciência
Co-supervisor: José Luis Passos Coelho, Faculdade de Ciências Médicas

I dedicate my work to breast cancer patients

I want to thank,

My family and my patients for the desire to learn

My mentors, colleagues and students for teaching me

Table of contents

Glossary 4

Publications that are part of this work 5

Synopsis 6

Synopsis in Portuguese 10

Introduction: Breast cancer care in the past 14

Part 1: Who to treat with adjuvant systemic therapy? 18

Chapter 1: TNM is not dead in breast cancer 18

Chapter 2: Redefining breast cancer prognosis: The predictive power and mechanism of centrosome alterations in breast cancer 34

Part 2: How to treat breast cancer subtypes? 53

Chapter 3: How many diseases is triple negative breast cancer? 53

Chapter 4: Systemic treatment for triple negative breast cancer 65

Chapter 5: Randomized Phase II Study of the Anti-Epidermal Growth Factor Receptor Monoclonal Antibody Cetuximab With Cisplatin Versus Cisplatin Alone in Patients With Metastatic Triple-Negative Breast Cancer 82

Chapter 6: Blocking angiogenesis to treat breast cancer 90

Chapter 7: Does hypoxic response mediate primary resistance to sunitinib in untreated locally advanced breast cancer? 103

Part 3: When to stop systemic treatment of breast cancer patients? 123

Chapter 8: The aggressiveness of cancer care in the last three months of life: a retrospective single centre analysis 123

Chapter 9: Is breast cancer treatment in the end of life less aggressive? 130

Chapter 10: Why do our patients get chemotherapy until the end of life? 139

Conclusion: What is the future of breast cancer care? 144

Glossary

ABC Advanced breast cancer
AC Doxorubicin cyclophosphamide
ALND Axillary lymph node dissection
BC Breast cancer
BRCA Breast cancer associated
CMF Cyclophosphamide, methotrexate and 5 fluouracil
CT Chemotherapy
DDCT Dose dense chemotherapy
DDFS Distant disease free survival
DFS Disease free survival
DMFS Distant metastasis free survival
EGF Epidermal growth factor
EGFR Epidermal growth factor receptor
ER Estrogen receptor
EoL End of life
Her2 Epidermal growth factor receptor type 2
HR Hazard ratio
IDFS Invasive disease free survival
IGF Insulin growth factor
IGFR Insulin growth factor receptor
LHRH Luteinizing hormone releasing hormone
MBC Metastatic breast cancer
OS Overall survival
ORR Overall response rate
pCR Pathologic complete response
PFS Progression free survival
RR Response rate
SLN Sentinel lymph node
TN Triple negative
TNBC Triple negative breast cancer
TK Tirosine kinase
TKI Tirosine kinase inhibitor
VEGF Vascular endothelial growth factor
VEGFR Vascular endothelial growth factor receptor

Publications that are part of this work

Published

Chapter 5: Randomized Phase II Study of the Anti-Epidermal Growth Factor Receptor Monoclonal Antibody Cetuximab With Cisplatin Versus Cisplatin Alone in Patients With Metastatic Triple-Negative Breast Cancer

Chapter 8: The aggressiveness of cancer care in the last three months of life: a retrospective single centre analysis.

Chapter 10: Why do our patients get chemotherapy until the end of life?

Submitted/Undergoing rebubmisson

Chapter 3: How many diseases is triple negative breast cancer?

Chapter 4: Systemic treatment for triple negative breast cancer

Chapter 6: Blocking angiogenesis to treat breast cancer

Chapter 7: Does hypoxic response mediate primary resistance to sunitinib in untreated locally advanced breast cancer?

Chapter 9: Is breast cancer treatment in the end of life changing?

Authorship review

Chapter 1: TNM is not dead in breast cancer

FInal paper under discussion

Chapter 2: Redefining breast cancer prognosis: The predictive power and mechanism of centrosome alterations in breast cancer

Synopsis

Introduction: Breast cancer care in the past
This work starts with an overview of the treatment of breast cancer (BC). From the first reports of patients ill with BC until 1950. From 1950 until 2000, there is a more detailed account on how BC patients were treated with emphasis on the different modalities, local, regional and systemic treatments and their evolution.

Part 1: Who to treat with adjuvant systemic therapy?

Chapter 1: TNM is not dead in breast cancer
It has been said that the current TNM staging system might not be suitable for predicting breast cancer (BC) outcomes and for making therapeutic decisions, especially for patients with screen detected BC which is smaller. The reason for this is also due to the non inclusion of tumor biology parameters in the current TNM system. We hypothesize that in a population where there is still a large abundance of non screen detected BC, with a low median age of incidence and abundance of high TNM staged lesions, biology is still second to classical staging in predicting prognosis.
We analyzed a population of consecutive BC patients from a single institution during ten years. We characterized current established prognostic factors, classical staging variables included in the current TNM staging system and biological variables, currently not included in the TNM system. We quantified the capacity of individual prognostic factors to predict survival. We analyzed a population of 1699 consecutive BC patients. We found that individually both the TNM system prognostic factors and the biological prognostic factors are differing among BC survivors and dead patients in a statistically significant distribution. Explicitly, patients with larger tumors, positive nodes, higher stage lesions, ER negative, HER2 positive, TN or lower differentiation tumors show decreased survival.
In the multivariate analysis we can conclude that in a population such as ours classical TNM staging variables, irrespective of tumor biological features, are still the most powerful outcome predictors.

Chapter 2: Defining breast cancer prognosis: The predictive power and mechanism of centrosome alterations in breast cancer
We performed a systematic analysis of the literature and compiled an extensive data set of gene expression data originated in primary tumours of BC patients with prognostic information. We analysed this data seeking for genes consistently up or down regulated in poor prognosis BC, i.e. that relapsed after initial treatment. In the course this bioinformatics analysis our lab identified 65 genes statistically significant across multiple datasets that can discriminate between relapsed and non-relapsed BC patients. Among the identified genes, we have detected genes such as MKI67, a marker of mitotic activity which is routinely used in the clinic. Unexpectedly, we also discovered several genes found to be involved in centrosome clustering, The most prominent of these is the kinesin KIFC1, also called HSET, and previously identified as regulator of centrosome clustering. Centrosome abnormalities (numerical, structural) have been observed in cancer. Indeed, compelling data has shown that cells from many cancers have multiple and abnormal centrosomes, that are either correlated with tumour malignancy or considered an early tumorigenesis event. However, extra centrosomes come at a cost and cells must be able to handle such abnormalities or otherwise die. Thus our results suggested a new mechanism of breast cancer progression with negative prognostic value. We aimed at quantifying the predictive power of centrosome clustering in BC clinical setting and at detecting this process in BC patient material. We validated the centrosome clustering genes KIFC1 and TACC3 in formalin fixed paraffin embedded (FFPE) BC patient material, using quantitative real-time PCR (RT-qPCR) technology. Our results indicate that the tested KIFC1 has a clear IHC signal (1) and that the protein expression patterns and levels correlate with prognosis, with relapsing patients having increased expression and nuclear localisation of this kinesin (2). Next we were able to show that centrosome clustering does occur in vivo. We identified centrosome amplification and clustering in breast cancer samples, and we established a fluorescence microscopy-based IHC approach by staining FFPE samples with centrosomal markers. Using this approach we have observed centrosome amplification and clustering in a small set of poor prognosis samples. By expanding the number of samples in which we have characterised the number of centrosomes, we were able to confirm our preliminary observation that centrosomes are clustered in relapsed BC.

Part 2: How to treat breast cancer subtypes?

Chapter 3: How many diseases is triple negative breast cancer? (review)
Triple negative breast cancer is a subtype of breast cancer that does not express the estrogen receptor, the progesterone receptor and the epidermal growth factor receptor type 2 (Her2). These tumors are not yet

treated with targeted therapies probably because no positive markers have been described to reliably classify them - they are described for what they are not. Perhaps for this reason, they are among the most aggressive of breast carcinomas, albeit with very heterogenous clinical behavior. The clinical observation that these patients do not carry a uniformly dismal prognosis, coupled with data coming from pathology and epidemiology, suggests that this negative definition is not capturing a single clinical entity, but several. We critically evaluate this evidence in this paper, reviewing clinical and epidemiological data, as well as molecular data. There is evidence for heterogeneity, but it is not clear how many diseases are grouped into triple negative breast cancer. Answering this question, and identifying the molecular basis of heterogeneity will help define prognosis and, eventually, the identification of new targeted therapies.

Chapter 4: Systemic treatment for triple negative breast cancer (review)
Chemotherapy remains the backbone of treatment for triple negative breast cancer (TNBC). Despite the appearance of new targeted and biologic agents there has been no targeted therapy validated for TNBC, possibly because the biology of TNBC has not been conclusively elucidated. Many studies have shown that TNBC derive significant benefit of chemotherapy in the neoadjuvant, adjuvant and metastatic treatment, possibly more benefit than other BC subtypes. Neoadjuvant chemotherapy studies have repeatedly shown higher response rates in TNBC than non-TNBC. Pathologic complete response has been shown to predict improved long term outcomes in BC. Although specific adjuvant regimens for TNBC are under study, third generation chemotherapy regimens utilizing dose dense or metronomic polychemotherapy are among the most effective tools presently available. The role of specific chemotherapy agents, namely platinum salts, in the treatment of TNBC remains undefined. Taxanes and anthracyclines are active in TNBC and remain important agents, but have not shown specific benefit over non-TNBC. TNBC is itself a heterogeneous group in which subgroups like basal like BC defined by higher proliferation and including those TNBC arising in BRCA1 mutation carriers may be more sensitive to platinum agents and relatively less sensitive to taxanes. The molecular characterization of TNBC is lacking and therefore the search for targeted therapy is still ongoing.

Chapter 5: Randomized phase II study of the anti-epidermal growth factor receptor monoclonal antibody cetuximab with cisplatin versus cisplatin alone in patients with metastatic triple-negative breast cancer
Epidermal growth factor receptor is overexpressed in metastatic triple-negative breast cancers, an aggressive subtype of breast cancer. Our randomized phase II study investigated cisplatin with or without cetuximab in this setting.
Patients who had received no more than one previous chemotherapy regimen were randomly assigned on a 2:1 schedule to receive no more than six cycles of cisplatin plus cetuximab or cisplatin alone. Patients receiving cisplatin alone could switch to cisplatin plus cetuximab or cetuximab alone on disease progression. The primary end point was overall response rate (ORR). Secondary end points studied included progression-free survival (PFS), overall survival (OS), and safety profiles. The full analysis set comprised 115 patients receiving cisplatin plus cetuximab and 58 receiving cisplatin alone; 31 patients whose disease progressed on cisplatin alone switched to cetuximab-containing therapy. The ORR was 20% with cisplatin plus cetuximab and 10% with cisplatin alone (odds ratio, 2.13). Cisplatin plus cetuximab resulted in longer PFS compared with cisplatin alone (median, 3.7 v 1.5 months; hazard ratio, 0.67. Corresponding median OS was 12.9 versus 9.4 months. While the primary study end point was not met, adding cetuximab to cisplatin doubled the ORR and appeared to prolong PFS and OS, warranting further investigation in mTNBC.

Chapter 6: Blocking angiogenesis to treat breast cancer (review)
Angiogenesis is a hallmark of cancer because tumors larger than 1mm need new vessels to sustain their growth. Since the discovery of the molecular players of this process and some inhibitors, that angiogenesis became a promising therapeutic target. Bevacizumab was the first molecular-targeted antiangiogenic therapy approved by the FDA and is used as first-line therapy in metastatic breast cancer. A second class of approved inhibitors (sunitinib, sorafenib, pazopanib and axitinib) include oral small-molecule tyrosine kinase inhibitors that target vascular endothelial growth factor receptors, platelet-derived growth factor receptors, and other kinases including KIT, Ret, BRAF and Flt-3, but none of these have gained approval to treat breast cancer.
This review analyzes and summarizes data from clinical trials of anti-angiogenic agents in the treatment of BC. Phase III trials of bevacizumab in advanced BC have demonstrated a reduction in disease progression (22–52%), increased response rates and improvements in progression-free survival of 1.2 to 5.5 months, but no improvements in OS. Bevacizumab phase III trials in early BC have both been negative. Bevacizumab combined with chemotherapy is associated with more adverse events. Phase III trials of the tyrosine kinase inhibitor sunitinib were negative, while randomized phase II trials of sorafenib and pazopanib have improved

some outcomes. Endostatin has been tested in neoadjuvant clinical trials in combination with anthracycline-based chemotherapy in treatment-naive patients and has increased the clinical response rate, but more trials are needed to establish this drug. Most trials of anti-angiogenic agents in BC have reported improved RR and PFS but no increase in OS compared to chemotherapy alone, leading to skepticism towards blocking angiogenesis. Selected trials in selected BC populations with translational endpoints related to harvested tumor tissue and other biological material samples, preferentially at several timepoints, will be crucial if antiangiogenesis is to survive as a strategy to treat BC.

Chapter 7: Does hypoxic response mediate primary resistance to sunitinib in untreated locally advanced breast cancer?

The antiangiogenic drug sunitinib has never been evaluated as single agent in untreated BC patients. We aimed to characterize the activity of sunitinib, alone and with docetaxel, in untreated locally advanced or operable BC, and, to uncover the mechanisms of response. Twelve patients were treated with an upfront window of sunitinib followed by four cycles of sunitinib plus docetaxel. Response, resistance and toxicity were evaluated according to standard clinical parameters, magnetic resonance imaging, positron emission tomography, pathology characterization and gene expression profiling. We detected primary resistance to sunitinib upfront window in untreated BC, as evidenced by four non-responding patients. At surgery, five patients had viable disease in the breast and axilla, four had viable tumor cells in the breast alone and three were taken off study due to unacceptable toxicity and thus not evaluated. Early functional imaging was useful in predicting response. There were no pathologic complete responses (pCR). Comparison of gene expression profiling tumor data between early responders and non-responders allowed us to identify up-regulation of VEGF and angiogenic pathways in non responders. Specifically, in tumors resistant to the single-agent sunitinib we detected a transcriptional response to hypoxia characterized by over-expression of several HIF1α target genes. In this report of single-agent sunitinib treatment of untreated localized BC patients, we found molecular evidence of primary resistance to sunitinib likely mediated by up-regulation of hypoxia responsive genes.

Part 3: <u>When to stop</u> systemic treatment of breast cancer patients?

Chapter 8: The aggressiveness of cancer care in the last three months of life: a retrospective single centre analysis.

All adult patients with solid tumors who died in our hospital in 2003 and received chemotherapy for advanced cancer, were included. Detailed data concerning chemotherapy and toxicity, in the last three months of life, were collected from patients' clinical charts. A total of 319 patients were included. Median age was 61 years. Median time from diagnosis of metastatic disease to death was 11 months. The proportion of patients who received chemotherapy in the last three months of life was 66% (n=211), in the last month 37% and in the last two weeks 21%. Among patients who received chemotherapy in the last three months of life, 50% started a new chemotherapy regimen in this period and 14% in the last month. There was an increased probability of receiving chemotherapy in the last three months of life in younger patients and in patients with breast, ovarian and pancreatic carcinomas. There was a large proportion of patients who received chemotherapy in the last three months of life, including initiation of a new regimen within the last 30 days. Thus, further study is needed to evaluate if such aggressive attitude results in better palliation of symptoms at the end of life.

Chapter 9: Is breast cancer treatment in the end of life changing?

We aimed to characterize the shifting trends in use of anti-cancer chemotherapy and palliative care approaches in the end of life of BC patients in different institutions and times. For this, we selected women that died of BC during six years, from 2007 to 2012, and were treated in a central acute care general hospital and compared it with the BC patients that died in 2003 and were treated in a large cancer center. We analyzed a total of 232 patients: the more recent group has 114 women and the older cohort has 118. We used descriptive statistics to characterize CT in the EoL and use of palliative care resources. Both populations were similar in terms of BC characteristics. We observed more palliative care resources, pain clinic, palliative care teams and palliative radiotherapy, involved in the care of MBC patients and a shift towards more deaths at hospices. Systemic anti cancer treatments continue to be prolonged until very late in patients' lives, notwithstanding, we could show a decrease in the use of such treatments. Other indicators of aggressiveness, namely hospital admissions, also show a decrease. We confirmed our hypothesis that there is more integration of multidisciplinary palliative care and less aggressiveness in the treatment of metastatic cancer patients, specifically, use of palliative anti-cancer treatment and hospital admissions. Nonetheless, we use systemic therapy until too late with underutilization of palliative medicine.

Chapter 10: Why do our patients get chemotherapy until the end of life? (editorial)
The editorial starts with a clinical case of a 21 year old patient that lives three months after starting palliative chemotherapy for the first time, a case that illustrates therapeutic futility at the end of life. Why are we not ceasing chemotherapy when it is useless, toxic, logistically complex and expensive? Are we prescribing chemotherapy until too late in solid tumor patients' lives? Medical oncologists have overly optimistic predictions and, excessive, treatment-prone attitude and they are criticized by other health care providers for this. Increasingly, patients, their families, advocacy groups, policy makers, journalists and society at large dwell on this topic, which is a perplexing conundrum, because sometimes they are the ones demanding not to stop aggressive systemic anticancer treatments, when it comes to their loved ones. There is a growing culture of awareness toward preserving quality of life, palliative care, symptom-directed care, hospice referral and end of life issues regarding terminal cancer patients. Sadly, this issue is gaining momentum, not because oncologists are questioning their practice but because health care costs are soaring. Whatever the motive, the reasons for administering chemotherapy at the end of life should be known. There are few and conflicting scientific data to guide treatments in this delicate setting and we review this evidence in this paper.

Conclusion: What is the future of breast cancer care?
This work ends with a view into the future of BC care. Looking into the different areas from prevention, screening, hereditary BC, local, regional and systemic treatments of adjuvant and metastatic patients. The last three paragraphs are a final comment where the story of a patient with Her2 positive locally advanced breast cancer is used as paradigm of evolution, heterogeneity and dynamism in the management of BC.

Synopsis in Portuguese

Introdução: Tratamento do carcinoma da mama
Este trabalho inicia-se com a história do tratamento do carcinoma da mama, desde os primeiros documentos que descrevem doentes com carcinoma da mama até 1950. Desde 1950 até 2000 o diagnóstico, risco e as modalidades terapêuticas usadas no tratamento das doentes são mais detalhadas com ênfase nas terapêuticas locais, regionais e sistémicas.

Parte 1:Quem tratar com terapêutica sistémica adjuvante

Capítulo 1: A classificação TNM não está morta no carcinoma da mama
Tem sido dito que a classificação TNM não é adequada para usar como ferramenta de prognóstico e decisão terapêutica no carcinoma da mama, especialmente em doentes com carcinoma detectado através de rastreio, que tem geralmente menores dimensões. A razão desta classificação não ser adequada prende-se com o facto de não estarem incluidos parâmetros biológicos na classificação TNM atual. Pusemos a hipótese de que numa população com alta percentagem de carcinoma da mama não detectado em exames de rastreio, com uma mediana de idade baixa e com alta percentagem de estadios II e III, o estadiamento clássico, pela classificação TNM, é mais descriminatório que as características biológicas na determinação do prognóstico.
Para isto analisámos uma população de doentes com carcinoma da mama tratados consecutivamente na mesma instituição, durante 10 anos. Caracterizámos os fatores de prognóstico do estadiamento clássico incluídos na classificação TNM e as variantes biológicas, presentemente não incluídas na classificação TNM. Quantificámos a capacidade de cada um dos factores de prognóstico para para prever a sobrevivência. A população é de 1699 doentes com carcinoma da mama que foram tratádos com terapêutica sistémica adjuvante. Individualmente, cada um dos fatores de prognostico, clássicos ou biológicos, diferem significativamente entre doentes que sobrevivem e que não sobrevivem. Explicitamente, como previsto, doentes com tumores maiores, envolvimento dos gânglios axilares, estadios TNM mais avançados, que não expressam recetor de esrogéneo, com amplificação do gene Her2, triplos negativos ou de menor diferenciação têm menor sobrevida. Na análise multivariada, só os fatores de prognostico da classificação TNM, o grau histológico e a amplificação do gene Her2, esta última com menos significância estatistica são preditores independentes de sobrevivência.

Capítulo 2: Em busca de novos factores de prognostico: Poder preditivo e mecanismo das alterações de centrossomas em carcinoma da mama
Compilámos inúmeros grupos de experiências de genómica feitas em tumores primários de doentes com carcinoma da mama para as quais existe informação prognóstica. Estas experiências são feitas com o objectivo de descobrir novos factores de prognóstico. Reanalisámos os dados, repetindo a mesma pergunta: Quais são os genes com expressão diferencial estatisticamente significativa entre doentes que recaíram e doentes que não recaíram. Identificámos 65 genes nestas condições e o MKI67, o gene que codifica a proteina Ki67, estava nesse grupo. Identificámos vários genes que se sabe estarem envolvidos no processo de agregação de centrossomas. O gene que considerámos mais promissor foi a kinesina KiFC1, que já tinha sido identificada como regulador da agregação de centrossomas. Anomalias cetrossomais numéricas e estruturais têm sido observadas em neoplasias. Há dados correlacionando anolmalias centrossomais estruturais e e numéricas com o grau de malignidade e os eventos precoces da carcinogénese. Mas estas anomalias centrossomais têm um peso para a célula que deve adapatar-se ou entrará em apoptose. Os nossos resultados sugerem que existe um mecanismo adaptativo, a agregação de centrossomas, com impacto prognóstico negativo. O nosso objetivo foi quantificar o valor prognóstico das anomalias centrossomais no carcinoma da mama. Para isto usámos material de doentes dos quais sabemos a história natural. Avaliámos os genes de agregação de centrossomas, KIFC1 e TACC3, nas amostras tumorais arquivadas em parafina: primeiro com PCR (polymerase chain reaction) quantitativa e depois com imunohistoquímica (IHQ). Apenas a proteína KIFC1 foi discriminatória em IHQ, não se tendo conseguido otimizar o anticorpo da TACC3. Os níveis proteicos de KIFC1 correlacionam-se com mau prognóstico. Nas doentes que recaíram observámos, no tumor primário, maior abundância desta proteína com localização nuclear. Em seguida, demonstrámos que a agregação de centrossomas é um fenómeno que ocorre in vivo. Identificámos centrossomas agregados em amostras de tumores primários de doentes que recaíram. Tecnicamente usámos microscopia de fluorescência e IHQ contra proteínas centrossomais que avaliámos nos tumores primários arquivados em blocos de parafina. Observámos agregação de centrossomas num pequeno número de doentes que recaíram, não validámos, ainda, este fenótipo celular em larga escala.

Parte 2: Como tratar com terapêutica sistémica os vários subtipos de carcinoma da mama

Capítulo 3: Quantas doenças estão englobadas na definição carcinoma da mama triplo negativo? (revisão)

O carcinoma da mama triplo negativo é um tumor que não expressa três proteínas: recetor de estrogénio, recetor de progesterona e o recetor do fator de crescimento epidermico tipo 2 (Her2). As doentes com estes tumores não são ainda tratadas com terapêutica dirigida, possivelmente porque esta definição negativa não tem ajudado. Sabemos apenas as alterações genéticas que estes tumores não têm, não as que eles têm. Talvez por esta razão, estes tumores são o subtipo mais agressivo de carcinoma da mama. No entanto, na prática clínica observamos que estas doentes não têm sempre mau prognóstico, além de que dados de histopatologia e epidemiologia sugerem que esta definição negativa não está a capturar um único subtipo de carcinoma da mama, mas vários. Avaliámos criticamente esta evidência, clínica, histopatológica, epidemiológica e molecular. Há evidência de heterogeneidade, mas não é claro quantos subtipos estão englobados nesta definição de carcinoma da mama triplo negativo. A resposta a esta pergunta, e a identificação do fundamento molecular desta heterogeneidade vai ajudar a melhor definir o prognóstico e eventualmente a definir novos alvos terapêuticos nesta população difícil.

Capítulo 4: Terapêuica sistémica em carcinoma da mama triplo negativo (revisão)

A quimioterapia é a única terapêutica sistémica disponível para as doentes com carcinoma da mama triplo negativo, ao contrário dos outros dois subtipo de carcinoma da mama que têm com a terapêutica antiestrogénica e anti Her2, importantes benefícios. Apesar de terem surgido várias opções terapêuticas para estes doentes nennhuma terapêutica dirigida foi validada pelos ensaios clínicos conduzidos, possivelmente porque a biologia deste carcinoma ainda não foi elucidada. Muitos ensaios demonstram que os tumores triplos negativos beneficiam com quimioterapia e que as mais altas taxas de resposta patológica completa à terapêutica neoadjuvante são observadas precisamente nestes tumors. A resposta patológica completa correlaciona-se com a sobrevivência. Estamos a estudar regimes adjuvantes específicos para doentes com estes tumors, mas, neste momento, regimes de terceira geração com taxanos e antraciclinas são os mais promissores. O papel de subgrupos de fármacos específicos, como os sais de platina, mantém-se mal definido. Quanto às antraciclinas e taxanos, estes grupos não mostraram beneficio específico em carcinoma da mama triplo negativo quando comparado com os outros subtipos. Os próprios carcinomas da mama triplos negativos são heterogéneos e carcinomas da mama basais triplos negativos com elevada taxa de proliferação e carcinomas da mama triplos negativos surgidos em doentes com mutação germinal BRCA1 poderão ser mais sensíveis a sais de platino e menos sensíveis a taxanos. Como a definição molecular ainda não foi explicada a busca de terapêutica dirigida vai continuar.

Capítulo 5: Ensaio randomizado de fase II do anticorpo monoclonal contra o recetor do fator de crescimento epidérmico tipo 1 combinado com cisplatino versus cisplatino em monoterapia em doentes com carcinoma da mama triplo negativo metastizado

O recetor do fator de crescimento epidérmico tipo 1 está sobre expresso nos tumores das doentes com carcinoma da mama triplo negativo metastizado, um subtipo agressivo de carcinoma da mama. Este ensaio investigou a combinação de cetuximab e cisplatino versus cisplatino isolado em doentes deste tipo.

Doentes em primeira ou segunda linha de terapêutica para doença metastizada foram randomizadas, num sistema de 2 para 1, para receber até 6 ciclos da combinação de cisplatino e cetuximab ou cisplatino isolado. Às doentes randomizadas para o braço de monoterapia podiamos, após progressão, acrescentar cetuximab ou tratá-las com cetuximab isolado. O objetivo primário foi a taxa de resposta global. Os objetivos secundários foram a sobrevivência livre de doença, a sobrevivência global e o perfil de segurança dos fármacos.

A população em análise foram 115 doentes tratadas com a combinação e 58 doentes tratadas com cisplatino em monoterapia, 31 destas em quem se documentou progressão passaram a ser tratadas com um regime que incluía cetuximab, isolado ou em combinação. A taxa de resposta global foi de 20% no braço da combinaçao e de 10% no braço da monoterapia (odds ratio, 2.13). A sobrevivência livre de doença foi de 3.7 meses no braço da combinação e de 1.5 meses no braço em monoterapia (hazard ratio, 0.67). A sobrevivência global foi de 12.9 meses no braço da combinação versus 9.4 meses no braço de cisplatino. Conclui-se que, apesar de não ter sido alcançado o objectivo primário, acrescentar cetuximab, duplica a resposta e prolonga tanto a sobrevivência livre de doença como a sobrevivência global.

Capítulo 6: Bloquear a angiogénese para tratar o carcinoma da mama (revisão)

A angiogénese é uma característica que define a neoplasia, porque tumores com mais de 1mm precisam de formar novos vasos para poderem crescer. Desde que se descobriram as moléculas que orquestram esta transformação, que se têm procurado desenvolver e testar fármacos que interfiram com este processo. No

carcinoma da mama o bevacizumab foi o primeiro fármaco aprovado pela FDA em primeira linha para tratar doença metastática. Depois foram estudados um grupo de inibidores de tirosina cinase associados aos recetores transmembranares envolvidos na angiogénese como o VEGFR, PDGFR, KIT, RET, BRAF e Flt3: sunitinib, sorafenib, pazopanib e axitinib

Neste capítulo, analisaram-se e resumiram-se os dados dos ensaios clínicos das drogas anti-angiogénicas no tratamaneto do carcinoma da mama. Os ensaios de fase III do bevacizumab em carcinoma da mama mostraram uma redução na progressão de doença de 22 a 52% e aumento da sobrevivência livre de doença de 1.2 a 5.5 meses mas nunca foi demonstrado prolongamento de sobrevivência. Os ensaios de fase III em carcinoma da mama adjuvante com bevacizumab são dois e foram ambos negativos.

O ensaio de fase III com o inibidor da tirosina cinase, sunitinib foi negativo, enquanto que os ensaios de fase II com os inibidores da tirosina cinase sorafenib e pazopanib melhoraram alguns indicadores de resposta e sobrevivência. A endostatina foi testada no contexto neoadjuvante com antraciclinas e melhorou a taxa de resposta, mas, mais ensaios são necessários para estabelecer este fármaco. A maioria dos ensaios clínicos dos agentes antiangiogénicos em carcinoma da mama reportaram aumento da taxa de resposta e de sobrevivência livre de doença mas nunca aumento da sobrevivência global quando comparado com quimioterapia isolada o que levou ao cepticismo a que assistimos atualmente em relação ao bloqueio da angiogénese.

Ensaios clínicos selecionados em doentes específicas com objetivos translacionais relacionados com material biológico colhido, preferefencialmente em diferentes intervalos da terapêutica, serão cruciais para o bloqueio da angiogénese sobreviver como estratégia terapêutica em carcinoma da mama.

Capítulo 7: A resposta à hipoxia medeia a resistência primária ao sunitinib em carcinoma da mama localmente avançado

O sunitinib é um fármaco antiangiogénico que nunca foi avaliado isolado em doentes com carcinoma da mama não tratadas. O nosso objetivo foi caracaterizar a atividade do sunitinib isolado e em combinação com o docetaxel em carcinoma da mama não tratado, localmente avançado ou operável, mas de dimensão superior a 2 cm, para compreender os mecanismos de resposta. Doze doentes foram tratadas com duas semanas iniciais de sunitinib seguido de quatro ciclos de combinação de sunitinib e docetaxel. A resposta, a reistência e a toxicidade foram avaliadas de acordo com parametros clínicos, ressonância magnética nuclear, tomografia de emissão de positrões, histopatologia e perfis de expressão genómica.

Detetámos resistência primária ao sunitinib na janela inicial de duas semanas, evidenciada em quatro doentes que não responderam. À data da cirurgia, cinco doentes tinham tumor viável na mama e axila, quatro tinahm tumor viável na mama e três foram retiradas do ensaio. Não houve respostas patológicas completas.

A comparação dos perfis de expressão genómica entre os respondedores e os não respondedores, aos quinze dias iniciais, permitiu-nos identificar sobre expressão de VEGF e outras vias angiogénicas nos não respondedores. Especificamente, em tumores resistentes ao sunitinib isolado detectámos uma resposta transcricional à hipoxia caracterizada por sobre expressão de vários dos genes alvo do HIF1α. Neste ensaio de sunitinib isolado em doentes não tratadas com carcinoma da mama localmente avançado, encontrámos evidência molecular de resistência primária ao sunitinib possivelmente mediada por sobre expressão de genes que respondem à hipoxia.

Parte 3: <u>Quando parar</u> a terapêutica sistémica às doentes com carcinoma da mama

Capítulo 8: Agressividade terapêutica ns últimos três meses de vida num estudo retrospetivo dum centro único

Incluímos todos os adultos que morreram com tumores sólidos na instituição em 2003 e foram tratados com quimioterapia para tratar neoplaias metastizadas. Colhemos dados detalhados relacionados com quimioterapia e toxicidade nos últimos três meses de vida a partir do processo clínico. Trezentas e dezanove doentes foram incluídos, a mediana de idade foi 61 anos. A mediana de sobrevivência de doença metastática foi de 11 meses. 66% (211) dos doentes foram tratados com QT nos últimos 3 meses de vida, 37% foram tratados com QT no úlimo mês de vida e 21% nas últimas duas semanas. Nos doentes que foram tratados com QT nos últimos três meses de vida, 50% começaram um novo regime terapêutico neste período e 14% começaram um novo regime no último mês. Identificámos como determinantes de tratamento com QT no fim de vida a idade jovem, o carcinoma da mama, do ovário e do pâncreas.

Concluímos que administrámos QT no fim de vida frequentemente e iniciámos novos regimes terapêuticos no último mês de vida em 14% dos casos. Precisamos de aprofundar este trabalho para compreender se esta atitude agressiva resulta em melhor paliação de sintomas e qualidade de vida no fim de vida dos doentes com neoplasias disseminadas.

Capítulo 9: O tratamento do carcinoma da mama no fim de vida está a mudar?
Quisémos caracterizar a modificação da tendência no uso de QT e de estratégias paliativas no fim de vida das doentes com carcinoma da mama em diferentes instituições e em intervalos de tempo diferentes. Para isto selecionámos doentes que morreram de carcinoma da mama durante 6 anos, entre 2007 e 2012, num hospital geral e comparámos com as doentes que morreram de carcinoma da mama em 2003 num centro oncológico. Avaliámos um total de 232 doentes. O grupo mais recente tem 114 doentes e o grupo anterior tem 118 doentes. Usámos estatística descritiva para caracterizar QT no fim de vida e o uso de estratégias paliativas. Ambas as coortes são comparáveis em termos das características do carcinoma da mama. Observámos aumento do uso de estatégias paliativas: consulta da dor, consulta de cuidados paliativos e radioterapia paliativa no cuidado das doentes com carcinoma da mama metastizado. Evidenciámos aumento do número de mortes em serviços de cuidados paliativos. No entanto, a QT paliativa continua a ser prolongada até aos últimos meses de vida, embora tenhamos mostrado uma diminuição desta prática. Outros indicadores de agressividade como a admissão hospitalar também mostraram diminuição. Confirmámos a nossa hipótese de que há maior integração da medicina paliativa multidisciplinar e menos agressividade na terapêutica sistémica das doentes com carcinoma da mama nos últimos meses de vida.

Chapter 10: Porque é que os nossos doentes são tratados com quimioterapia até ao fim da vida? (editorial)
Este capítulo começa por dar o exmeplo duma jovem de 22 anos que viveu três meses após começar QT paliatva. Este caso epitomiza a futilidade terapêutica e é usado como ponto de partida para explorar as razões pelas quais administramos QT no fim de vida aos doentes quando é inútil, tóxica, logisticamente complexa e cara. Será que estamos a prescrever QT até tarde demais? Os oncologistas fazem previsões excessivamente otimistas e têm uma atitude pró terapêutica excessiva e são criticados por outros intervenientes nas instituições de saúde por isto. Crescentemente doentes, familiares, associações de doentes, definidores de políticas de saúde, jornalistas e a sociedade em geral afloram este tema mas tornam-se inconsistentes quando se trata dum doente próximo em que se modifica o discurso para que se façam terapêuticas sitémicas agressivas. Há uma crescente cultura de preservação da qualidade de vida, paliação, abordagem sintomática, referenciação a unidades de cuidados paliativos e outros temas do fim de vida dos doentes oncológicos terminais. Infelizmente, este tema tem ganhado momentum não porque os oncologistas estejam a refletir criticamente sobre a sua prática, mas porque os custos dos cuidados de saúde são crescentes e incomportáveis. Seja qual fôr o motivo, as razões que levam os oncologistas a administrar QT no fim de vida devem ser criticamente elucidadas. Mas há poucos dados para nos guiar nesta fase delicada da vida dos doentes e os que existem são por vezes irreconciliáveis, é uma revisão destes dados que foi feita neste capítulo.

Conclusão: A abordagem do carcinoma da mama no futuro?

Na conclusão, tenta-se olhar para o futuro e prever como será a tomada a cargo dum doente com carcioma da mama amanhã. Faz-se uma avaliação das várias àreas desde prevenção, rastreio, suscetibilidade genética e comportamental e terapêutica. Na terapêutica separa-se a terapêutica locoregional, sistémica adjuvante e da doença metastizada. Nos três últimos parágrafos a história duma mulher com um carcinoma localmente avançado que sobre expressa o recetor Her2, serve como ilustração de como devemos estar preparados para incorporar evolução, heterogeneidade e dinamismo no cuidado de doentes com carcinoma da mama.

Introduction: Breast cancer care in the past

Egypt, Mesopotamia, India, Greece, Rome and Medieval Europe

The Edwin Smith surgical papyrus, which dates from 3000–2500 BC, is believed to be the first report of breast cancer, it describes forty eight cases of tumors or ulcers of the breast for which there was no treatment. It collects the teachings of Imhotep a great Egyptian physician, a "Rennaissance" man at the center of Egyptian "Rennaissance", he knew medicine architecture and astronomy. The Greeks, centuries later, in Egypt, fused Imhotep into their medical god Asclepius. Imhotep writes with surgical clarity about broken bones, collapsed vertebrae, shattered skulls and abscesses of the skin, that he describes to be cool, with no fever, granulations or fluid, they are large, spreading and hard. Imhotep advises no treatment, only ointments. Assyrian cuneiform tables from 2000 BC, also mention breast cancer and Indian reports mention surgery and cautery as treatment approaches. The Greek historian Herodotus (400 BC), describes a surgical cure when he reports the story of the queen of Persia who had a red swollen mass in her breast with axillary lymph node invasion. Hippocrates only mentions breast cancer twice, and advises no treatment. Hippocrates coined the word cancer from the Greek word karkinos, that he described as large, superficial tumors, visible to the eye, like breast, epidermal or head and neck cancers. Karkinos means, in fact, crab. A better word is onkos, also greek, but meaning burden, mass or load. This word has a less physical tone and denotes a burden carried by the body, more concordant with the systemic nature of cancer.

The early Romans performed extensive surgery with removal of the pectoralis major muscle, but Celsus (40 AD), advised against surgery. Galen (200 AD), was very pessimistic and attributed breast cancer to a particular humor that prevails in the body, "an excess of black bile, without boiling". Despite this systemic theory, he advocated surgical removal of early lesions. This black bile without boiling is reminiscent of chronic inflammation or a cortisol or catecholamine response to aggression, curiously in unison to modern thinking. In the Middle Ages, surgical approaches were discouraged by the church. It was in the Middle Ages that the high incidence of breast cancer in nuns was first described; today, we understand the higher incidence of breast cancer in nulliparous women. In the Middle Ages this phenomenon was called the convent plague and breast cancer in nuns was seen as a punishment.

Renaissance to XXth Century

Ambroise Paré (1510-90), excised small tumors and treated advanced lesions with ointments. He was the first to advocate removal of the axillary lymph nodes en bloc with the breast, recognizing they were part of the malignant process. In the nineteenth century, great advances were made in surgery: the introduction of general anesthesia, antisepsis and microscopic pathology.

A memorable legacy of the nineteenth century was the discovery that breast cancer was a hormone dependent cancer. The growth of breast lesions, in certain premenopausal patients, was observed to increase in certain phases of the menstrual cycle, and, it was observed that the disease grew more slowly in postmenopausal women. Beatson, in 1896, showed that breast cancer was hormonally dependent in a very famous paper where he describes the regression of breast lesions in two premenopausal breast cancer patients after removal of the ovaries. Today, ovariectomy remains a very useful and cheap treatment for adjuvant and advanced breast cancer with the added benefit of lowering ovarian carcinoma risk.

The results of surgery for breast cancer at this time were still poor, partly because of a high operative mortality of 20%. The patients that survived, rarely lived longer than two years. Paget, the famous British surgeon, confessed to never having seen a cure. German pathologists, a very important school before the World Wars, with microscopists dedicated to pathology and cell biology like Virchow and Boveri, were instrumental in documenting involvement of the axillary nodes and the pectoralis fascia in mastectomy samples. One of Vichow's students, Muller, was the first to report that cancers were composed of living cells. More interestingly, he reported the similarity of cells in a breast lesion and its metastases in the ribs and noted that cancer cells had lost the proportions of normal cells. Theodor Boveri's prescient depth of understanding of cell biology of cancer led one of his colleagues to write ten years after his death that, ''Boveri was the greatest cytologist of his generation, a man so keen, so careful and so cautious, that any least suggestion from him deserves most thorough consideration. Boveri's work should be the starting point for any studies of causes, inheritance or cure of cancer''. Remarkably, this is still true today.

Aware of the results reported by the Germans, in the United States, Halstead, advocated the removal en bloc of the breast, the axillary contents and the pectoralis major, a surgery called radical mastectomy. In his series of 50 patients the local recurrence was 6%, compared to other series with 80%. However, with longer follow-up, this number was closer to 20%. Nevertheless, it was a great achievement that changed the management of breast cancer. Today, there are still women in follow-up at our institutions that have no pectoral muscle, leading to an impressive loss of functionality of the arm. The worldwide popularity of radical mastectomy led to even more aggressive approaches with internal mammary, supraclavicular, mediastinal lymphadenectomies and even arm amputation. Fortunately, these were abandoned for high operative

mortality and morbidity, and similar results to Halstead's radical mastectomy. Halstead wrote that radical mastectomy was performed in "old" patients: "their average age is 55 years, and, they are no longer very active members of society". This is shocking today but, at the time, the average life expectancy was 47 years.
In the end of the century, X-rays and radium were discovered. Marie Curie won the Nobel Prize in chemistry in 1911, for the discovery of radium and polonium and the characterization of their chemical properties. In 1937, a british surgeon, implanted radium needles into inoperable tumors and reported on a series of 200 patients. The five-year survival was 29%, a result as good as that of radical mastectomy, but, the limited availability of radium, handling problems and fibrosis, precluded its wide use. In 1932, a report was published of 1022 patients that were irradiated, either because of early disease, frailty or surgery refusal. The 5-year survival of 80%, was better than in historical surgical controls. With this in mind, researchers started adding radiotherapy to the supraclavicular, internal mammary and axillary nodes to patients that had simple mastectomy - adjuvant radiotherapy. The long term toxic effects of radiotherapy, that were less predictable than those of surgery, were the reason for less enthusiasm for radiotherapy, as well as the increased availability of surgery. It was successfully demonstrated that inoperable cancers could be eliminated by 70 Gy of radiotherapy over 3 months. We still have patients alive and in follow up that had inoperable breast cancers treated by radiation alone.

1950-2000
The pace of change of breast cancer treatment in these 50 years is only surpassed by the even greater pace of change in the years 2000 to 2014. The evolution witnessed is truly more like a revolution. In 1954, the International Union Against Cancer devised the TNM classification, an integral part of cancer care. But two other changes were even more relevant: First, the discovery of x-rays which provided the basis for diagnosis by mammography and for treatment by radiotherapy. Second, the discovery of hormones by Starling in 1905. The discovery of estrogen changed breast cancer. It changed the concepts of carcinogenesis, incidence, risk factors and, most fundamentally, it changed breast cancer treatment. Estrogen had been responsible for Beatson's surgical success while performing oophorectomy, and surgeons entered into a growing belief in endocrine surgery for controlling metastatic breast cancer. However, the second half of the twentieth century brought the rise and fall of endocrine surgery for metastatic disease. The importance of the hormonal milieu was subsequently confirmed by the use of adrenalectomy in 1951 and hypophysectomy in 1953. In the one-third of patients who benefited, the mechanism by which this occurred was thought to be oestrogen deprivation, and the scientific foundation for this, was confirmed by the discovery of the oestrogen receptor (ER) in breast tumours, by Jensen, in 1967. Ablative endocrine surgery has now largely been superseded by the development of medical endocrine therapies. Thus, the estrogen antagonist, tamoxifen, has mostly replaced surgical oophorectomy, the aromatase inhibitors (which block peripheral synthesis of estrogen) have replaced adrenalectomy, and the luteinizing hormone releasing hormone (LHRH) agonists have replaced hypophysectomy, in the management of patients with adjuvant and metastatic breast cancer.
Regarding surgery, Patey recommended preserving the pectoralis major muscle, unless it was directly involved by cancer, an operation called "conservative" radical mastectomy. This operation eventually prevailed, since 1979, as the "modified" radical mastectomy, and is still a surgical standard today. But the change from radical to modified mastectomy was not the only change. The intellectual leadership of Bernard Fisher and Umberto Veronesi dominated the landscape of breast cancer since 1970. They convincingly showed that lymph nodes were not an effective barrier to the spread of cancer and the seed for breast conservation was planted. Fisher wrote that "either the original surgical principles have become anachronistic or, if they are still valid, they were conceived originally for the wrong reasons". This was written only 70 years after the reign of Halstead's radical mastectomy swept the world. Fisher asserted that breast cancer was a systemic disease and viable cancer cells always, or almost always, disseminated before diagnosis. If this was true (1) variations in local treatment will not influence cure, and (2) effective systemic treatment was necessary to improve cure rates. Beginning at Guy's Hospital, in London, controlled clinical trials of breast conservation, would change breast surgery. These were also started in Milan, in 1973, and by the NSABP, in 1976, in the USA. These trials established that "lumpectomy", followed by whole breast irradiation was as effective as total mastectomy for both local and distant disease control of most patients with early-stage breast cancers, and this was an obvious cosmetic improvement. Based on these outcomes, since 1990, breast-conserving surgery is the preferred treatment of stage I and II breast cancer.
If breast cancer is a systemic disease, the axilla should not be subjected to extensive surgical dissection, if the sentinel lymph node was found to be negative. Since the 1990s, the innovation of sparing axillary dissection has been reliably established. Radiotherapy, in the current setting, of minimal surgery, has won a very relevant position, but, the rethinking of the role of surgery in breast cancer has not yet come into acceptance. It would be the shattering of a dogma, but as anal, laryngeal and rectal carcinomas are treading that unchartered territory, soon the question will be put to breast cancer physicians. Meanwhile radiotherapy

is indicated in every single breast conserving surgery as well as for DCIS and there are still mastectomy patients that must undergo radiotherapy.

What about chemotherapy? The first experiments with mustard gas to treat humans were done, in Yale, in 1942, but wartime secrecy prevented it's dissemination until 1946. To most, the birth of chemotherapy was in December 1943, in Bari harbor, Italy, during the II World War, when there was an explosion of mustard gas. The sailors were later diagnosed with bone marrow aplasia showing that the gas interfered with hematopoietic cell proliferation. This was thought to be useful in the treatment of acute leukemia, and, it gave mustard gas the wide dissemination that was lacking in the experiments done in small labs. Continued research produced alkylating agents such as busulfan, cyclophosphamide and chlorambucil, all still used today. None proved toxic exclusively for cancer cells or free of undesirable side effects, and none cured clinical breast cancers, but their judicious use proved clinically useful. These drugs administered systemically often produced temporary regression and occasionally complete disappearance of advanced breast cancers. Initial trials of intravenous, perioperative triethlylene-thiophos-phoramide (Thio-TEPA) in the late 1950s, intended to destroy tumor cells released during mastectomy, were failures, but extended adjuvant treatment with L-phenylalanine mustard, directed against occult micrometastases, improved the survival of patients with early stage breast cancer. These studies were published in early sixties and established the rationale for adjuvant treatment. The 1970s brought the concept of combination chemotherapy for adjuvant treatment, joining several drugs with different mechanisms of action, cyclophosphamide, fluorouracil, and methotrexate in the acronym CMF, that we still use today. The end of the 1970s brought doxorubicin. Anthracyclines are still essential drugs in the adjuvant treatment of breast cancer today. Despite the long term toxicity profile ,that includes cardiac failure and secondary leukemias. After several clinical trials asking questions about anthracycline sparing regimens, this group of drugs is still unavoidable. In the early 1960s, paclitaxel was discovered as part of the North American National Cancer Institute (NCI) program in which extracts of thousands of plants and natural products were screened. In vitro, the crude extract of the bark of the Pacific yew, *Taxus brevifolia*, a slow growing evergreen, was found to have broad antineoplastic activity. The active component was only isolated in 1969, and, the structure, essential for synthesizing the molecule, was elucidated in 1971. Clinical trials started in 1977, and, because of its broad but not impressive activity, it was not given high priority. To make matters worse, paclitaxel, as we know today, is poorly soluble in water, and, at the time, due to its origin, the molecule was scarce. This meant procurement and preparation of quantities sufficient for large-scale clinical development would be difficult. In the 1980s paclitaxel was shown to have a 60% response rate in metastatic breast cancer and the race started. Academia, pharmaceutical companies, chemists, botanists and environmentalists discussed how to move forward with such an effective drug in breast cancer that was menacing Pacific northwest forests. In the initial trials, and, due to its scarcity, paclitaxel was purified from the urine and feces of one patient to give to the next, as had happened with antibiotics in the II World War. In Europe, Rohne Poulenc, a chemical company, screened *Taxus baccata*, and was able to produce by semi synthesis an analogue of paclitaxel prepared from a non cytotoxic precursor extracted from the needles of the yew. This drug, docetaxel, entered trials in the begining of the 1990s. One of the major toxicities of docetaxel are several effusions, pleural, pericardial and peritoneal. This fact could have jeopardized an efficacious drug, because, in the Phase I studies, the effusions were at first thought to be disease progression, and, only after closer scrutiny, were they finally interpreted as toxicity. This story is important, because, in drug development, we should be aware of serendipitous, unexpected toxicity; it may jeopardize the development of promising drugs.

In the 1980s, in the lab of a formidable cancer scientist named Robert Weinberg, researchers were changing the paradigm in the new direction of drug discovery: identify the oncogene then design the drug. They cloned oncogenes from cell lines and animal models. There, in 1982, an oncogene was isolated in a rat neuroblastoma and named neu after neuroblastoma. In 1983, in a paper in Science, Dennis Slamon demonstrated that Her2/neu amplification was a negative prognostic factor in breast cancer. Her2 is an enormous transmembrane receptor protein securely fastened to the cell membrane, not unpredictably motile in the cytoplasm, like the untargetable oncogene, myc. Soon after, it was shown that an antibody could bind and inactivate this molecule. This ignited the effort to synthesize antibodies against Her2 blocking its action. Genentech, synthesized trastuzumab, and, in the beginning of the 1990s, trials in metastatic trials performed with this monoclonal antibody showed unquestionable activity.

Regarding the old question of heritable breast cancer, the clustering of breast cancer cases in families had been observed for decades, but only in 1990 was early-onset breast cancer linked to chromosome 17q21. In the subsequent years, there was a race to identify the gene in that region that was responsible for the phenotype. Twenty two genes were cloned and sequenced, but, mutations in BRCA1, appeared to explain most of inherited cases of breast and ovarian cancer. In the initial series of familial breast cancer, close to

70% of the affected individuals had mutations in BRCA1. Mutations in BRCA2 were later associated with inherited female and male breast cancer. But half of the families with aggregation of breast cancer cases remain without identifiable mutations. Since then, families with affected young patients and with multiple cases in the family, undergo genetic testing for BRCA1 and BRCA2, and, if a mutation is found, an intensive screening program, consisting of mammography, sonography and MRI is recommended, and, interventions such as chemoprevention with tamoxifen or prophylactic surgery with oophorectomy or mastectomy are discussed. In fact tamoxifen was validated as a preventive strategy in a trial with 14000 women who had increased risk of breast cancer. The eligibility criteria allowed the randomization of patients over 60 years of age, or aged 35 to 59 years but with increased risk based on the Gail model, or women diagnosed with lobular breast cancer. Despite the 50% reduction in incidence of invasive breast cancer, the widespread use of tamoxifen in every women aged 60 has not been implemented.

Another very attractive strategy is population-based screening given the frequency and mortality of BC. An early randomized trial of screening with mammography and physical examination, in New York, in 1963, demonstrated that 30% of cancers could be detected by mammography alone, and deaths from cancer among screened women were reduced by 30%. The Breast Cancer Detection Demonstration Project (BCDDP), begun in 1973, and sponsored by the NCI and the American Cancer Society (ACS), screened 283,222 asymptomatic women. The BCDDP established the feasibility of mass population screening. Multiple randomized clinical trials of mammographic screening followed, showing that regular mammograms could detect 85 to 90% of asymptomatic breast cancers, with a reduction of breast cancer mortality. Periodic mammograms and physical examinations for detection of breast cancer in asymptomatic women aged 50 years of age and older received endorsement by the NCI, ACS, and numerous professional groups, although the recommended age to start screening remains controversial.

Part 1: Who to treat with adjuvant systemic therapy?

Chapter 1: TNM is not dead in breast cancer

Conception and design: Sofia Braga

Provision of study materials or patients: Instituto Português de Oncologia, Lisboa

Collection and assembly of data: Nuno Guerra-Pereira, Jorge Fernandes, Joana Monteiro Dias, Sofia Braga

Data analysis and interpretation: Nuno Guerra-Pereira, Jorge Fernandes, Joana Monteiro Dias, Sofia Braga

Manuscript writing: Sofia Braga

Final approval of manuscript: All authors

Funding: Sofia Braga was funded during three years (from October 2008 until October 2011) by the Fundação Calouste Gulbenkian and worked at the Computational Genomics Laboratoty (CGL) at Instituto Gulbenkian de Ciência (IGC)

Ethical Committee approval: Ethical committee of Instituto Português de Oncologia, Lisboa

TNM is not dead in breast cancer

Nuno Guerra-Pereira[1], Jorge Fernandes[1], Joana Monteiro Dias[1], António Moreira[2], Sofia Braga[1 2 3]

1- Departamento de Ciências Biomédicas e Medicina, Universidade do Algarve, Faro, Portugal
2- Instituto Português de Oncologia, Lisboa, Portugal
3- Currently at José de Mello Saúde, Lisboa, Portugal. Corresponding author
sofia.braga@jmellosaude.pt
Av. do Forte 3, Ed Suecia 3, piso 2, 2790-073 Lisboa

Summary

Background:
It has been said that the current TNM staging system might not be suitable for predicting breast cancer (BC) outcomes and for making therapeutic decisions, especially for patients with screen detected BC which is smaller. The main reason being the non inclusion of tumor biology parameters in the current TNM system. We hypothesize that in a population where there is still a large number of non screen detected BC, with a low median age of incidence and frequent high TNM stage lesions, biology is still second to classical staging in predicting prognosis.

Patients and methods:
We analyzed a population of consecutive BC patients from a single institution during ten years. The patients were treated according to institutional protocols, and followed indefinitely. We characterized current established prognostic factors, classical staging variables included in the current TNM staging system and biological variables, currently not included in the TNM system. We quantified the capacity of individual prognostic factors to predict survival.
Furthermore, we performed multivariate regression analysis to study the power to predict survival of each individual prognostic factor in a model where all other factors are taken into account.

Results:
We analyzed a population of 1699 consecutive patients diagnosed between January 1st 2000 and 31st December 2009. In the univariate analysis, increased tumor size, involved axillary nodes, higher stage lesions, estrogen receptor negativity, HER2 positivity, triple negative status or lower differentiation tumors are significant predictors of decreased survival. However, in the multivariate regression analysis, only axillary lymph node involvement, TNM stage and histological grade are independent predictors of survival.

Conclusion:
We have concluded that, in a population such as ours, classical TNM staging variables, irrespective of tumor biological features, are still the most powerful predictors of patient survival.

Introduction

Breast cancer (BC) is a heterogenous disease {Polyak, 2011, The Journal of Clinical Investigation, 121, 3786}. It is still an important public health problem since it is the most frequent cancer in women and the second cause of death by cancer in women {Igene, 2008, Breast J, 14, 428-34}. Screening mammography allows the diagnosis of earlier stage breast cancer with smaller lesions and less frequent axillary lymph node involvement, leading to lower mortality {Puliti and Zappa, 2012, BMC Med, 10, 106} {Bleyer and Welch, 2012, The New England Journal of Medicine, 367, 1998-2005}. BC originates in the glandular tissue of the mammary gland, the primary tumor (T), then, usually disseminates, first to the axillary nodes (N), and then to distant organs, where it forms metastases (M). With this in mind, sixty years ago, a French surgeon proposed that these three characteristics should be stratified in categories, integrated into a staging system to predict prognosis and tailor therapy. During the second half of the last century the TNM system was validated as a predictor disease free and overall survival {Gospodarowicz et al., 1998, Cancer Prev Control, 2, 262-8}. The utility of the TNM system for predicting survival has been more useful in patients treated with curative intent, i.e., stage I-III BC, because stage IV BC is generally fatal with an expected median survival of 2 to 3 years {Chung and Carlson, 2003, Oncologist, 8, 514-20}.

For the last half century we have known there are active systemic therapies for metastatic and adjuvant BC {Fisher et al., 1968, Ann Surg, 168, 337-56}{Fisher et al., 1975, The New England Journal of Medicine, 292, 117-22}. Since 1970, several discoveries changed BC and showed that it is a heterogenous disease in treatment and prognosis. These include the unequivocal demonstration of the prognostic and predictive power of the estrogen receptor (ER) {Jensen, 1962, Perspect Biol Med, 6, 47-59} and antiestrogenic therapy {Jordan, 1976, Eur J Cancer, 12, 419-24} and the unravelling of epidermal growth factor biology as oncogenic in BC, namely Her2 {Slamon et al., 1987, Science, 235, 177-82}. Endocrine therapy is extremely relevant because ER is the main therapeutic target in BC. Her2 amplification is prognostic and predictive to Her2-directed therapy and its strength is independent of ER {Piccart-Gebhart et al., 2005, N Engl J Med, 353, 1659-72}. Since 2005, trastuzumab is standard adjuvant treatment for Her2-amplified BC. About fifteen percent of BC that do not express ER and do not have Her2 amplification are identified as Triple negative (TN). Despite several years of effort, no targeted therapy for TNBC has emerged, and, possibly because of this, it is associated with worse prognosis. The evaluation of the proliferative rate of BC cells, through histological grade or Ki67 nuclear staining, has also been integrated into patient care. The relevance of all these determinants, collectively termed BC biology, have aroused criticism of TNM {Veronesi et al., 2006, Breast (Edinburgh, Scotland), 15, 3-8; Veronesi et al., 2009, The Breast Journal, 15, 291-5}{Yi et al., 2011, Journal of Clinical Oncology, 29, 4654}{Park et al., 2011, Ann Oncol, 22, 1554-60}. It has been proposed that TNM should be modified to include BC biology.

Others, however, recognize the utility of TNM in BC even without incorporating biologic features {Uehiro et al., 2013, Breast cancer (Tokyo, Japan)}. Work from Japan, looking at smaller tumors and the identification of micrometastatic axillary disease, as reflected in the 7th edition of the TNM {Greene FL., 2009, TNM classification of malignant tumours. 7th ed., p. 181–93.} concludes that the TNM classification is still useful in predicting prognosis. For the present work, different from the Japanese series, that focuses on small tumors, we have hypothesized that TNM is still the most important prognostic indicator when axillary involvement is present (stage II and III BC).

To test this hypothesis, we analyzed a consecutive population of BC patients from a single institution, treated with curative intent, mainly constituted by stage II and III BC. In the comprehensive cancer center, patients are treated according to institutional protocols for local and systemic therapy, with all treatment modalities delivered in the institution where patients are followed indefinitely. In the analysis, with the included TNM stage variables as well as standard biologic prognostic variables, we performed univariate survival analysis followed by multivariate regression analysis where we studied the contribution of each factor for survival.

Patients and methods

We performed retrospective analysis of ten years of BC cases in Instituto Português de Oncologia, Lisboa, this study was submitted and approved by the institutional ethics committee, informed consent from patients was waived. Sequential BC cases from 1st January 2000 until 31st December 2009 that were treated in the institution and, followed indefinitely, were analyzed. We chose these years to allow a median follow up time of over five years. We also wanted to evaluate the effect of adjuvant anti Her2-directed therapy, instituted in 2005, although we do not include these data in the current report. Data was collected from patient clinical charts, pathological reports and electronic medical records. When necessary, surgical protocols, or specific pharmacy, day hospital or radiotherapy records were reviewed. Relapse information was obtained from

primary care physicians, in the rare cases where it was necessary. Vital status was confirmed in the national online database system of death certificates when there was no such information in the institution medical records.

We collected demographic data on age at diagnosis and sex. The disease was classified as localized, locally advanced or metastatic at diagnosis. Data on clinical and pathological T and N staging were recorded. Tumor samples were classified according to standard pathology criteria for histological subtype, differentiation, hormonal receptor expression and Her2 expression and/or amplification. Primary antibodies used here were estrogen receptor (clone SPI; Ventana Roche cat. 790-4324), progesterone receptor (clone 1E2; Ventana Roche cat. 790-2223) and Her2 expression or amplification was evaluated by fluorescence in situ hybridization (FISH) with pathway HER-2/neu, clone 4B5; Ventana Roche cat. 780-001, in Ventana BENCHAMRK ULTRA instrument. Ki67 was not systematically evaluated. ER end PgR were evaluated by immunohistochemistry and the cutoff for positivity is 10%. Her2 positivity is considered when the IHC score is 3 or, in case of IHC score 2, if there is amplification of the gene by FISH.

Patients were treated with systemic therapy, in the neoadjuvant or adjuvant setting, as recommended by the institutional multidisciplinary clinic, according to institutional, national, european and international guidelines. Endocrine therapy was administered to ER or PgR positive BC cases. Since 2005, Her2 positive BC patients were treated with the monoclonal antibody trastuzumab. The data on therapy for advanced disease were not collected because it was not part of the study objective which was to evaluate the ability of the TNM classification to predict survival of patients with early stage BC and also due to its individuality and variability.

We collected data on the systemic neoadjuvant or adjuvant treatment administered. But this is not included in this report. In general, all but T1N0M0 BC patients were routinely treated with adjuvant chemotherapy. Some high risk patients with T1b or T1cN0 carcinomas were equally treated. Most patients were treated with 6 cycles of FEC: 5 fluouracil 500 mg per square meter, epirubicin 100 mg per square meter and cyclophosphamide 500 mg per square meter every three weeks for 6 cycles, in the majority of neoadjuvant and adjuvant cases. For low risk patients, without involved axillary nodes, 4 cycles of AC: doxorubicin 60 mg per square meter and cyclophosphamide 600 mg per square meter every three weeks for four cycles. Since 2005, with the demonstration of the value of adjuvant taxanes in node positive patients, we changed the systemic chemotherapy protocol for node positive patients, to incorporate taxanes. Several options were available: 80 mg of paclitaxel per square meter by intravenous infusion weekly for 12 doses or 3 cycles of docetaxel 100 mg per square meter, after 3FEC. Regarding outcome variables, we collected data regarding date on local relapse and distant recurrence, location of distant disease which we do not present in this report, death from BC or other causes and date of last follow-up.

Statistical analysis was performed with PASW statistics version 19 (SPSS Inc., Chicago, IL, USA). We used T-tests to compare continuous variables and the Chi-squared test to compare categorical variables. All tests were two-sided and the results were considred significant if the *p* value was lower than 0.05.

Patient survival analysis, was performed the Kaplan-Meier (KM) method. Comparison of survival curves was evaluated by the log rank test. We performed multivariate analysis with categorical and continuos variables with logistic regression model.

Results

One thousand nine hundred (N=1900) patients were treated between 1st of January 2000 and 31st of December 2009, of which 41 are men (2%). The median age of the population is 56 years with a range between 15 and 87 years, but 75% of the sample had between 40 and 70 years at diagnosis, with a similar distribution of patients between these three decades i.e. 25% of the subjects in each decade. In 49 patients (2.5%) the disease was disseminated ab initio, these patients did not undergo BC surgery. Two hundred and twelve (212 - 11%) other patients did not undergo surgery for various reasons that included inoperability, refusal, comorbidities and old age. These patients were treated with several combinations of systemic treatments, mainly endocrine therapy in patients with comorbidities and old age and radiotherapy was used for local control in more aggressive local disease.

The analysis includes 1639 patients that underwent BC surgery and were treated with curative intent. Because of the recruitment profile of the institution this cohort has a large percentage of locally advanced disease, 30% of the patients were treated with neoadjuvant systemic therapy. Eighty five percent (1398) of the patients had axillary lymph node dissection (ALND), in 61% there was axillary involvement. The remaining 241 (15%) patients did not undergo ALND, were staged with a sentinel lymph node biopsy (SLNB) which was negative, therefore, no further axillary exploration was deemed necessary.

Histologically, invasive ductal carcinoma currently denominated carcinoma not otherwise specified, was the most frequent histological subtype, comprising 92% of the cases. We collected pTNM and biological profile data of 1639 patients who underwent BC surgery (Table 1). Regarding tumor size, the sample has the same amount of T1 and T2 lesions, it has 61% of axillary lymph node involvement. Survival analysis according to TNM variables is shown graphically with KM survival curves (Figures 1 to 4). The survival analysis according to biology variables is similarly depicted with KM curves (Figures 5 to 8). The *p* value of the Log Rank test performed to show that the survival curves are not superimposable is similarly shown. TNM staging variables and biology variables are predictive of survival.

Two thirds of the cases express ER, 9% were not evaluated. Nearly half of the cases had no PgR evaluation. Regarding HER2, in 16% of the cases had higher abundance of this oncogene, either at the protein or gene level, however, 30% of the cases were not evaluated. There were 13% of TN cases.

Regarding outcome variables, the median follow-up is 67 months for the whole population and 73 months for living patients. Four hundred and twenty one patients died, 361 deaths (22%) from metastatic breast carcinoma, 3,6% from other causes. The causes of death, other than BC, are known, but, we did not collect the data.

The logistic regression analysis contains data from the three TNM variables and the four biological variables included in the model. We conducted logistic regression to determine which variables are independent predictors of survival. In the model, for each survival predictor, we have a statistic and associated probabilities. These provide an index of the significance of each predictor in the equation. For assessing each predictor, we take the significance values, and, if the value of *p* is lower than 0.5 we reject the null hypothesis. We conclude the variable contributes significantly for predicting survival. The third column of table 2 shows the change in odds. If the value exceeds 1 then the odds of dying increase, if the figure is less than 1 any increase in the predictor leads to a drop in the odds of death, this is the case for Her2 negative status. The odds ratio (OR), estimates the change in the odds of survival if any given patient has BC with the characteristic in study. The OR is calculated by using the regression coefficient of the variable as exponent. In the case of TNM variables, the regression coefficient of stage is 2, thus the OR is $\exp^2$, or 7.4. The odds of dying from BC are 7.4 times greater for a patient who has higher stage disease. In case of the other significant TNM variables in the model, the regression coefficient of involved axillary nodes is 1.42, therefore the odds of dying is 4 times greater for a patient with involved axillary nodes. Regarding the biological variables, in our model, built with the data from our population, we show that the odds of survival are doubled in patients with Her2 negative BC and that increasing histological grade increases the odds of death 4 times. We stress that adjuvant trastuzumab was administered since 2005.

Discussion

The cohort has a majority (61%) of stage II disease, i.e., with axillary lymph node involvement, this value is high {Turner and Leo, 2013, 2013 ASCO Educational Book, 3-8}. There are two possible reasons: First, the median age of the cohort is lower in the patients of this institution, when compared to the median age of incidence of breast cancer in the Western world {Leong et al., 2010, World J Surg, 34, 2308-24} {Turner and Leo, 2013, 2013 ASCO Educational Book, 3-8}. As has been said, this institution recruits younger patients with more advanced disease. Second, on a socioeconomic perspective, this institution serves mainly the region of southern portugal that has a less mature screening program than the north and center, and mainly serves patients without private insurance {Dourado et al., 2012, Eur J Public Health}. The high axillary lymph node involvement, and the low median age of the cohort, are the two most important findings of our work. These influence decisively of our results and conclusions.

Fifteen percent (241) patients that did not have axillary dissection because they were staged with a SLNB and ALND was deemed unnecessary. After the results of ACOSOG Z11 and other SLN trials, this figure is low, but, up to 2008, the SLN procedure was undergoing institutional validation. The main questions in the population spared ALND are about outcome measures, which we have addressed, and quality of life, which we have not. The incidence of lymphedema is not reported because the retrospective nature of data collection might induce bias of underreporting. In the institution, all patients are systematically geared towards an experienced team of physiotherapists in the immediate post surgical period where they stay until arm health and mobility is established, generally for some months. Furthermore, patients are taught about the need of lymphedema prevention and warning signs throughout life. Others have shown that these measures reduce the incidence of lymphedema {Boccardo et al., 2009, Lymphology, 42, 1-9; Erickson et al., 2001, J Natl Cancer Inst, 93, 96-111}.

The 2% metastatic (M) BC cases ab initio is lower than other series {Chung and Carlson, 2003, Oncologist, 8, 514-20}. There are two reasons for this. First, stage III BC in 2009 were not routinely staged with CT scans and PET therefore the true MBC rate might be underestimated. Second, because the institution is a tertiary referral center it might not receive so many MBC cases. Referring physicians, once MBC is an incurable condition, prefer to treat patients nearer to their homes, in an effort to preserve quality of life.

Her2 was not evaluated routinely until 2005 because trastuzumab was not approved for adjuvant therapy of Her2 positive cases. PgR was not evaluated routinely because its prognostic and predictive role has not been unequivocally established. The positivity of PgR in ER negative cases is the only situation where patient management might change, favoring the use of endocrine therapy. In such cases, PgR determination by IHC was done. Nine percent of non evaluated ER is similar to other reports and preferentially happens in locally advanced or inoperable cases that are diagnosed by cytology. The lack of comprehensive assessment of HER2 and ER might underestimate the true percentage of Her2 positive and triple negative cases which is lower than expected for this population.

In this sample there are 41 men, this is twice the expected number of male BC cases in such a sample size. The reason for this is the recruitment profile of the institution as a tertiary center. The institution has an active familial BC clinic where more than 1000 families are followed, this may bias the case mix, towards younger median age and abundance of male BC. Regarding hereditary syndromes of breast and ovarian carcinomas, there is a founder mutation in BRCA2 in the Portuguese population and the family risk clinic has strong abundance of such families {Machado et al. 2007, JCO}. The phenotypic expression of this mutation includes male BC.

In this work, our findings support our hypothesis, that in a population with more than half of axillary lymph node involvement, the strength of classical TNM prognostic factors is greater than biology-based prognostic factors. We were able to show in univariate analysis that all prognostic factors are individually predictive of survival. It is graphically apparent, but we then asked the question if the several KM curves are different. We used the Log Rank statistical test to answer this question and the null hypothesis of the test, i.e. is that the curves are superimposable, was rejected. All KM survival curves are statistically significantly different, this is shown by significant Log Rank tests.

Histological grade is not evaluated in tumors that were treated with neoadjuvant chemotherapy that is why the KM curve with worse survival is the curve of non evaluable grade. This survival curve is that of locally advanced BC that naturally has worse survival.

In the multivariate model, the classical TNM variables are independent predictors of survival, except tumor size, and, of the biology-based variables, only HER2 positivity and histological grade remain independent predictors of survival. The adverse prognostic feature of Her2 positivity in this population, only partially treated with adjuvant trastuzumab, is apparent. Furthermore, Her2 positive metastatic BC patients did not, at the time, have the current availablity of Her2 blocking agents like trastuzumab, lapatinib, pertuzumab or TDM1. It should be extremely interesting to perform the Her2-stratified survival analysis in more modern cohorts, comprehensively treated with adjuvant trastuzumab, and, upon relapse, further treated with anti Her2 agents.

The main message of this paper is that in populations with stage II and III BC, classic prognostic factors are still more important than biology in predicting survival, and, necessarily, that the TNM classification is not outdated in BC. Our data is in accordance to the EBCTCG overview data which included nearly 100.000 early BC cases randomized in 123 trials. Our questions are not the same as the overview, where the focus is on treatment. In the overview, the proportional benefits of adjuvant chemotherapy, in the analyzed population, were independent of age, nodal status, ER status and histological grade. But the EBCTCG metaanalysis, like our population, has over 50% node positive patients, in all trials combined. In the overview data, for the questions of anthracyclines versus taxanes, there are nearly 90% of node positive patients. Furthermore, the EBCTCG, like our data, has suboptimal characterization of BC biology. Our data, has issues that limit the generalization of our findings to current BC clinical practice where we are taking care of patients with small, screen detected BC, in which we thoroughly assess tumour biology parameters {Turner and Leo, 2013, 2013 ASCO Educational Book, 3-8}. In such populations, it may be that the TNM classification is obsolete.

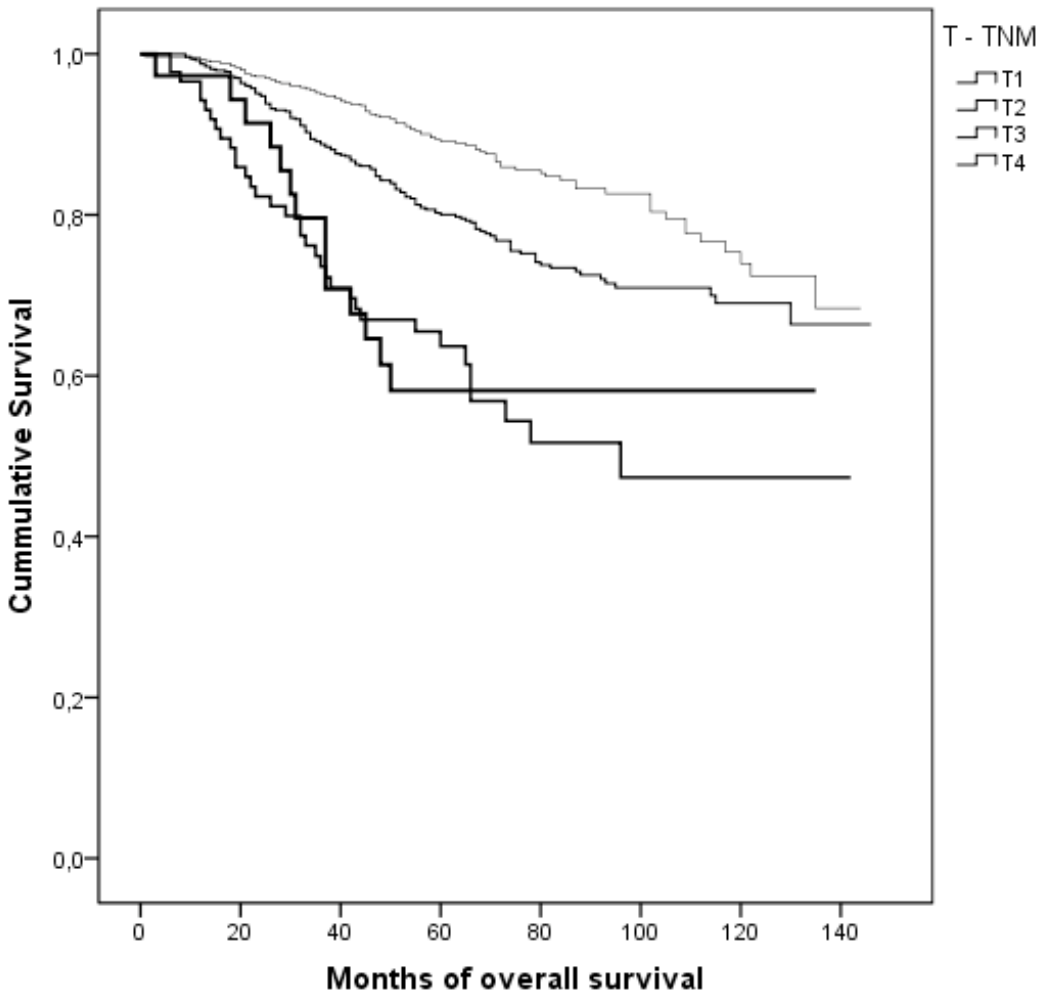


Figure 1 Guerra et al.
Overall survival stratified according to primary tumour size as categorized by the TNM classification. (P value $4x10^{-13}$)

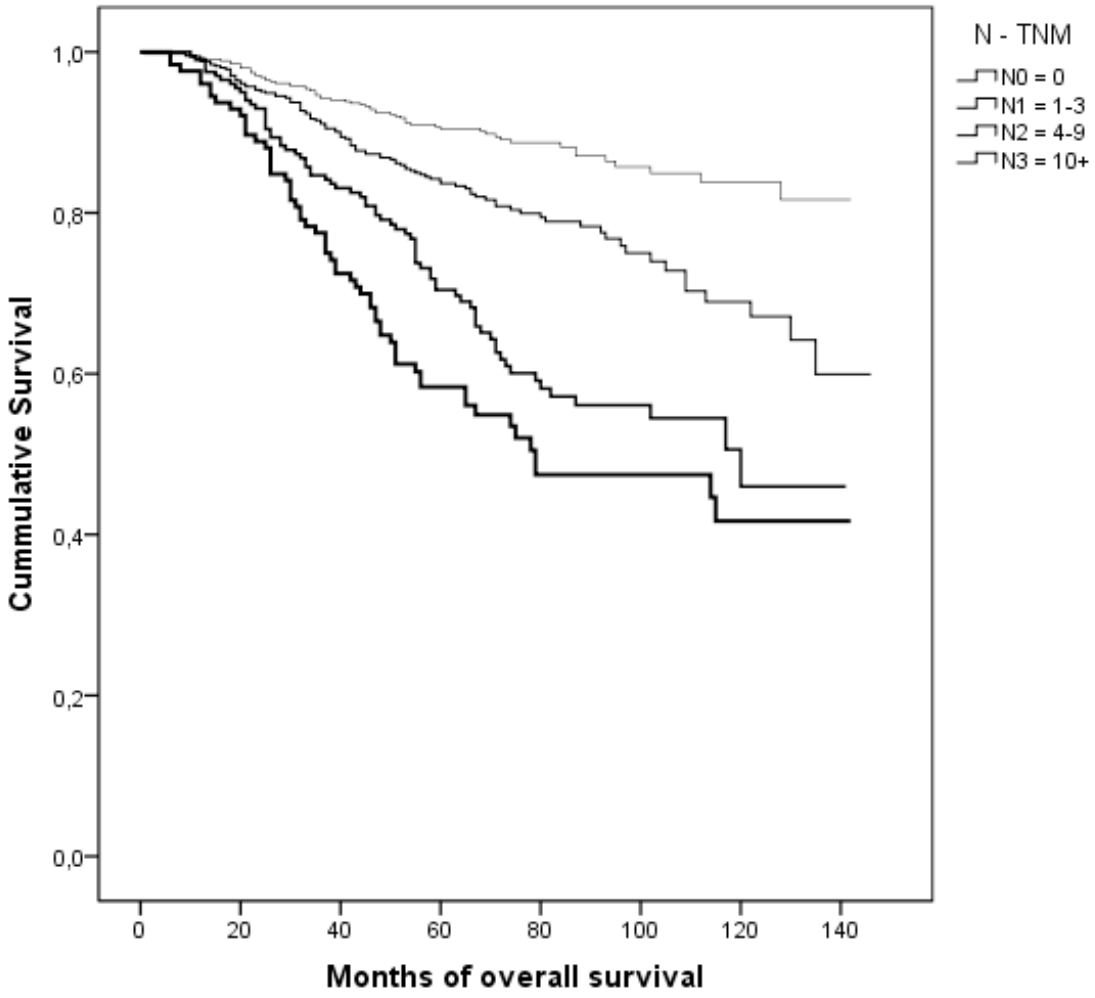


Figure 2 Guerra et al.
Overall survival stratified according to number of involved axillary lymph nodes as categorized by the TNM classification. Patients with no involved axillary nodes have a higher probality of survival. (P value $2x10^{-25}$).

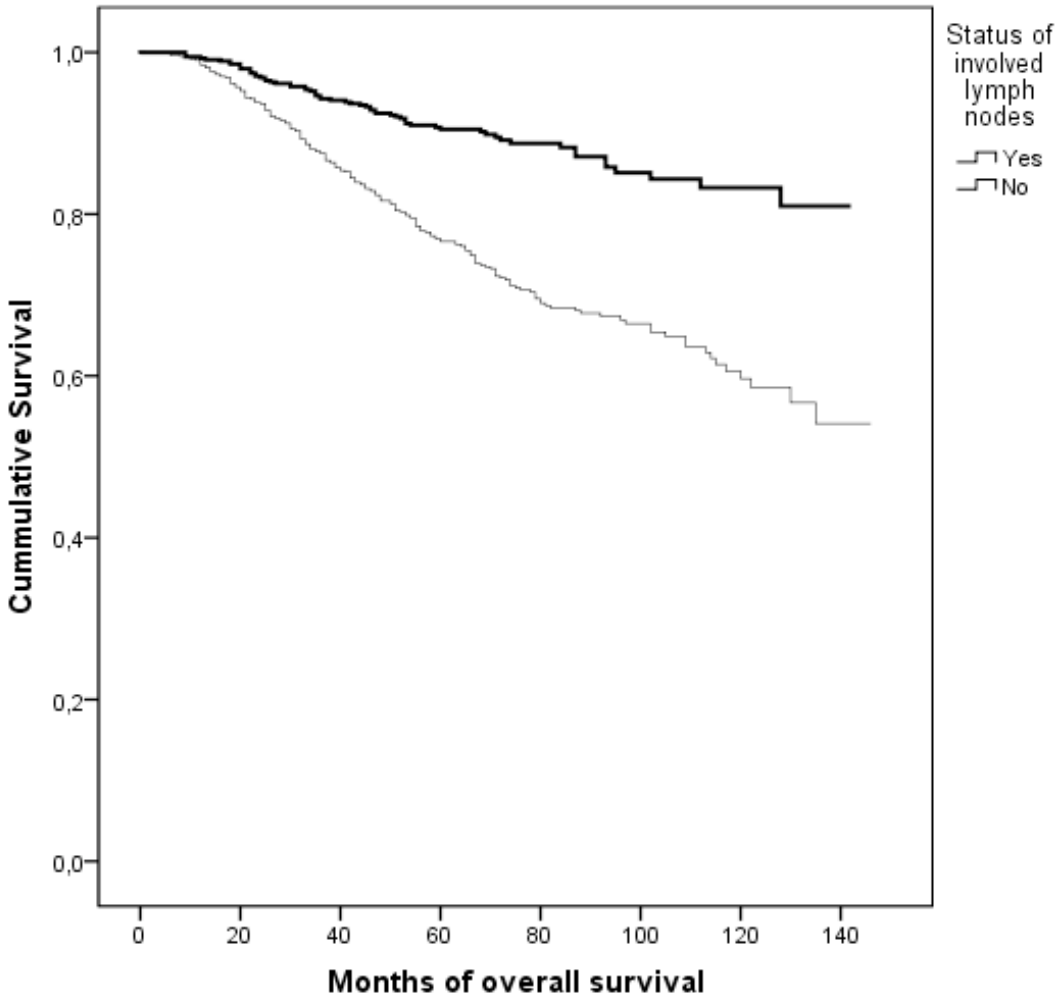


Figure 3 Guerra et al.
Overall survival stratified according to lymph node involvement. Patients with no involved axillary nodes have a higher probality of survival. (P value $1x10^{-13}$).

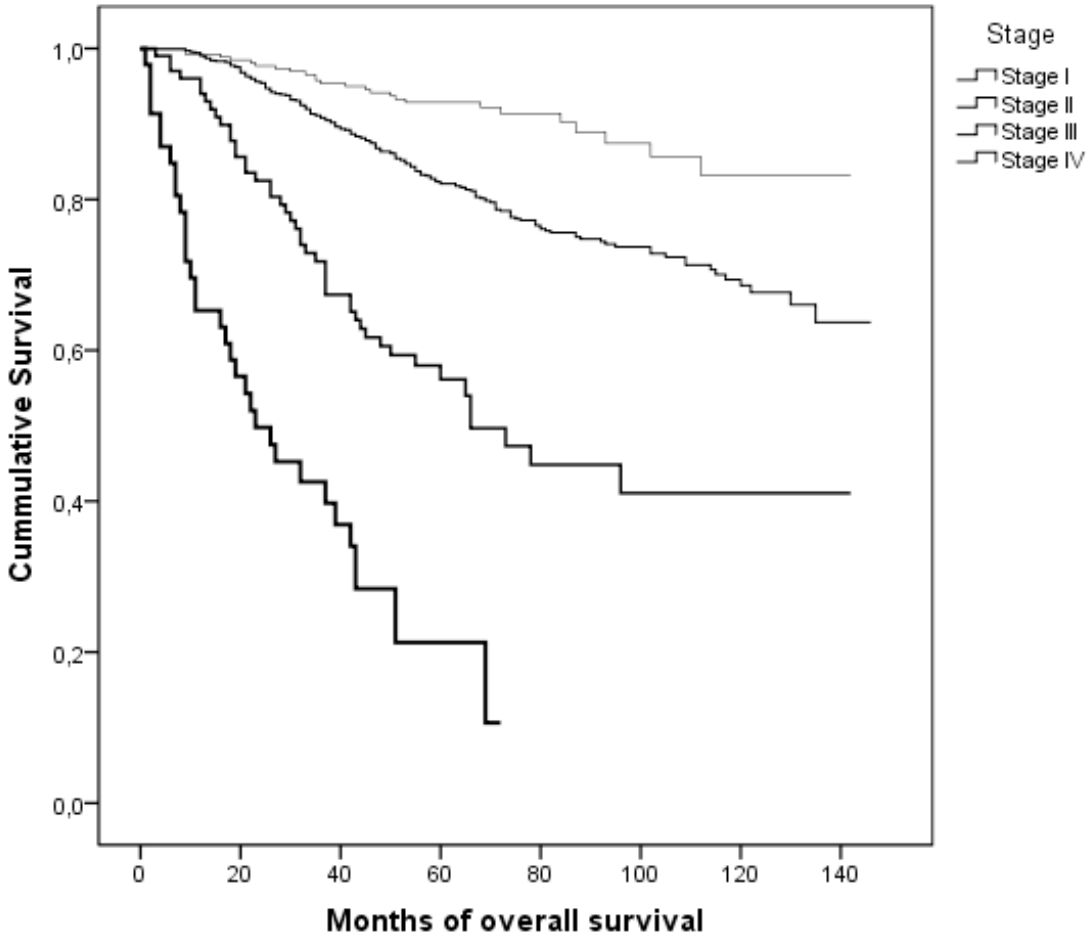


FIgure 4 Guerra et al.
Overall survival stratified according to TNM stage. (P value 3x10-[66])

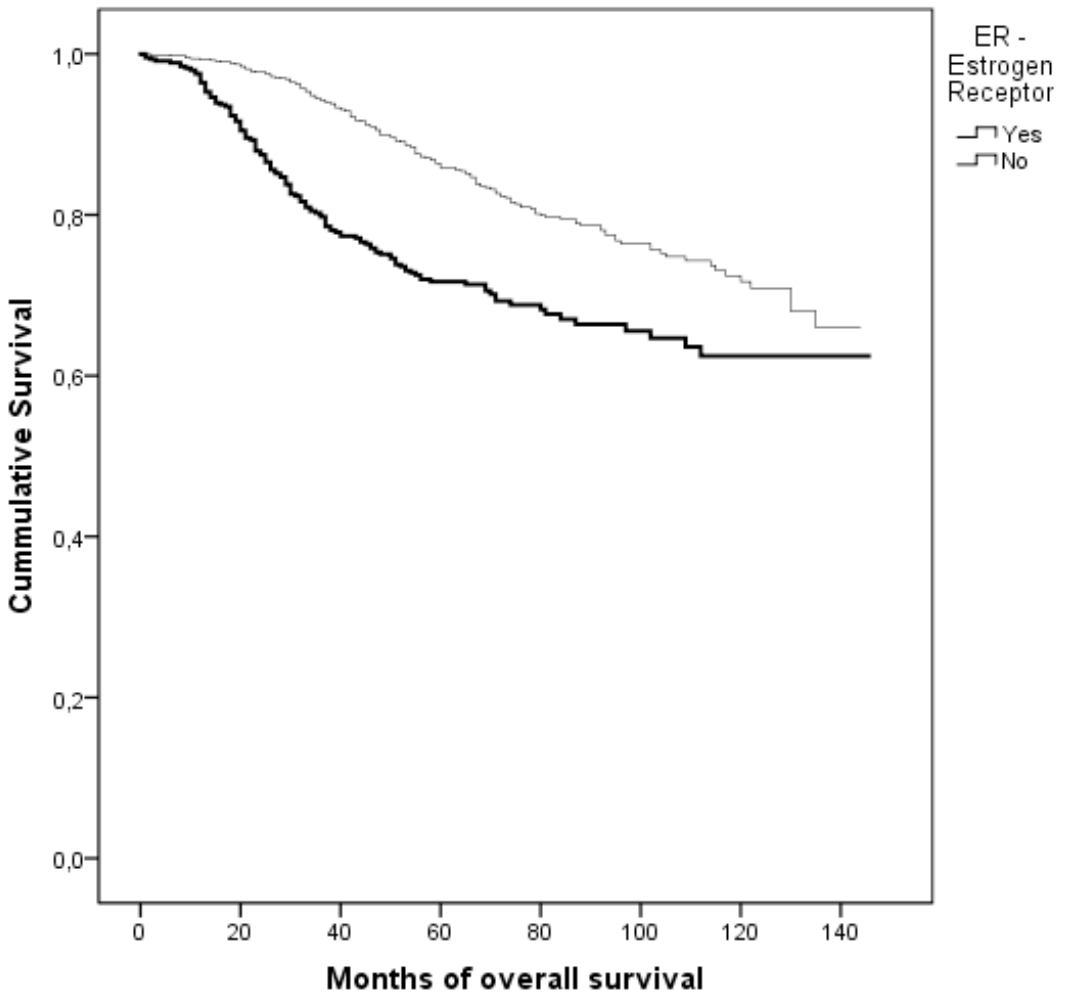


Figure 5 Guerra et al.
Overall survival stratified according to ER status. ER positive cases have higher probality of survival (p vaue $6x10^{-9}$)

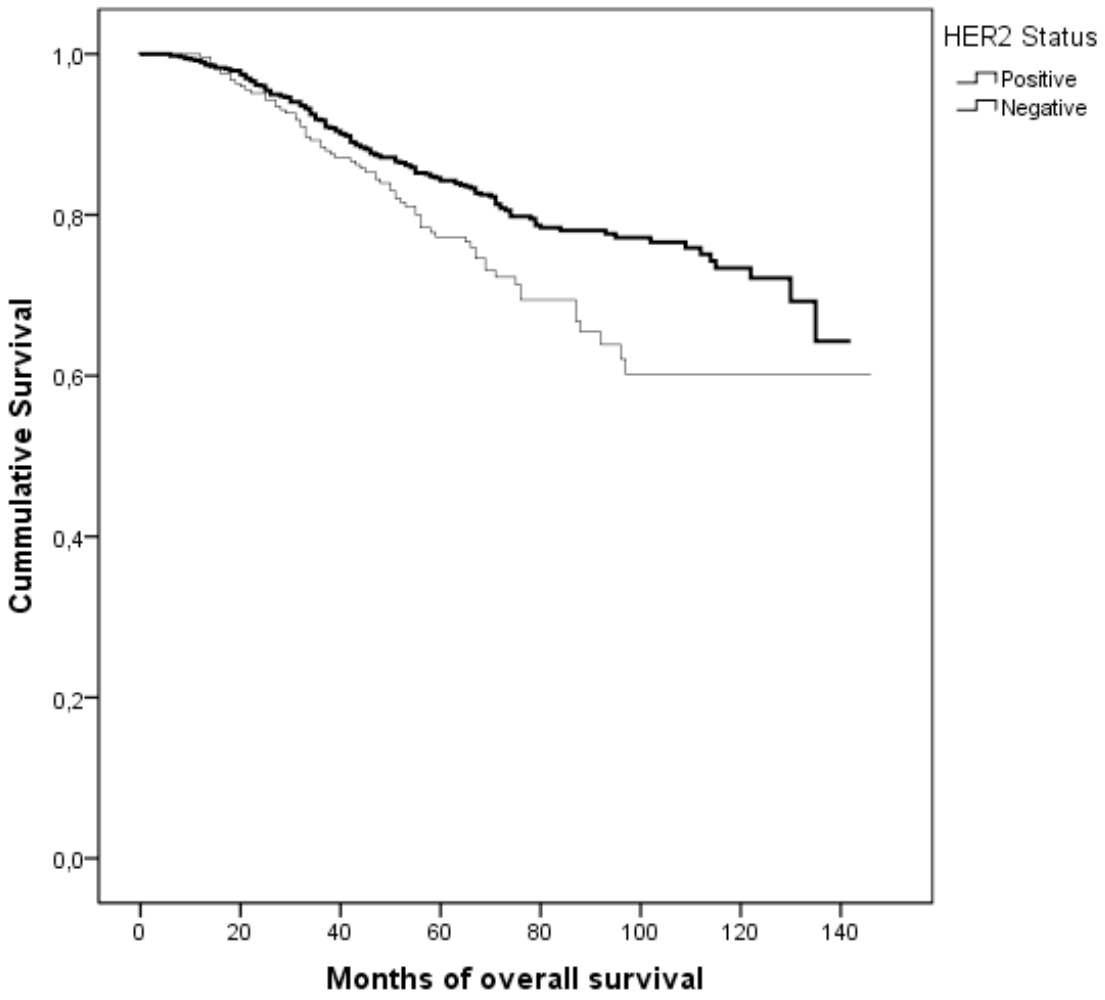


Figure 6 Guerra et al.
Overall survival stratified according to HER2 status. The lighter curve corresponds to the patients with HER2 positive tumours. Patients with HER2 positive tumours are more likely do die (P value 0.003)

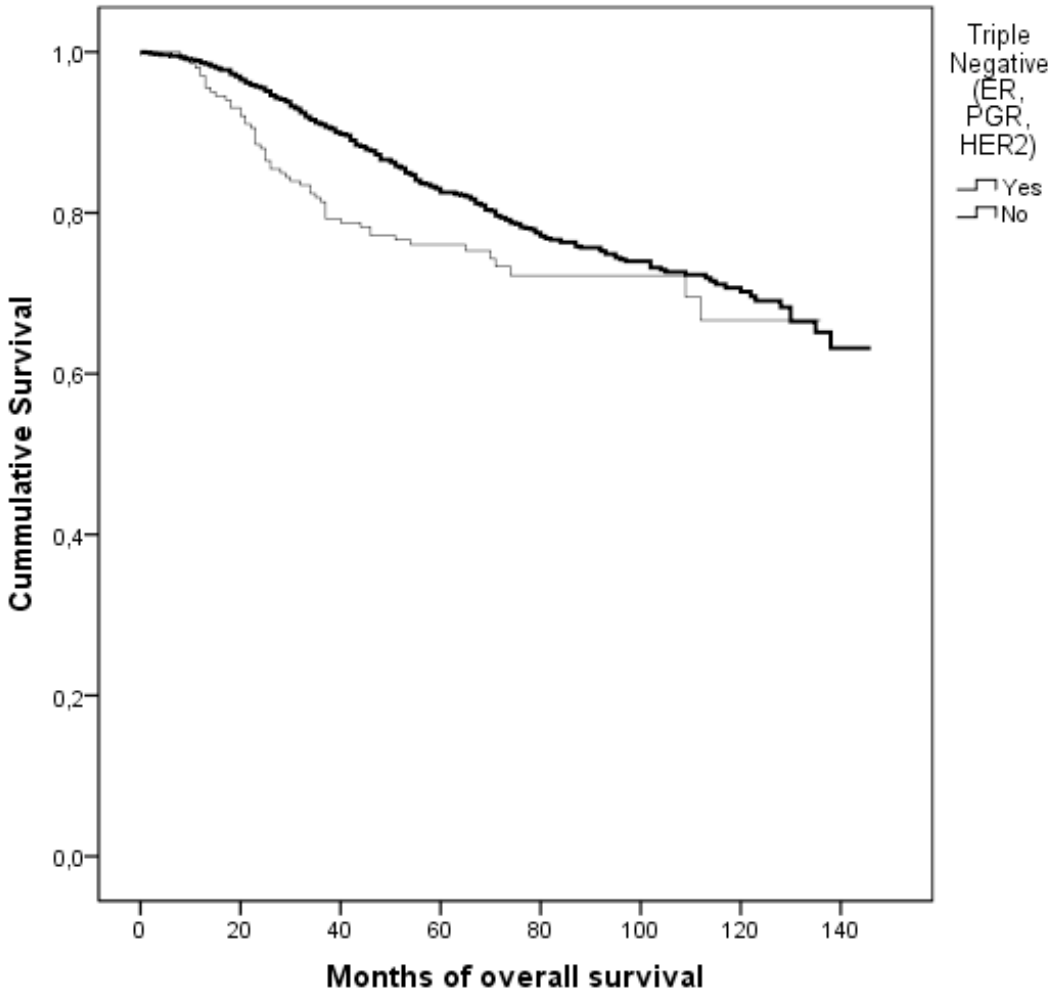


Figure 7 Guerra et al.
Overall survival stratified according to TN status. The lighter curve corresponds to the patients with TN tumours. Patients with TN tumours are more likely do die (P value 0.042)

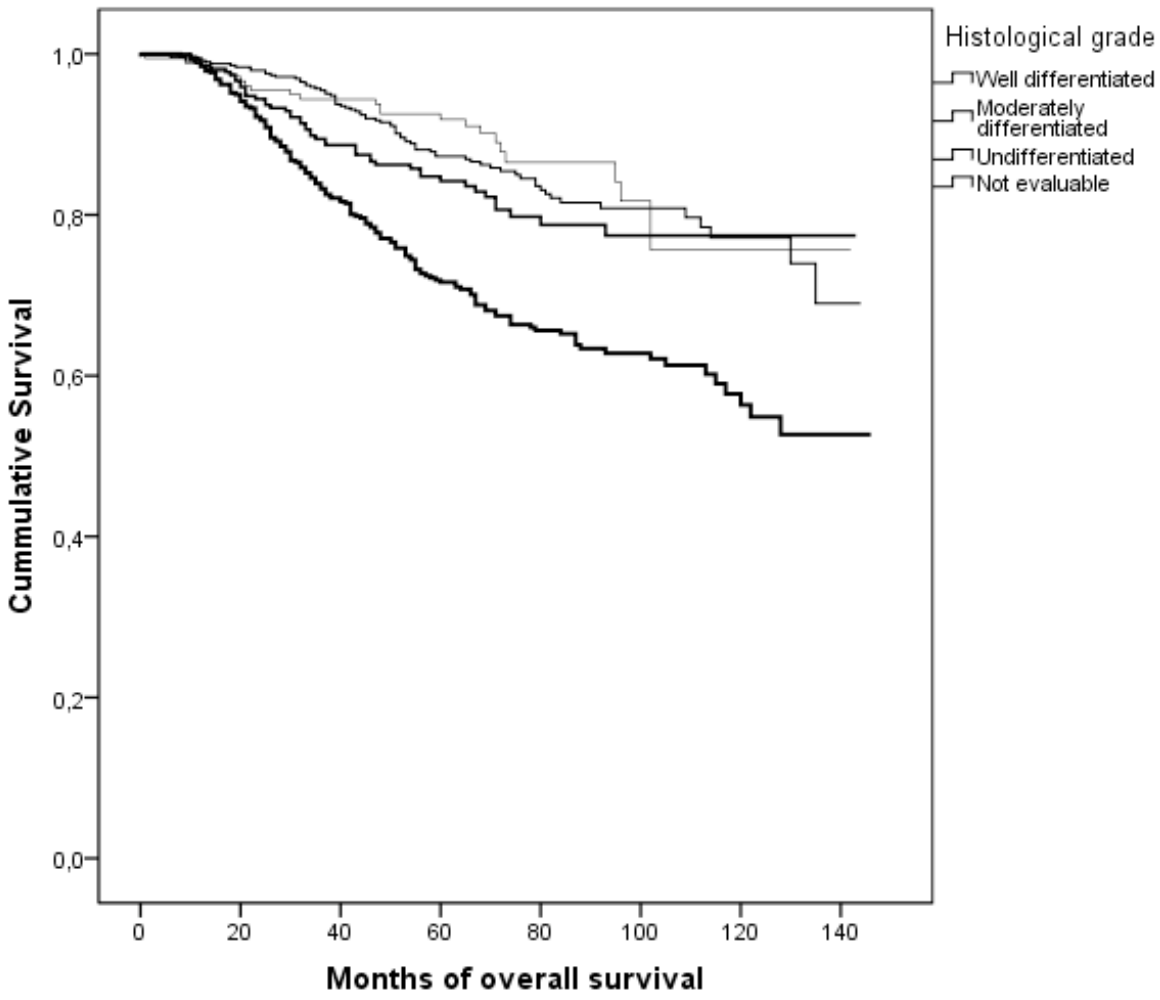


Figure 8 Guerra et al.
Overall survival according to histological grade. Well differentiated tumours had a higher probability of survival. (P value $2x10^{-12}$). Histological grade is not evaluable in tumors treated with neoadjuvant chemotherapy.

Distribution of the analyzed variables in the population	
Variable	n (%) (n=1639)
Histological classification	
Ductal	1517 (92,6)
Lobular	36 (2,2)
Other	86 (5,2)
T - pTNM	
T1	613 (37,3)
T2	637 (38,9)
T3	87 (5,3)
T4	36 (2,2)
Pathological complete response	14 (0,9)
Not evaluated surgically	252 (15,4)
Involved axillary nodes	(n=1398)
Yes	847 (60,6)
No	551 (39,4)
N - pTNM	(n=1398)
N0 (0)	551 (39,4)
N1 (1-3)	517 (37)
N2 (4-9)	204 (14,6)
N3 (10+)	126 (9)
Histological grade	
Well differentiated	245 (15)
Moderately differentiated	611 (37,3)
Undifferentiated	319 (19,5)
Not evaluable (neoadjuvant chemotherapy)	464 (33,2)
ER status	
Positive	1034 (63,1)
Negative	451 (27,5)
Not evaluated	154 (9,4)
PR status	
Positive	376 (23)
Negative	492 (30)
Not evaluated	771 (47)
HER-2 status	
Positive	258 (15,7)
Negative	876 (53,5)
Not evaluated	505 (30,8)
“Triple Negative”	
Yes	205 (12,5)
No	1434 (87,5)
*Chi-squared test; **Binomial test Binomial	

Table 1 Distribution of analyzed prognostic factors in the population

Multivariable Cox proportional hazards regression model for overall survival				
Variables		Regression coefficient	*p* value	95% confidence interval
TNM Variables	Tumour size (continuous)	1.14	0.25	0.91-1.43
	Involved axillary nodes	1.42	0.0000001	1.19-1.69
	TNM stage (continuous)	2.00	0.0000001	1.36-2.95
Biology-based variables	ER positivity	1.22	0.41	0.76-1.96
	HER2 positivity	0.68	0.05	0.46-0.99
	“Triple Negative” status	0.68	0.18	0.38-1.20
	Histological grade (continuous)	1.38	0.000	1.20-1.58

Table 2 Multivariable Cox proportional hazards regression model for overall survival including the eight variables that are shown in Figures 1 to 8

Chapter 2: Redefining breast cancer prognosis: The predictive power and mechanism of centrosome alterations in breast cancer

Conception and design: Sofia Braga, Joana Vaz, José Pereira-Leal

Provision of study materials or patients: Instituto Português de Oncologia, Lisboa

Collection and assembly of data: Sofia Braga, Joana Vaz, Telma Carrilho, Saudade André, Marta Mesquita

Data analysis and interpretation: Sofia Braga, Joana Vaz

Manuscript writing: Sofia Braga

Final approval of manuscript: All authors

Funding: Sofia Braga was funded during three years (from October 2008 until October 2011) by the Gulbenkian Foundation and worked at the Computational Genomics Laboratoty (CGL) at Instituto Gulbenkian de Ciência (IGC).
Laboratory funding from the Terry Fox scholarship of Liga Portuguesa Contra o Cancro and Collaborative research grant of the Harvard Medical School-Portugal Program 2009 funded by the Fundação para a Ciência e Tecnologia

Ethical Committee approval: Ethical committee of Instituto Português de Oncologia, Lisboa

Redefining breast cancer prognosis: The predictive power and mechanism of centrosome alterations in breast cancer

Sofia Braga[1 3], Joana Cardoso[1], Marta Mesquita[2], Telma Carrilho[2 4], Saudade André[2], Zé Pereira-Leal[1]

1- Instituto Gulbenkian de Ciência, Oeiras, Portugal
2- Instituto Português de Oncologia, Lisboa, Portugal
3- Currently at José de Mello Saúde, Lisboa, Portugal. Corresponding author
sofia.braga@jmellosaude.pt
Av. do Forte 3, Ed Suecia 3, piso 2, 2790-073 Lisboa

Abstract
It has been known that breast cancers harbor centrosome changes, namely centrosome amplification, and, more precisely, increased centrosome numbers. It has also been shown that increased centrosomes are an initiator of chromosomal instability, through inadequate spindle formation. Multipolar mitoses, endanger cell viability, by generating aneuploidy. However, cells with multiple centrosomes have an adaptive mechanism to protect them from multipolar mitoses which is centrosome clustering. We hypothesized that centrosome clustering is a negative prognostic factor in breast cancer. With our work in silico we showed there were two genes involved in centrosome clustering and that these were also negative prognostic factors. In patient samples we were able to show that increased abundance of these gene and protein products correlate with relapse and with the centrosome clustering phenotype at cellular level.

Introduction
The centrosome is the major microtubule organizing center (MTOC) of the cell {Bettencourt-Dias et al., 2011, Trends Genet, 27, 307-15}. The MTOC is responsible for orchestrating mitosis in dividing cells and thus centrosome replication is tightly regulated, duplicating once and only once per cell cycle {Bettencourt-Dias et al., 2011, Trends Genet, 27, 307-15}. In quiescent cells, centrosomes are involved in maintaining cell shape and polarity {Bettencourt-Dias et al., 2011, Trends Genet, 27, 307-15}. As first observed by Theodore Boveri in 1902 {Theodore Boveri, 2008, J Cell Sci, 121, 1-84}, many cancers harbor numerical and structural centrosome abnormalities. More than a century has passed and no one has ever answered the question if such abnormalities are a cause or a consequence of carcinogenesis. This is the case of human breast cancers where 80% of breast carcinomas present centrosome amplification {Salisbury et al., 2004, Journal of mammary gland biology and neoplasia, 9, 275-83}. Characterization of centrosome abnormalities in breast cancer fine-needle aspiration biopsies was done by other groups and found to be present in only 10% of the cases. In these carcinomas, centrosome amplification correlated with genomic instability {Kronenwett et al., 2005, Br J Cancer, 92, 389-95}. Centrosome amplification, morphologically, includes, increase in centrosome number and volume, increase in pericentriolar material, increased centrioles, and increased phosphorylation of centrosome proteins. Functional abnormalities include, inappropriate centrosome duplication and nucleation of larger microtubule arrays. This may be the cause of chromosomal instability through the development of multipolar mitotic spindles {Lingle et al., 2002, Proceedings of the National Academy of Sciences of the United States of America, 99, 1978-83}. In addition, centrosome amplification may affect cell polarity in interphase because cytoplasmic architecture and directional vesicular trafficking may be disorganized in a cell with multiple MTOCs {Lingle et al., 2002, Proceedings of the National Academy of Sciences of the United States of America, 99, 1978-83}.
Increased centrosomes are associated with changes in cell polarity, changes in cell and tissue differentiation, and chromosome missegregation through abnormal and multipolar mitoses. However, cancer cells cannot survive undergoing multipolar mitosis, and, when harboring multiple centrosomes, cells cluster them in order to undergo bipolar mitosis. It has been described that, in cancer cells, with centrosome amplification, centrosome clustering prevented cell multipolarity and it was shown that clustering centrosomes is a survival advantage for the breast cancer cell but these experiments have been carried out in cell line models {Quintyne et al., 2005, Science, 307, 127-9} {Kwon et al., 2008, Genes & Development, 22, 2189-203} {Fielding et al., 2011, Oncogene, 30, 521-34}

We focused on centrosome clustering mechanisms, and hypothesized, that through the survival advantage this mechanism provides the cell, centrosome clustering may be an adverse prognostic feature in breast cancer patients. In our work, we first worked in silico, and we used public gene expression datasets of patient material and cell line data of centrosome clustering screens. For the individual marker work we

focused on three molecules: PLK4 which is a gene involved in centrosome amplification, and in two genes involved in centrosome clustering: TACC3 and KIFC1.

Materials and methods

Data collection, pre-processing and graphical display

We mined Gene Expression Omnibus (GEO) repository (http://www.ncbi.nlm.nih.gov/geo) {Barrett T, 2009, Nucleic Acids Res., 37, D885-90}{R Development Core Team., 2009, R Foundation for Statistical Computing} according to the criteria: 1) Breast gland tissue, simple hyperplasia, atypical hyperplasia, ductal carcinoma in situ (DCIS), invasive ductal carcinoma and axillary node metastases tissue; 2) Primary breast cancers that had been operated and treated with curative intent according to international guidelines and had a median follow up of five years and 3) for both prior conditions, microarray experiments performed in the Affymetrix® Human Genome U133A microarray platform (HGU133a). (Table 1)

Data analysis was performed with R Statistical Computing software {R Development Core Team., 2009, R Foundation for Statistical Computing} complemented with Bioconductor packages {Gentleman RC, 2004, Genome Biol, 5, R80}. Heatmaps and Venn-diagrams were plotted using gplots (http://CRAN.R-project.org/package=gplots) and VennDiagram (http://CRAN.R-project.org/package=VennDiagram) packages, respectively. Affy {Gautier L, 2004, Bioinformatics, 20, 307-15} and frma {McCall MN, 2010, Biostatistics, 11, 242-5} packages were used for raw data uploading and normalization. The R script used is available upon request.

Differential expression analysis

We have used a Bayesian differential expression analysis approach implemented in the R package limma {Smyth GK, 2004, Stat Appl Genet Mol Biol, 3: Article 3} to define differentially expressed genes. Threshold for selection of differentially expressed probe sets was set to a B-statistic parameter Lods (already adjusted for multiple testing) 5 and a log2 ratio + 0.58 or - 0.58. The very conservative Lods>5 was based on differential expression analysis results between breast cancer samples from {Nagalla et al., 2013, Genome Biol, 14, R34) datasets, where several significant differentially expressed probe sets were found. For our experiment we used a conservative threshold, expecting less differentially expressed genes, to control for inter-dataset variability noise.

Assessment of Significance of Study Overlap

To determine if the level of overlap among the studies was significant, we performed a Monte Carlo simulation that generates random numbers to model a process {Chan et al., 2008, Cancer Epidemiol Biomarkers Prev, 17, 543-52}. Python scripts were created to perform Monte Carlo simulations. In each of the 10,000 permutations, the appropriate number of Entrez Gene IDs from the total gene list of each study was randomly chosen and each ID was randomly labeled as ‘‘UP’’ for up-regulated or ‘‘DOWN’’ for down-regulated. We used an ‘‘all-or-none’’ approach in which the level of overlap for a particular gene was only considered if all the independent studies reporting its differential expression agreed on the direction. The level of overlap among studies in each permutation was counted as in the real analysis. On completion of the permutations, a distribution of overlap results from the simulations was determined and a p value was estimated by comparing the overlap from the simulations to the actual level of overlap in the real data. Significance was defined at $p < 0.05$. We used hypergeometric testing to assess gene set enrichment when gene lists derived from BC analysis were intersected with lists of genes involved in centrosome clustering.

Samples and clinical data

FFPE breast cancer samples from primary surgery of patients that were treated with curative intent and continued follow-up indefinitely at Instituto Português de Oncologia Lisboa (IPOL) were used for validation (Table 2). For this initial validation we used 14 patient samples 8 had relapsed and 5 of these have died and 6 were in follow up without evidence of relapse. Hematoxylin-and eosin-stained sections were used by an experienced breast pathologist (SA) for histopathological characterization and area selection.

RNA extraction and cDNA synthesis

Archived breast cancer surgical blocks in FFPE were cut into tissue sections of 5 μm. These were deparaffinized and counterstained with Mayer’s hematoxylin and eosin. Cancer-enriched areas were needle microdissected under the breast pathologist (SA) guidance. Total RNA was extracted with the RNeasy FFPE kit (Qiagen), according to manufacturer’s instructions with a slight modification: proteinase K cell-lysis at 56°C was performed overnight. The RNase-Free DNase Set (Qiagen) “on column” DNA digestion procedure was included. Each extracted RNA was reverse-transcribed with the First-Strand cDNA Synthesis kit (GE Healthcare), using a 1:1 mixture of random primers (pd(N)6) and oligo-dT primers (NotI-d(T)18). cDNA from

control samples (high-quality RNA from HCT-116 and a primary skin fibroblasts cell lines) was synthesized from 3 μg of total RNA.

RT-qPCR

RNA concentration and integrity could not be assessed using standard methods due to known FFPE degradation issues and to the small amounts of extracted samples. Thus, to indirectly check the amount of each isolated total RNA FFPE sample and its RT-qPCR downstream performance, we prepared two standards dilution series using cDNA from the two control cell lines, corresponding to 100 ng, 10 ng, 1 ng, 0.1 ng, 0.01 ng, 0.001 ng and 0.0001 ng of the original total RNA. These series were subsequently used to calculate a RT-qPCR standard curve for the non-differentially expressed gene MAPKAPK2 (Lods=-2.7). Primer sets were designed with the NCBI Primer-BLAST tool {Ye J, 2012, BMC Bioinformatics, 13, 134}, to work at 59 °C and with an amplicon length of 70–100bp. Duplicates of each breast cancer sample were analyzed by RT-qPCR using SsoFast™ EvaGreen® Supermix (Bio-Rad, Hercules CA, USA) reagent in 10 L of reaction mixture containing template (2 L, ~200pg/ L) and primers (0.5 M each). Samples were processed in a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, Hercules CA, USA) according to the cycling program: 95 °C for 60 s, 50 cycles of 95°C for 10s and 59°C for 15s. Fluorescence data collection occurred at 59°C. Relative differential expression analysis of target genes by RT-qPCR was based on the 2-ΔΔCt methodology from {Livak KJ, 2001, Methods, 25, 402-8} using mean quantification cycle of duplicates as cycle threshold (Ct).

Immunohistochemistry

In breast cancer samples (3 μm thick tissue sections), IHC was performed according to standard protocols. Primary antibodies were diluted in Bond Primary Antibody Diluent (Leica Microsystems) plus background-reducing components at the dilutions: KIFC1 (1:150, mouse monoclonal, Abnova, 53-152). Slides were incubate with Polymer Kit-Envision (HRP) DAKO 150 μl for 30 minutes. Washed and sequentially stained with diaminobenzidine, Mayer's hematoxilin and ethanol. Nuclei were counterstained with Mayer's hematoxylin. Images were acquired on a Leica DM5500 microscope.

Fluorescence microscopy

From each paraffin block, 3 !m-thick tissue sections were obtained using a conventional microtome and transferred to positively charged glass slides and oven dried (70º C) for a minimum of one hour. Sections were then deparaffinized in xylene and placed in 100% ethanol. Then, in order to block the endogenous peroxidase, the slides were treated with a 2% hydrogen peroxide in methanol solution for 10 minutes and washed in running water. Antigen retrieval was done in a pressure cooker in a 0.01M sodium citrate buffered solution (pH 6) for sixteen minutes, followed by incubation with a blocking buffer (triphosphate-buffered saline (TBS) with 5% bovine serum albumin) for 10 minutes at room temperature to avoid nonspecific binding by primary and secondary antibodies. The slides were then incubated with primary antibodies diluted in Bond Primary Antibody Diluent (Leica Microsystems) with background-reducing components for one hour at room temperature. Primary antibodies used were against polyglutamylated tubulin (GT335, 1:800, clone, Firm) and pericentrin (1:250, Abcam). The sections were then washed three times with TBS (5 minutes/ wash) before incubation with the fluorochrome-conjugated secondary antibodies fluorescein isothiocyanate (FITC)-conjugated donkey anti-rabbit IgG and rhodamine red-conjugated donkey anti-mouse IgG (1:50, Jackson ImmunoResearch) for thirty minutes at 37ºC. The sections were then washed extensively with TBS and dehydrated through gradient alcohols. Finally, the sections were counterstained and mounted with 4,6-diamidino-2-phenylin-dole (DAPI) for nuclear DNA (Vectashield Vector with DAPI, Burlingame, CA, USA) and examined by fluorescence microscopy using a Ti-E inverted microscope (Nikon Eclipse), using an ORCA ER2 CCD camera (Hamamatsu) and Nikon software.

Mitotic and multipolar index

In at least two independent experiments, cells were screened and a minimum of 500 cells were counted for the mitotic index (mitoses per cells counted in %) and up to 20 mitoses per tumor were analyzed for the mitotic profile (cells in prophase, prometaphase, metaphase, anaphase and telophases) and the occurrence of multipolar mitosis (multipolar mitosis per cells counted; range of total mitoses counted).

Statistical analysis

Data analysis was performed with R language for Statistical Computing {R Development Core Team., 2009, R Foundation for Statistical Computing}. Expression differences between different conditions of breast cancer progression and relapsed and non relapsed BC patients microarray data were determined with a Bayesian T-test implemented in the R package limma {Wettenhall and Smyth, 2004, Bioinformatics, 20,

3705-6}. Statistical significance of RT-qPCR data was calculated with Wilcoxon Rank Sum test (confidence level=0.95). IHC categorical data was analyzed with Pearson's Chi-squared test.

Mitotic control

To find over-represented GO biological processes among specific sets of genes we used the GSEA tool from InnateDB (http://www.innatedb.ca/) using Entrez ID as gene identifier. There was high abundance of mitotic genes, therefore, we collected 16 datasets of cells that could serve as an internal control for mitotic genes. Two senescent cancer cell lines treated with chemotherapy. Two cell lines with replicative senescence. Normal breast tissue in parous and nulliparous women. Four datasets of mammosphere cells in different proliferative stages. We then used microarray signatures from patient cancer samples . Finally we used controls of proliferating *Drosophila melanogaster* cells and umbilical cord progenitors. The methodology is, for the studied centrosomal genes, to check if they are present in these signatures, if present, if ''UP'' (red) for up-regulated or ''DOWN'' (blue) for down-regulated genes. The finding of MKI67 as prognosis predictor prompted us to specifically perform linear correlation of the expression vector of MKI67 with the KIFC1 expression vector in each of the datasets (Fig 6).

Results

Centrosome abnormalities in breast cancer progression

Centrosome amplification has been detected in DCIS, suggesting that centrosome amplification is an early event in these lesions. Therefore we compiled datasets of breast carcinoma precursor lesions and their expression profile and assessed whether there was overlap between these genes and the genes in centrosome proteome and the genes involved in centrosome clustering. Breast cancer presents precursor lesions: simple hyperplasia, atypical hyperplasia and DCIS. There are publicly available expression datasets of normal mammary gland as well as precursor lesions and invasive carcinomas of the breast and metastatic sites, namely axillary lymph node metastasis. The Vogelstein coloretal carcinogenesis model {Goyette et al., 1992, Mol Cell Biol, 12, 1387-95} is applicable in BC. There is continuum of genetic aberrations and cancer defining properties throughout these stages (Figure 1). The change in expression of the genes in the centrosome proteome across several states of breast cancer progression including normal breast, hyperplasia, DCIS and invasive cancer in the breast as well as metastatic lesions. There is a statistically significant change in expression across the continuum of breast cancer pre invasive, invasive and metastatic lesions for centrosome proteome genes and the centrosome clustering proteome genes (Figure 2). We were able to detect the stepwise increase in expression for three selected genes Plk4, TACC3 and KIFC1. Plk4 a gene involved in centrosome amplification. For centrosome clustering we have assessed TACC3 and KIFC1 molecules involved in centrosome clustering. KIFC1 (also denominated HSET) is involved in clustering centrosomes in acentrosomal cells as well as in cells with centrosome amplification.

Quantifying the prognostic impact of centrosomal genes in expression datasets of breast cancer

We performed a systematic analysis of the literature and compiled an extensive data set of gene expression data for >4000 of Breast Cancer (BC) patients with prognostic information (relapsed versus non-relapsed), individual datasets are shown in Table 1 Eighteen datasets were retrieved but only 14 were used because some datasets had no informative genes at the statistical cutoffs. Setting stringent cutoffs enables reduction of interdataset variability and background noise. The expression arrays were from untreated primary breast cancers at the time of primary surgery. In this analysis there is no expression data of relapsed material, i.e. distant organ metastasis. We analysed this data seeking for genes consistently up or down regulated in poor prognosis BC, i.e. that relapsed after initial treatment. In the course this bioinformatics analysis we identified 65 genes statistically significant across multiple datasets (≥2) that can discriminate between relapsed and non-relapsed BC patients. We used the B statistic with a false discovery rate of 0.05, lods score of 0 and an adjusted p-value of 0.05. To test whether 65 genes in common was more than should be expected by chance we did a Monte Carlo simulation where we were able to show that in a random simulation there would be less genes in common (Figure 3A). Working with whole genome expression arrays and comparing the discriminant genes for two conditions among two datasets by chance only one gene would be in common and we found 65. For the same simulation in three, four or five datasets in the Monte Carlo Simulation there are no common genes and we found 18, 2 and 2 common genes, respectively, in these intersection experiments.

Among the identified genes, we have detected genes such as MKI67, a marker of mitotic activity and routinely used in the clinic. In the intersection of the 65 BC prognostic genes with the list of centrosome clustering genes found in the screens we have previously curated, there are 6 common genes in the intersection of the 65 and in the 158 centrosome clustering genes. These gens are KIFC1, TACC3, BUB1, PRC1, CENPA, and BIRC5. The gene intersection was not due to chance. We performed a statistical

hypergeometric test where we show a *p*-value of 8.5 e-7 for this intersection of 6 genes not being due to chance. In other words, 6 genes involved in BC prognosis and centrosome clustering are more than should be expected by chance (Figure 3B). We looked specifically the absolute difference in expression of KIFC1, TACC3 and PLK4 in the microarray data (Figure 3C). We were able to show that the differences in gene expression levels were statistically significant for the genes involved in centrosome clustering, KIFC1 and TACC3, but not for the gene involved in centrosome amplification. For this phenotype we tested only PLK4, largely responsible for this phenotype, it was not significantly changed between patients with different outcomes (Figure 3D).

The prognostic value of centrosomal genes in different breast cancer subtypes

Since the year 2000, we are increasingly viewing BC as three different diseases, possibly with even more subtypes. With clinical relevance and implications there are primarily ER positive tumors, Her2 positive tumors and triple negative tumors. In the clinic we assess proliferation markers and these have been specifically important in discriminating between ER positive disease. In the seminal publication {Perou et al., 2000, Nature, 406, 747-52}, gene expression profiling separated BC into five subtypes: Tumors that expressed ER but had low proliferation rate (Luminal A) or high proliferative rate (Luminal B). Tumors that express Her2 and those that do not express any receptor (triple negative) and, finally, in the initial classifier, tumors that are normal or have no specific subtype. We assessed if centrosomal genes were more or less able to predict prognosis in the different BC subtypes. Because the initial PAM50 classifier is expression based it is possible to separate into subtypes all the datasets we used, even if data on ER, PgR, Her2, histological grade and Ki67 are not available comprehensively in all datasets. Our results show that the six chosen genes are not different throughout breast cancer subtypes in their capacity to predict prognosis. This result is shown for dataset GSE1456, on of the most informative datasets we worked with (Figure 4).

Validate the centrosome clustering genes by non high throughput method in patient samples

Our work was performed in silico up to this point. In this step we have sought to validate our findings in FFPE embedded patient samples. The methodology includes designing of primers for qPCR for the evaluated genes MKI67, KIFC1 and TACC3. We selected a consecutive breast cancer case series and tested in relapsed and in non relapsed primaries. We tested by qPCR using the methodology of extracting mRNA from the FFPE blocks (Figure 5). There is a statistically difference in mRNA abundance for the tested genes. For generalizability and clinical applicability it is best to validate the use of antibodies for IHC because qPCR is not routinely available in pathology departments. Therefore, we worked on the validation and optimization of antibodies against KIFC1 and TACC3. The TACC3 antibody was not discriminative and we could not validate the findings of the qPCR with IHC. On the other hand, the results for KIFC1 are coherent with the qPCR results and retain clinical significance.

Identify and quantify the phenotype of centrosome clustering in relapsed breast cancers

Despite higher levels of genes involved in centrosome clustering we tested if we have evidence of this phenotype in relapsed patient samples. For this, we used immnunoflourescence confocal microscopy detecting centrosome molecules. We stained gamma tubulin and pericentrin. We studied metaphase plates in the primary tumor FFPE from relapsed breast cancer patients. The objective is to find: 1) amplified centrosomes, 2) clustered centrosomes. The blue staining shows DNA stained with DAPI and the green and red markers show pericentrin that is abundant in pericentriolar material and gamma tubulin which is the main protein of centrosomes. In breast carcinomas mitosis are not abundant. Generally less than 10 mitotic figures per high power field, therefore this work is particularly painstaking. We were able to find two such mitotic figures in tumor samples with increased numbers of centrosomes, increased levels of KIFC1 and TACC3 and where we were able to find the centrosomes clustered in the metaphase plate.

Controlling for proliferation markers

Finally, we were struck by the enormous abundance of proliferation genes in our initial 65 gene group. In fact it is known that the BC prognosis signatures, derived from the datasets we used, were mainly proliferation driven. This begged the question if with our metaanalysis we were not discovering new biology but simply validating the fact that tumors that proliferate more have worse prognosis. This has been known to pathologists and clinicians since the XIXth century. To answer this, we compared our 65 gene group with gene groups that were said to represent proliferation signatures. We used several proliferation signatures from senescent breast cancer cell lines, then from normal mammary gland cells from reduction mammoplasties and then from breast cancer datasets. We also used non human and non breast tissue controls fro proliferation signatures, where we saw that proliferation genes are highly conserved. There is some overlap for the proliferation genes as can be seen (Figure 6A). The results in the figure show a subset of genes involved in centrosome amplification and clustering. After this, we focused on MKI67, as has been

said MKI67 is one of the genes that is assessed routinely in breast cancer samples as a proliferation marker. The question we asked is if the signal of bad prognosis being captured by high Ki67 is the same as the signal we are detecting assessing centrosome clustering. As is shown graphically there is no correlation between the two molecules and numerically the r^2 is 0.38.

Conclusion

In this study, we were able to show that a phenomenon that was known to occur in breast cancer, centrosome clustering, occurs, and is correlated with worse prognosis. We have shown this in two ways: First, using an unbiased method, with differential expression analysis, the differentially expressed genes that predicted for relapse had an enrichment of centrosome genes. We detected genes involved in centrosome amplification and genes involved in centrosome clustering. Then, using a different experimental approach, with patient samples, single markers and confocal microscopy. We these techniques, were able to show that in patients with worse prognosis there was increased amount of mRNA of KIFC1 and TACC3. We were able to validate the KIFC1 increase at the protein level, with IHC, showing increased staining for the protein. Finally in these tumors with higher abundance of proteins involved in centrosome clustering we detected multiple centrosomes, and, most importantly, we were able to detect mitotic figures where the supranumerary centrosomes appear clustered.

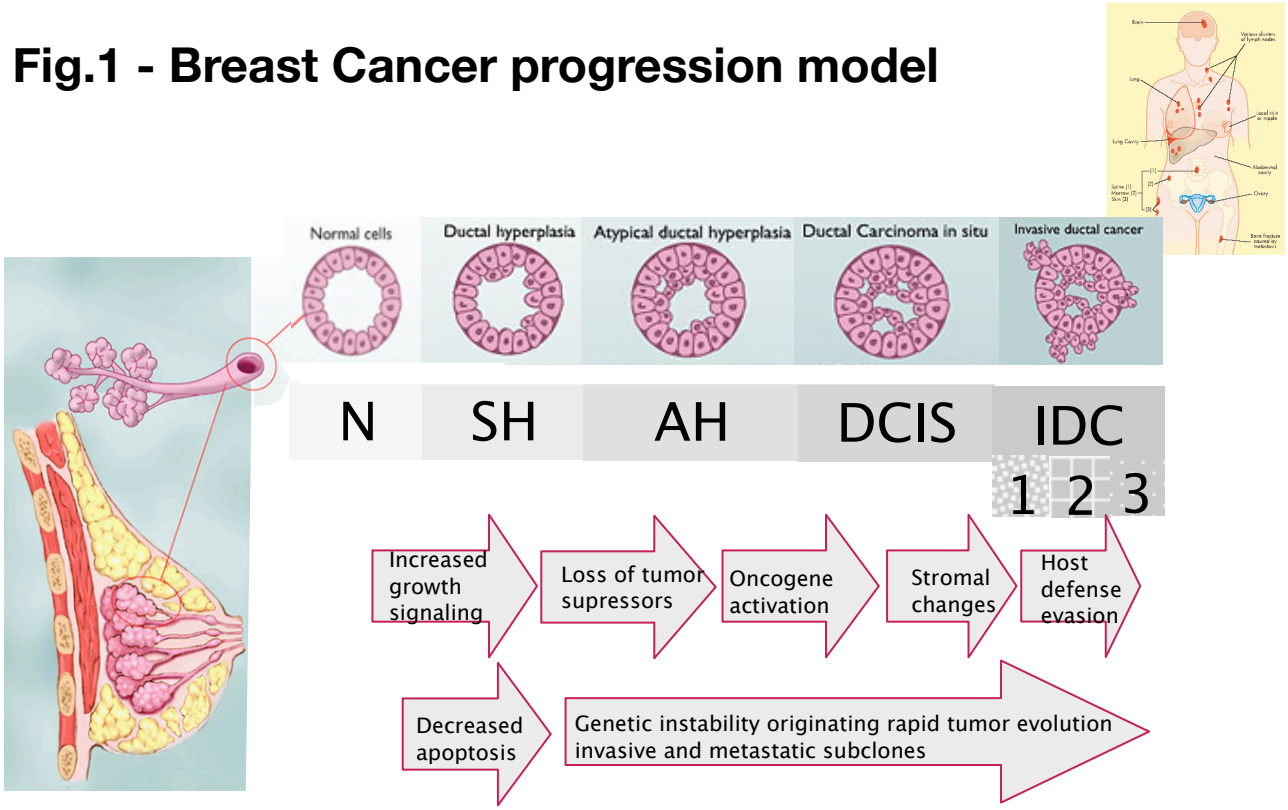
GEO	n pts	Location	Citation
GSE1456	159	Stockholm	Pawitan et al., 2005, Breast Cancer Research : BCR {Pawitan et al., 2005, Breast Cancer Research : BCR, 7, R953-64}
GSE2109	812	Arizona	Microarray Quality Control Consortium {Consortium et al., 2010, Nat Biotechnol}
GSE2429	8	Washington	Poola et al., 2005, Nat Med {Poola et al., 2005, Nat Med, 11, 481-3}
GSE3744	47	Boston	Richardson et al., 2006, Cancer Cell {Richardson et al., 2006, Cancer Cell, 9, 121-32}
GSE5460	129	Boston	Lu et al., 2008, Breast Cancer Res Treat {Lu et al., 2008, Breast Cancer Res Treat, 108, 191-201}
GSE5764	30	Czech Republic	Turashvili et al., 2007, BMC Cancer{Turashvili et al., 2007, BMC Cancer, 7, 55}
GSE7390	198	Brussels	Desmedt et al., 2007, Clinical cancer research {Desmedt et al., 2007, Clinical cancer research : an official journal of the American Association for Cancer Research, 13, 3207-14}
GSE10780	185	Florida	Chen et al., 2010, Breast Cancer Res Treat {Chen et al., 2010, Breast Cancer Res Treat, 119, 335-46}
GSE10810	58	Granada	Pedraza et al., 2010, Cancer {Pedraza et al., 2010, Cancer, 116, 486-96}
GSE15852	86	Malaysia	Pau Ni et al., 2010, Pathol Res Pract {Pau Ni et al., 2010, Pathol Res Pract, 206, 223-8}
GSE16873	40	Boston	Emery et al., 2009, Am J Pathol {Emery et al., 2009, Am J Pathol, 175, 1292-302}
GSE17907	109	Marseille	Sircoulomb et al., 2010, BMC Cancer {Sircoulomb et al., 2010, BMC Cancer, 10, 539}
GSE19615	115	Boston	Li et al., 2010, Nat Med {Li et al., 2010, Nat Med, 16, 214-8}
GSE20194	278	Arizona	Popovici et al., 2010, Breast Cancer Research {Popovici et al., 2010, Breast Cancer Research : BCR, 12, R5}
GSE21422	19	Berlin	Kretschmer et al., 2011, Mol Cancer {Kretschmer et al., 2011, Mol Cancer, 10, 15}
GSE22544	20	Georgia (USA)	Hawthorn et al., 2010, BMC Cancer {Hawthorn et al., 2010, BMC Cancer, 10, 460}
GSE23177	116	Leuven	Smeets et al., 2011, Breast Cancer Res Treat {Smeets et al., 2011, Breast Cancer Res Treat, 129, 767-76}
GSE23593	50	Duke (NC)	Barry et al., 2010, J Clin Oncol {Barry et al., 2010, J Clin Oncol, 28, 2198-206}
GSE29431	66	Barcelona	Unpublished
GSE14017	29	Houston	Zhang et al., 2009, Cancer Cell {Zhang et al., 2009, Cancer Cell, 16, 67-78}
GSE20565	172	Paris	Meyniel et al., 2010, BMC Cancer {Meyniel et al., 2010, BMC Cancer, 10, 222}
GSE11078	23	Paris	Landemaine et al., 2008, Cancer Research {Landemaine et al., 2008, Cancer Research, 68, 6092-9}
GSE14018	36	Houston	Zhang et al., 2009, Cancer Cell {Zhang et al., 2009, Cancer Cell, 16, 67-78}
GSE12630	276	California	Monzon et al., 2009, J Clin Oncol {Monzon et al., 2009, J Clin Oncol, 27, 2503-8}
GSE44408	44	Barcelona	Unpublished
GSE12093	136	Rotterdam	Zhang et al., 2009, Breast Cancer Res Treat {Zhang et al., 2009, Breast Cancer Res Treat, 116, 303-9}
GSE11121	200	Mainz	Schmidt et al., 2008, Cancer Research {Schmidt et al., 2008, Cancer Research, 68, 5405-13}
GSE9195	77	Brussels	Loi et al., 2008, BMC Genomics {Loi et al., 2008, BMC Genomics, 9, 239}
GSE7390	198	Brussels	Desmedt et al., 2007, Clinical cancer research {Desmedt et al., 2007, Clinical cancer research : an official journal of the American Association for Cancer Research, 13, 3207-14}
GSE6532	741	Brussels	Loi et al., 2007, J Clin Oncol {Loi et al., 2007, J Clin Oncol, 25, 1239-46}
GSE5327	58	Rotterdam	Minn et al., 2007, PNAS {Minn et al., 2007, Proceedings of the National Academy of Sciences of the United States of America, 104, 6740-5}
GSE4922	249	Stockholm	Ivshina et al., 2006, Cancer Research {Ivshina et al., 2006, Cancer Research, 66, 10292-301}
GSE3494	236	Stockholm	Miller et al., 2005, PNAS {Miller et al., 2005, Proceedings of the National Academy of Sciences of the United States of America, 102, 13550-5}
GSE2990	187	Brussels	Sotiriou et al., 2006, J Natl Cancer Inst {Sotiriou et al., 2006, J Natl Cancer Inst, 98, 262-72}
GSE2034	286	Rotterdam	Wang et al., 2005, Lancet {Wang et al., 2005, Lancet, 365, 671-9}
GSE1456	159	Rotterdam	Pawitan et al., 2005, Breast Cancer Research
GSE20194	278	Houston	Popovici et al., 2010, Breast Cancer Research
GSE4779	102	EORTC	Farmer et al., 2009, Nat Med

Table 1 Datasets available in public databases used for the in silico metaanalysis. GEO gene Expression Omnibus

Identification and tumor data							Follow up data	
age breast cancer	tumor (mm)	nº positive nodes	er	pgr	her2	grade	years to metastasis	months to death
62	60	17	100	na	0	3	3	17m
57	27	20	100	na	0	3	3	11m
47	15	0	focal	0	3	2	5	
76	35	4	100	na	0	2	4	24m
67	25	19	100	na	na	2	2	24m
46	22	0	100	0	0	1	0	
27	11	0	100	na	0	3	1	30m
31	23	0	100	na	0	3	6	
35	5	0	100	na	0	2		
75	40	12	100	na	0	2		
63	15	0	100	na	0	2		
66	28	1	100	na	0	2		
93	22	0	100	na	0	2		
71	25	1	100	na	0	2		

Table 2. - Patient clinical data. The patient material used for experimental studies was of a sample of consecutive breast cancer cases. Shown are clinical and pathological data of the primary tumor. ER estrogen receptor, PGR progesterone receptor, HER2 epidermal growth factor receptor type 2, grade as assessed by Elston and Ellis.

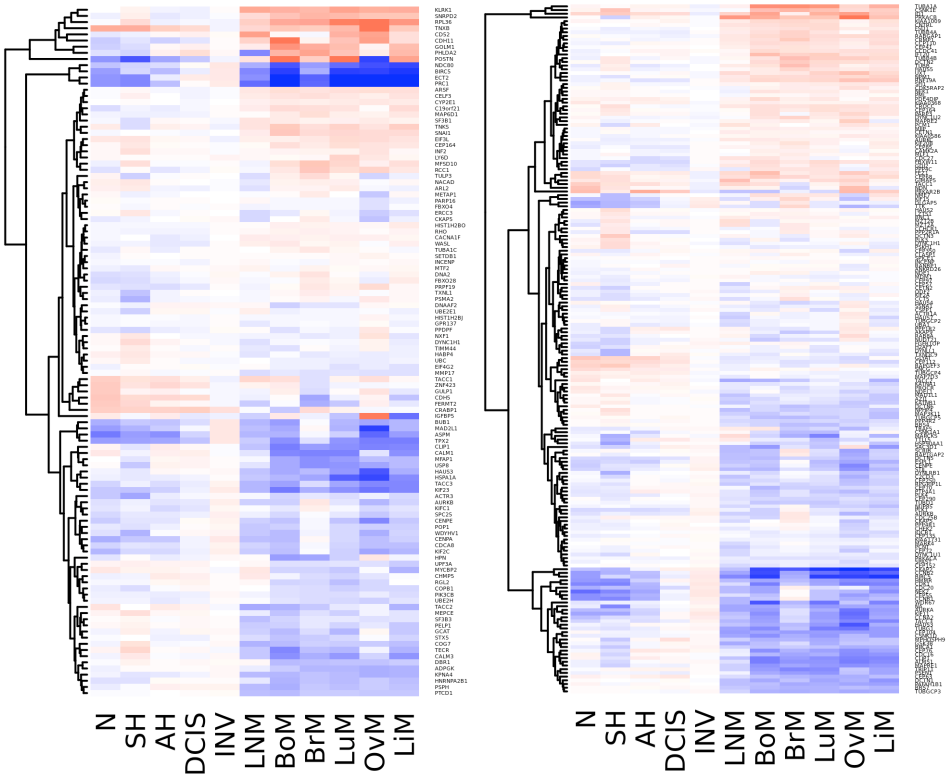
Fig.1 - Breast Cancer progression model



N normal
SH simple hyperplasia
AH atypical hyperplasia
DCIS ductal carcinoma in situ
IDC infiltrating ductal carcinoma. Grades according to Ellston and Ellis

Fig.2 – Centrosome and centrosome clustering genes are changed along Breast Cancer malignant progression

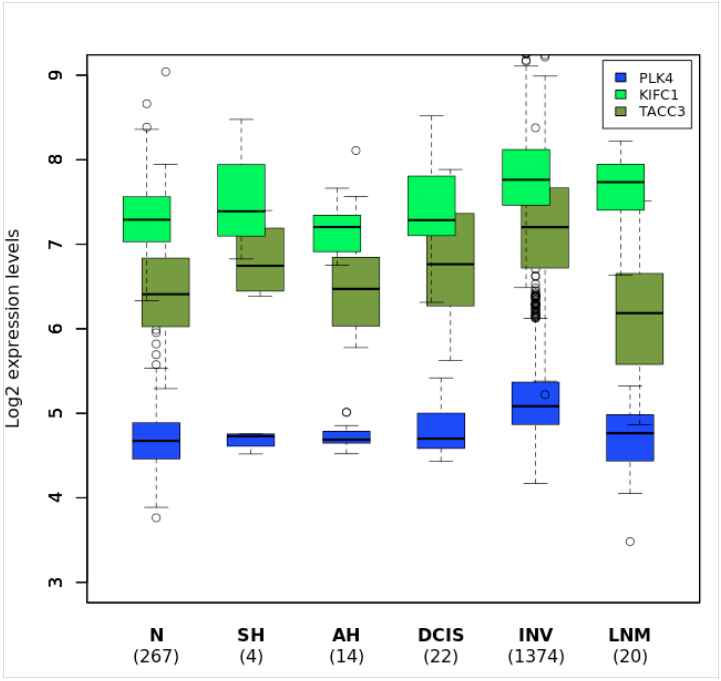
A.



Centrosome proteome
([Jackobsen, 2011] and Centrosome 3D

Centrosome clustering proteome
([Leber, 2010] and [Kwon & Godinho, 2008]

B.


PLK4
KIFC1
TACC3
Log2 expression levels
3
4
5
6
7
8
9
N
(267)
SH
(4)
AH
(14)
DCIS
(22)
INV
(1374)
LNM
(20)

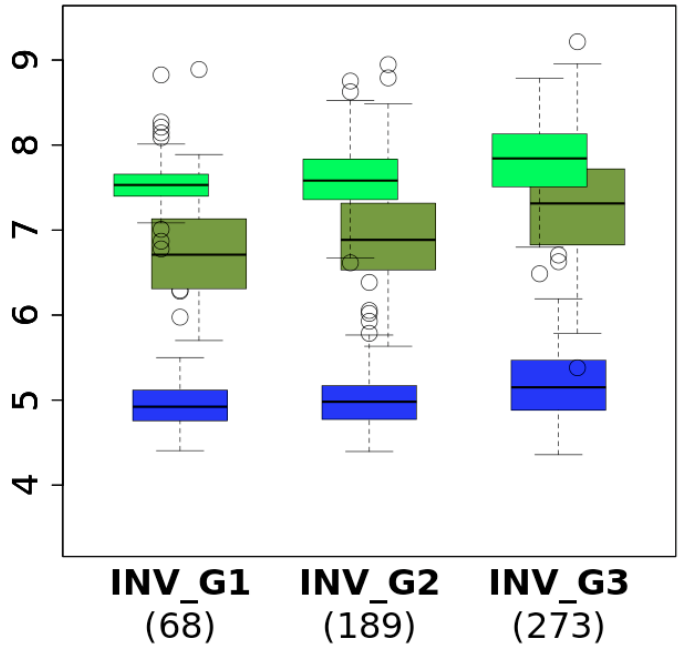
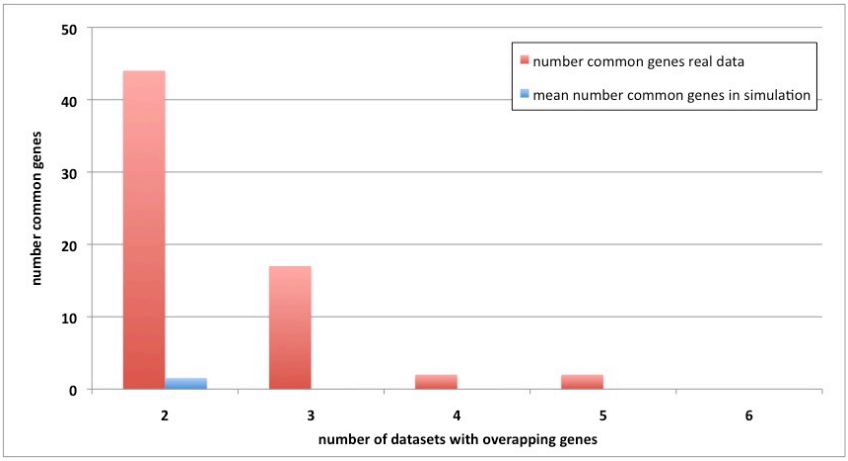

4
5
6
7
8
9
INV_G1
(68)
INV_G2
(189)
INV_G3
(273)

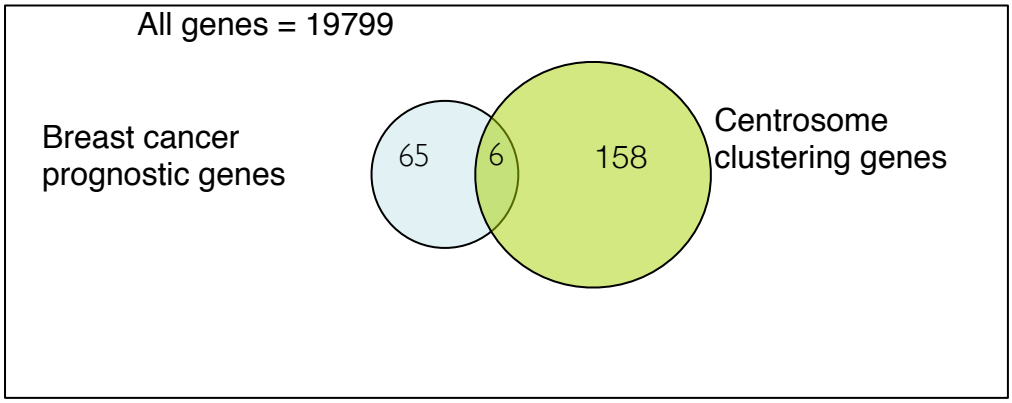
Fig.3 - Discovery of centrosome clustering genes as prognostic markers of breast cancer relapse by in silico metaanalysis

A.



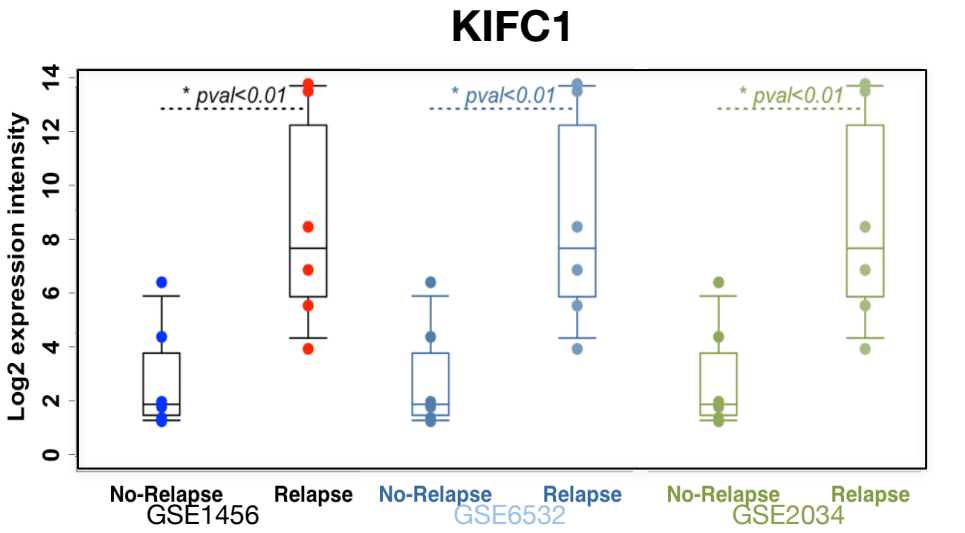
65 genes are significant, by Monte Carlo simulation

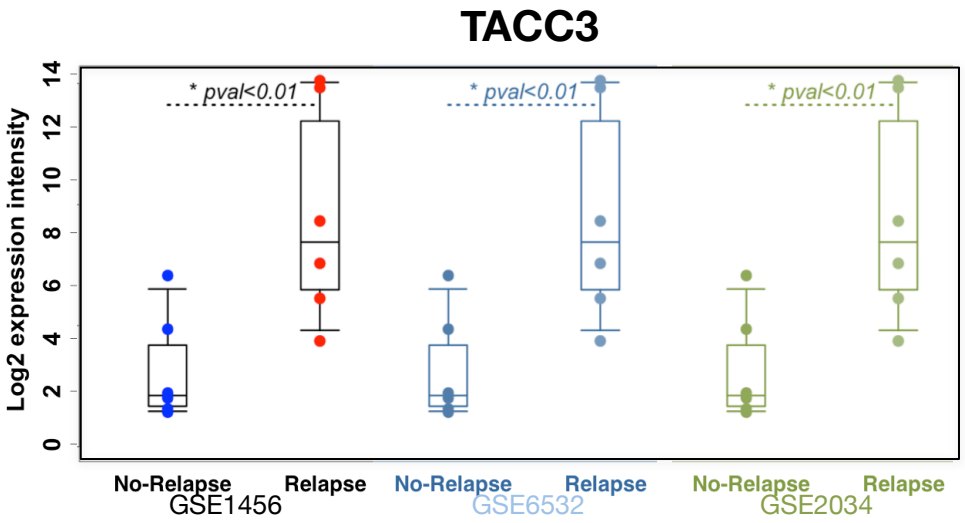
B.



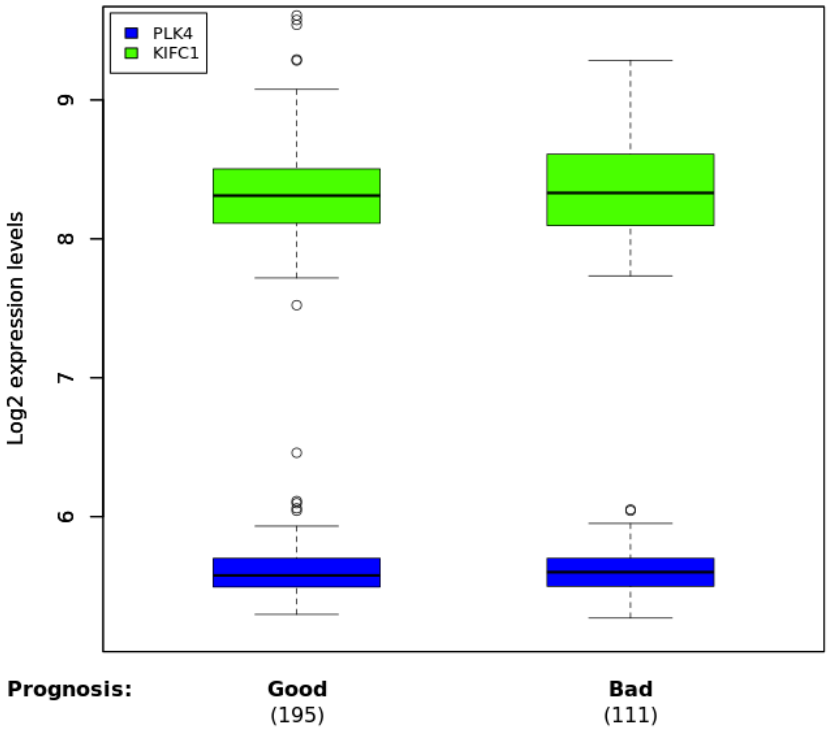
6 genes* BIRC5, BUB1, CENPA, KIFC1, PRC1, TACC3
*more than expected by change (Hypergeometric Test p value=8.51821e-07)

C.


KIFC1
Log2 expression intensity
0
2
4
6
8
10
12
14
* pval<0.01
* pval<0.01
* pval<0.01
No-Relapse
Relapse
No-Relapse
Relapse
No-Relapse
Relapse
GSE1456
GSE6532
GSE2034


TACC3
Log2 expression intensity
0
2
4
6
8
10
12
14
* pval<0.01
* pval<0.01
* pval<0.01
No-Relapse
Relapse
No-Relapse
Relapse
No-Relapse
Relapse
GSE1456
GSE6532
GSE2034

D.


PLK4
KIFC1
Log2 expression levels
9
8
7
6
Prognosis:
Good
(195)
Bad
(111)

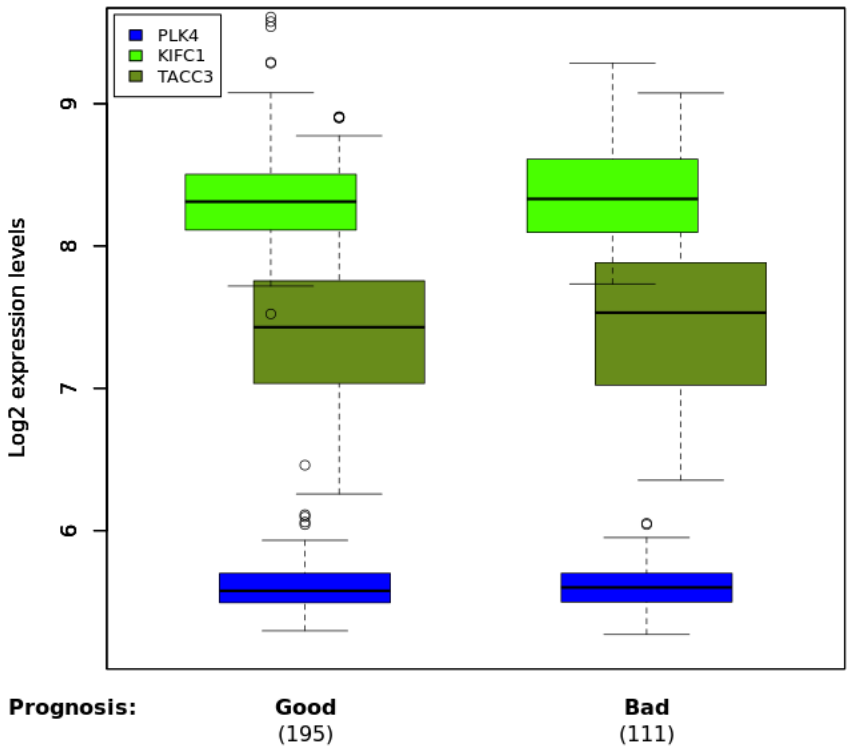
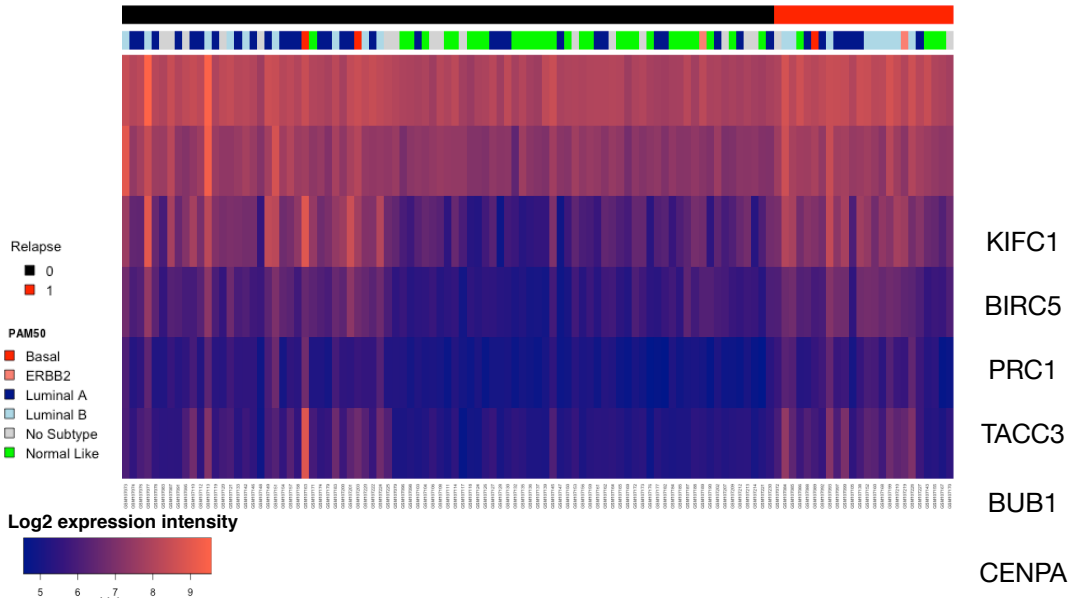

PLK4
KIFC1
TACC3
Log2 expression levels
9
8
7
6
Prognosis:
Good
(195)
Bad
(111)

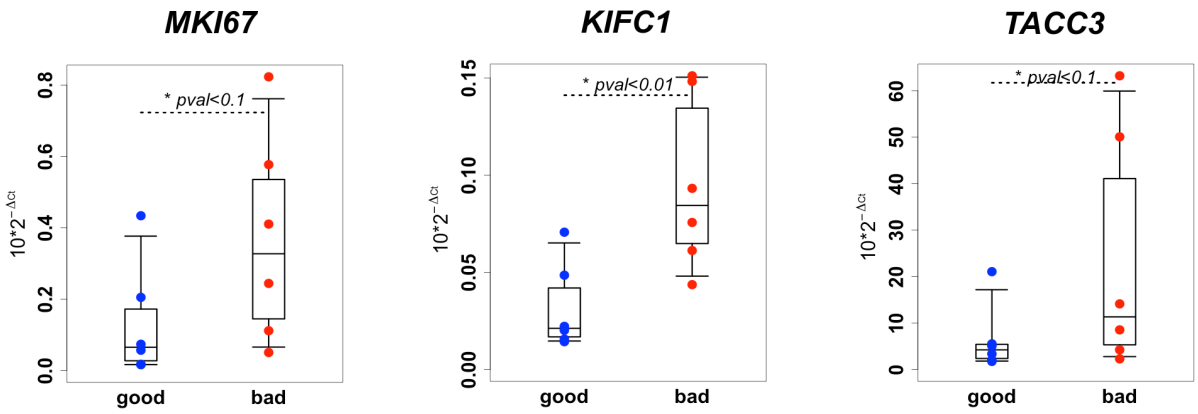
Fig.4 - Centrosome clustering genes are prognostic in breast cancer molecular computational subtypes assessed by the PAM50 classifier



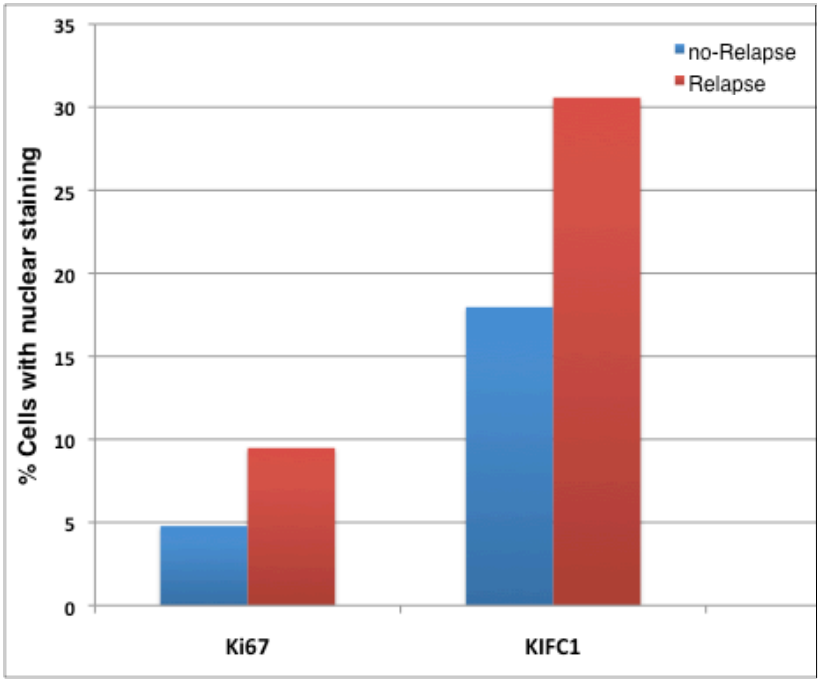
GSE1456

Fig.5 – The two centrosome clustering genes KIFC1 and TACC3 are significantly up-regulated in retrospective breast cancer samples associated with bad prognosis (Relapse), by RT-qPCR and IHC

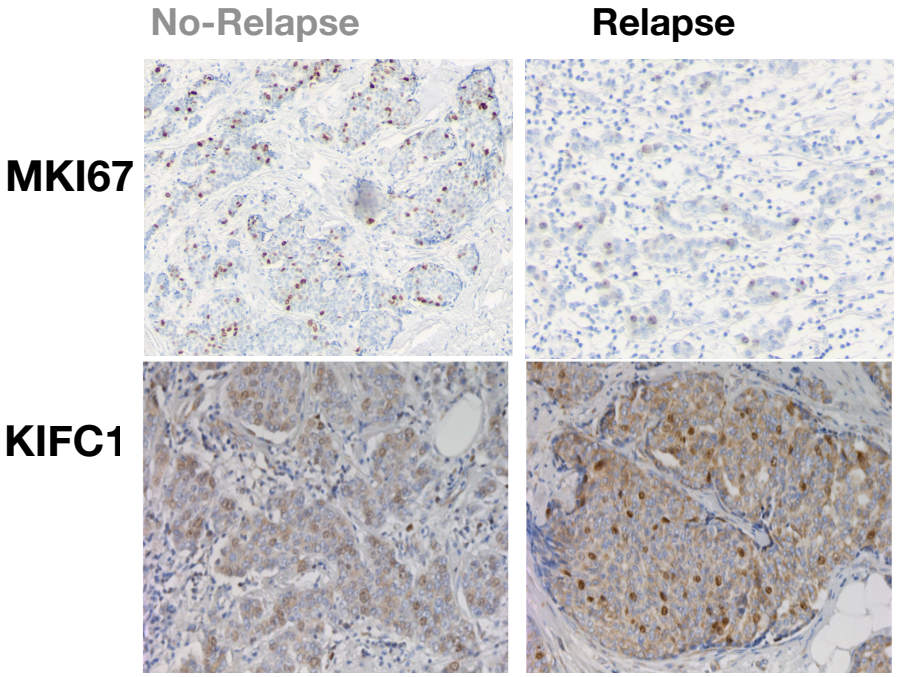
A.



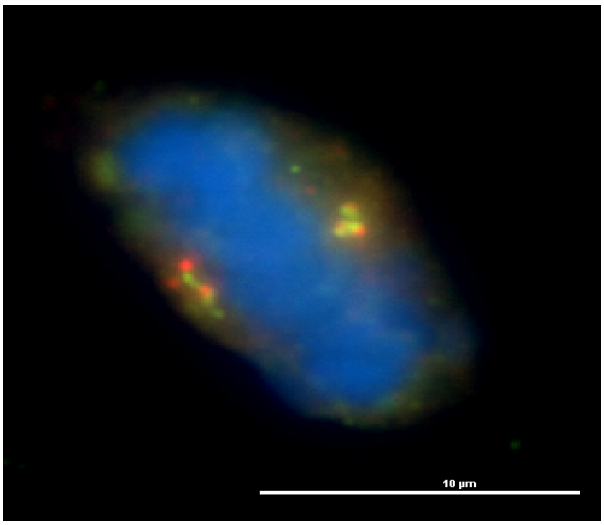
B.



C.



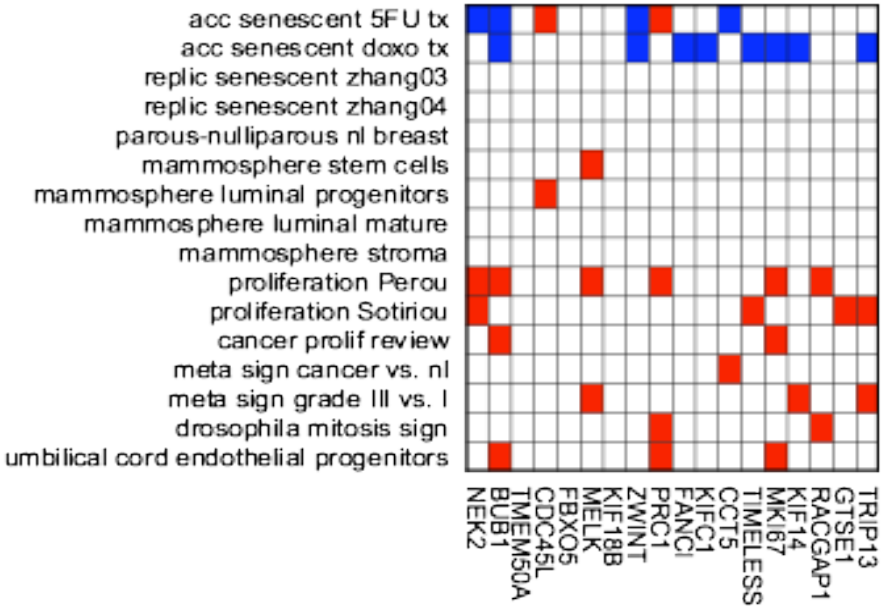
D.



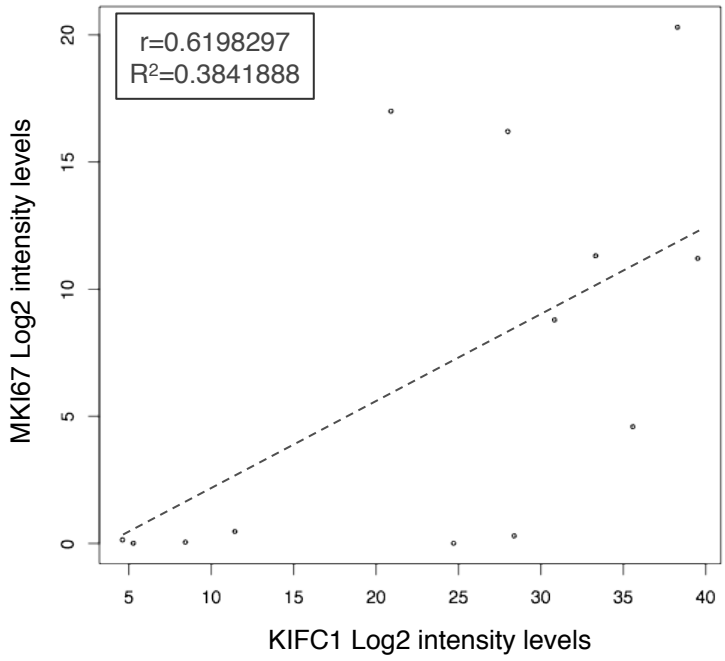
Blue DAPI
Green (FITC) Pericentrin
Red (Rhodamine) GT-335

Fig.6 – The value of centrosome clustering genes is not just a mitotic signature

A.



B.



Part 2: How to treat breast cancer subtypes?

Chapter 3: How many diseases is triple negative breast cancer?

Conception and design: Sofia Braga

Provision of study materials or patients: Sofia Braga

Collection and assembly of data: Sofia Braga

Data analysis and interpretation: Sofia Braga

Manuscript writing: Sofia Braga, José Pereira-Leal

Final approval of manuscript: Both authors

Funding: Sofia Braga was funded during three years (from October 2008 until October 2011) by the Gulbenkian Foundation and worked at the Computational Genomics Laboratoty (CGL) at Instituto Gulbenkian de Ciência (IGC)

Ethical Committee approval: Not required

How many diseases is Triple Negative Breast Cancer?

Sofia Braga[1,2], José B. Pereira-Leal[1]
[1]Instituto Gulbenkian de Ciência, Oeiras, Portugal
[2]José de Mello Saúde, corresponding author sofia.braga@jmellosaude.pt

Abstract

Triple negative breast cancer is a subtype of breast cancer that does not express the estrogen receptor, the progesterone receptor and the epidermal growth factor receptor type 2 (Her2). These tumors are not yet treated with targeted therapies, probably because no positive markers have been described to reliably classify them - they are described for what they are not. Perhaps for this reason, they are among the most aggressive of breast carcinomas. The clinical observation that these patients do not carry a uniformly dismal prognosis, coupled with data coming from pathology and epidemiology, suggests that this negative definition is not capturing a single clinical entity, but several. We critically evaluate this evidence in this paper, reviewing clinical and epidemiological data, as well as molecular data. There is evidence for heterogeneity, but it is not clear how many diseases are grouped into triple negative breast cancer. Answering this question, and identifying the molecular basis of heterogeneity will help define prognosis and, eventually, the identification of new targeted therapies.

Preface

The sources of information used in this article are articles indexed to PubMed retrieved after a search with keywords: triple negative breast cancer, basal like breast cancer, african american, PARP inhibitors and chemotherapy resistance. Major conference proceedings were searched for unpublished reports. Clinical trials were searched for at http://clinicaltrials.gov.

Introduction

Breast cancer (BC) is the leading cause of death in women aged 35 to 55 years, in developed countries, and the most frequent cancer in women. The incidence of BC is increasing. Over the twenty five year period 1982-2006 the incidence rate increased by 51% (Cancer Statistics: Registrations of cancer diagnosed in 2006, England). There are several clinical types of BC, defined by amplification of specific markers. Steroid hormone receptor overexpression (estrogen and/or progesterone receptors: ER, PgR) define the most abundant type of BC. Roughly 70% of BC is ER and/or PgR positive {Ravdin et al., 2007, The New England Journal of Medicine, 356, 1670-4; Marques et al., 2009, Breast Cancer Res Treat, 114, 223-32}. Her2 receptor amplification, defines a second type, with an incidence of roughly 20% {Slamon et al., 1987, Science, 235, 177-82}. Her2+ BC can be either ER+ or ER-, but its dominant biological driver and clinical feature is traceable to Her2 gene amplification, a potent oncogene. The remaining cases are termed triple negative BC (TNBC), breast carcinomas that neither express the ER nor the PgR and do not have overexpression of Her2. TNBC can represent between 10 to 82% of the cases in published series, a variability due to sampling issues and population heterogeneity {Stark et al., 2010, Cancer; Dent et al., 2007, Clin Cancer Res, 13, 4429-34; Cleator et al., 2007, The Lancet Oncology, 8, 235-44; Huo et al., 2009, J Clin Oncol, 27, 4515-21}.

TNBC is the clinical subtype of breast cancer with the worse prognosis when compared to ER (and/or PgR) positive disease and Her2 positive disease {Onitilo et al., 2009, Clinical Medicine & Research, 7, 4; Dent et al., 2007, Clin Cancer Res, 13, 4429-34}. This may be due in part to the fact that it is the only clinical subtype of BC for which there is no validated adjuvant targeted therapy. Patients with BC overexpressing ER and/or PgR benefit from antiestrogenic therapy and those overexpressing Her2 benefit from anti-HER2 therapy. In fact, in the pre anti-Her2 therapy era, TNBC and Her2 positive BC had a similar dismal survival {Spitale et al., 2009, Ann Oncol, 20, 628-35}. Validated treatments for TNBC are surgery, radiotherapy and chemotherapy. However, not all TNBC respond to chemotherapy {Eralp et al., 2008, Ann Oncol, 19, 669-74; Wysocki et al., 2008, Med Sci Monit, 14, SC7-10; Ivanov et al., 2008, Breast Cancer Res Treat, 111, 411-7}. This exemplifies that TNBC may be more than a single disease. Here we critically review the clinical, therapeutical, epidemiological and molecular evidence supporting this claim. Our objective is to systematically address the potential sources of heterogeneity and give the practicing clinician some clues to prognosis and prediction when faced with a TNBC patient.

Clinical heterogeneity

The aggressive behavior of TNBC, with presentation of de novo metastatic BC, large locally advanced breast lesion or metastatic disease developing shortly after adjuvant chemotherapy is a hallmark of breast oncology {Seewaldt and Scott, 2007, N Engl J Med, 356, e12; Collett et al., 2005, Cancer

Epidemiol Biomarkers Prev, 14, 1108-12; Vona-Davis et al., 2008, Cancer Epidemiol Biomarkers Prev, 17, 3319-24}. TNBC frequently metastasizes to the viscera, liver, lung or brain {Dent et al., 2008, Breast Cancer Res Treat; Rodríguez-Pinilla et al., 2006, Clin Cancer Res, 12, 1533-9; Pinilla et al., 2006, Breast Cancer Res Treat, 99, 85-90; Gadiyaram et al., 2009, Cancer Research, 69, 6159; Tsang et al., 2009, Cancer Research, 69, 3072}. However, this is not always the case (Figure 1 and Table 1). TNBC can also present as a slow growing lesion, similar to the other ER+ BC {Yin et al., 2008, Breast Cancer Res Treat}. TNBC may also have an oligometastatic phenotype, similar to some ER+ BC, with lymph node and bone disease {Wei et al., 2008, Hum Pathol, 39, 1809-15}.

TNBC is also heterogeneous in terms of time of recurrence. Unlike ER+ BC whose recurrence curve is linear, TNBC has a high rate of recurrence in the first two years followed by a plateau {Lee et al., 2010, Breast Cancer Res Treat, 123, 177-87}. Despite frequent recurrences in the first two years, there appears to be a second delayed peak of TNBC recurrences {Park et al., 2009, Cancer Research, 69, 6032; Yin et al., 2008, Breast Cancer Res Treat; Lee et al., 2009, Cancer Research, 69, 4044}. The pattern of late recurrence is generally associated with less aggressive disease, frequently with bone metastases {Dent et al., 2007, Clin Cancer Res, 13, 4429-34; Wei et al., 2008, Hum Pathol, 39, 1809-15}, whereas the one of early recurrence is associated with widely metastatic phenotype and dismal prognosis.

The data above, suggest the existence of at least two natural histories in TNBC (Figure 1 and Table 1). However, we need to consider two potential confounding factors. First, that these differences are not just a proxy for age of incidence, i.e. that we are just seeing bad prognosis TNBC in younger women and good prognosis TNBC in older women {Tse et al., 2009, Histopathology, 55, 441-51}. Secondly, that the late recurrence, good prognosis group, may include the 20% of tumors that are false negatives for ER {Gong et al., 2007, The Lancet Oncology, 8, 203-11}. The false negativity rate for ER is getting lower as pathologists use reproducible techniques and are increasingly aware of international guidelines and present quantitative readouts of hormone receptors {Hammond et al., 2010, J Clin Oncol, 28, 2784-95}. The lower false negative rate for ER determination will naturally lower the incidence of TNBC.

Epidemiological heterogeneity

There is increasing evidence that TNBC may have a bimodal distribution, with the first incidence peak in pre-menopausal patients and a second peak after 70 years of age {Muguti, 1993, Journal of the Royal College of Surgeons of Edinburgh, 38, 75-8; Anderson et al., 2002, Breast Cancer Res Treat, 76, 27-36; Lund et al., 2009, Breast Cancer Res Treat, 113, 357-370; Yin et al., 2008, Breast Cancer Res Treat}. Prognosis of stage-matched pre-menopausal TNBC is worse than older age TNBC. One can speculate on the underlying biology that explains this difference in outcome. Premenopausal TNBC would be a disease with a few very powerful molecular drivers, more akin to single hit neoplasms, whereas geriatric TNBC would be a disease of generalized chromossomal instability, a hallmark of aging tissues and of geriatric cancer {Curtin et al., 2005, The New England Journal of Medicine, 353, 2135-47}. In fact, such genomic heterogeneity has been observed in TNBC using deep sequencing {Stephens et al., 2009, Nature, 462, 1005-10} (Figure 2).

Race is perhaps the most striking source of heterogeneity in TNBC, both in the natural history of the disease and its incidence. In the 1990s, before BC subtypes were part of our thought process, it was known that BC in women of African ancestry was more frequently ER negative, affected younger women and, when stage and age matched, had worse prognosis {Swanson and Lin, 1994, J Natl Cancer Inst Monographs, 69-77; Crowe et al., 1986, Surgery, 100, 599-605; Natarajan et al., 1985, Cancer, 56, 1704-9}. Indeed, in the United States African American women have more frequently pre-menopausal aggressive TNBC {Huo et al., 2009, J Clin Oncol, 27, 4515-21; Carey et al., 2006, JAMA, 295, 2492-502; Lund et al., 2009, Breast Cancer Res Treat, 113, 357-370; Zaky et al., 2009, Cancer Research, 69, 6045}. In contrast, Caucasian and Asian women tend to have TNBC with a later age of onset and less aggressive clinical course {Yin et al., 2008, Breast Cancer Res Treat; Kim et al., 2009, Cancer Research, 69, 4065; Kwong et al., 2009, Cancer Research, 69, 3071}, but still with higher recurrence rate in the first two years that subsequently decreases to the same hazard rate as ER, PgR positive and Her2 negative breast cancer {Nishimura and Arima, 2008, Breast Cancer, 15, 303-308; Lee et al., 2010, Breast Cancer Res Treat, 123, 177-87}. Women of African ancestry are less responsive to neoadjuvant chemotherapy and have worse prognosis when diagnosed with locally advanced TNBC {Frasci et al., 2009, Ann Oncol, 20, 1185-92}. Knowing that BRCA1 germline mutations are not frequent in black women one might suspect that TNBC arising in these women is not deficient in DNA repair and is, therefore, chemoresistant {Pegoraro et al., 2003, Int J Gynecol Cancer, 13, 444-9} (figure 2). It is known that BC in BRCA deficient patients has better prognosis and is chemosensitive. The genome of the primary tumor and metastasis of a 44-year old, African American woman that presented with locally advanced chemorresistant disease that relapsed 8 months after primary treatment with widespread metastasis, was sequenced. The material analyzed was primary tumour, brain metastasis, a xenograft derived from the primary lesion and peripheral blood for control {Ding et al., 2010, Nature, 464,

999-1005}. It revealed that the mutational evolution is scarce, suggesting that the chemorresistance and metastatic phenotype is already present in a few cells in the primary lesion. In contrast, in lobular, ER+, breast cancer deep sequencing revealed that new mutations are acquired in the metastasis, after 9 years of disease free interval, that were not present in the primary lesion {Shah et al., 2009, Nature, 461, 809-13} (figure 2). Both these studies remarkably reconcile the different behavior of these diseases in the clinic: On one hand, aggressive TNBC, that quickly relapses with chemorresistant disease, and, lobular breast cancer, that relapses generally after five years and is sensitive to tamoxifen.

Regarding incidence, TNBC has been defined as a rare disease in northern Europe and in some parts of USA {Linn and Van 't Veer, 2009, Eur J Cancer, 45 Suppl 1, 11-26}, accounting for less than 15% of BC cases, 10% in some series {Cleator et al., 2007, The Lancet Oncology, 8, 235-44}. In contrast, in states within the United States where more than 20% of the population is of African ancestry, the frequency of TNBC is substantially higher. Black, pre-menopausal, women were twice as likely to have TNBC than white women in North Carolina (39% vs. 14%) {Carey et al., 2006, JAMA, 295, 2492-502} and Atlanta (47% vs. 22%) cohorts {Lund et al., 2009, Breast Cancer Res Treat, 113, 357-370}. Being of African ancestry increases three times the risk of a women developing TNBC, independent of other risk factors for breast cancer {Stead et al., 2009, Breast Cancer Res, 11, R18}. An epidemiological study from Nigeria reported that 59% of BC are TNBC {Huo et al., 2009, J Clin Oncol, 27, 4515-21} and a study from Peru reported a frequency of 20% {Alarcon-Rozas et al., 2009, Cancer Research, 69, 2072}. A recent study that compared TNBC in the United States and in Ghana showed that TNBC appears in 82% of Ghanian women, 32% of African Americans and in 10% of white Americans {Stark et al., 2010, Cancer}.

A possible explanatory factor for these ethnicity-based differences are intrinsic physiological differences. For example, breast density which is greater in black women {El-Bastawissi et al., 2001, Ann Epidemiol, 11, 257-63}. Since cancer in dense breasts is harder to diagnose by mammography {Boyd et al., 2007, The New England Journal of Medicine, 356, 227; Barlow et al., 2006, JNCI Journal of the National Cancer Institute, 98, 1204}, this could mean that the worse prognosis of black women might be due to a delay in diagnosis. However, stage matching in epidemiological studies eliminates this hypothesis. Alternatively, denser mammary glands may have distinct growth factor profiles that predispose women to more aggressive cancer with estrogen independence. Indirect support for this scenario comes from the observation of different growth factor profiles, IGF-1 and HGF, and different incidence of non-cancer endpoints, like metabolic syndrome, in cohorts of black vs. white obese women {Baird and Travlos, 2007, Cancer Epidemiol Biomarkers Prev, 16, 1526 author reply 1526; Henderson et al., 2006, Cancer Epidemiol Biomarkers Prev, 15, 2298-302}. In fact, the metabolic syndrome is more prevalent in TNBC patients {Maiti et al., 2010, Breast Cancer Res Treat, 121, 479-83}. Obesity has long been recognized as a risk factor for BC {Vona-Davis et al., 2007, Obesity reviews : an official journal of the International Association for the Study of Obesity, 8, 395-408; Vona-Davis et al., 2008, Cancer Epidemiol Biomarkers Prev, 17, 3319-24; Montazeri et al., 2008, BMC Cancer, 8, 278}. Since women of black ethnicity are more frequently obese {Trivers et al., 2009, Cancer Causes Control; Jones et al., 1997, American Journal of Epidemiology, 146, 394-404; Coates RJ, 1990, J Natl Cancer Inst.}, this could constitute a confounding factor. However, there is still no evidence that obesity alone is a specific risk factor for TNBC.

Social and economic status have been claimed to have prognostic value. Women of low social and economic status have worse prognosis BC {Vona-Davis and Rose, 2009, Journal of women's health (2002), 18, 883-93}. They are less educated, have less access to screening and health care. There is an association between being of black race and lower social and economic status (U.S. Bureau of the Census, Aug. 2008 supplement to the Current Population Survey (CPS): www.census.gov.), which suggests that the race-based heterogeneity may be a surrogate for social rather than biological heterogeneity. An analysis of outcome of 25,000 cancer patients enrolled in the Southwest Oncology Group phase III clinical trials {Albain et al., 2009, JNCI Journal of the National Cancer Institute, 101, 984} showed that ethnicity does not affect outcome of carcinoma of the lung or colon nor for lymphoma, leukemia or multiple myeloma, but that it affects sex-hormone responsive carcinomas of the breast, ovary and prostate. These unbiased observations, albeit not specifically addressing TNBC, support that, for breast cancer, there might be a true ethnicity-based heterogeneity. (see Table 1)

Molecular heterogeneity

Histologically, the majority of TNBC are grade 3 or poorly differentiated, infiltrating ductal carcinoma not otherwise specified (IDC NOS) (Figure 3A). These tumors have poor prognosis {Maehle et al., 1982, Br J Cancer, 46, 95-100; O'Reilly et al., 1990, Br J Cancer, 61, 671-4}. The few remaining cases are rare histological types like adenoid-cystic, medullary, apocrine, metaplastic or inflammatory BC. There is prognostic heterogeneity associated with these subtypes: the first three have good prognosis and the last two bad {Reis-Filho and Tutt, 2008, Histopathology, 52, 108-18; Hance et al., 2005, J Natl Cancer Inst, 97, 966-75; Rizzo et al., 2009, Cancer, 115, 3009-15; Jung et al., 2010, Breast Cancer Res Treat}.

Five breast cancer subtypes have been identified based on gene expression termed Luminal A, Luminal B, Normal, Her2 and Basal {Perou et al., 2000, Nature, 406, 747-52}. There is some overlap between immunohistochemical (IHC) and the molecular subtypes. Basal BC, thus defined because of the expression of the basal cytokeratins 5/6, 14 and 17 {Pintens et al., 2009, Journal of Clinical Pathology, 62, 624-8}, is similar to TNBC, but not totally overlapping. About twenty percent of TNBC are not basal-like and, similarly, about 20% of basal-like BC are not TNBC {Tan et al., 2008, Breast Cancer Res Treat, 111, 27-44} (Figure 3B). Those TNBC that are not basal generally belong to the normal molecular subtype. The TNBC that are basal-like BC generally have bad prognosis, worse than TNBC per se, which suggests that the molecular classification might not be able to further subtype TNBCs and that it captures a group of TNBC with bad prognosis. However, this group of tumours must be further classified.

The molecular classification of TNBC based on BRCA1 mutations is informative for a small number of TNBC cases (Figure 2, 3C). A proportion of the women with TNBC carry BRCA1 germline mutations, this proportion varies with age of diagnosis and family history but was as high as 40% in an unpublished series {Kandel et al., 2006, ASCO Meeting Abstracts, 24, 508}. The role of BRCA1 null TNBC arising in non-BRCA1 germline mutation carriers is not yet clear, in part on methodological grounds, because in BRCA1 mutation carriers the BRCA1 protein, which is malfunctioning, is detected by IHC. The most frequent mechanism for somatic inactivation of the BRCA1 is promoter methylation, this happens in 10 to 15% of sporadic breast cancer but the immunohistochemical readout of the BRCA1 protein has not been established {Vaz et al., 2007, Journal of Histochemistry and Cytochemistry, 55, 1105; Esteller et al., 2000, JNCI Journal of the National Cancer Institute, 92, 564}. Furthermore, 80% of the BC arising in BRCA1 germline mutation carriers are TNBC but with good prognosis {Rennert et al., 2007, The New England Journal of Medicine, 357, 115-23}. Deep sequencing of breast cancer genomes revealed that the BRCA1 null TNBC shows less genomic instability than the BRCA1 wild type TNBC {Stephens et al., 2009, Nature, 462, 1005-10}, suggesting a disease caused by less genomic alterations, the BRCA1 mutation being particularly relevant. BRCA1 status may thus be suggesting two distinct TNBCs. BRCA1 deficient TNBC, germline or somatic, is likely a different biological entity, deficient in DNA repair, and therefore responsive to therapy with PARP inhibitors and platinum salts that other TNBC, without BRCA1 deficiency, are not {J. O'Shaughnessy, 2009, J Clin Oncol 27:18s, 2009 (suppl abstr 3); A. Tutt, 2009, J Clin Oncol 27:18s, 2009 (suppl abstr CRA501); Venkitaraman, 2009, Cancer Cell, 16, 89-90; Koshy et al., 2010, Breast (Edinburgh, Scotland); Farmer et al., 2005, Nature, 434, 917-21}. PARP1 is an enzyme involved in repair of double strand DNA breaks induced by platinum salts.

There have been attempts to establish a relationship between normal mammary gland development and occurrence of BC, i.e. to map different types of BC into different stages of the mammary gland development {Stingl and Caldas, 2007, Nature Reviews Cancer, 7, 791-9; Stratford et al., 2010, Expert Rev Mol Med, 12, e22}, as has been done for acute myeloid leukemia {Krivtsov and Armstrong, 2007, Nature Reviews Cancer, 7, 823-33}. TNBCs would correspond to a more primitive subtype of tumor, closer to the most undifferentiated BC progenitor cell (stem cell) {Pece et al., 2010, Cell, 140, 62-73}. This reasoning is supported by the observation that the putative BC stem cells in in vitro models are ER- and that, as they subsequently differentiate into mammary gland luminal cells, acquire ER {Liu et al., 2008, Proceedings of the National Academy of Sciences of the United States of America, 105, 1680–1685; Dontu et al., 2004, Trends Endocrinol Metab, 15, 193-7}. Thus some aggressive TNBC might be more similar to the BC stem cell phenotype, showing capacity to undergo epithelial to mesenchymal transition and reprogramming of embryonic genes whereas others would not {Ginestier et al., 2009, Cell Stem Cell, 5, 229-30; Ben-Porath et al., 2008, Nature genetics, 40, 499-507}. This hypothesis would be concordant with the data on chemoresistance of some TNBC. The link between breast development and tumorigenesis is conceptually appealing. However, there are still limited data to support it {Pece et al., 2010, Cell, 140, 62-73; Lim et al., 2009, Nat Med, 15, 907-13}.

Regarding individual markers that are assayed by immunohistochemistry or PCR (Table 2): p53 protein expression is a predictor of shorter time to relapse in two independent european TNBC populations {Alberti et al., 2009, Cancer Research, 69, 2018}. Higher expression of EGFR protein appears to predict adverse prognosis in TNBC {Viale et al., 2009, Breast Cancer Res Treat, 116, 317-28}. This data is coherent with the expression of basal cytokeratins that separates TNBC in basal and non-basal, because EGFR seems to segregate with the basal-like breast cancers {Thike et al., 2010, Am J Surg Pathol, 34, 956-64; Dogu et al., 2009, Med Oncol}. It is still debatable whether EFGR is truly overexpressed in carcinomas versus their normal tissues of origin because the majority of reports do not measure EGFR expression in non-neoplastic control tissue, probably the erratic response to EFGR monoclonal antibodies and tyrosine kinase inhibitors is an illustration of this fact {Gusterson and Hunter, 2009, The Lancet Oncology, 10, 522-7}. Regarding anti-EGFR therapy and breast cancer, cetuximab has been evaluated in TNBC {Baselga et al., 2013, J Clin Oncol, 31, 2586-92}, and, in fact, EGFR positivity was not found to be predictive of response but one of it's downstream effectors, PTEN positivity, was {Khambata-Ford et al., 2010, ASCO Meeting

Abstracts, 28, 1056}. c-Kit protein expression was reported to be predictive of poor outcome {Park et al., 2009, Cancer Research, 69, 6032} albeit being a targetable molecule with imatinib, the results in unselected breast cancer patients were disappointing {Cristofanilli et al., 2008, Ann Oncol, 19, 1713-9}. The loss of E-cadherin, a well characterized phenomenon in breast and other carcinomas, because it correlates with loosing epithelial cohesiveness, is a marker of bad prognosis in TNBC {Kashiwagi et al., 2010, Br J Cancer, 103, 249-55}. The presence of the androgen receptor has been reported to be frequent in TNBC, it is a hallmark of apocrine carcinoma, with good prognosis and possibly a targetable molecule albeit with a number of undesirable side effects {Farmer et al., 2005, Oncogene, 24, 4660-4671}.

There are some reports on the molecular heterogeneity of TNBC that assess pathways or groups of genes instead of single markers making use of genome-wide gene expression profiles. As we have seen the deficiency in DNA double strand break repair mechanisms also called the “BRACness of TNBC” has been tested and is being exploited in clinical trials. The observation that TNBC more frequently exhibit stem cell characteristics, i.e. the “stemness of TNBC”, can also be tested therapeutically because it should correlate with chemoresistance. Lastly, some authors have described the activation of immune response as a good prognostic marker in ER negative BC {Teschendorff et al., 2007, Genome Biol, 8, R157}. This can be called the “immunogenicity of TNBC”, a reasonable surrogate of another TNBC subgroup, this, coupled to a specific antigenic profile of TNBC cells is being exploited therapeutically with vaccination strategies {Curigliano et al., 2010, Annals of Oncology}

Therapeutic heterogeneity

There is a perplexing feature of TNBC, termed the “TN paradox”: chemoresponsiveness and chemoresistance {Carey et al., 2007, Clin Cancer Res, 13, 2329-34} (Fig 2). On one hand, TNBC are among the most chemoresponsive breast cancers: data from neoadjuvant studies show that the fraction of tumours experiencing pathologic complete response (pCR) is mostly comprised of TNBC, with a pCR rate generally over 50% {Carey et al., 2007, Clin Cancer Res, 13, 2329-34; Wang et al., 2009, Gan To Kagaku Ryoho, 36, 255-8; von Minckwitz et al., 2008, J Natl Cancer Inst, 100, 552-62; von Minckwitz et al., 2008, J Natl Cancer Inst, 100, 542-51; Liedtke et al., 2008, J Clin Oncol, 26, 1275-81; Esserman et al., J Clin Oncol 27:18s, 2009 (suppl abstr LBA515); Chang et al., 2010, Cancer; Colleoni et al., 2010, J Clin Oncol, 28, 2966-73}. On the other hand, TNBC are sometimes chemoresistant, as documented by the short survival of patients with metastatic disease in published series {Ding et al., 2010, Nature, 464, 999-1005; Lin et al., 2008, Cancer, 113, 2638-45; Kassam et al., 2009, Clinical Breast Cancer, 9, 29-33}. One could argue that the apparent chemorresponsiveness found in neoadjuvant studies, evolves into a chemorresistant phenotype. However, this does not seem to be the case because in neoadjuvant trials with long follow up, those women that obtain a pathological complete response consistently maintain survival advantage over the years {Bear et al., 2006, J Clin Oncol, 24, 2019-27}, and the same observation has been done for TNBC that achieve pCR {Liedtke et al., 2008, J Clin Oncol, 26, 1275-81}. This suggests that chemosensitivity is a hard-wired feature of a particular tumor {Hüsemann et al., 2008, Cancer Cell, 13, 58-68; Ding et al., 2010, Nature, 464, 999-1005}.

Since chemossensitive TNBC tends to be deficient in DNA repair, this suggests that reduced expression of genes involved in homologous recombination repair predict better response to antracyclines, that cause double strand breaks in DNA, than to taxanes which interfere with microtubule polimerization. This is indeed the case, as the DNA repair deficient TNBC had better response to anthracyclines than to taxanes {Rodriguez et al., 2010, Breast Cancer Res Treat, 123, 189-196}.

Regarding apoptosis, two members of the p53 familly, p63/p73 and their network, have been shown to be upregulated in TNBC and predictive of response to cisplatin in vitro {Leong et al., 2007, The Journal of Clinical Investigation, 117, 1370-80}. This hypothesis was tested in a clinical trial and a surrogate of this network, a gene expression signature of E2F3 activation was found to predict response to cisplatin in TNBC {Silver et al., 2010, Journal of Clinical Oncology}. E2F3 is a transcription factor frequently upregulated and associated with bad prognosis in bladder and prostate carcinomas {Oeggerli et al., 2006, Oncogene, 25, 6538-43; Foster et al., 2004, Oncogene, 23, 5871-9}. In this trial, those TNBC that had mutations in p53 also responded better to cisplatin {Silver et al., 2010, Journal of Clinical Oncology}.

Besides these markers, what other marker could be more readily used in the clinic that captures the heterogeneity regarding pCR in TNBC? A study reported that, in TNBC, those that were basal-like had a 23% pCR rate and those that were non-basal-like had a 62% pCR rate. The chemotherapy regimen evaluated was standard fluorouracil, epirrubicin at 100mg/m² and cyclophosphamide (FEC100) and the definition of basal subtype was based on positivity for EGFR and basal cytokeratin 5/6 {Masuda et al., 2010, Cancer Chemotherapy and Pharmacology}. These assumptions are being tested in current randomized clinical trials in TNBC where PARP inhibitors, platinum salts, alquilating agents and anthracyclines are part of the investigational arms {Silver et al., 2010, Journal of Clinical Oncology; Petrelli et al., 2009, Expert opinion on investigational drugs, 18, 1467-77}.

Future directions

There is no doubt that TNBC presents as a therapeutic problem. For therapy to be tackled, the underlying diagnostic challenge has to be met, and this is, in our opinion, the key unsolved issue, for we still do not know what TNBC is. We outlined the existing evidence pointing to different diseases present in this negatively defined entity. It is plausible that we face at least two distinct diseases or possibly several, but numerous questions remain unanswered.

Firstly, it is imperative that the epidemiological characterization of TNBCs arising in different populations is done. The hypotheses that there are different TNBCs in different racial, age, body mass index and genetic backgrounds must be tested. We need to quantify the specific prognostic value associated with each clinical and epidemiological variables to better choose therapy and inform patients.

Secondly, we need to define the molecular drivers for each subtype of TNBC so that we can identify markers with definite prognostic value, that can be used by clinicians to decide with confidence whether the tumor is chemoresponsive, or whether other therapies must be tested. These markers may co-exist with existing ones, the same way that tumors may be simultaneously ER+ and HER2+. Currently, there are several promising leads, including immune response activation, p53 and EGFR protein expression, mesenchymal and stem cell features and androgen receptor expression {Lehmann et al., 2011, The Journal of Clinical Investigation, 121, 2750-67}. Determining the association of these potential markers with clinical parameters such as race, age of incidence and their predictive value for relapse and drug sensitivity, are also necessary steps to apply them in the clinic.

Finally, we need to identify targeted therapies for TNBC. This will happen if we are able to overcome the second challenge. Multiple targeted therapies that already explore potential differences in subtypes of TNBC are currently being tested in the clinic for TNBC with encouraging results.

We have come a long way since the first sub-typing of breast cancer, and the development of the first targeted therapy, tamoxifen. TNBC is today ‘the edge of our ignorance’, those tumors that we still have not segmented into classes for which we know the underlying molecular basis. Soon we will be defining quadruple-negative, or quintuple negative breast cancers, as we identify new molecular drivers in breast cancer leading to new diagnostic markers and therapeutic targets.

Figure legends & tables

Figure 1 - Heterogeneity in the natural history of triple negative breast cancer {Muguti, 1993, Journal of the Royal College of Surgeons of Edinburgh, 38, 75-8; Anderson et al., 2002, Breast Cancer Res Treat, 76, 27-36; Lund et al., 2009, Breast Cancer Res Treat, 113, 357-370; Yin et al., 2008, Breast Cancer Res Treat; Seewaldt and Scott, 2007, N Engl J Med, 356, e12; Collett et al., 2005, Cancer Epidemiol Biomarkers Prev, 14, 1108-12; Vona-Davis et al., 2008, Cancer Epidemiol Biomarkers Prev, 17, 3319-24; Dent et al., 2008, Breast Cancer Res Treat; Rodríguez-Pinilla et al., 2006, Clin Cancer Res, 12, 1533-9; Pinilla et al., 2006, Breast Cancer Res Treat, 99, 85-90; Gadiyaram et al., 2009, Cancer Research, 69, 6159; Tsang et al., 2009, Cancer Research, 69, 3072; Yin et al., 2008, Breast Cancer Res Treat; Lee et al., 2009, Cancer Research, 69, 4044; Dent et al., 2007, Clin Cancer Res, 13, 4429-34; Wei et al., 2008, Hum Pathol, 39, 1809-15}.

Figure 1
Braga et al

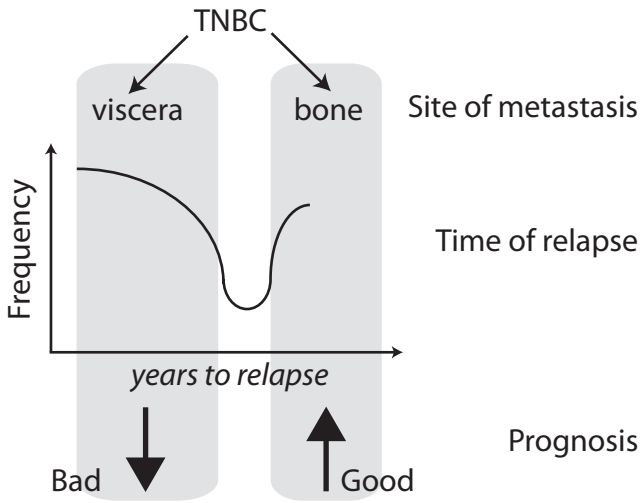


Figure 2 - Hypothesis of genomic stability and TNBC heterogeneity {Stephens et al., 2009, Nature, 462, 1005-10; Ding et al., 2010, Nature, 464, 999-1005; Curtin et al., 2005, The New England Journal of Medicine, 353, 2135-47}. DSB, double strand breaks

Figure 2
Braga et al

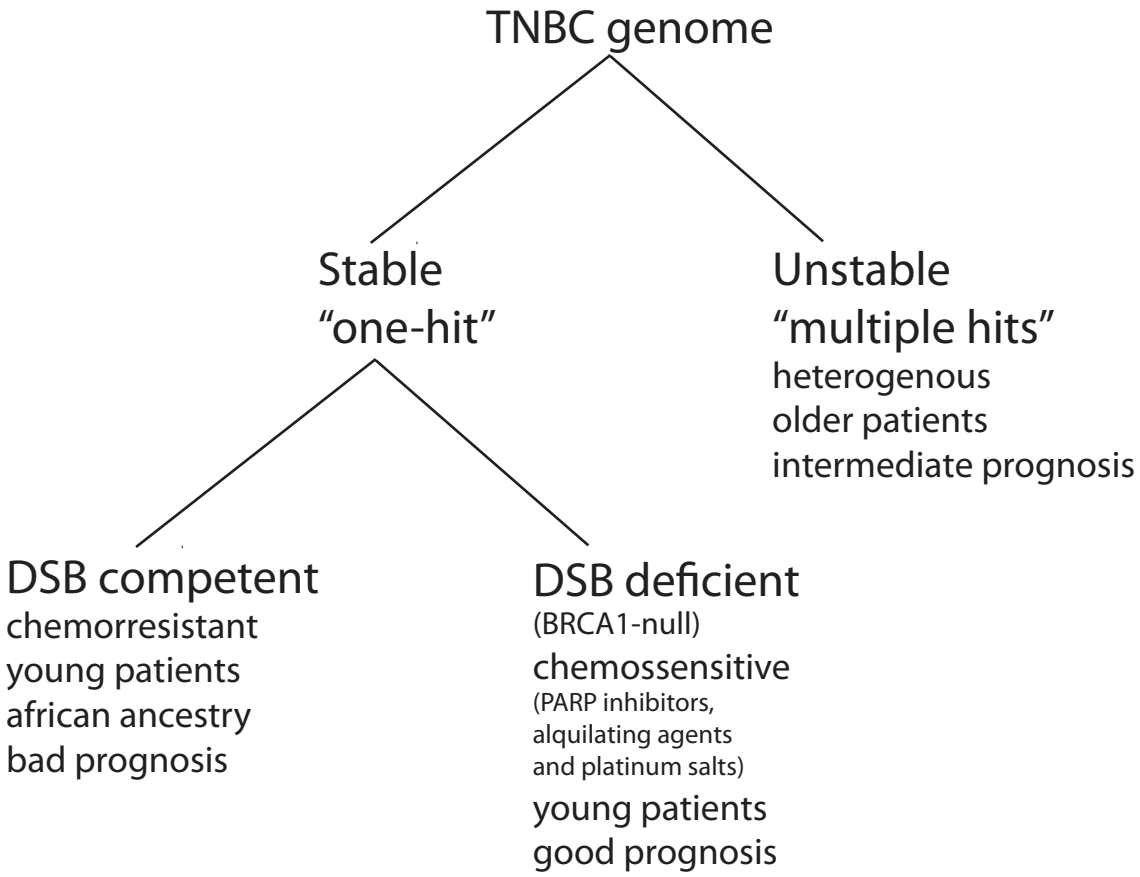


Figure 3 - Histological and molecular heterogeneity of triple negative breast cancer and other subtypes of breast cancer. IDC NOS - Infiltrating Ductal Carcinoma Not Otherwise Specified.

Figure 3
Braga et al

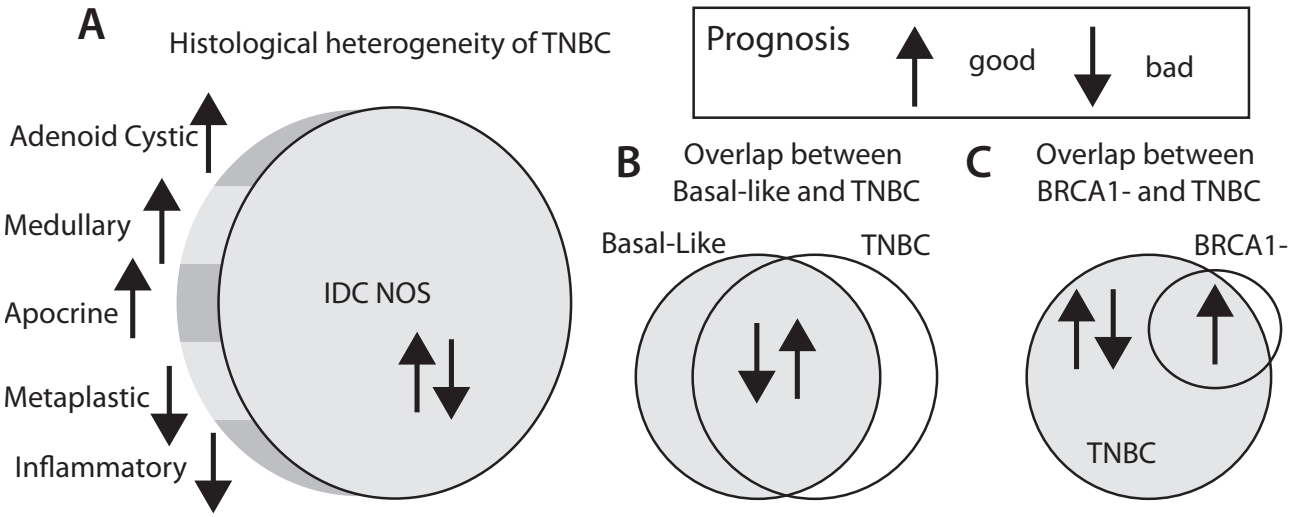


Table 1 - Clinical, epidemiological and therapeutic heterogeneity of triple negative breast cancer.

Characteristics	Worse outcome	Better outcome
Age of presentation	Young	Old
Stage at presentation	Advanced	Early
Growth rate	Fast	Slow
First site metastasis	Liver & brain	Lymph nodes & bone
Chemotherapy response	Resistant	Exquisitely sensitive
Body mass index	High	Low
Race	Black	White
Social deprivation	Yes	No

Table 2 - Molecular heterogeneity of triple negative breast cancer according to differences in expression and their correlation to outcome.

Molecule or pathway	Worse outcome	Better outcome
p53	+	-
EGFR	+	-
CK5/6	+	-
E-cadherin	-	+
BRCA1	+	-
c-kit	+	-
Androgen receptor	-	+
DSB repair deficiency	+	-
Stem cell markers	+	-
Immune response activation	-	+

Chapter 4: Systemic treatment for triple negative breast cancer

Conception and design: Sofia Braga

Provision of study materials or patients: Sofia Braga

Collection and assembly of data: Sofia Braga

Data analysis and interpretation: Sofia Braga

Manuscript writing: Sofia Braga

Final approval of manuscript: Not applicable

Funding: Not applicable

Ethical Committee approval: Not applicable

Systemic treatment for TNBC

Abstract
Chemotherapy remains the backbone of systemic therapy for triple negative breast cancer (TNBC). Despite the validation of targeted agents, there is no such therapy for TNBC, possibly because the biology of TNBC has not been conclusively elucidated, this tumor is still defined by what it is not. Data shows that these tumors benefit from chemotherapy in the neoadjuvant, adjuvant and metastatic setting, possibly even more than other BC subtypes. Neoadjuvant chemotherapy trials show higher response rates in TNBC than non-TNBC. Pathologic complete response (pCR) has been shown to predict improved long term survival in BC. Specific adjuvant regimens for TNBC are under study, third generation chemotherapy regimens utilizing dose dense or metronomic polychemotherapy are some tools presently available. The role of specific chemotherapy agents, namely platinum salts, remains poorly defined. Taxanes and anthracyclines are active in TNBC and remain important agents, but have not shown specific benefit over non-TNBC. Targeted agents have been similarly difficult to study and approve for TNBC treatment. Trials with anti epithelial growth factor agents, antiangigenic agents and, more recently, PARP inhibitors have not met their primary endpoints. TNBC is itself a very heterogenous group of tumors, in this paper we attempt to review data and point directions in the systemic treatment of TNBC.

Triple negative breast cancer subtypes
Since 2005, when the widespread use of anti Her2 therapy started to modify the natural history of Her2 positive BC that TNBC became the subtype of BC for which there is no targeted therapy. Since then, several trials have been performed to try to understand what is targetable in TNBC and if so, if the clinical trials designed to test these hypothesis meet their endpoints. TNBC has a negative definition, it is defined by what it is not, therefore, we do not know what TNBC is. This is one of the important problems. We do not know what disease we are treating, with what alterations, in which genes.
TNBC is itself a heterogeneous group in which subgroups like basal like BC, defined by higher proliferation, and, including those TNBC arising in BRCA1 mutation carriers, may be more sensitive to platinum agents and relatively less sensitive to taxanes. Lehmann et al, after several other experiments, have put forward a convincing classification of four subtypes with histological, genetic, epidemiological, therapeutic, and, therefore, practical implications {Lehmann et al., 2011, The Journal of Clinical Investigation, 121, 2750-67}. First, the basal-like 1 and 2 (BL1 and BL2): these are the TNBC that were in PAM50 classified as basal-like. They are the tumors that develop frequently in BRCA1 and BRCA2 mutation carriers. These tumors are susceptible to classical chemotherapy because they have mutations in DNA repair genes, these are the tumors that respond well to neoadjuvant chemotherapy. These tumors are genomically unstable and have a number of other alterations in proliferative genes which also concur for chemossensitivity. Histologically, they are invasive carcinomas NOS. They appear more frequently in young women.
Next, is the group of TNBC that has genomic evidence of immune modulation. The activation of immune response has been another feature frequently present in TNBC, apparently exclusive from the typical proliferation driven basal-like TNBC. These tumors are frequently medullary histologically, they have good prognosis. These tumors were first identified in a large neoadjuvant trial that aimed to stratify the benefit of chemotherapy according to P53 mutation status {Farmer et al., 2009, Nat Med, 15, 68-74}{Teschendorff et al., 2007, Genome Biol, 8, R157}.
The third group are TNBC associated with mesenchymal and stem cell features. These tumors are histologically anaplastic, displastic, metaplastic or sarcomatoid. These tumors have lost their epithelial features, they express non epithelial markers and stem cell markers like P-cadherin. These carcinomas are chemorresistant and account for the bad prognosis of TNBC. There do not seem to be preferential age groups for appearance of mesenchymal TNBC. These non epithelial features have been linked to a stem like phenotype and cancer stem cells have been postulated to be chemorresistant {Pece et al., 2010, Cell, 140, 62-73}. These tumors might be susceptible to growth factor inhibition: EGFR, anti Src, anti angiogenesis, and anti IGF therapy.
Finally, the TNBC that expresses the androgen receptor. These tumors are histologically apocrine, have tendency towards local relapse and might be susceptible to anti androgens {Farmer et al., 2005, Oncogene, 24, 4660-4671}.
This provoking classification has recently been put forward, but for the last decade we have been trying to retrospectively gather data and prospectively test specific agents in these breast tumors. This is a simplified classification that uses molecular and histological biomarkers to predict prognosis and response to specific systemic treatments. The Lehmann classification and other similar classifications, should start to be studied prospectively. Such studies will be particularly informative for this difficult subgroup of BC.

In this paper, we have gone through trial data and retrospective series trying to understand how best to treat TNBC with the currently available evidence, and, when possible, correlating trial data with the biological basis of TNBC.

Classical chemotherapy

Adjuvant setting

BC adjuvant clinical trials have generally been conducted in unselected BC cases, therefore, to study adjuvant outcomes of TNBC one must perform retrospective analysis of adjuvant trials. Regarding the most important body of data on adjuvant therapy, the ECBTCG published a study looking specifically at the benefit of anthracycline or alquilating agent based adjuvant chemotherapy versus not, in ER poor patients. There was a reduction in the risk of BC death from 32% to 24% (hazard ratio 0.73, p=0·0002) in women aged less than 50 years. In women in the fifth and sixth decades the reduction in the risk of dying of BC was 36% in those treated versus 42% in those not treated {EBCTCG, 2008, Lancet, 371, 29-40}.

Very relevant in TNBC, is dose dense chemotherapy (DDCT) with peripheral blood stem cell rescue (PBSCR), because increased genomic instability and DNA repair pathway mutations are hallmarks of TNBC and these characteristics are thought to be harbingers of chemosensitivity. A West German Group trial of DDCT with PBSCR conducted in unselected patients, was reanalyzed and the benefit was greatest in patients with histological grade 3 TNBC, an interesting correlate of particular CT sensitivity {Gluz et al., 2008, Ann Oncol, 19, 861-70}. Similarly the NKI group led by Rodenhuis continues to explore a DNA repair deficiency signature as predictive of response to DDCT.

Neoadjuvant setting

Neoadjuvant chemotherapy (NACT) is administered before surgery, irrespective of the overall duration of treatment. It has been shown that clinical midcourse response predicts the achievement of pathological complete response (pCR) and long term outcome. Patients with tumors showing no response at midcourse might be considered as chemoresistant, patients without response at midcourse treatment have a pathologic response rate of less than 5%. Patients with indication for NACT represent 30% of all patients, and these patients have, due to high initial staging, higher risk of recurrence and death.

NACT seems to be of particular interest in TNBC: There are several studies retrospectively showing that some TNBC patients respond better than other BC subtypes to NACT. This said, the survival advantage is only seen in those that attain pCR. The MDAnderson Cancer Center analyzed retrospectively their cohort of 1118 patients treated with NACT and was able to show that the pCR rate in TNBC was twice the pCR rate of non-TNBC (22 vs. 11%). Regarding survival, TNBC vs non-TNBC have a 3 year OS of 74% vs 89%, but TNBC with pCR have a similar 3 year survival to non-TNBC with pCR (94 vs 98%) {Liedtke et al., 2008, J Clin Oncol, 26, 1275-81}. This is why NACT is of specific interest in these patients because if pCR is reached prognosis is similar to other BC subtypes. A retrospective series of 107 patients treated with anthracycline and alquilating agents showed TN tumors had a pCR rate of 35%. Curiously, the TNBC patients that achieved pCR did not recur, the TNBC that recurred had a distant metastasis free survival (DMFS) of 60% {Carey et al., 2007, Clin Cancer Res, 13, 2329-34}. This study similarly shows that if patients with TNBC are treated with NACT and obtain pCR they will have similar outcome than non-TNBC patients. Conversely, TNBC patients that do not, are a subgroup of TNBC with worse survival. Due to CT resistance profile, these TNBC that do not respond to NACT might overlap with TNBC with mesenchymal features.

The National Adjuvant Breast and Bowel Project (NSABP) B27 trial randomized 2411 patients to an anthracycline vs. taxane question. The results show that ER negative tumors respond better to chemotherapy: pCR rate in the anthracycline arm (AC) was 14% in ER negative tumors, compared to 6% for ER positive tumors. With the addition of docetaxel the pCR increased in both subsets (23% vs. 14%). This trial did not detect differences in survival. It has been shown in adjuvant trials that sample sizes needed to detect a taxane survival benefit were over 3000, so this is possibly the reason for B27 never having showed survival advantage. There were two weaker points in B27. First, the definition of pCR did not consider lymph nodes and second, tamoxifen was given concurrently with CT. The non randomized, I-SPY1 Trial also studied the same drug regimen, TNBC had a 35% pCR rate {Esserman et al., 2012, J Clin Oncol, 30, 3242-9}.

Another single arm trial used taxanes followed by triple therapy with anthracycline, alquilating and anti folate (FEC). Here patients were studied by gene expression profiling according to the PAM50 classifier, the basal like subgroup and the Her2 subgroup had a pCR rate of 45%, whereas the luminal subgroups had a pCR rate of 6% and there was no pCR in the normal BC. In this trial, there is currently no data regarding outcome {Rouzier et al., 2005, Clin Cancer Res, 11, 5678-85}. There is a paradox in TNBC: The high pCR rate translates into a high DMFS, but as a group, including all women, those with pCR and those without, TNBC

has a DMFS of 60%, which is poor. This was similarly seen in two retrospective series of 145 and 107 BC, where the TNBC patients had 17% and 27% pCR rate, from the latter, none of the patients with pCR relapsed {Keam et al., 2007, BMC Cancer, 7, 203}{Carey et al., 2007, Clin Cancer Res, 13, 2329-34}
The German Breast Group (GBG) has contributed extensively performing NACT trials, called Gepar trials. Gepar Duo asked three questions: 1) if we could spare alquilating agents 2) if DDCT has a role and 3) if sequential taxanes are better than the combination of anthracyclines and taxanes. It randomized 902 women to a dose dense two drug regimen (ADOC) verus a sequential two drug regimen followed by docetaxel (AC-DOC). In the analysis relevant for TNBC, the pCR rate in ER negative disease was 23% versus 6% in ER positive. {von Minckwitz et al., 2005, J Clin Oncol, 23, 2676-85}.
Gepar Trio, the next trial from the German group, was designed for randomization after two cycles of TAC, and stratified patients according to response. Responders were randomized to four or six more cycles of TAC. Non responders were randomized to four more cycles of TAC or vinorelbine and capecitabine {von Minckwitz et al., 2008, J Natl Cancer Inst, 100, 552-62}{von Minckwitz et al., 2008, J Natl Cancer Inst, 100, 542-51}. Differences in pCR were not significant in either comparison. This is an extremely provoking result: in patients that do not respond to anthracyclines, taxanes and alquilating agents, the use of fluoropyrimidines and vinca alkaloids seems futile. The expression of cytoplasmic poly(adenosine diphosphate-ribose) polymerase that has been correlated with TNBC and is a potential target of PARP inhibitors, was seen to be predictive of response to chemotherapy but also prognostic of survival {von Minckwitz et al., 2011, J Clin Oncol, 29, 2150-7}.
Gepar Quattro asks a question in Her2 positive locally advanced BC patients, if the addition of trastuzumab to chemotherapy, increases pCR {Untch et al., 2010, J Clin Oncol, 28, 2024-31}. Chemotherapy regimens include anthracyclines (EC), taxane (docetaxel) and fluoropirimidines (capecitabine). Patients with Her2 positive tumors had 40% pCR, defined by no invasive breast cancer in the breast and lymph nodes, and a near 32% pCR rate considering disappearance also of ductal carcinoma in situ, while in the patients with Her2 negative tumors had a pCR rate of 15%. In a retrospective analysis of this and the previous trial, it was possible to find that thymosin beta 15A, a protein binds to and sequesters G actin monomers, and, therefore, inhibits actin polymerization, is a predictor of pCR, only in TNBC, but not in other biological subtypes. The evaluation of TMBS15A divided TNBC into a low and a high expression group (pCR rate 16.0% vs 47.2%) {Darb-Esfahani et al., 2012, Br J Cancer, 107, 1892-900}. This molecule was found in another study to be predictive of response to CT, in P53 mutated tumors {Bertheau et al., PLoS Med, 4}. The biologic rationale is thought to be the capacity of cells to repair damage induced by chemotherapy or not.
Gepar Quinto tested the addition of either bevacizumab or everolimus to conventional anthracycline and taxane CT, in Her2 negative BC, and lapatinib or trastuzumab are added to a similar CT backbone in Her2 positive BC patients. In the German trials pCR is defined as ypT0N0. The conclusion is that neither lapatinib nor everolimus can “rescue” non responding patients to CT plus bevacizumab or CT plus trastuzumab. The addition of bevacizumab did not increase pCR rate 15% vs. 17.5%. However in the subgroup of 684 TNBC patients pCR rate was 27.8% vs. 36.4% (p = 0.021) {von Minckwitz et al., 2012, The New England Journal of Medicine, 366, 299-309}.
Ten years ago the role of platinum salts specifically in TNBC started to be investigated. Gepar Sixto used carboplatin as neoadjuvant CT in addition to taxanes in Her2 positive and TNBC patients. Women were treated for 18 weeks with weekly paclitaxel and non-pegylated-liposomal doxorubicin. Her2 positive patients received concurrently trastuzumab and lapatinib. TNBC patients received concurrently bevacizumab. All patients were randomized 1:1 to receive concurrently weekly carboplatin or not, stratified by subtype. Results show an increase in pCR from 37% without carboplatin to 44% with carboplatin. In TNBC patients the increase in pCR with the platinum addition was from 37% to 53%. In Her2 positive BC patients the addition of carboplatin decreased pCR from 37% to 33%. The authors concluded that adding platinum salts is beneficial only in TNBC patients and that bevacizumab did not change pCR rate in these patients {von Minckwitz et al., 2014, Lancet Oncol}.

Specific drugs

Platinum salts
In 1988, Sledge et al reported a 47% response rate to cisplatin in first line metastatic (M)BC. This trial was conducted in 20 patients {Sledge et al., 1988, J Clin Oncol, 6, 1811-4}, the generalization to larger sample sizes that subsequently happened reduced response rate. The response rate in 2nd and 3rd line MBC, after anthracyclines and alquilating agents, has been shown to be close to 10% or less {Yap et al., 1978, Cancer treatment reports, 62, 405-8}{Baselga et al., 2013, J Clin Oncol, 31, 2586-92}. This, and the development of taxanes, more efficacious and less toxic, was the reason platinum salts were not developed in BC. However, in ovarian cancer platinum salts were always the backbone of systemic therapy. With the Cancer Genome

Atlas dataset, TNBC has molecular similarities to ovarian caner, and, this, together with findings in BRCA deficient BC, has reignited the interest in platinum salts. TNBC and BRCA-deficient BC overlap, and there is cell line data showing that BRCA-/- cell lines are more sensitive to DNA-damaging agents, particularly those able to induce double strand breaks, such as cisplatin or carboplatin, when compared to doxorubicin or paclitaxel. This sensitivity is 3 fold higher than in the BRCA competent lines and is reversed with either BRCA1 upregulation or restoration {Tassone et al., 2003, Br J Cancer, 88, 1285-91} {Husain et al., 1998, Cancer Research, 58, 1120-3}. BRCA-null tumors are deficient in genes that encode for proteins critical in DNA integrity, genomic stability, and DNA repair, these tumors are basal-like in the PAM50 classification.

Researchers first characterized platinum salt activity in a retrospective analysis of 802 MBC patients, 67 had measurable disease and received a platinum and paclitaxel based regimen in the first or second line. The overall response rate (ORR) in these was 39%. In the TNBC patients, the ORR was 37.5%, and there was no difference compared to the hormone receptor positive subgroup {Uhm et al., 2009, Int J Cancer, 124, 1457-62}.

The use of platinum salts in NA treatment of BRCA1 mutated TNBC was elegantly demonstrated by Byrski et al. (Table 1). The Polish group treated 10 women with BRCA1-associated breast cancer, 9 of which had TNBC, with 4 cycles of preoperative single-agent cisplatin (75 mg/m2) and 8 patients obtained pCR {Byrski et al., 2009, Breast Cancer Res Treat, 115, 359-63}. Silver et al administered NACT with cisplatin in TNBC, not necessarily BRCA deficient, the pCR rate was only 22% (6/28) with 36% of the tumors with a Miller-Payne score of 4 or 5. Concordant with the Polish results, the two patients that were BRCA1 mutation carriers achieved pCR {Silver et al., 2010, Journal of Clinical Oncology}. The expression of p63/p73 as a readout of apoptosis in these tumor blocks, showed pCR in 3 of 9 (33%) positive patients but only 1 of 13 (7%) negative patients. This group studied the addition of the antiangiogenic agent bevacizumab to cisplatin in the NA treatment of 51 patients this resulted in a lower pCR rate of 16%, with 37% achieving a Miller-Payne score of 4 or 5 {Ryan et al., 2009, J Clin Oncol, 27 suppl, abstr 551}

An Italian group, reported a very high pCR rate of 65% in 74 patients with TNBC treated with cisplatin 30mg/m2, epirubicin 50mg/m2 and paclitaxel 120mg/m2 weekly for 8 weeks with GCSF support. Adjuvant alquilating therapy with 4 cycles of CMF was administered to all patients, and those with four or more positive nodes after preoperative therapy received an additional 4 cycles of CMF. Those patients who achieved a pCR had a 3- and 5- year disease-free survival (DFS) of 97% and 90%, respectively, compared with 3- and 5-year DFS rates of 61% and 56% in those with residual disease after preoperative therapy. Showing once more the correlation of pCR with survival {Frasci et al., 2009, Ann Oncol, 20, 1185-92}.

A similar italian trial of 30 TNBC patients treated with 4 preoperative cycles of epirubicin, cisplatin and fluorouracil (ECF) followed by weekly paclitaxel resulted in a pCR rate of 43% {Torrisi et al., 2008, Cancer Chemotherapy and Pharmacology, 62, 667-72}. An Italian retrospective analysis of NA, adjuvant and metastatic BC trials looking at the activity of any platinum-based chemotherapy in TNBC patients, showed pCR of 50%, 5 year survival of around 60% and ORR of 40% {Sirohi et al., 2008, Ann Oncol, 19, 1847-52}

A North American cooperative group, CALGB, is testing in 40603 trial carboplatin and bevacizumab in TNBC. This a randomized Phase II NA study where patients are enrolled in one of 4 arms in a 2 by 2 factorial design. The CT backbone is 12 weekly administrations of paclitaxel followed by 4 cycles of dose dense doxorubicin and cyclophosphamide (AC). The factorial design either adds bevacizumab or carboplatin or both. Von Mincwitz et al seem to have already conclusively answered this question in Gepar Sixto trial {von Minckwitz et al., 2014, Lancet Oncol}.

GEICAM, a Spanish cooperative group, conducted a NACT trial in TNBC positive for basal markers EGFR, CK5 and CK7 with the same regimen. The pCR rate only changed from 30 to 35% suggesting that carboplatin is not specifically useful in this setting, whereas in the BRCA1 null tumors platin salts seem to increase pCR rates {Alba et al., 2012, Breast Cancer Res Treat, 136, 487-93}. This results are concordant with the Polish by Byrski et al. and the New England results by SIlver et al.

Platinum salts have been extensively studied in MTNBC, BALI-1 is a multicentric trial that randomized 173 patients to receive either cisplatin alone versus cisplatin in combination with cetuximab. Final analysis demonstrated a modest improvement in PFS among patients who received combination therapy, 1.5 versus 3.7 months (HR 0.675 CI 0.470–0.969, P=0.032). Despite the doubling of ORR in the combination arm from 10% to 20%, the study failed to meet its primary endpoint of greater than a 20% response {Baselga et al., 2013, J Clin Oncol, 31, 2586-92}.

A North American trial asked the same question with a carboplatin combination, and had similar 20% ORR, additionally, the genomic patterns of response did not show consistent inhibition of EGFR signaling {Carey et al., 2012, J Clin Oncol, 30, 2615-23}. A follow up to this trial, will use either cisplatin or carboplatin, asking which is more active in this context, and will ask prospectively the question whether p63 expression assessed by RT-PCR is predictive of response to platinum agents in TNBC patients {Isakoff SJ, 2007, J Clin Oncol, 25 , 10522}.

The Polish group, due to the high prevalence of BRCA1 founder mutations in Poland, showed that the response rate in BRCA1-associated MBC is 80% with 45% complete response {Byrski et al., 2012, Breast Cancer Research : BCR, 14, R110}.
A large randomized phase III Triple Negative Breast Cancer Trial (TNT), with approximately 400 patients, in the UK, is underway, comparing carboplatin with docetaxel for MTNBC. Patients may receive up to 6 cycles of treatment and will crossover to the other arm. The TNT study is designed to detect a 15% improvement in response to carboplatin compared to docetaxel. This trial will provide the answer to an important daily problem: What is the first line treatment, outside a clinical trial, to administer a MTNBC patient?

Anthracyclines
There is evidence that chromosomal instability correlates with anthracycline response in BC. In a retrospective study, fluorescent in situ hybridisation for chromossomes 1, 7, 11, 17 and 18, as a test for chromossomal instability, was used to identify patients with unstable tumors and study response to anthracyclines. In 322 patients recruited to BR9601 clinical trial, high tumor instability correlates with reduced DFS and overall survival. However, patients with unstable tumors, had better survival if treated with anthracyclines {Munro et al., 2012, Br J Cancer, 107, 71-4}. Instability seems to be predictive of response but simultaneously to indicate worse prognosis.
As a corollary, possibly anthracyclines might not be so useful in genomically stable subtypes of TNBC. There are only two suggestions that BRCA1 associated TNBC may be less sensitive to anthracycline-based therapy {Foulkes et al., 2003, J. Natl. Cancer Inst., 95, 1482-1485}. The first is that among 55 TNBC patients who received 6 cycles of fluorouracil, epirubicin 100mg/m2 and cyclophosphamide (FEC100), 12 BRCA1 carriers were identified. The pCR rate for the 12 triple negative BRCA1 carriers was 17% compared with 42% in the 55 sporadic TNBC patients non BRCA carriers {Petit T, 2007, J Clin Oncol, 25, 580}. The other, is a retrospective analysis of one of the Canadian adjuvant trials (MA5) comparing cyclcophosphamide, epirubicin, fluoruracil (CEF) to CMF, showed an improvement in 5-year overall survival from 51% to 71% for TNBC patients randomized to the CMF arm, while the anthracycline combination was superior in all other subgroups {Cheang M, 2009, J Clin Oncol, 27 }. The role of anthracyclines in TNBC is not clarified: On one hand, all trials have shown that TNBC patients benefit from anthracyclines and only two retrospective analyses do not confirm the findings but one of the studies defines TNBC differently, looking specifically at BRCA mutation carriers. A BRCA1 mutation, might affect anthracycline sensitivity like it has been shown for platinum salts, in this case negatively.

Taxanes
The data regarding taxanes in TNBC seems to be as unclear as the data for anthracyclines. It is perplexing how such conflicting data exist regarding the two most important therapeutic groups in the treatment of BC, concerning such a difficult BC subtype. A French retrospective analysis of NACT used to treat TNBC patients showed that FEC100 gave higher (58%) response rate than docetaxel (25%) {Petit T, 2007, J Clin Oncol, 25, 580}.
The MD Anderson conducted a retrospective analysis of 1079 patients in NACT clinical trials with or without taxanes. In the ER negative subgroup, pCR was achieved in 15% without adding taxanes, and in 29% with taxanes {Mazouni et al., 2007, Ann Oncol, 18, 874-80}. A preoperative study of paclitaxel, followed by FAC, resulted in a 45% pCR rate among the basal-subgroup of patients {Rouzier et al., 2005, Clin Cancer Res, 11, 5678-85}
As with platinum and anthracycline agents, the BRCA1 population may demonstrate distinct patterns of response to taxanes compared to sporadic TNBC. In a Polish restrospective analysis, 44 BRCA1 carriers out of 3500 patients, only 6 of 15 who received docetaxel and doxorubicin had a complete or partial response, compared to 29 of 29 who received the non-taxane combination with DNA damaging drugs like anthracyclines or aquilating agents {Byrski et al., 2009, Breast Cancer Res Treat, 115, 359-63}.
The clinical trial CALGB 9344 was conducted more than 10 years ago but is still a very informative adjuvant trial. CALGB 9344 showed that the addition of paclitaxel to AC increased survival in circa 3000 patients. A retrospective analysis demonstrated that in Her2 negative patients, those that were ER negative, derived most benefit from taxanes {Hayes et al., 2007, The New England Journal of Medicine, 357, 1496-506}.
The BCIRG001 trial tested the benefit of TAC vs. FAC in 1500 patients {Martin et al., 2005, The New England Journal of Medicine, 352, 2302-13}. In the retrospective analysis of this trial, the benefits of docetaxel were independent of hormone receptor status {Hugh et al., 2009, J Clin Oncol, 27, 1168-76}. The NSABP B28 trial compared doxorubicin and cyclophosphamide with or without four cycles of paclitaxel, in 3000 patients, and found no statistically significant difference in the relative risk of recurrence and overall survival, based on hormone receptor status {Mamounas et al., 2005, J Clin Oncol, 23, 3686-96}.
Another Polish retrospective analysis, evaluated 175 patients with MBC treated with docetaxel-based regimens and identified 19 with primary resistance to docetaxel {Wysocki et al., 2008, Med Sci Monit, 14,

SC7-10}. Mutations in BRCA1, were found in 5 of the 19 (26%). The hypothesis that BRCA1 deficient BC do not respond well to taxanes needs to be prospectively tested, this will be done in the UK-based BRCA-trial, comparing carboplatin and docetaxel for first line treatment of MBC, like the TNT Trial but only in BRCA carriers.
Further data suggesting lack of benefit for taxanes for TNBC comes from a large trial in MBC using differing taxane regimens. The CALGB9342 trial, which evaluated three different doses of paclitaxel for metastatic breast cancer, showed no statistically significant difference in response rate or time to treatment failure, in general. CALGB9243 did not meet its primary endpoint and it did not show response differences between TNBC and hormone receptor positive tumors. However, as expected, in CALGB9243, the overall survival was significantly worse for the TNBC compared to hormone receptor positive group {Harris et al., 2006, Breast Cancer Research : BCR, 8, R66}. The inconclusive results of CALGB9342 were the main reason for the adjuvant trial ECOG trial 1199. This very important trial, that has definitely clarified the use of taxanes in the adjuvant treatment of BC, randomized nearly 5000 adjuvant BC patients to AC followed by docetaxel or paclitaxel given weekly or once every three weeks. This trial showed superiority of weekly paclitaxel in general and in a retrospective analysis on TNBC patients which showed better survival with weekly paclitaxel albeit bad survival when compared to other BC subgroups (Sparano NEJM 2008).
Another, later, ECOG study, the 2100 trial randomized 722 first line MBC patients to paclitaxel with or without bevacizumab {Miller et al., 2007, The New England Journal of Medicine, 357, 2666-76}. Because of trastuzumab efficacy, over 90% of the patients were Her2 negative, and more than a third were ER and PR negative. Therefore, it is possible that close to a third of ECOG 2100 patients had, in fact, MTNBC. The benefit of adding bevacizumab was seen across the board. In fact the ECOG2100 results were the basis for the approval by the FDA and EMA of the first line MBC regimen of paclitaxel and bevacizumab to treat BC. Interestingly, however, the progression free survival was only 4.6 months for the hormone receptor negative subset in the paclitaxel alone arm, compared to 8.0 months in the hormone receptor positive group. This data from the ECOG 2100 control arm shows that TNBC might not be so sensitive to single agent paclitaxel. But similarly to other trials, we cannot dissect the subtypes of TNBC in ECOG 2100. In fact, there is preclinical data demonstrating that functional BRCA1 might be required for anti-microtubule agent sensitivity {Quinn et al., 2003, Cancer Research, 63, 6221-8}. It is possible that a fraction of sporadic TNBC also has functional deficiency of BRCA1, therefore in this subgroup we might be able to demonstrate more resistance to taxanes, this is some of the data that will be available after the report of the TNT Trial. From this data we can once more appreciate the necessity of subtyping TNBC into a meaningful classification.
TNBC was shown to have more frequent expression of Caveolin-1 {Pinilla et al., 2006, Breast Cancer Res Treat, 99, 85-90}. One mechanism of cellular uptake of nanoparticle-albumin bound paclitaxel (nab-paclitaxel) is via Caveolin-1 dependent receptor mediated transcytosis. Therefore, the use of nab-paclitaxel may warrant further testing for TNBC with high Caveolin-1 expression {Altundag et al., 2007, J Clin Oncol, 25, 1294-5 author reply 1295-6}. EndoTAG®-1 (ET) is a novel combination of paclitaxel with neutral and positive lipids. It attacks activated negatively charged endothelial cells that are needed for the formation of new tumor blood vessels {Strieth et al., 2008, Clinical cancer research, 14, 4603-11}. ET is also being tested in TNBC for the same reason of nab-paclitaxel.
In fact, the amazing activity of taxanes in BC, possibly has to do with the activity of these agents in ER positive BC, which comprises 60 to 70% of the BC population. Neither in Her2 positive nor in TN have we got conclusive evidence that anthracyclines can be spared. As for platinum salts, as we have seen, there is renewed interest in TNBC, mostly fueled by their important activity in BRCA1 carriers, unfortunately, not undoubtedly seen in sporadic TNBC. In this analysis of classical chemotherapy for TNBC patients, there are several results showing that the replacement of alquilating agents by anthracyclines and taxanes that we have witnessed in the XXIst century, as well as the lack of development of platinum salts, might not have been beneficial for TNBC patients. Most importantly, taxanes are possibly less active in TNBC. This history of classical chemotherapy development in BC might have been deleterious specifically for TNBC patients.

Fluoropypirimidines

The activity of fluoropirimidines can only be inferred from retrospective subgroup analyses because they have not been studied prospectively. Several trials are underway to evaluate capecitabine in TNBC patients. CALGB49907, is a non inferiority adjuvant trial with either CMF or AC compared to capecitabine in women over age 65, after 600 patients were enrolled, the trial found capecitabine was inferior to standard chemotherapy with a hazard ratio (HR) higher than 2 {Muss et al., 2009, The New England Journal of Medicine, 360, 2055-65}. A planned subgroup analysis, showed the benefit of standard chemotherapy was higher in hormone receptor negative patients (HR 3.04 for relapse-free survival, 2.62 for overall survival).
In the metastatic setting, two randomized phase III trials compared capecitabine plus ixabepilone to capecitabine monotherapy, in 1712 patients treated with prior anthracycline and taxane therapy {Rugo et al., 2009, Cancer Research, 69, 3057-0}. Nearly 860 patients received capecitabine, 208 of these had TNBC,

the ORR was 25% and the progression free survival (PFS) 4.2 months whereas it dropped to 15% and 1.7 months in TNBC. A single arm phase 2 study of capecitabine with bevacizumab in MBC found nearly double the ORR, time to progression and overall survival in patients with ER positive tumors compared to patients with TNBC {Sledge et al., 2007, J Clin Oncol, 25, 1013}. Similarly to the taxanes, these data suggest that fluoropirimidines might be most efficacious in ER positive BC. The most evident benefit of classic combination chemotherapy in TNBC is in the neoadjuvant and adjuvant setting. In the metastatic context, when TN tumors have been shown to be more unstable (Ding et al, Nature 2010), and undergo new mutations at increased rate, it may be that single agent chemotherapy might not be suitable in TNBC.

Targeted agents

PARP inhibitors

BRCA gene products are non functional in a subset of sporadic TNBCs, generally through promoter hypermethylation or by other means. Additionally, these tumors might have DNA repair defects in homology-directed repair pathways, not BRCA dependent. This is the reason PARP inhibitors were investigated in TNBC in general. Poly ADP ribose polymerase is an enzyme involved in base excision repair of DNA. If this enzyme is blocked, there will be double strand breaks that will not be repaired in case of homologous recombination deficiency, which is the case in BRCA null tumors. This phenomenon is called synthetic lethality, that two alterations concur to produce a phenotype {Farmer et al., 2005, Nature, 434, 917-21} {Bryant et al., 2005, Nature, 434, 913-7}. Possibly as a compensatory feature, PARP1 is over expressed in TNBC {Fong et al., 2009, The New England Journal of Medicine, 361, 123}

Olparib, an oral PARP inhibitor, was shown to be active in BRCA null breast cancer. In a phase II trial where 54 patients with germline mutations of BRCA1 or BRCA2 had locally advanced or metastatic BC, the response rate was 41%, and, 57% of these patients had TNBC {Tutt et al., 2010, Lancet, 376, 235-44}. The activity of olaparib in wild type BRCA TNBC, was shown in another trial. Here, olaparib was used in 23 patients with MBC, where only 8 were BRCA mutation carriers, in this trial, there were no objective responses {Gelmon et al., 2011, Lancet Oncol, 12, 852-61}. These results suggest that isolated iPARP is not active in WT BRCA TNBC. There is another trial with veliparib, in combination with temozolomide where responses were observed only on BRCA carriers {Isakoff et al., 2010, J Clin Oncol, 28, 1019}. Olaparib is now being planned to be studied in a very challenging adjuvant trial in BRCA mutation carriers (Table 4).

A phase II trial randomized 123 patients with metastatic TNBC to carboplatin and gemcitabine, alone or in combination with iniparib. Response rate increased from 16 to 48%, clinical benefit increased from 21 to 62%, progression free survival increased from 3,6 to 6 months and survival increased from 7,7 to 12,3 months. The results of this trial were better than expected. This trial was conducted in unselected TNBC and had an overall survival benefit, uncommon in randomized phase II studies, generally not designed with power to demonstrate survival benefits {O'Shaughnessy et al., 2011, The New England Journal of Medicine, 364, 205-14}. These results were not confirmed by the ensuing phase III trial, where 519 patients with metastatic TNBC, in first to third line, were randomized to the same therapeutic regimen. In this trial, neither response nor survival were increased {O'Shaughnessy J, J Clin Oncol 29:81s, 2011 (suppl abstr 1007)}. In a retrospective analysis, it was seen that due to the heterogeneity of TNBC, only 25% of these patients had basal like TNBC and their BRCA status was unknown. Additionally the capacity of iniparib to inhibit PARP was put in question {Maegley et al., 2011, J Clin Oncol, 29, e13576}{Mateo et al., 2013, Nature reviews Clinical oncology, 10, 688} and today iniparib is a defunct drug {Sinha, 2014, J Natl Cancer Inst, 106}.

Anti Src

There is increased activity of the src tyrosine kinase in TNBC, and, promising results were reported in cell lines {Finn et al., 2009, Cancer Research, 69 }. A Phase I was conducted in non selected MBC patients, the cohort had 60% of ER positive disease. Dasatinib combined with capecitabine were associated with clinical benefit in 56% of patients. Biomarker changes were consistent with what has been observed with bevacizumab, with significant decreases in plasma VEGF-A and increases in VEGFR-2 and collagen-IV, suggesting dasatinib might have an antiangiogenic effect {Somlo et al., 2013, Clinical cancer research, 19, 1884-93}. Despite enthusiasm, the phase II trial as single agent in pretreated metastatic TNBC had a 5% response rate and a PFS of 2 months which are not encouraging results {Finn et al., 2011, Clinical cancer research, 17, 6905-13}.

Antiandrogenic therapy

Two independent research groups have found that about 12% of TNBC expresses the androgen receptor (AR). The phase II trial with bicalutamide in metastatic, unselected, TNBC had a clinical benefit rate of 19% {Gucalp A, ASCO Meet Abstr 2012 30: 1006}.The same concept is being tested in a similar phase II trial, with abiraterone, but if tumors are not included based on AR expression, these trials might be futile efforts.

Ongoing trials with testing enzalutamide and other drugs used in castrate resistant prostate cancer might bring interesting results.

Anti angiogenic therapy:

Bevacizumab

It has been said that highly proliferative lesions need increased angiogenesis {Schneider and Miller, 2005, J Clin Oncol, 23, 1782-90}, the aggressiveness of TNBC has been correlated to the levels of VEGF. There are increased levels of intratumoral VEGF in TNBC {Linderholm et al., 2009, Ann Oncol} and there are frequent gains in the VEGF gene in TNBC genome {Andre et al., 2009, Clin Cancer Res, 15, 441-51}.

The phase III clinical trials in MBC with bevacizumab in combination with taxanes or capecitabine did not prolong survival and approval of this drug in MBC has been withdrawn. The retrospective analysis aiming to test an increased efficacy in TNBC patients randomized in the investigational arm of E2100 trial suggested the addition of bevacizumab to paclitaxel reduced the risk of progression in first-line by 51% and doubled the PFS {OShaughnessy et al., 2009, Cancer Research, 69, 207-207}. A similar reduction in the risk of progression in TNBC patients, was reported in the AVADO trial, combining bevacizumab with docetaxel, although in RiBBOn-1 trial, which added bevacizumab to a chemotherapy regimen of choice, there was no benefit. A meta-analysis of the 621 TNBC patients from the three trials, revealed a reduction in 35% of the risk of progression and a PFS increase of 2.7 months, when bevacizumab was added to chemotherapy regimens {O'shaughnessy et al., 2010, Cancer Research, 70, P6-12-03-P6-12-03}. Similar improvements were observed in the second-line setting: The 159 TNBC patients in RiBBOn-2 trial {Brufsky et al., 2012, Breast Cancer Res Treat}, demonstrated a 51% reduced risk of progression and a doubling of median progression free survival, among patients treated with the bevacizumab combination, compared with chemotherapy alone (2.7 versus 6.0 months), along with a trend towards improved survival (median, 17.9 versus 12.6 months). With these encouraging results as backbone, numerous trials in neoadjuvant and adjuvant setting aimed to test the efficacy of bevacizumab specifically in TNBC patients.

The German Breast Group that has contributed heavily in NACT trials, designed Gepar Quinto. In the United States, the NSABP also designed a very similar trial. Both trials specifically tested the addition of bevacizumab to a third generation neoadjuvant chemotherapy backbone. GeparQuinto and NSAPB B40, combined bevacizumab with anthracycline–taxane chemotherapy in Her2 negative patients, and demonstrated overall improvements in pCR rates for patients receiving bevacizumab compared with CT alone (GeparQuinto, 18.4% versus 14.9%, P = 0.04; B40, 34.5% versus 28.4%, P = 0.027) {von Minckwitz et al., 2012, The New England Journal of Medicine, 366, 299-309}{Bear et al., 2011, J Clin Oncol, 29, LBA1005}. A prespecified analysis of the 663 TNBC patients, in the German trial, revealed an improvement in pCR rates (39% versus 28%, P = 0.003) for patients receiving bevacizumab compared with chemotherapy. The B40 study showed that adding bevacizumab to chemotherapy did not improve pCR rates in the 479 TNBC patients (51% versus 47%, P =0.44). The conflicting results of the metastatic trials (E2100, AVADO and RIBBON) and of the neoadjuvant trials (GEPAR Quinto and B40) could have been an indication that the tumors classified as TNBC might be very different diseases, nevertheless, the adjuvant trial was launched in 2008, at initiation, not all the current data were available. Due to the inconsistency of previous trials, the results of the adjuvant trial in TNBC questioning the addition of bevacizumab to standard chemotherapy regimens which were published recently were eagerly awaited.

In the Beatrice trial 2591 patients with TN operable primary invasive BC were randomly assigned to receive a minimum of four cycles of adjuvant CT alone or with 5mg/kg weekly bevacizumab for 1 year. Similar proportions of patients received anthracycline and taxane therapy (59% and 58%), nontaxane anthracycline-containing therapy (36% and 37%), nonanthracycline taxane-containing therapy (5% of both), and radiation therapy (74% and 73%). The study showed that the addition of bevacizumab did not improve invasive disease–free survival (IDFS), which was the primary endpoint, specifically excluding all in situ cancer events. An exploratory biomarker analysis suggested benefit of bevacizumab in patients with high pretreatment vascular endothelial growth factor receptor 2 (VEGFR-2) levels. The HR for IDFS for bevacizuma-treated versus chemotherapy-alone patients were 0.61 among patients with VEGFR-2 levels above the median value and 1.24 for those with levels below median. Adjuvant bevacizumab was completed as planned in only 68% of patients, suggesting a compliance issue. In fact toxicity data show, grade 3 or 4 adverse events in 72% of patients in the bevacizumab group and 57% of the CT group, with increased frequency of grade 3 or 4 hypertension (12% vs 1%), severe cardiac events (1% vs < 0.5%), and treatment discontinuation (20% vs 2%). The 3-year IDFS was 84% with bevacizumab and 83% with CT alone. After 200 deaths, there was no difference in overall survival. Sites of recurrence were similar in the two treatment groups, with the most common being distant recurrence. The most common sites of distant recurrence were lung (28% and 27%), liver (20% and 15%), and bone (17% and 20%). The pattern of recurrence in TNBC patients is very different from hormone receptor positive disease with decreased bone events. Central nervous system disease was seen in 7% of patients in the bevacizumab group and in 12% in the chemotherapy group.

Despite the fact that bevacizumab did not meet its endpoint, the final survival analysis was not reported and will occur after a median follow-up of 5 years. This was a considerable effort, to randomize 2500 TNBC patients, that failed in this important group of patients. Several explanations have been put forward. It has been known that tumors need new vessels to grow larger than 2 mm, however, after tumors have been completely removed, the size of undetectable disease should be smaller than the 2 mm threshold. The longest administration of bevacizumab in this trial is mainly without CT, during adjuvant treatment, while we have observed that bevacizumab is active combined with CT and not isolated. These might be two reasons for this negative trial. There is a reassuring point, the accelerated metastatic disease after cessation of anti angiogenesis observed in mouse models was not observed here {Cameron et al., 2013, Lancet Oncol, 14, 933-42}.

Tirosine kinase inhibitors

Cell surface receptor tyrosine kinases (TK), including those linked to VEGF receptors, are relevant to angiogenesis stimulation, the effects of two TK inhibitors on endothelial cell proliferation have been evaluated in TNBC. Neoadjuvant and metastatic phase II and III trials have shown limited activity of sunitinib {Burstein et al., 2008, J Clin Oncol, 26, 1810-6}{Wildiers et al., 2010, Breast Cancer Res Treat, 123, 463-9} {Curigliano G, 2010, Cancer Res, 70, Abstr P6-12-02}, significant toxicity and increased deaths {Mayer et al., 2010, Ann Oncol, 21, 2370-6}{Crown J, 2010, J Clin Oncol, 28, LBA1011}{Bergh et al., 2012, Journal of Clinical Oncology}. Sorafenib has equally modest single-agent activity {Moreno-Aspitia et al., 2009, J Clin Oncol, 27, 11-5}{Bianchi et al., 2009, Anticancer Drugs, 20, 616-24}; however, three randomized phase IIb trials have demonstrated improved overall outcomes for sorafenib–CT combinations in first- and second- line MBC {Gomez P, 2010, Cancer Res, 70, Abstr P2-16-01}{Hudis C, 2011, J Clin Oncol, 29, Abstr 1009} {Bondarde S, 2010, Cancer Res, 70, Abstr P2-16-03}. A prespecified subgroup analysis of TNBC patients in the SOLTI-0701 (N = 53) trial showed an improvement of almost two months in median PFS with the addition of sorafenib to capecitabine (2.5 versus 4.3 months) {Gomez P, 2010, Cancer Res, 70, Abstr P2-16-01}. Similarly, in a North Amrican trial in the same setting a trend towards improved PFS was detected {Hudis C, 2011, J Clin Oncol, 29, Abstr 1009}. The unconvincing results of sunitinib, and the conflicting results of sorafenib, will possibly jeopardize the further development of VEGF bound TK inhibitors in TNBC patients.

EFGR inhibitors:

Monoclonal antibody

EGFR has been implicated as a molecular target for treatment of TNBC based on its frequent IHC expression (27%–57%) {Kreike et al., 2007, Breast Cancer Res, 9, R65}{Tan et al., 2008, Breast Cancer Res Treat, 111, 27-44}{Viale et al., 2009, Breast Cancer Res Treat, 116, 317-28}{Rakha et al., 2007, Cancer, 109, 25-32}. Cetuximab has demonstrated efficacy in two prospective studies and one retrospective analysis of randomized phase II trials in advanced TNBC. The largest EGFR trial, the international BALI-1, prospectively evaluated the addition of cetuximab to cisplatin to the first or second-line treatment of 173 MTNBC patients {Baselga et al., 2013, J Clin Oncol, 31, 2586-92}. The trial did not meet its primary endpoint of PFS prolongation. The addition of cetuximab to irinotecan and carboplatin in first- and second-line treatment of MTNBC patients in the USOR-04-070 trial, a North American endevour {O'Shaughnessy J, 2007, Breast Cancer Res Treat, 106, S32}, that randomomized 72 patients, resulted in improved response rates in TNBC patients (30% versus 49%) with no improvements in PFS or OS. A third cetuximab trial, conducted in North America, added carboplatin to cetuximab in 102 heavily pretreated MTNBC patients and resulted in a response rate of 17%. Prolonged PFS (2 versus 8 months) was seen in responders compared with the overall trial population {Carey LA, 2008, J Clin Oncol, 26, Abstr 1009}. None of these trials have proceeded to phase III and the development of cetuximab to treat TNBC patients has been abandoned.

Tirosine kinase inhibitors

A randomised phase II trial assessed the combination of erlotinib with carboplatin and docetaxel in the neoadjuvant treatment of 30 TNBC patients and demonstrated promising activity with pCR of 40%. Retrospective data from two randomized phase II trials, demonstrated modest activity for gefitinib (N = 82) in combination with standard neoadjuvant chemotherapy, and a lack of activity for lapatinib (N = 131) in combination with paclitaxel in advanced TNBC patients {Finn et al., 2009, J Clin Oncol, 27, 3908-15}. Similarly the development of either TKI inhibitors linked to EGFR or to HER2 have both been abandoned.

Anti HSP90: Ganetespib

Heat Shock protein 90 (Hsp90), is a molecular chaperone protein essential for the stability and function of multiple cellular client proteins, a number of which have been implicated in the pathogenesis of BC. These are kinases and transcription factors {Friedland et al., 2013, Invest New Drugs}. The ENCHANT-1 trial (NCT01677455) was an open label, multicenter phase II proof of concept study evaluating first-line

ganetespib monotherapy in women with metastatic Her2-positive or TNBC but there are no efficacy results available yet. It is possible that smaller molecules like these with different mechanisms of action might produce interesting results.

Apoptosis inducers

Frequently the hardest property to attack in cancer cells is their capacity to evade apoptosis, because highly proliferative cells are susceptible to chemotherapy. One apoptosis inducer is LCL161 is a small molecule mimetic of the Smac mitochondrial protein. Like Smac, it binds to inhibitor of apoptosis proteins and prevents their interaction with active caspases. In a phase 1b in BC combined with paclitaxel, 61% of the responses were seen in TNBC patients and therefore this molecule is considered promising in this particular subgroup {Dienstmann et al., 2012, Cancer Research, 72, P6-11-06}. Given this observation LCL161 will be studied with paclitaxel in the neoadjuvant setting, which has been shown to be a particularly favorable experimental set up for TNBC patients.

Conclusion

The attempt to show which of the most frequently employed therapeutic classes of conventional CT is most useful in TNBC has not yet been solved. It seems clearer today that alquilating agents and anthracyclines should not be replaced by taxanes, in neoadjuvant and adjuvant regimens. It is established that TNBC has consistently high pCR rates with modern combination CT in the neoadjuvant setting. Today it is unquestionable that those patients achieving pCR have better survival, approaching that of ER positive BC patients. A lot of data with platinum salts has shown that BRCA null TNBC patients might be the main subgroup to benefit from these drugs, whereas wild type BRCA TNBC patients might not benefit with such magnitude. If we could daily adopt the Lehmann classification, which has not been reliably translated to the clinic, genomically unstable MTNBC and mesenchymal TNBC are possibly driving chemorresistance. The search for targeted systemic therapy for TNBC has met with three big setbacks in the last half a decade: PARP inhibition, epidermal growth factor and angiogenesis targeting. The way forward with iPARP is BRCA null TNBC, this will be tested in the upcoming adjuvant trial with olaparib. The way forward with anti angiogenesis and epidermal growth factor inhibition will be a clinically useful readout of mesenchymal TNBC. Genomically unstable TNBC, should be treated with aggressive combination chemotherapy, possibly even dose dense or high dose regimens. This story of failures in the systemic treatment of TNBC represents a failure of scientists, pharma, clinical researchers and society at large. It is time for a paradigm shift in BC drug development. BC oncologists will have to think 300 times smaller. The 9000 patient trials we developed, as in no other cancer, will now give rise to 30 patient trials. Efficacy has been shown in BCR-ABL mutated CML, BRAF mutated melanoma, C-KIT mutated GIST and ALK-mutated lung adenocarcinoma, with few patients. We will now prepare for trials in BC patients with a specific mutation, specifically susceptible to a given drug. The deception of TNBC treatment has paved the way forward.

Table 1 Neoadjuvant trials with platinum salts in TNBC

Question and design of trialTrial	Phase	Rand	n	Endpoints and/or results
Cisplatin 75mg/m2 x4 q3w {Silver et al., 2010, Journal of Clinical Oncology}	II	No	28	22% pCR (6/28). Both BRCA1 mutation carriers had pCR
Combination of cisplatin and bevacizumab {Ryan et al., 2009, J Clin Oncol, 27 suppl, abstr 551}	II	No	51	26% pCR
Cisplatin 75mg/m2 x4 q3w {Byrski et al., 2009, Breast Cancer Res Treat, 115, 359-63}.	II	No	25	72% pCR
Paclitaxel weekly +/- carboplatin followed by AC +/- bevacizumab	II	Yes	362	NA
Metronomic CT: Weekly doxorubicin 24 mg/m2 x12 and daily oral cyclophosphamide 60mg/m2 followed by weekly paclitaxel (80mg/m2) and carboplatin (AUC=2) x 12	II	No	28	NA
Cisplatin 25 mg/m2 IV weekly + everolimus 5 mg PO daily for 1 week followed by Cisplatin 25 mg/m2 IV + Paclitaxel 80 mg/m2 IV weekly + everolimus for 11 weeks	II	Yes	96	Inhibition of P53 family of proteins (P63 and P73)
Six cycles of TAC or TC in TN or HER2 positive BC	II	Yes	70	NA
Weekly paclitaxel with or without everolimus followed by FEC	II	Yes	50	Inhibition of PI3K/PTEN/ AKT Pathway
Four cycles EC followed by four cycles of docetaxel vs. the combination of docetaxel and carboplatin {Alba et al., 2012, Breast Cancer Res Treat, 136, 487-493}	II	Yes	100	Standard arm (docetaxel) 35%pCR Investigational arm (docetaxel/carboplatin) 30% pCR
Four cycles of carboplatin and paclitaxel followed by four cycles of cyclophosphamide	II	No	60	NA
Weekly cisplatin, epirubicin and paclitaxel eight cycles and 4 adjuvant cycles of CMF to pts w/ less than 4 positive nodes or 8 to those w/ more (Frasci et al)	II	No	74	65% pCR DFS at 3 and 5y: 97% and 90% for those w/ pCR, 61% and 56% for those w/o pCR
Four cycles of ECF followed by three cycles of weekly paclitaxel	II	No	30	43% pCR
Neoadjuvant Sunitinib (S) Administered with Weekly Paclitaxel (P)/Carboplatin(C) in Patients (Pts) with Locally Advanced Triple-Negative Breast Cancer (TNBC)	II	No	22	9% pCR. Only 4 pts completed neoadjuvant CT, there was 68% grade 3/4 hematological toxicity{Yardley et al., 2012, Cancer Research, 72, Supplement 3}

Question and design of trialTrial	Phase	Rand	n	Endpoints and/or results
Predictors of Response to Neoadjuvant Docetaxel-Carboplatin Chemotherapy for Patients With Stage II and III disease	II	No	75	Predictors of pCR and of chemoresistance, response by MRI, pCR in BRCA carriers

Table 3: Clinical trials testing the efficacy of platins and/or targeted agents, excluding PARP inhibitors, in the management of metastatic/locally advanced TNBC. These trials were all initiated between November 2006 and September 2007. Source: http://clinicaltrials.gov/

PI, ID Trial	Phase	Rand	Line	n	Intervention	1ary endpoint	2ary endpoint	result
Isakoff NCT 00483223	II	No	1	39	Cisplatin and Evaluation of p63/ p73 as a Biomarker of Response	ORR	CBR, PFS, OS, p63/ p73 marker of response	Recruiting
Tutt ISRCTN 97330959	III	Yes	1	450	Carboplatin vs. docetaxel (crossover)	Efficacy	Response RECIST, PFS, OS	Recruiting
Baselga BALI1	II	Yes	1	180	Cetuximab and cisplatin or cisplatin alone	ORR	PFS, OS, TTR	Investigational arm: 20% Control arm: 10%
Carey NCT 00492375	II	Yes	3 or more	100	Cetuximab alone or in combination with carboplatin	ORR	PFS, OS, targets EGFR inhibition	Waiting report
NCT 00448305	II	Yes	1	135	Combination: EndoTAG-1 paclitaxel Monotherapy: EndoTAG-1 Control: paclitaxel	4-month PFS	PFS, OS, pain, QoL	Combination: 59% (26/44) Montherapy: 34% (13/38) Control:48% (12/25)
NCT 00371254	II	No	2	45	Dasatinib	ORR	PFS, disease control rate	Limited efficacy: ORR 4.7%(2/43) {Finn et al., 2009, Cancer Research, 69 }
NCT 00479674	II	No	1	70	Abraxane, bevacizumab and carboplatin	safety tolerability	PFS	Waiting report
NCT 00472693	II	Yes	2	37	Abraxane, bevacizumab	PFS	OS, toxicity	Waiting report
NCT 00246571	II	Yes	2	200	Sunitinib vs. standard of care chemotherapy	PFS	ORR, OS, QoL, safety, toxicity	Trial was closed w/o patients

ORR, overall response rate; PFS, progression-free survival; OS, overall survival; RECIST, Response Evaluation Criteria in Solid Tumours; EGFR epidermal growth factor receptor

Table 4: Clinical trials testing PARP inhibitors in TNBC or in BRCA-mutated BC. Source: http://clinicaltrials.gov/. NCT numbers are provided for the trials registered in clinicaltrials.gov.

Trial	Stage	P	R	Intervention	1ary endpoint	n	2ary endpoint	result/ estimated report
NCT 00707707	Metastatic	I/II	No	Olaparib and paclitaxel	Safety	19	Response rate	Response: 53% (10/19)
NCT 00540358	Metastatic	II	Yes	Iniparib, gemcitabine, carboplatin vs. gemcitabine and carboplatin (crossover allowed), q3w	CBR, safety	123	ORR, PFS, OS	OS: 9.2 versus 5.7 months
NCT 00938652	Metastatic	III	Yes	Iniparib, gemcitabine, carboplatin vs. gemcitabine and carboplatin (crossover allowed), q3w	PFS, OS	420	RR	May 2012
NCT 00813956	I-IIIA (operable)	II	No	Neoadjuvant iniparib, gemcitabine and carboplatin, q3w	pCR	36	NS	May 2012
NCT 01045304	Metastatic	II	Yes	Iniparib, gemcitabine and carboplatin qw or q2w	ORR	80	CBR, PFS, OS	Oct 2011
NCT 01173497	Brain metastasis	II	No	Intravenous iniparib and irinotecan	TTP	45	RR	Jan 2013
Astra Zeneca sponsored	Adjuvant BRCA mutation carriers	III	Yes	Adjuvant chemotherapy with or without olaparib	OS	200	OS	Jan 2020
Astra Zeneca sponsored	Metastatic BRCA mutation carriers	III	Yes	Chemotherapy for MBC with or without olaparib	OS	200	OS	Jan 2020

q3w, every 3 weeks; CBR, clinical benefit rate; ORR, overall response rate; PFS, progression free survival; OS, overall survival; qw, weekly; q2w, every 2 weeks; RR, response rate; pCR, pathological complete response; NS, not specified; TTP, time to progression

Table 5 Adjuvant trials in TNBC

Trial	P	R	Intervention	1ary endpoint	n	2ary endpoint	Result/ Estimation
NCT 00528567 Beatrice	III	Yes	Standard adjuvant chemotherapy concurrent with bevacizumab followed by bevacizumab until 1 year vs standard adjuvant chemotherapy	invasive DFS	2530	OS, safety, biomarkers	{Cameron et al., 2013, Lancet Oncol, 14, 933-42}
NCT 01112826 Chinese study	III	Yes	Standard adjuvant chemotherapy followed by 1 year of capecitabine vs. standard adjuvant chemotherapy alone	DFS	684	NS	Ongoing
NCT 01097642 Neoadjuvant	II	Yes	Ixabepilone (I) Vs. Ixabepilone Plus Cetuximab	pCR	118	ORR, safety, toxicity	Ongoing
NCT 00630032 PACS08 (France)	III	Yes	3 cycles FEC100 followed by 3 cycles of docetaxel or followed by 3 cycles of ixabepilone	5 year DFS	2500	5 year: DMFS EFS, OS translation QoL	Waiting events
NCT 00789581 (USA)	III	Yes	4 cycles AC followed by 12 cycles of paclitaxel qw or followed by 4 cycles of ixabepilone. Very similar question to PACS08.	DFS	1800	OS, safety	Waiting events
NCT 01150513 Chinese study	III	Yes	4 cycles epirubicin plus cyclophosphamide followed by 4 cycles docetaxel vs 6 cycles docetaxel plus carboplatin*	DFS	500	Safety	Ongoing
NCT 01057069 Dutch neoadjuvant	II/III	No	In HRD: Testing dose dense AC vs. PBPC harvest, intensified alquilating agents with PBPC reinfusion. In nonHRD: Testing, after 3 cycles of ddAC, 3 cycles of ddAC vs. 3 cycles of docetaxel and capecitabine, according to response	pCR	270	RFS, OS	Ongoing
NCT 00472693	II	Yes	Abraxane, bevacizumab	PFS	37	OS, toxicity	Completed no results available
Adjuvant trial in BRCA mutated patients	III	Yes	Adjuvant olaparib vs. observation after a backbone of adjuvant CT at physicians choice	OS	300	OS, DFS, toxicity	Center selection

FEC100, fluorouracil, epirrubicin at 100mg/m2 and cyclophosphamide; DMFS, distant metastasis free survival; EFS, event free survival; * the dose of epirrubicin and docetaxel are 75mg/m2, which are lower than standard and the investigational arm has two cycles less than the comparator arm

Chapter 5: Randomized Phase II Study of the Anti-Epidermal Growth Factor Receptor Monoclonal Antibody Cetuximab With Cisplatin Versus Cisplatin Alone in Patients With Metastatic Triple-Negative Breast Cancer

Conception and design: Jose Baselga, Sofia Braga

Provision of study materials or patients: Jose Baselga, Patricia Gomez, Sofia Braga, Richard Greil, Miguel A. Climent, Andrew M. Wardley, Bella Kaufman, Andreas Schneeweiss, Ahmad Awada

Collection and assembly of data: Patricia Gomez, Richard Greil, Sofia Braga, Miguel A. Climent, Andrew M. Wardley, Bella Kaufman, Salomon M. Stemmer, Antonio Pego, Jean-Charles Goeminne, Marie-Pascale Graas, Eva Maria Ciruelos Gil, Andreas Schneeweiss, Angela Zubel, Ahmad Awada

Data analysis and interpretation: Jose Baselga, Richard Greil, Salomon, M. Stemmer, Arlene Chan, M. John Kennedy, Eva Maria Ciruelos Gil, Angela Zubel, Jutta Groos, Helena Melezínkova, Ahmad Awada

Manuscript writing: All authors

Final approval of manuscript: All authors

Funding: Merck

Ethical Committee approval: Comissão de Ética para a Investigação Clínica (Portuguese National Ethics Committee) approved the trial in Portugal

VOLUME 31 · NUMBER 20 · JULY 10 2013

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Randomized Phase II Study of the Anti–Epidermal Growth Factor Receptor Monoclonal Antibody Cetuximab With Cisplatin Versus Cisplatin Alone in Patients With Metastatic Triple-Negative Breast Cancer

José Baselga, Patricia Gómez, Richard Greil, Sofia Braga, Miguel A. Climent, Andrew M. Wardley, Bella Kaufman, Salomon M. Stemmer, António Pêgo, Arlene Chan, Jean-Charles Goeminne, Marie-Pascale Graas, M. John Kennedy, Eva Maria Ciruelos Gil, Andreas Schneeweiss, Angela Zubel, Jutta Groos, Helena Melezínková, and Ahmad Awada

José Baselga, Memorial Sloan-Kettering Cancer Center, New York, NY; Patricia Gómez, Vall D'Hebron Institute of Oncology, Barcelona; Miguel A. Climent, Instituto Valenciano de Oncologia, Valencia; Eva Maria Ciruelos Gil, Hospital Universitario 12 de Octubre, Madrid, Spain; Richard Greil, Paracelsus Medical University Salzburg, Salzburg, Austria; Sofia Braga, Instituto Português de Oncologia de Lisboa, Lisbon; António Pêgo, Instituto Português de Oncologia de Coimbra Francisco Gentil E.P.E., Coimbra, Portugal; Andrew M. Wardley, The Christie NHS Foundation Trust, Manchester, United Kingdom; Bella Kaufman, Sheba Medical Center, Tel Hashomer; Salomon M. Stemmer, Davidoff Center, Petach Tiqva, Israel; Arlene Chan, Mount Hospital Perth, Perth, Australia; Jean-Charles Goeminne, Clinique et Maternité Sainte Elisabeth, Namur; Marie-Pascale Graas, Clinique St-Joseph, Liège; Ahmad Awada, Medical Oncology Clinic, Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium; M. John Kennedy, Trinity College and St James' Hospital, Dublin, Ireland; Andreas Schneeweiss, National Center for Tumor Diseases, University Hospital, Heidelberg; Angela Zubel, Jutta Groos, Helena Melezínková, Merck KGaA, Darmstadt, Germany.

Published online ahead of print at www.jco.org on June 3, 2013.

Supported by Merck KGaA, Darmstadt, Germany.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Clinical trial information: NCT00463788.

Corresponding author: José Baselga, MD, PhD, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10065; e-mail: baselgaj@mskcc.org.

0732-183X/13/3120w-2586w/$20.00

DOI: 10.1200/JCO.2012.46.2408

ABSTRACT

Purpose
Epidermal growth factor receptor is overexpressed in metastatic triple-negative breast cancers (mTNBCs), an aggressive subtype of breast cancer. Our randomized phase II study investigated cisplatin with or without cetuximab in this setting.

Patients and Methods
Patients who had received no more than one previous chemotherapy regimen were randomly assigned on a 2:1 schedule to receive no more than six cycles of cisplatin plus cetuximab or cisplatin alone. Patients receiving cisplatin alone could switch to cisplatin plus cetuximab or cetuximab alone on disease progression. The primary end point was overall response rate (ORR). Secondary end points studied included progression-free survival (PFS), overall survival (OS), and safety profiles. Analyses included a significance level of $\alpha = .10$ with no adjustments for multiplicity.

Results
The full analysis set comprised 115 patients receiving cisplatin plus cetuximab and 58 receiving cisplatin alone; 31 patients whose disease progressed on cisplatin alone switched to cetuximab-containing therapy. The ORR was 20% (95% CI, 13 to 29) with cisplatin plus cetuximab and 10% (95% CI, 4 to 21) with cisplatin alone (odds ratio, 2.13; 95% CI, 0.81 to 5.59; $P = .11$). Cisplatin plus cetuximab resulted in longer PFS compared with cisplatin alone (median, 3.7 *v* 1.5 months; hazard ratio [HR], 0.67; 95% CI, 0.47 to 0.97; $P = .032$). Corresponding median OS was 12.9 versus 9.4 months (HR, 0.82; 95% CI, 0.56 to 1.20; $P = .31$). Common grade 3/4 adverse events included acne-like rash, neutropenia, and fatigue.

Conclusion
While the primary study end point was not met, adding cetuximab to cisplatin doubled the ORR and appeared to prolong PFS and OS, warranting further investigation in mTNBC.

J Clin Oncol 31:2586-2592. © 2013 by American Society of Clinical Oncology

INTRODUCTION

Triple-negative breast cancer (TNBC), which is defined as estrogen receptor (ER), progesterone receptor (PgR), and human epidermal growth factor receptor 2 (HER2) –negative disease, accounts for 11% to 17% of all breast cancers.[1,2] The incidence of this type of breast cancer is greater among young patients and African American patients.[2] TNBC has a more aggressive clinical course than other types of breast cancer, with a much shorter median time from recurrence to death.[3] In addition, these tumors lack expression of hormone receptors and HER2 and they are not responsive to hormonal or anti-HER2 therapy. The only available therapy for advanced TNBC is cytotoxic chemotherapy[4] and bevacizumab in combination with chemotherapy in European countries, but prognosis generally remains poor,[5] especially for metastatic disease. Thus, investigation of novel ways to treat patients with this subtype of breast cancer is of particular importance.

Epidermal growth factor receptor (EGFR) has been shown to be highly expressed in TNBC cell lines,[6] and TNBC cell lines overexpressing the receptor are growth inhibited by the anti-EGFR monoclonal antibody cetuximab.[7] In addition, a majority of

patients with TNBC comprise basal-like molecular characteristics and often have defects in BRCA1-regulated DNA repair pathways and thus may be particularly sensitive to DNA damaging agents such as cisplatin.[4,8] Clinical studies have shown that the use of platinum-based chemotherapy is associated with tumor response in both nonmetastatic[9] and metastatic TNBC (mTNBC).[10,11]

In vitro studies have demonstrated that the EGFR-targeting monoclonal antibody cetuximab potentiates the effects of oxaliplatin[12] and radiation[13] by impairing DNA repair. Cetuximab has also been shown to enhance the demonstrated antitumor activity of cisplatin and carboplatin.[14-16] The safety and efficacy of the combination of cetuximab with platinum-based regimens has been demonstrated clinically in other tumor types.[17-19]

Although at the molecular level, patients with TNBC comprise a heterogeneous subgroup, in the absence of predictive biomarkers to identify patients who would benefit from cetuximab treatment we investigated all patients with mTNBC.[4,20] Based on the high EGFR expression levels in TNBC, the sensitivity of these tumors to DNA-damaging agents, the observed impairment of DNA repair by cetuximab, and the strong preclinical evidence for a synergistic effect of cetuximab and cisplatin, we performed this randomized phase II study evaluating the efficacy and safety of cisplatin plus cetuximab versus cisplatin alone in patients with mTNBC.

PATIENTS AND METHODS

Patients

Eligible patients were ages 18 years or older with a histologically confirmed diagnosis of metastatic (stage IV) TNBC. ER-negative, PgR-negative, and HER2-negative status were determined locally, and tumor receptor status of the primary lesion was permitted for inclusion. Other main inclusion criteria were: no more than one previous chemotherapeutic regimen for the treatment of metastatic breast cancer, at least one measurable lesion by magnetic resonance imaging or computed tomography according to Response Evaluation Criteria in Solid Tumors (RECIST, version 1.0),[21] Eastern Cooperative Oncology Group (ECOG) performance status 0 to 2, and tumor tissue available for EGFR expression assessment. Main exclusion criteria were: prior therapy with a platinum agent or mitomycin; previous exposure to monoclonal antibody therapy, signal transduction inhibitors, or EGFR-targeting therapy; known history of brain metastases; and other cancers except for basal-cell skin carcinoma or preinvasive cervical carcinoma.

The protocol was approved by independent ethics committees of the participating centers and the study was carried out in accordance with the Declaration of Helsinki. All patients provided written informed consent.

Study Design

This open-label, randomized phase II study was conducted in Europe, Australia, and Israel. Eligible patients were randomly assigned in a 2:1 ratio to receive cisplatin with or without cetuximab. Randomization was performed centrally using an Interactive Voice Response System with stratification according to the line of treatment (first or second).

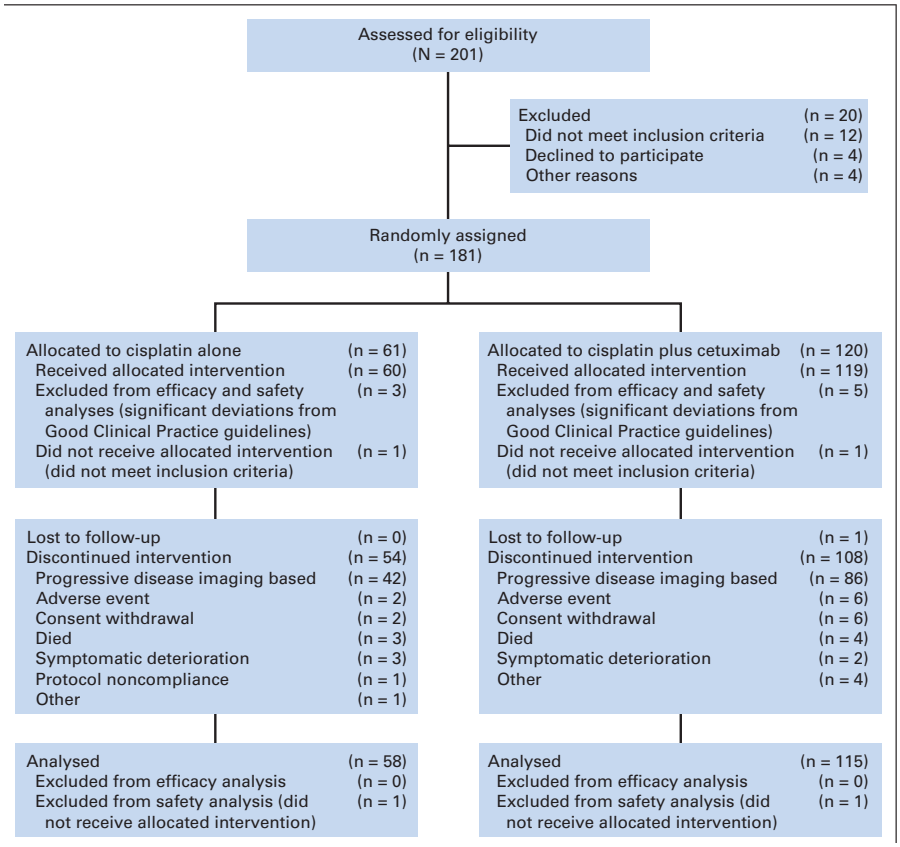


Fig 1. CONSORT diagram.

The primary end point was the best overall response rate (ORR), defined as the proportion of patients with a confirmed complete response or partial response according to RECIST version 1. Secondary end points included progression-free survival (PFS), overall survival, time to response, and safety. The rate of disease control, defined as the proportion of patients with a complete response, partial response, or stable disease for at least 6 weeks as best response, was assessed in a post hoc analysis. Planned exploratory subgroup analyses were used to investigate the association between best ORR and baseline patient and disease characteristics and any association between first-cycle acne-like rash and response in patients receiving cisplatin plus cetuximab who were undergoing treatment at day 21.

Treatment and Assessments

In the cisplatin plus cetuximab group, patients were to receive an initial dose of cetuximab (Merck KGaA, Darmstadt, Germany) 400 mg/m^2 by intravenous infusion followed by 250 mg/m^2 once weekly. Patients were also to receive intravenous cisplatin 75 mg/m^2 on day 1, every 3 weeks, for six cycles. Patients benefiting from treatment could continue to receive weekly cetuximab 250 mg/m^2 alone following the six cycles of cisplatin until first relapse, unacceptable toxicity, or withdrawal of consent.

Patients in the cisplatin-alone group were to receive cisplatin 75 mg/m^2 on day 1 every 3 weeks for six cycles. On disease progression, patients had the option to switch to cisplatin plus cetuximab as above or cetuximab alone, depending on whether the patient's disease progression was reported during or after the six cisplatin cycles, respectively.

Tumor response was assessed by local investigators every 6 weeks until disease progression according to RECIST version, 1.0. After disease progression, data on survival status and further anticancer treatment use was collected systematically every 3 months after the end of study visit. Adverse events were recorded at treatment visits, the final tumor assessment, and the end of study visit and were graded using the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0.

ER, PgR, and HER2 expression status was determined by immunohistochemistry of patient tumor sections. The IHC cutoff for ER-negative and PgR-negative status was $\leq$ 10% of staining in the nuclei. HER2-negative status was less than 3+ by immunohistochemistry (based on staining intensity).[22] HER2-negative status was confirmed in 1+ or 2+ expressing cells by fluorescence in situ hybridization analysis (confirming the absence of gene amplification).[23]

Statistical Analysis

The statistical analysis was based on the objective of showing superiority of cisplatin plus cetuximab over cisplatin alone in terms of overall response together with showing that the overall response for cisplatin plus cetuximab was above a prespecified clinically relevant threshold of 0.2. Two null hypotheses were tested simultaneously, that is, to test whether the ORR was equal in both treatment groups and to test if the ORR was less than or equal to 20% in the cisplatin plus cetuximab group. The simultaneous null hypothesis would be rejected if both single null hypotheses were rejected. A planned sample size of 180 was based on the following assumptions: an increase of at least 18% in ORR with cisplatin plus cetuximab compared with cisplatin alone (from 14% to 32%); an ORR of more than 20% in the cisplatin plus cetuximab group; 2:1 randomization ratio for cisplatin plus cetuximab versus cisplatin alone; a two-sided α of .10; an 80% power for the rejection of both single null hypotheses; and a 5% dropout rate. A randomization ratio of 2:1 was applied to increase both exposure in the experimental group and the power for testing the ORR in this group against 20%.

Efficacy analyses were performed on the full analysis set. Because of the exploratory nature of the study, all statistical tests used a significance level of α = .10, and no adjustments were made for multiplicity. The ORR in the cisplatin plus cetuximab group was tested against .20 using a one-sided z test at a significance level of $\alpha/2$ = .05.

Best ORRs were compared using a two-sided Cochran-Mantel-Haenszel test stratified by line of treatment. The odds ratio (cisplatin plus cetuximab v cisplatin alone) between treatment groups and ORR per group are presented with the corresponding 95% CI.

Time-to-event variables were compared using a two-sided log-rank test stratified by line of treatment. Kaplan-Meier curves (except for time to response) and median values with corresponding two-sided 95% CIs are presented. Hazard ratios (cisplatin plus cetuximab v cisplatin alone) were calculated by Cox's proportional hazards model stratified by line of treatment and presented with 95% CI.

Data cutoff times for analyses were 6 months after the last patient had been randomly assigned for best overall response, PFS, and time to response; times for analyses for overall survival were after two thirds of the randomly assigned patients had died.

The safety population included all patients to whom any dose of study treatment was administered. Analysis of safety end points was performed according to the actual treatment received. Adverse events were categorized according to the Medical Dictionary for Regulatory Activities (version 12.0) system organ classes and preferred terms, as well as predefined special adverse event categories in which the preferred terms were pooled.

RESULTS

From June 2007 to February 2009, 181 patients were randomly assigned from 47 sites (Fig 1). Significant deviations from the Good

Table 1. Baseline Patient Characteristics

Characteristic	Cisplatin Plus Cetuximab (n = 115)		Cisplatin Alone (n = 58)	
	No. of Patients	%	No. of Patients	%
Age, years				
Median	53.0		52	
Standard deviation	12.5		10.7	
< 65	93	81	51	88
≥ 65	22	19	7	12
ECOG performance status				
0	72	63	39	67
1	37	32	17	29
2	6	5	2	3
Line of treatment*				
First line	84	73	42	72
Second line	31	27	16	28
Time to metastasis, months†				
Median	15.7		15.4	
No. of patients	108		55	
Interquartile range, Q1-Q3	5.0-26.4		1.8-29.7	
Site of metastasis‡				
Liver	36	31	17	29
Lung	64	56	26	45
Bone	37	32	20	34
Lymph nodes	49	43	22	38
Skin	20	17	8	14
Other	15	13	9	16
Previous anticancer therapy§	101	88	53	91
Neoadjuvant	46	40	12	21
Adjuvant	75	65	41	71
Local therapy	53	46	26	45
No previous chemotherapy	19	17	10	17
Naturally postmenopausal	64	56	36	62

Abbreviation: ECOG, Eastern Cooperative Oncology Group.
*According to Interactive Voice Response System.
†Duration from initial diagnosis to date of metastasis, Q1-Q3 interquartile range (25% quartile to 75% quartile).
‡Patients could have metastases at more than one site.
§Patients could have received more than one type of therapy.

Table 2. Efficacy of Study Treatment

Response	Cisplatin Plus Cetuximab (n = 115) No. of Patients	%	Cisplatin Alone (n = 58) No. of Patients	%
Complete response	2	2	1	2
Partial response	21	18	5	9
Stable disease	48	42	18	31
Progressive disease	34	30	31	53
Not evaluable	10	9	3	5
Best overall response rate	23	20	6	10
95% CI	13 to 29		4 to 21	
Odds ratio*	2.13			
95% CI	0.81 to 5.59			

NOTE. Complete responses, partial responses, and stable disease were defined according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0.
*Stratified Cochran-Mantel-Haenszel test, $P = .11$.

Clinical Practice guidelines were identified at one site that resulted in a lack of credibility of the data and a subsequent mandate from the national health authority to exclude all their eight patients from the analysis. This gave a full analysis set of 173 patients. One hundred and fifteen patients were randomly assigned to receive cisplatin plus cetuximab and 58 were assigned to receive cisplatin alone. Thirty-one (53%) of 57 patients in the cisplatin-alone group switched to cetuximab on disease progression: 21 patients switched to cisplatin plus cetuximab and 10 switched to cetuximab alone. Two patients who did not receive study treatment were excluded from the safety population.

In the safety population, the cisplatin plus cetuximab group had a median duration of 13.1 weeks and 13.6 weeks of cetuximab and of cisplatin treatments, respectively. A relative dose intensity of at least 90% for cetuximab and cisplatin was attained in 81 (71%) of 114 patients and 90 (80%) of 113 patients, respectively. The cisplatin-alone group had a median treatment duration of cisplatin of 12.7 weeks. Patients switching to cetuximab after progression had a median treatment duration of cetuximab of 5.9 weeks.

The study groups were well balanced for performance status, line of treatment, and median time to metastasis (Table 1). Overall, the majority of patients had infiltrating ductal carcinoma (150 of 173; 86%), had received study treatment as first-line therapy (126 of 173; 73%), and had an ECOG performance status of 0 (111 of 173; 64%). The patients' median age was 52.0 years. Altogether, 82 (71%) of 115 patients in the cisplatin plus cetuximab group and 35 (60%) of 58 patients in the cisplatin-alone group received anticancer therapy post-study treatment.

The best ORR was 20% (95% CI, 13 to 29; 23 of 115) and 10% (95% CI, 4 to 21; six of 58) in the cisplatin plus cetuximab and cisplatin-alone groups, respectively (odds ratio, 2.13; 95% CI, 0.81 to 5.59; $P = .11$). Thus, the primary end point was not met (Table 2).

Median PFS was significantly longer in the cisplatin plus cetuximab group than in the cisplatin-alone group (3.7 months *v* 1.5 months; hazard ratio [HR], 0.67; 95% CI, 0.47 to 0.97; $P = .032$; Fig 2A). Median overall survival was 12.9 months in the cisplatin plus cetuximab group and 9.4 months in the cisplatin-alone group (HR, 0.82; 95% CI, 0.56 to 1.20; $P = .31$; Fig 2B). Median time to response was 1.4 months and 1.3 months in the cisplatin plus cetuximab and cisplatin-alone groups, respectively (HR, 0.75; 95% CI, 0.26 to 2.17; $P = .60$).

In the exploratory subgroup analyses regarding ORR, odds ratios in favor of cisplatin plus cetuximab over cisplatin alone seemed highest in postmenopausal patients and in patients receiving study treatment as second-line therapy (Fig 3). Among patients undergoing treatment at day 21 in the cisplatin plus cetuximab group with (n = 64) and without (n = 43) first-cycle acne-like rash, there seemed to be no difference in the ORR (with acne-like rash, 20%; 13 of 64; without acne-like rash, 23%; 10 of 43).

All 171 patients experienced at least one adverse event. Altogether, 69 (61%) of 114 patients in the cisplatin plus cetuximab group and 24 (42%) of 57 patients in the cisplatin-alone group experienced

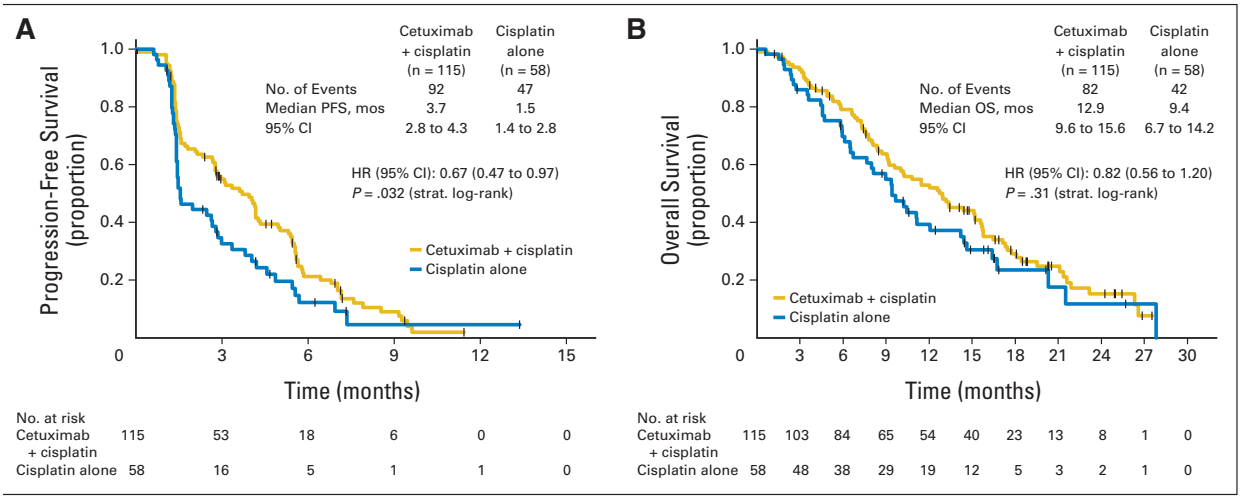


Fig 2. Kaplan-Meier estimates of progression-free survival (PFS) and overall survival (OS). (A) Median PFS was significantly longer in patients who received cisplatin plus cetuximab compared with patients who received cisplatin alone (3.7 months [mos] *v* 1.5 months; hazard ratio [HR], 0.67; 95% CI, 0.47 to 0.97; $P = .032$). (B) Median OS was 12.9 months in the cisplatin plus cetuximab group and 9.4 months in the cisplatin-alone group (HR, 0.82; 95% CI, 0.56 to 1.20; $P = .31$).

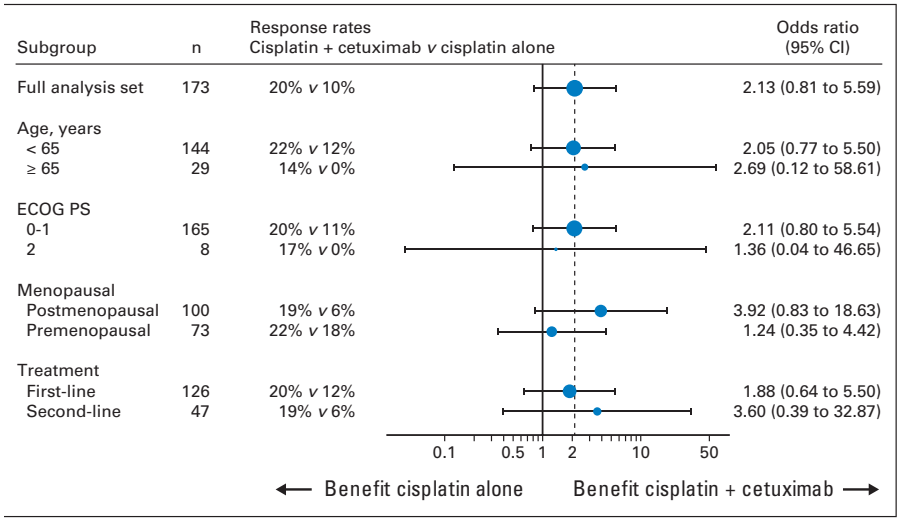


Fig 3. Odds ratios for best overall response rate in subgroup analyses. ECOG PS, Eastern Cooperative Oncology Group performance status.

at least one grade 3 or 4 adverse event. Grade 3 or 4 adverse events occurring in at least 5% of patients in the cisplatin plus cetuximab or cisplatin-alone groups are listed in Table 3 and included neutropenia (11 [10%] of 114 *v* three [5%] of 57), fatigue (10 [9%] of 114 *v* four [7%] of 57), dyspnea (seven [6%] of 114 *v* one [2%] of 57), and acne-like rash (17 [15%] of 114 *v* 0%). In the cisplatin plus cetuximab group, grade 3 or 4 dyspnea was associated with clinical deterioration and disease progression in all patients. Other grade 3/4 adverse events with cisplatin plus cetuximab and cisplatin alone were sepsis (two [2%] of 114 *v* 0%), hypertension (four [4%] of 114 *v* 0%), and hypomagnesemia (four [4%] of 114 *v* one [2%] of 57). Any grade and grade 3 infusion-related reactions occurred in 15 (13%) of 114 and three (3%) of 114 patients in the cisplatin plus cetuximab group, respectively, and in no patients in the cisplatin-alone group. There were no treatment-related adverse events leading to death.

Table 3. Grade 3 or 4 Adverse Events

Event	Cisplatin Plus Cetuximab (n = 114)		Cisplatin Alone (n = 57)	
	No. of Patients	%	No. of Patients	%
Any grade 3 or 4 event	69	61	24	42
Any grade 4 event	12	11	4	7
Grade 3 or 4 adverse events in ≥ 5% patients in either treatment arm				
Neutropeni a	11	10	3	5
Fatigue	10	9	4	7
Dyspnea	7	6	1	2
Nausea	5	4	3	5
Vomiting	5	4	3	5
Asthenia	3	3	3	5
General physical health deterioration	0		3	5
Special adverse events*				
Acne-like rash	17	15	0	
Infusion-related reaction	3	3	0	
Cardiac event (arrhythmia)	0		1	2

*Special adverse events are of composite categories of preferred terms that were prospectively defined in the study protocol according to Medical Dictionary for Regulatory Activities (MedDRA; version 12); all were grade 3.

DISCUSSION

This randomized phase II study demonstrated that cisplatin plus cetuximab doubled the ORR achieved with cisplatin alone (from 10% to 20%) in patients with mTNBC. The primary end point of the study was not met and this is therefore a negative trial. However, it is possible that this may not accurately represent the true activity of this combination regimen as, unlike other randomized phase II studies, the primary end point was based on two null hypotheses; on the one hand, superiority of the cetuximab arm and, on the other, an ORR of greater than 20%. Therefore, the observed doubling of the ORR with the addition of cetuximab to cisplatin should not be ignored when considering the potential of anti-EGFR agents in mTNBC.

The addition of cetuximab significantly prolonged median PFS from 1.5 months with cisplatin alone to 3.7 months with cisplatin plus cetuximab. There was a nonsignificant improvement in median survival of 3.5 months observed with cisplatin plus cetuximab, although the sample size was small and 31 (53%) of 57 patients in the cisplatin-alone group switched to a cetuximab-containing therapy on disease progression. It is not possible to accurately assess the impact of this cross-over on outcome, as response was not protocol-specified in these patients.

The tolerability profile of cisplatin plus cetuximab was as expected, with no new safety concerns. The more frequent grade 3 or 4 adverse events in the cisplatin plus cetuximab compared with the cisplatin alone group are mainly owing to grade 3 acne-like rash associated with cetuximab, which was generally manageable. In addition, patients in the cisplatin plus cetuximab group had a slightly higher incidence of grade 3 or 4 neutropenia than patients in the cisplatin-alone group as well as some grade 3/4 infusion-related reactions. Infusion-related reactions are a known adverse effect of

cetuximab treatment and severe neutropenia or neutropenic complications, such as sepsis, have been noted to occur more frequently in patients receiving cetuximab plus platinum-based therapy compared with platinum-based therapy alone in phase III trials in other tumor types.[18,24,25]

Other phase II studies have reported clinical activity for cetuximab in mTNBC, supporting the concept that this may be a worthwhile approach for the treatment of this disease. In a randomized phase II trial conducted by the Translational Breast Cancer Research Consortium (TBCRC 001), in which 54% of patients had received prior chemotherapy for mTNBC, though cetuximab alone (n = 31) demonstrated low activity with a response rate of 5%, the combination of cetuximab and carboplatin (n = 71) led to a response rate of 17% and a clinical benefit rate of 31%.[26] In a second study of patients with metastatic breast cancer who were randomly assigned to receive irinotecan followed by carboplatin with or without cetuximab as first- or second-line treatment, among a subgroup of 78 patients with mTNBC, a higher response rate was reported in the group receiving cetuximab (49% *v* 30%).[27] These data suggest that the addition of cetuximab to platinum-based chemotherapy may offer clinical benefit for patients with mTNBC. The data also highlight the importance of an appropriate control group in cetuximab studies. For example, the 18% response rate reported for the combination of cetuximab and carboplatin in TBCRC 001 may appear disappointingly low, particularly when compared with historical data of response rates with platinum salts in mTNBC.[10,11] However, the response rate with the combination is similar to the response rate that was observed in our cetuximab-containing arm and superior to the one observed with cisplatin alone in our study.

A limitation of our study is the unavailability of data from the analysis for predictive biomarkers of cetuximab activity. The identification of patients with tumors most likely to respond to cetuximab is clearly an area deserving intense investigation. A retrospective analysis of data from patients who received subsequent-line cetuximab for colorectal cancer reported that high gene expression levels of the EGFR ligands epiregulin and amphiregulin were found to be associated with improved disease control compared with lower levels.[28] In addition, disease control following treatment with cetuximab was significantly higher among patients without tumor mutations in the *KRAS* gene,[28] an observation subsequently confirmed by analyses of other studies of cetuximab in combination with standard first-line therapy.[19,29] In breast cancer, however, the *KRAS* gene is not frequently mutated[30] and so is unlikely to be a useful predictive marker.

In conclusion, the primary end point of the study was not met. However, the addition of cetuximab to cisplatin was associated with a numerical doubling of the response rate and an increase in PFS when compared with cisplatin alone in patients with mTNBC who had received no more than one line of palliative chemotherapy. These results suggest that EGFR may be a suitable target in the treatment of TNBC and that cetuximab might be a potentially important addition to treatment strategies for the management of patients with TNBC. These findings warrant further investigation. Future approaches might include the identification of predictive markers for the treatment with cetuximab and the development of a more active chemotherapy-cetuximab combination regimen in mTNBC.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.
Employment or Leadership Position: Angela Zubel, Merck KGaA (C); Helena Melezínková, Merck KGaA (C) **Consultant or Advisory Role:** Richard Greil, Merck KGaA (C) **Stock Ownership:** None **Honoraria:** Richard Greil, Merck KGaA **Research Funding:** None **Expert Testimony:** None **Other Remuneration:** None

AUTHOR CONTRIBUTIONS

Conception and design: José Baselga, Sofia Braga, Ahmad Awada
Provision of study materials or patients: José Baselga, Patricia Gómez, Richard Greil, Miguel A. Climent, Andrew M. Wardley, Bella Kaufman, Andreas Schneeweiss, Ahmad Awada
Collection and assembly of data: Patricia Gómez, Richard Greil, Miguel A. Climent, Andrew M. Wardley, Bella Kaufman, Salomon M. Stemmer, António Pêgo, Jean-Charles Goeminne, Marie-Pascale Graas, Eva Maria Ciruelos Gil, Andreas Schneeweiss, Angela Zubel, Ahmad Awada
Data analysis and interpretation: José Baselga, Richard Greil, Salomon M. Stemmer, Arlene Chan, M. John Kennedy, Eva Maria Ciruelos Gil, Angela Zubel, Jutta Groos, Helena Melezínková, Ahmad Awada
Manuscript writing: All authors
Final approval of manuscript: All authors

REFERENCES

1. Cheang MC, Voduc D, Bajdik C, et al: Basal-like breast cancer defined by five biomarkers has superior prognostic value than triple-negative phenotype. Clin Cancer Res 14:1368-1376, 2008
2. Kwan ML, Kushi LH, Weltzien E, et al: Epidemiology of breast cancer subtypes in two prospective cohort studies of breast cancer survivors. Breast Cancer Res 11:R31, 2009
3. Dent R, Trudeau M, Pritchard KI, et al: Triple-negative breast cancer: Clinical features and patterns of recurrence. Clin Cancer Res 13:4429-4434, 2007
4. Isakoff SJ: Triple-negative breast cancer: Role of specific chemotherapy agents. Cancer J 16:53-61, 2010
5. Kassam F, Enright K, Dent R, et al: Survival outcomes for patients with metastatic triple-negative breast cancer: Implications for clinical practice and trial design. Clin Breast Cancer 9:29-33, 2009
6. Corkery B, Crown J, Clynes M, et al: Epidermal growth factor receptor as a potential therapeutic target in triple-negative breast cancer. Ann Oncol 20:862-867, 2009
7. Baselga J, Norton L, Masui H, et al: Antitumor effects of doxorubicin in combination with anti-epidermal growth factor receptor monoclonal antibodies. J Natl Cancer Inst 85:1327-1333, 1993
8. Turner N, Tutt A, Ashworth A: Hallmarks of 'BRCAness' in sporadic cancers. Nat Rev Cancer 4:814-819, 2004
9. Silver DP, Richardson AL, Eklund AC, et al: Efficacy of neoadjuvant cisplatin in triple-negative breast cancer. J Clin Oncol 28:1145-1153, 2010
10. Staudacher L, Cottu PH, Dieras V, et al: Platinum-based chemotherapy in metastatic triple-negative breast cancer: The Institut Curie experience. Ann Oncol 22:848-856, 2011
11. Uhm JE, Park YH, Yi SY, et al: Treatment outcomes and clinicopathologic characteristics of triple-negative breast cancer patients who received platinum-containing chemotherapy. Int J Cancer 124:1457-1462, 2009
12. Balin-Gauthier D, Delord JP, Pillaire MJ, et al: Cetuximab potentiates oxaliplatin cytotoxic effect through a defect in NER and DNA replication initiation. Br J Cancer 98:120-128, 2008

13. Dittmann K, Mayer C, Rodemann HP: Inhibition of radiation-induced EGFR nuclear import by C225 (Cetuximab) suppresses DNA-PK activity. Radiother Oncol 76:157-161, 2005

14. Aboud-Pirak E, Hurwitz E, Pirak ME, et al: Efficacy of antibodies to epidermal growth factor receptor against KB carcinoma in vitro and in nude mice. J Natl Cancer Inst 80:1605-1611, 1988

15. Fan Z, Baselga J, Masui H, et al: Antitumor effect of anti-epidermal growth factor receptor monoclonal antibodies plus cis-diamminedichloroplatinum on well established A431 cell xenografts. Cancer Res 53:4637-4642, 1993

16. Hoadley KA, Weigman VJ, Fan C, et al: EGFR associated expression profiles vary with breast tumor subtype. BMC Genomics 8:258, 2007

17. Baselga J, Trigo JM, Bourhis J, et al: Phase II multicenter study of the antiepidermal growth factor receptor monoclonal antibody cetuximab in combination with platinum-based chemotherapy in patients with platinum-refractory metastatic and/or recurrent squamous cell carcinoma of the head and neck. J Clin Oncol 23:5568-5577, 2005

18. Vermorken JB, Mesia R, Rivera F, et al: Platinum-based chemotherapy plus cetuximab in head and neck cancer. N Engl J Med 359:1116-1127, 2008

19. Bokemeyer C, Bondarenko I, Makhson A, et al: Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. J Clin Oncol 27:663-671, 2009

20. Carey LA: Directed therapy of subtypes of triple-negative breast cancer. Oncologist 15:49-56, 2010 (suppl 5)

21. Therasse P, Arbuck SG, Eisenhauer EA, et al: New guidelines to evaluate the response to treatment in solid tumors: European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 92:205-216, 2000

22. Regan MM, Viale G, Mastropasqua MG, et al: Re-evaluating adjuvant breast cancer trials: Assessing hormone receptor status by immunohistochemical versus extraction assays. J Natl Cancer Inst 98:1571-1581, 2006

23. Carlson RW, Moench SJ, Hammond ME, et al: HER2 testing in breast cancer: NCCN Task Force report and recommendations. J Natl Compr Canc Netw 4:S1-S22, 2006; quiz S23-S24 (suppl 3)

24. Lynch TJ, Patel T, Dreisbach L, et al: Cetuximab and first-line taxane/carboplatin chemotherapy in advanced non-small-cell lung cancer: Results of the randomized multicenter phase III trial BMS099. J Clin Oncol 28:911-917, 2010

25. Pirker R, Pereira JR, Szczesna A, et al: Cetuximab plus chemotherapy in patients with advanced non-small-cell lung cancer (FLEX): An open-label randomised phase III trial. Lancet 373:1525-1531, 2009

26. Carey LA, Rugo HS, Marcom PK, et al: TBCRC 001: Randomized phase III study of cetuximab in combination with carboplatin in Stage IV triple-negative breast cancer. J Clin Oncol 30:2615-2623, 2012

27. O'Shaughnessy J, Weckstein D, Vukelja S: Preliminary results of a randomized phase II study of weekly irinotecan/carboplatin with or without cetuximab in patients with metastatic breast cancer. Breast Cancer Res Treat 106:S32-S33, 2007 (suppl 1; abstr 308)

28. Khambata-Ford S, Garrett CR, Meropol NJ, et al: Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab. J Clin Oncol 25:3230-3237, 2007

29. Van Cutsem E, Kohne CH, Hitre E, et al: Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N Engl J Med 360:1408-1417, 2009

30. Khambata-Ford S, O'Shaughnessy J, Brickman D, et al: Candidate predictive biomarkers of cetuximab benefit in triple-negative breast cancer. J Clin Oncol 28:127s, 2010 (abstr 1056)

Chapter 6: Blocking angiogenesis to treat breast cancer

Conception and design: Sofia Braga

Provision of study materials or patients: Sofia Braga

Collection and assembly of data: Sofia Braga

Data analysis and interpretation: Sofia Braga

Manuscript writing: Sofia Braga

Final approval of manuscript: Not applicable

Funding: Not applicable

Ethical Committee approval: Not applicable

Blocking angiogenesis to treat breast cancer

Abstract

Angiogenesis is a hallmark of cancer because tumors larger than 1mm need new vessels to sustain their growth. Since the discovery of molecular players of this process and its inhibitors, that angiogenesis became a promising therapeutic target. This review will summarize and analyze data from clinical trials of anti-angiogenic agents in the treatment of breast cancer (BC).

Before the bevacizumab era, matrix metaloproteinase inhibitors, thalidomide and endostatin were tested, as well as classic chemotherapy, administered metronomically. Bevacizumab was the first molecularly-targeted antiangiogenic therapy approved and extensively studied. Phase III trials of bevacizumab in advanced BC have demonstrated a reduction in disease progression (22–52%), increased response rates (RR) and improvements in progression-free survival (PFS) of 1 to 5 months, but no improvements in overall survival (OS). Bevacizumab phase III trials in early BC have been closed or negative. Bevacizumab combined with CT is associated with more adverse events.

A second class of approved inhibitors (sunitinib, sorafenib, pazopanib and axitinib) include oral small-molecule tyrosine kinase inhibitors (TKI) that target vascular endothelial growth factor receptors (VEGFR), platelet-derived growth factor receptors, and other kinases including KIT, Ret, BRAF and Flt-3 but none of these have gained approval to treat BC. Phase III trials of the TKI, sunitinib, were negative, while randomized phase II trials of sorafenib and pazopanib have improved some outcomes. Toxicity is less predictable with these agents, first, the expected vascular class effects, but the “off-target” side effects, common to small molecule inhibitors, are of concern.

Most trials of anti-angiogenic agents in BC have reported improved RR and PFS but no increase in OS compared to CT alone, leading to skepticism towards blocking angiogenesis. Selected trials in selected BC populations with translational endpoints related to harvested tumor tissue and other biological material samples, preferentially at several timepoints, will be crucial if antiangiogenesis is to survive as a strategy to treat breast cancer.

Basic and translational research

Angiogenesis, the development of vessels from pre existant vessels is an indispensable process for tumor development, it is a hallmark of cancer [1]. In BC, this was shown in preclinical in vivo and in vitro models and in tumor samples. MCF7 cell lines transfected with VEGF gene or fibroblast growth factor (FGF) gene, develop more aggressive tumors in mice than the non modified cell line [2, 3]. On the other hand, an antiangiogenic treatment in the same model, can prevent the development of such tumors [4-6]. A similar experiment showed that cell lines that could not develop cancer in mice could do so after acquiring an antiangiogenic phenotype characterized among others by VEGF and overexpression [7].

An experiment where the T47D cell line was transfected with a conditional expression vector with VEGF gene, showed, that, at implantation or shortly after, turning off VEFG expression had significant impact on tumor growth, whereas in a more advanced stage such reduction was not impactful. This model shows that the timing in cancer natural history when antiangiogenic therapy is administered is relevant [8].

There is crosstalk between antiangiogenic proteins and breast cancer carcinogenic proteins, namely, steroid hormones and their receptors and epithelial growth factors and their receptors. Intracytoplasmatic signaling via Her2 induces expression of HIF1α (Hypoxia inducing factor) and VEGF. Blocking Her2 with trastuzumab reduces VEGF expression [9, 10]. VEGF is an ERα target gene, while ERβ decreases VEGF transcription [11, 12]. In cell lines, P53 inhibits VEGF expression by forming a complex with the transcriptional factor SP1 [13]. This might be one of the reasons P53 mutated tumors have worse prognosis [14].

Data from patient samples has shown the relevance of angiogenesis in BC. One of the first studies searching angiogenic molecules in patient material was done precisely in BC. This study showed correlation between increased vascular density and worse prognosis in 49 patients, this was one of the first confirmations of the theory Judah Folkman put forward in 1971 [15, 16]. Judah Folkman was a pioneer, he postulated the existence of the proangiogenic molecules, years before they were known or detectable. Confirmatory studies ensued as well as the demonstration of this prognostic value in lymph node negative BC and ductal carcinoma in situ (DCIS) [17-19]. The correlations between increased angiogenesis in the primary tumor and worse prognosis have mainly been done with VEGF levels measured by trasnscript, immunohistochemistry or western blot in the primary tumor [20, 21]. Tumor VEGF levels have been established as an independant predictor of survival [22]. Furthermore, tumor VEGF level has also been shown to be a negative predictor of response to first line metastatic endocrine therapy and CT [23]. Regarding tumor VEGF2, there is a study showing that, in early breast cancer, high expression of VEGFR2 in the primary tumor correlates with no efficacy of 2 year tamoxifen [24]. Other authors have looked at the

crosstalk between the downstream molecules of VEGF in BC development namely HIF1α [25]. Polymorphisms of germline VEGF have been correlated with BC risk and prognosis [26, 27].
Circulating VEGF studies have been difficult to interpret, because platelet VEFG is released into the circulation upon coagulation and the levels are very different if evaluation is done in plasma or serum and IT also depends on the anticoagulant used. Possibly because of this, studies have not shown correlation between increased circulating VEGF in MBC patients and survival [28-30]. Which was unexpected, due to the results in the primary tumors. Serum VEGF levels, do not have a standard measurement and have not been efficiently exploited for assessing response. But, in other tumor types, circulating VEGF level, predicts worse prognosis and correlates with the findings of primaries [22, 31]. Several hypothesis have been put forward for this inconsistency [32]: Could it be that VEGF is crucial in the primary tumor and looses its prognostic value in MBC due to numerous redundant pathways. Could this be the reason for the inefficacy of bevacizumab in MBC. In fact, angiogenesis is a differential of proangiogenic and anti angiogenic molecules in an equilibrium. Neovessels are the result of more proangiogenic molecules and there are antiangiogenic molecules detected in BC, namely endostatin [33]. Surprisingly, circulating endostatin is increased in more aggressive and in MBC patients [34, 35]. These paradoxical results have also been shown in other tumor types, and might be linked to the fact that the expression of collagen XVIII, the precursor of endostatin is increased in aggressive cancer [36, 37]. This can be the result of a negative feedback loop or of a basal antiangiogenic activity of angiostatin that was shown in initial trials. Despite effort, this equilibrium between pro and anti angiogenic factors has not been conclusively elucidated in BC [38]. The procurement of high quality samples of metastatic lesions as well as serum samples during treatment and the necessity of using reliable and generalizable laboratory techniques has thwarted this research.

Clinical research

Sixty molecules with angiogenic activity are being evaluated clinically in BC with very different mechanisms of action. A large part of these molecules are VEGF antagonists, because VEGF is possibly the most important angiogenic molecule in cancer and undoubtedly the best studied. There are monoclonal antibodies anti-VEGF or anti-VEGF receptors, soluble receptors, inhibitors of the VEGF coupled tyrosine kinase or antisense oligonucleotides [39]. The other class of molecules are inhibitors of matrix metalloproteinases. Finally, are the molecules that are directly antiangiogenic like thalidomide, whose mechanism of action is not well characterized and endostatin or angiostatin [40]. The antiangiogenic action of classic chemotherapy is also of interest, such as the metronomic administration of low dose cyclophosphamide or weekly paclitaxel. This is thought to be due to their cytostatic effect on endothelial cells, precursors of new premetastatic niches, in tumors resistant to cytotoxic approaches because of few mitotic cells [41].

Matrix metaloproteinase inhibitors

The initial clinical trials, started in the late 1990‘s, prompted phase III trials that were negative [42, 43]. A randomized placebo-controlled phase III trial in first line MBC of maintenance marimastat after induction chemoptherapy, in 181 patients, did not prolong survival and was associated with grade 2-3 musculoskeletal toxicity, in 63% of the patients. Surprisingly, increased toxicity correlated with worse survival [44]. Two adjuvant studies with this drug were done. One phase II, where the objective was to obtain therapeutic plasma levels of marimastat. In this trial, 35% of the patients stopped the drug due to musuloskeletal toxicity while the maximum tolerated dose was only 25% of the dose predicted to be efficacious. These results did not give confidence to proceed to phase III [45]. The other, was a randomized double blind phase II trial with BMS275291, the objectives were toxicity and feasibility, it was stopped after the enrollment of 72 patients due to arthralgia and the development of the drug stopped [46]. The development of MMPi was stopped in cancer after these results.

Thalidomide

This drug was tested after arguably interesting preclinical data, in numerous solid tumors [47]. A phase II trial with three doses was performed in 28 MBC patients with no objective responses and only two stabilizations. These two patients, one had stable disease for 16 weeks and the other for 11 weeks, but this patient had to stop the drug due to neurologic toxicity [48]. Serum from thalidomide-treated patients did not show decrease in VEGF levels. Another study confirmed these results, and drug development was abandoned for this indication [49].

Natural inhibitors of angiogenesis: endostatin

Until recently, there were no clinical trials specifically testing the natural inhibitors of angiogenesis in BC. In the two phase I clinical trials reported there were 7 patients with MBC but there was no response. Translational research studies, done as components of these trials, with biopsies of the lesions before and after treatment, evaluating VEGF levels and tumor perfusion studies, did not show a strong antiangiogenic

effect compared to the same essays done on bevacizumab-treated lesions [50, 51]. Endostatin has been further tested in neoadjuvant clinical trials in combination with anthracycline-based chemotherapy in treatment-naive patients and has increased the clinical response rate, but more trials are needed to establish this drug. The third generation of angiogenesis inhibitors is represented by recombinant human endostatin that is being tested in phase III clinical trials, there are interesting results available in the neoadjuvant setting.

Metronomic chemotherapy

Metronomic chemotherapy in BC was evaluated in a phase II trial with 64 patients where metotrexate was given weekly and cyclophosphamide was given continuously. In 52 of these patients it was used as third line. There were 2 complete responses and 12 partial responses with a 22% RR, PFS was 3 months. Serum VEGF decreased but without correlation with response [52]. A randomized follow-up of this trial used the same regimen with or without thalidomide, in 175 patients. RR was 20%, the same as the previous study, and the addition of thalidomide did not increase RR, while it increased toxicity, similarly, the serum VEGF decreased after 2 months of therapy, but without correlation with response [53].

It is not clear from these trials if there is true antiangiogenic effect of metronomic chemotherapy, nor is it clear which are the cells affected, the microenvironment or the epithelial BC cells [54]. There are other empirical observations of the metronomic effect of protratcted 5FU, capecitabine and weekly paclitaxel.

Bevacizumab

Metastatic setting

Bevacizumab was developed on the rational basis of VEGF biology and has activity in numerous tumors with bearable toxicity, it was shown that using the drug, in part, blocks angiogenesis [55, 56]. Bevacizumab created great excitement at the time, in fact it validated that blocking angiogenesis was a therapeutic modality in human cancer, although results were not as promising as initially expected [57]. In Phase II trials with monotherapy in pretreated MBC patients a 9% response rate was observed and less than 20% stable disease at 6 months [58]. In combination therapy, the addition of bevacizumab to docetaxel or vinorelbine did not result in the expected increased RR [59]. Bevacizumab was combined with trastuzumab in patients progressing on the latter, there were responses and no additive toxicity [60]. This strategy of combination of bevacizumab and trastuzumab has intermittently been tested in MBC trials and some small neoadjuvant trials. The definitive phase III adjuvant trial named BETH, has recently reported results. Subsequent phase III trials suggesting improved activity when bevacizumab is administered in conjunction with CT were numerous and randomized thousands of MBC patients. Starting with second line MBC therapy, a phase III trial combined bevacizumab with capecitabine for patients previously treated with anthracycline and taxane therapy. The trial randomized 462 patients to receive capecitabine with or without bevacizumab, 25% of patients had Her2 positive MBC. There was an increase in overall RR with the addition of bevacizumab to capecitabine (9.1% vs. 19.8%), with no significant improvement in PFS or OS [61].

The most important phase III trial combined bevacizumab with taxanes as first-line treatment for patients with locally recurrent or MBC. It was an open label trial called ECOG 2100, 673 patients were randomized to paclitaxel plus or minus bevacizumab [63].In this ECOG trial the ORR more than doubled (22.2% vs. 48.9%) as well as PFS (median, 5.8 vs. 11.3 months) with no improvement in OS (median, 25.2 vs. 26.7 months) [63, 66]. Based on the ECOG2100 results, the FDA approved bevacizumab for first-line therapy of MBC patients, in the beginning of 2008. This was granted under accelerated approval program, which allowed bevacizumab to be approved based on the ECOG 2100 data that were not sufficiently complete to permit full approval. The accelerated approval program provided earlier access of BC patients to what was thought to be a promising new drug, while confirmatory trials of ECOG 2100 were being conducted.

The similar, placebo-controlled, AVADO trial, randomized 736 patients to three study arms, two dose levels of bevacizumab combined with docetaxel. At progression, patients could continue receive bevacizumab as second-line therapy. At a median follow-up of 25 months, results show that standard dose (15 mg/kg) bevacizumab, in combination with docetaxel, increased ORR modestly (46.4% vs. 64.1%), improvement in PFS (median, 8.2 vs. 10.1). The addition of low dose bevacizumab did not significantly improve ORR or PFS. The final analysis showed no improvement in OS [65]. AVADO was the first trial not to confirm the results of ECOG 2100 and AVADO was a larger trial.

RiBBOn 1 and 2 trials tested chemotherapy with or without bevacizumab in first and second-line setting in 1237 patients [64, 67, 68]. The RIBBON trials were placebo-controlled. Data were analyzed based on patients receiving: (i) taxane or anthracycline-based CT plus standard dose bevacizumab versus CT plus placebo and (ii) capecitabine plus bevacizumab versus capecitabine plus placebo. The addition of bevacizumab to CT resulted in improvements in PFS for both the taxane-anthracycline (median, 8.0 vs. 9.2 months) and capecitabine cohorts (median, 5.7 vs. 8.6 months). No significant differences in OS were observed between treatment arms in either the taxane–anthracycline (HR = 1.03, p = 0.83) or capecitabine

cohort (HR = 0.85, p = 0.27), although a trend toward improved 1-year survival was apparent when bevacizumab was added to capecitabine (74.4% vs. 81.0%, p = 0.076).
Chemotherapeutic treatment options in the RIBBON 2 trial included taxanes, gemcitabine, vinorelbine or capecitabine. A total of 684 previously-treated (3rd line or more) patients were randomly assigned to receive either bevacizumab (15 mg/kg, q3w or 10 mg/kg, q2w) or placebo [67, 68]. There was a statistically not significant trend toward improved RR (29.6% vs. 39.5%) in the bevacizumab-containing treatment arms [67]. The addition of bevacizumab resulted in a significant improvement in PFS (median, 5.1 vs. 7.2 months; HR = 0.78, p = 0.0072) [68]. An interim survival analysis showed no improvement in OS (median,16.4 vs. 18.0 months; HR = 0.90, p = 0.3741) with the addition of bevacizumab [68]. However, an exploratory subgroup analysis of patients with triple-negative breast cancer (TNBC) demonstrated a highly significant improvement in PFS (median, 2.7 vs. 6.0 months; HR = 0.49, p = 0.0006) and a trend toward improved OS (median, 12.6 vs. 17.9 months; HR = 0.62, p = 0.0534) [68].
Given the results of AVADO and RIBBON, the confirmatory trials of ECOG 2100, that only show small response rates with no survival benefit, the FDA announced that the agency recommended removing the BC indication from the label of bevacizumab in the end of 2010. In June 2011 there was a hearing with Genentech to re-appreciate data but the decision was revoked and in the end of 2011 the agency's accelerated approval of bevacizumab for BC was withdrawn. The explanation for the very different results of ECOG 2100 and the subsequent confirmatory trials will never be clear but the fact that ECOG 2100 was open-label and the other two trials were placebo-controlled has flared up an old discussion in cancer trials.

Neoadjuvant setting
Researchers are continuing to study this drug in other BC settings and BC subtypes, but, outside clinical trials, bevacizumab should not be administered to BC patients. The NSABP B-40 trial added bevacizumab (15 mg/kg) and/or antimetabolites to standard neoadjuvant CT in a randomized phase III trial of Her2-negative BC (n = 1206) [69]. Overall, the pCR rate (breast alone, [ypT0/Tis]) improved with the addition of bevacizumab (28.4% vs. 34.5%, p = 0.027) with the greatest impact observed in the hormone receptor-positive subset (15.2% vs. 23.3%, p = 0.008).
The phase III GeparQuinto trial randomized 1948 Her2-negative BC patients to anthracycline-taxane CT with or without bevacizumab (15 mg/kg). Adding bevacizumab did not significantly increase the pathological complete response rate (pCR in breast and axilla, 15% vs. 17.5% or pCR breast alone [ypT0/Tis], 21.3% vs. 23.9%) or rates of breast conserving surgery in patients overall, but did improve the pCR rate in a sub-population of 684 patients with TN disease (breast and axilla [ypT0, ypN0], 27.8% vs. 36.4%, p = 0.021) [70, 71, 72].
As is expected, adding bevacizumab to CT increases neutropenia, hand-foot syndrome, mucositis and hypertension. Again, with these two important trials that randomized more than 3000 neoadjuvant BC patients, B40 and Quinto, we see discordant results, not only in response but also in BC subtypes.

Adjuvant setting
The study of the drug in the adjuvant setting started by ECOG 2104, a large randomized phase II pilot trial that incorporated bevacizumab into dose-dense doxorubicin and cyclophosphamide followed by paclitaxel in patients with lymph node positive breast cancer. It was designed to evaluate the safety of two different strategies incorporating bevacizumab; into anthracycline or taxane containing adjuvant therapy, as a precursor to a definitive randomized phase III trial. Patients were treated with dose-dense doxorubicin and cyclophosphamide, followed by paclitaxel, all patients received bevacizumab (10 mg/kg every 2 weeks during 26 weeks), initiated either concurrently with AC or with paclitaxel. The primary end point was incidence of cardiac dysfunction; once the results of the MBC trials had suggested increased cardiac adverse events with the addition of bevacizumab. In 226 enrolled patients, grade 3 hypertension, thrombosis, proteinuria and hemorrhage were reported for 12%, 2%, 2% and less than 1% of patients, respectively. Two patients had grade 3 cerebrovascular ischemia. Three patients in each arm developed congestive heart failure. There was no significant difference between arms in the proportion of patients with an absolute decrease in left ventricular ejection fraction. Bevacizumab, combined with adjuvant therapy, was concluded not to result in prohibitive cardiac toxicity and the definitive, 5000 patient, phase III trial, ECOG 5103, was started in 2007. Fortunately, despite the optimistic safety signals coming from ECOG 2104, systematic and extensive cardiac monitoring was implemented and detected an excess of toxic cardiac events in the bevacizumab containing arm leading to premature termination of the adjuvant trial in 2009 [74, 75].
Beatrice, was an open-label phase III trial, where 2591 patients with triple-negative operable primary invasive breast cancer were randomly assigned to receive a minimum of four cycles of adjuvant chemotherapy alone (n = 1290) or with bevacizumab at an equivalent of 5 mg/kg every week for 1 year (n = 1301). Beatrice was started in 2008 and has just published final results [76]. Similar proportions of

patients received anthracycline and taxane therapy (59% and 58%), nontaxane anthracycline-containing therapy (36% and 37%), nonanthracycline taxane-containing therapy (5% of both), and radiation therapy (74% and 73%). Chemotherapy was completed as planned in 92% of the chemotherapy group and 93% of the bevacizumab group, and bevacizumab was completed as planned in 68%. After median follow-up of 32 months, there was no difference between the bevacizumab group and the chemotherapy group in invasive disease–free survival (IDFS). IDFS events occurred in 14% of the bevacizumab group vs 16% of the chemotherapy group; 3-year IDFS was 83.7% with bevacizumab and 82.7% with chemotherapy alone. Subgroup analyses showed no evidence of differences. After 200 deaths, there was no difference in OS. Sites of recurrence were similar in the two treatment groups, with the most common being distant recurrence. The most common sites of distant recurrence were lung (28% and 27%), liver (20% and 15%), and bone (17% and 20%). Distant central nervous system or meningeal recurrence accounted for 7% of recurrences in the bevacizumab group and 12% in the chemotherapy group. Exploratory biomarker assessment in approximately 45% of patients suggested that patients with high pretreatment plasma VEGFR-2 levels might benefit from the addition of bevacizumab. The hazard ratios for invasive disease–free survival for bevacizumab vs chemotherapy were 0.61 among patients with levels above the median value and 1.24 for those with levels below median (P = .0291 for interaction). Grade 3 or higher adverse events occurred in 72% of patients in the bevacizumab group and 57% of the chemotherapy group. The bevacizumab group had an increased frequency of grade 3 or worse hypertension (12% vs 1%), severe cardiac events occurring at any point during the 18-month safety-reporting period (1% vs < 0.5%), and treatment discontinuation (bevacizumabor chemotherapy, 20% vs 2%) [76]. In Beatrice, unlike ECOG 5103, the safety data did not require premature termination of the trial, therefore efficacy results of bevacizumab in the adjuvant setting in the TNBC subtype are available. With respect to Her2 positive breast cancer, the Beth trial randomized 3509 women who had either node-positive or high-risk node-negative disease, with the latter group making up 41% of the population. These results were recently presented at San Antonio Breast Cancer Symposium in December 2013. Patients were enrolled in 1 of 2 chemotherapy regimens: 6 cycles of docetaxel/carboplatin plus trastuzumab (TCH) with or without bevacizumab (n = 3231) or an anthracycline-based regimen involving 3 cycles of docetaxel plus trastuzumab given with or without bevacizumab followed by 3 cycles of 5-fluorouracil, epirubicin, and cyclophosphamide (n = 278). In both regimens, patients continued trastuzumab with or without bevacizumab after chemotherapy to complete 1 year of targeted therapy. For the primary outcome of the study, which was IDFS, there was no statistically significant difference between the patients who received bevacizumab and those who did not. At a median follow-up of 38 months, IDFS rates were 92% for both groups of the TCH cohort (ie, those treated with or without bevacizumab) A secondary endpoint compared IDFS in patients in the anthracycline-based vs the TCH-based cohorts and also found no significant differences between the regimens, whether with or without bevacizumab. However, the study was not designed to compare these different chemotherapy approaches, and only 278 patients received anthracyclines, less than 5%. The IDFS of 92% is in striking contrast with the IDFS of BCIRG 006 Trial of 86% that had the same investigational arm without bevacizumab. These results will question the use of anthracyclines in the adjuvant treatment of Her2 BC. In grade 3 or 4 adverse events, hypertension was higher in the bevacizumab group (19% vs 4%; $p < .001$). There was also a trend for more congestive heart failure with bevacizumab (2.1% vs <1%; $p = .0621$), and a difference in hemorrhage (2% vs <1%; $p < .0001$). Proteinuria and gastrointestinal perforations were also more common in the bevacizumab group. Contrary to the favorable toxicity results Beatrice trial the Beth trial has again showed important toxicity with adjuvant bevacizumab.

Inhibitors of VEGFR coupled tyrosine kinases: Sunitinib and Sorafenib

Small molecule oral TKIs target the intracellular catalytic function of the VEGFR family linked tyrosine kinases. The kinases are coupled to the intracellular portion of the transmembrane receptors (e.g., VEGFR-1, 2 and 3). VEGFR-2 is the primary signaling receptor for VEGF-mediated angiogenesis. Sunitinib is a multi-targeted inhibitor of VEGFR-1, 2 and 3, platelet-derived growth factor receptor, c-Kit, FMS-like tyrosine kinase-3 and RET. A recent phase III trial comparing sunitinib (37.5 mg/day) to capecitabine and a small randomized phase II trial evaluating sunitinib as consolidation therapy following induction chemotherapy [77, 78] have both demonstrated inferior outcomes for single agent sunitinib compared with controls in pretreated MBC. Additionally, two randomized phase III trials in the advanced setting evaluating the addition of sunitinib (37.5 mg/day) to either capecitabine or docetaxel compared with the respective chemotherapies alone demonstrated increased toxicity and comparable PFS with the addition of sunitinib [79, 80]. The definitive first line phase III trial of sunitinib in BC was done comparing docetaxel with or without sunitinib in the first line treatment of MBC. The combination increased response rate but had no effect in PFS and OS [81]. Based on these findings, and the early termination of the phase III sunitinib trial in first line MBC, due to a lack of feasibility due to increased toxicity and weak efficacy results [82] the clinical development of sunitinib in BC was stopped.

Sorafenib, a small molecule TKI, has both anti-angiogenic and anti-proliferative effects [83]. Sorafenib has shown single agent activity in pretreated patients [84]. Three randomized, phase IIb trials have shown that sorafenib in combination with standard chemotherapy significantly improved outcomes from first-line MBC treatment (PFS; median, 5.6 vs. 6.9 months; HR = 0.79, p = 0.09 and TTP; median 5.6 vs. 8.1 months; HR = 0.67, p = 0.017) and second-line (PFS; median, 4.1 vs. 6.4 months; HR = 0.58, p = 0.0006). Sorafenib showed activity after bevacizumab resistance (PFS; median, 2.7 vs. 3.4 months; HR = 0.65, p = 0.01) [85-88]. A multivariate analysis of these trials suggested sorafenib to be an interesting drug to pursue development [89]. The toxicity profile includes minimal grade 3/4 hypertension but high rates of grade 3/4 hand-foot syndrome. A placebo-controlled phase III trial in MBC evaluating capecitabine in combination with sorafenib is currently underway (NCT01234337). Other ongoing randomized trials will evaluate sorafenib in combination with standard chemotherapy (NCT00499525 and NCT01320111), metronomic chemotherapy and/or endocrine therapy (NCT00573755, NCT00954135) in the advanced setting. There are obvious advantages to an oral drug in this setting, that is why these phase III trials are designed with oral regimens.

Other tirosine kinase inhibitors: Vandetanib and axitinib

Not a lot of trials were subsequently run with small molecules. There was a phase II trial in anthracycline and taxane treated patients with vandetanib an inhibitor of VEGFR2 and HER1. Forty patients were included, there was manageable diarrhea, but no responses, the best result was one patient with stable disease during six months [90]. There are two more trials with vandetanib: vandetanib with metronomic CT by the Dana Farber and a phase II in first line MBC combined with docetaxel. The results were not enthusiastic and the development of vandetanib in BC has been abandoned.

Axitinib inhibits VEGFR2 and PDGFR. Phase I-II trials were reported in combination with docetaxel. The largest trial, randomized 168 first line MBC to docetaxel combined with axitinib or placebo. There was no difference in TTP and toxicity is considerable with 10% diarrhea, fatigue, stomatitis and mucositis and hematological toxicity in 15% of patients. There were three serious thromboembolic events and one resulted in death [91]. Due to these results, the development of axitinib, as all other TKIs, except sorafenib, has been abandoned.

Discussion

Numerous trials have been done and some are still underway, testing the concept of blocking angiogenesis in BC. Several thousands of patients were treated with antiangiogenic agents in clinical trials, but their results have not been convincing enough for the widespread treatment of patients with advanced BC with antiangiogenic agents. Bevacizumab should not be used in the treatment of MBC patients, despite continued regulatory approval by some agencies, including the European Medicines Agency. As for early BC, there is no positive adjuvant trial to back up this treatment strategy and there is no regulatory approval. What has failed, why has the promise of blocking angiogenesis not held up in BC, while it is useful in several other solid tumors? The urgent effort to prospectively collect biological material has not been done. There have been responses to these drugs, but no data has been produced to know why and in which patients. Prospective collection of blood and biopsies at different time points across the continuum of MBC progression should have been mandatory.

It might be due to the fact that BC seems to be more heterogenous than coloretal or renal cancer. But BC heterogeneity is not solved by the current BC subtype classification, namely, ER positive, Her2 positive and TNBC. As we have seen, after the meta-analysis of the bevacizumab phase III trials in MBC, the PFS advantage in TNBC patients has not been meaningful, and, finally, the Beatrice adjuvant trial, in TNBC, was a negative trial. Possibly because this is a negative definition, that brings us no closer to the driving biology of the disease. Alternative angiogenic pathways have not been sufficiently explored in the datasets of non responding patients. It should be established, in any given patient, what are the neoangiogenic molecules at play in that disease state, there are dozens molecules that could play such roles [92-97]. There is a report showing upregulation of hypoxic response mediators in BC patients resistant to sunitinib, and, several preclinical models similarly showing this rebound response [98]. The Kerbel lab showed increased metastasis in mice models upon sunitinib withdrawal.

If there is still opportunity to go forward with antiangiogenic therapy in BC, drugs must be studied in lab models to further understand the mechanism of action and resistance to different anti-angiogenic agents [99, 100]. The hypoxic response through HIF1α inhibition in combinatorial treatment strategies with antiangiogenic drugs could be tested [101,102] as well as low dose metronomic chemotherapy in combination with antiangiogenic agents [103]. Antiangiogenic strategies have not impacted survival, and, they present a challenge in terms of safety issues. If there are no new results coming from specific subgroups or specific molecular targets or new drugs, antiangiogenic treatment in BC should be abandoned. We have done a poor job at performing and demanding more thorough research in one of the hallmarks of

cancer. We have lost our time, resources, failed drug development, but, much more seriously, failed our patients.

1. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med 1995 ; 1: 27-31.
2. Zhang HT, Craft P, Scott PA, Ziche M, Weich HA, Harris AL, et al. Enhancement of tumor growth and vascular density by transfection of vascular endothelial cell growth factor into MCF-7 human breast carcinoma cells. J Natl Cancer Inst 1995 ; 87 : 213-9.
3. Ruohola JK, Viitanen TP, Valve EM, Seppanen JA, Loponen NT, Keskitalo JJ, et al. Enhanced invasion and tumor growth of fibroblast growth factor 8b-overexpressing MCF-7 human breast cancer cells. Cancer Res 2001; 61 : 4229-37.
4. Zhang W, Ran S, Sambade M, Huang X, Thorpe PE. A monoclonal antibody that blocks VEGF binding to VEGFR2 (KDR/Flk1) inhibits vascular expression of Flk-1 and tumor growth in an orthotopic human breast cancer model. Angiogenesis 2002 ; 5 : 35-44.
5. Wedge SR, Ogilvie DJ, Dukes M, Kendrew J, Chester R, Jackson JA, et al. ZD6474 inhibits vascular endothelial growth factor signaling, angiogenesis, and tumor growth following oral administration. Cancer Res 2002 ; 62: 4645-55.
6. Sacco MG, Cato EM, Ceruti R, Soldati S, Indraccolo S, Caniatti M, et al. Systemic gene therapy with anti-angiogenic factors inhibits spontaneous breast tumor growth and metastasis in MMTVneu transgenic mice. Gene Ther 2001 ; 8 : 67-70.
7. Naumov GN, Bender E, Zurakowski D, Kang SY, Sampson D, Flynn E, et al. A model of human tumor dormancy : an angiogenic switch from the nonangiogenic phenotype. J Natl Cancer Inst 2006 ; 98 : 316-25.
8. Yoshiji H, Harris SR, Thorgeirsson UP. Vascular endothelial growth factor is essential for initial but not continued in vivo growth of human breast carcinoma cells. Cancer Res 1997 ; 57 : 3924-8.
9. Laughner E, Taghavi P, Chiles K, Mahon PC, Semenza GL. HER2 (neu) signaling increases the rate of hypoxia-inducible factor 1alpha (HIF-1alpha) synthesis : novel mechanism for HIF-1-mediated vascular endothelial growth factor expression. Mol Cell Biol 2001 ; 21 : 3995-4004.
10. Petit AM, Rak J, Hung MC, Rockwell P, Goldstein N, Fendly B, et al. Neutralizing antibodies against epidermal growth factor and ErbB-2/neu receptor tyrosine kinases down-regulate vascular endothelial growth factor production by tumor cells in vitro and in vivo : angiogenic implications for signal transduction therapy of solid tumors. Am J Pathol 1997 ; 151 : 1523-30.
11. Hyder SM, Nawaz Z, Chiappetta C, Stancel GM. Identification of functional estrogen response elements in the gene coding for the potent angiogenic factor vascular endothelial growth factor. Cancer Res 2000 ; 60 : 3183-90.
12. Buteau-Lozano H, Ancelin M, Lardeux B, Milanini J, Perrot-Applanat M. Transcriptional regulation of vascular endothelial growth factor by estradiol and tamoxifen in breast cancer cells : a complex interplay between estrogen receptors alpha and beta. Cancer Res 2002 ; 62 : 4977-84.
13. Pal S, Datta K, Mukhopadhyay D. Central role of p53 on regulation of vascular permeability factor/ vascular endothelial growth factor (VPF/VEGF) expression in mammary carcinoma. Cancer Res 2001 ; 61 : 6952-7.
14. Falette N, Paperin MP, Treilleux I, Gratadour AC, Peloux N, Mignotte H, et al. Prognostic value of P53 gene mutations in a large series of node-negative breast cancer patients. Cancer Res 1998 ; 58 : 1451-5.
15. Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis: correlation in invasive breast carcinoma. N Engl J Med 1991; 324 : 1-8.
16. Folkman J. Tumor angiogenesis : therapeutic implications. N Engl J Med 1971 ; 285 : 1182-6.
17. Weidner N, Folkman J, Pozza F, Bevilacqua P, Allred EN, Moore DH, et al. Tumor angiogenesis : a new significant and independent prognostic indicator in early-stage breast carcinoma. J Natl Cancer Inst 1992 ; 84: 1875-87.
18. Guidi AJ, Fischer L, Harris JR, Schnitt SJ. Microvessel density and distribution in ductal carcinoma in situ of the breast. J Natl Cancer Inst 1994; 86 : 614-9.
19. Uzzan B, Nicolas P, Cucherat M, Perret GY. Microvessel density as a prognostic factor in women with breast cancer : a systematic review of the literature and meta-analysis. Cancer Res 2004 ; 64 : 2941-55.
20. Relf M, LeJeune S, Scott PA, Fox S, Smith K, Leek R, et al. Expression of the angiogenic factors vascular endothelial cell growth factor, acidic and basic fibroblast growth factor, tumor growth factor beta-1, platelet-derived endothelial cell growth factor, placenta growth factor, and pleiotrophin in human primary breast cancer and its relation to angiogenesis. Cancer Res 1997 ; 57: 963-9.
21. Brown LF, Guidi AJ, Schnitt SJ, Van De WL, Iruela-Arispe ML, Yeo TK, et al. Vascular stroma formation in carcinoma in situ, invasive carcinoma, and metastatic carcinoma of the breast. Clin Cancer Res 1999 ; 5: 1041-56.
22. Gasparini G. Prognostic value of vascular endothelial growth factor in breast cancer. Oncologist 2000 ; 5 (Suppl. 1) : 37-44.
23. Foekens JA, Peters HA, Grebenchtchikov N, Look MP, Meijer-van Gelder ME, Geurts-Moespot A, et al. High tumor levels of vascular endothelial growth factor predict poor response to systemic therapy in advanced breast cancer. Cancer Res 2001 ; 61 : 5407-14.

24. Ryden L, Jirstrom K, Bendahl PO, Ferno M, Nordenskjold B, Stal O, et al. Tumor-specific expression of vascular endothelial growth factor receptor 2 but not vascular endothelial growth factor or human epidermal growth factor receptor 2 is associated with impaired response to adjuvant tamoxifen in premenopausal breast cancer. J Clin Oncol 2005 ; 23 : 4695-704.
25. Bos R, van der GP, Greijer AE, Shvarts A, Meijer S, Pinedo HM, et al. Levels of hypoxia-inducible factor-1alpha independently predict prognosis in patients with lymph node negative breast carcinoma. Cancer 2003 ; 97: 1573-81.
26. Krippl P, Langsenlehner U, Renner W, Yazdani-Biuki B, Wolf G, Wascher TC, et al. A common 936 C/T gene polymorphism of vascular endothelial growth factor is associated with decreased breast cancer risk. Int J Cancer 2003 ; 106 : 468-71.
27. Lu H, Shu XO, Cui Y, Kataoka N, Wen W, Cai Q, et al. Association of genetic polymorphisms in the VEGF gene with breast cancer survival. Cancer Res 2005 ; 65 : 5015-9.
28. Adams J, Carder PJ, Downey S, Forbes MA, MacLennan K, Allgar V, et al. Vascular endothelial growth factor (VEGF) in breast cancer : comparison of plasma, serum, and tissue VEGF and microvessel density and effects of tamoxifen. Cancer Res 2000 ; 60 : 2898-905.
29. Salven P, Perhoniemi V, Tykka H, Maenpaa H, Joensuu H. Serum VEGF levels in women with a benign breast tumor or breast cancer. Breast Cancer Res Treat 1999 ; 53 : 161-6.
30. Bachelot T, Ray-Coquard I, Menetrier-Caux C, Rastkha M, Duc A, Blay JY. Prognostic value of serum levels of interleukin 6 and of serum and plasma levels of vascular endothelial growth factor in hormone-refractory metastatic breast cancer patients. Br J Cancer 2003 ; 88 : 1721-6.
31. Poon RT, Fan ST, Wong J. Clinical implications of circulating angiogenic factors in cancer patients. J Clin Oncol 2001 ; 19 : 1207-25.
32. Miller KD, Chap LI, Holmes FA, Cobleigh MA, Marcom PK, Fehrenbacher L, et al. Randomized phase III trial of capecitabine compared with bevacizumab plus capecitabine in patients with previously treated metastatic breast cancer. J Clin Oncol 2005 ; 23 : 792-9.
33. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell 1996 ; 86 : 353-64.
34. Bachelot T, Ratel D, Menetrier-Caux C, Wion D, Blay JY, Berger F. Autoantibodies to endostatin in patients with breast cancer : correlation to endostatin levels and clinical outcome. Br J Cancer 2006 ; 94 : 1066-70.
35. Alba E, Llombart A, Ribelles N, Ramos M, Fernandez R, Mayordomo JI, et al. Serum endostatin and bFGF as predictive factors in advanced breast cancer patients treated with letrozole. Clin Transl Oncol 2006 ; 8 : 193-9.
36. Feldman AL, Pak H, Yang JC, Alexander HR, Libutti SK. Serum endostatin levels are elevated in patients with soft tissue sarcoma. Cancer 2001; 91 : 1525-9.
37. Iizasa T, Chang H, Suzuki M, Otsuji M, Yokoi S, Chiyo M, et al. Overexpression of collagen XVIII is associated with poor outcome and elevated levels of circulating serum endostatin in non-small cell lung cancer. Clin Cancer Res 2004 ; 10 : 5361-6.
38. Schneider BP, Miller KD. Angiogenesis of breast cancer. J Clin Oncol 2005 ; 23 : 1782-90.
39. Jain RK, Duda DG, Clark JW, Loeffler JS. Lessons from phase III clinical trials on anti-VEGF therapy for cancer. Nat Clin Pract Oncol 2006 ; 3 : 24-40.
40. O'Reilly MS, Boehm T, Shing Y, Fukai N, Vasios G, Lane WS, et al. Endostatin : an endogenous inhibitor of angiogenesis and tumor growth. Cell 1997 ; 88 : 277-85.
41. Kerbel RS, Klement G, Pritchard KI, Kamen B. Continuous low-dose anti-angiogenic/metronomic chemotherapy : from the research laboratory into the oncology clinic. Ann Oncol 2002 ; 13 : 12-5.
42. Edwards DR, Murphy G. Cancer proteases : invasion and more. Nature 1998 ; 394 : 527-8.
43. Bramhall SR, Schulz J, Nemunaitis J, Brown PD, Baillet M, Buckels JA. A double-blind placebo-controlled, randomised study comparing gemcitabine and marimastat with gemcitabine and placebo as first line therapy in patients with advanced pancreatic cancer. Br J Cancer 2002 ; 87: 161-7.
44. Sparano JA, Bernardo P, Stephenson P, Gradishar WJ, Ingle JN, Zucker S, et al. Randomized phase III trial of marimastat versus placebo in patients with metastatic breast cancer who have responding or stable disease after first-line chemotherapy : Eastern Cooperative Oncology Group trial E2196. J Clin Oncol 2004 ; 22 : 4683-90.
45. Miller KD, Gradishar W, Schuchter L, Sparano JA, Cobleigh M, Robert N, et al. A randomized phase II pilot trial of adjuvant marimastat in patients with early-stage breast cancer. Ann Oncol 2002 ; 13 : 1220-4.
46. Miller KD, Saphner TJ, Waterhouse DM, Chen TT, Rush-Taylor A, Sparano JA, et al. A randomized phase II feasibility trial of BMS-275291 in patients with early stage breast cancer. Clin Cancer Res 2004 ; 10 : 1971-5.
47. Eriksson T, Bjorkman S, Hoglund P. Clinical pharmacology of thalidomide. Eur J Clin Pharmacol 2001 ; 57 : 365-76.

48. Baidas SM, Winer EP, Fleming GF, Harris L, Pluda JM, Crawford JG, et al. Phase II evaluation of thalidomide in patients with metastatic breast cancer. J Clin Oncol 2000 ; 18 : 2710-7.
49. Morabito A, Carillio G, Longo R, Gasparini G. Thalidomide is inactive in heavily pretreated patients with metastatic breast cancer. Cancer J 2005; 11 : 248-51.
50. Herbst RS, Hess KR, Tran HT, Tseng JE, Mullani NA, Charnsangavej C, et al. Phase I study of recombinant human endostatin in patients with advanced solid tumors. J Clin Oncol 2002 ; 20 : 3792-803.
51. Eder Jr. JP, Supko JG, Clark JW, Puchalski TA, Garcia-Carbonero R, Ryan DP, et al. Phase I clinical trial of recombinant human endostatin administered as a short intravenous infusion repeated daily. J Clin Oncol 2002 ; 20: 3772-84.
52. Colleoni M, Rocca A, Sandri MT, Zorzino L, Masci G, Nole F, et al. Low-dose oral methotrexate and cyclophosphamide in metastatic breast cancer: antitumor activity and correlation with vascular endothelial growth factor levels. Ann Oncol 2002 ; 13 : 73-80.
53. Colleoni M, Orlando L, Sanna G, Rocca A, Maisonneuve P, Peruzzotti G, et al. Metronomic low-dose oral cyclophosphamide and methotrexate plus or minus thalidomide in metastatic breast cancer : antitumor activity and biological effects. Ann Oncol 2006 ; 17 : 232-8.
54. Kerbel RS, Viloria-Petit A, Klement G, Rak J. “Accidental” antiangiogenic drugs. anti-oncogene directed signal transduction inhibitors and conventional chemotherapeutic agents as examples. Eur J Cancer 2000 ; 36: 1248-57.
55. Ferrara N. Vascular endothelial growth factor as a target for anticancer therapy. Oncologist 2004 ; 9 (Suppl. 1) : 2-10.
56. Wedam SB, Low JA, Yang SX, Chow CK, Choyke P, Danforth D, et al. Antiangiogenic and antitumor effects of bevacizumab in patients with inflammatory and locally advanced breast cancer. J Clin Oncol 2006 ; 24: 769-77.
57. Kerbel RS. A cancer therapy resistant to resistance. Nature 1997 ; 390 :
335-6.
58. Cobleigh MA, Langmuir VK, Sledge GW, Miller KD, Haney L, Novotny WF, et al. A phase I/II dose-escalation trial of bevacizumab in previously treated metastatic breast cancer. Semin Oncol 2003 ; 30 : 117-24.
59. Ramaswamy S, Rhoades C, Kendra K, Hauger M, Allen J, Jhaveri S, et al. CTEP-sponsored phase II trial of bevacizumab (Avastin) in combination with docetaxel (Taxotere) in metastatic breast cancer. 26th Annual San Antonio Breast Cancer Symposium 2003 (abstr. 224).
60. Pegram M, Yeon C, Ku N, Gaudreault J, Slamon DJ. Phase I combined biological therapy of breast cancer using two humanized monoclonal antibodies directed against HER2 proto-oncogene and vascular endothelial growth factor (VEGF). 27th Annual San Antonio Breast Cancer Symposium 2004 (abstr 3039).
61. Miller KD, Chap LI, Holmes FA, et al. Randomized phase III trial of capecitabine compared with bevacizumab plus capecitabine in patients with previously treated metastatic breast cancer. J Clin Oncol 2005;23:792–9.
62. Cobleigh MA, Langmuir VK, Sledge GW, et al. A phase I/II dose-escalation trial of bevacizumab in previously treated metastatic breast cancer. Semin Oncol 2003;30:117–24.
63. Miller K, Wang M, Gralow J, et al. Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. N Engl J Med 2007;357:2666–76.
64. Robert NJ, Dieras V, Glaspy J, et al. RIBBON-1: randomized, double-blind, placebo-controlled, phase III trial of chemotherapy with or without bevacizumab for first-line treatment of human epidermal growth factor receptor 2-negative, locally recurrent or metastatic breast cancer. J Clin Oncol 2011;29:1252–60.
65. Miles DW, Chan A, Dirix LY, et al. Phase III study of bevacizumab plus docetaxel compared with placebo plus docetaxel for the first-line treatment of human epidermal growth factor receptor 2-negative metastatic breast cancer. J Clin Oncol 2010;28:3239–47.
66. Gray R, Bhattacharya S, Bowden C, Miller K, Comis RL. Independent review of E2100: a phase III trial of bevacizumab plus paclitaxel versus paclitaxel in women with metastatic breast cancer. J Clin Oncol 2009;27:4966–72.
67.Brufsky A, Bondarenko IN, Smirnov V, et al. RIBBON-2: A randomized, double-blind, placebo-controlled, phase III trial evaluating the efficacy and safety of bevacizumab In combination with chemotherapy for second-line treatment of HER2-negative metastatic breast cancer. Cancer Res 2009;69(Suppl.):24 [Abstr 42].
68. Brufsky A, Rivera RR, Hurvitz SA, et al. Progression-free survival (PFS) in patient subgroups in RIBBON-2, a phase III trial of chemotherapy (chemo) plus or minus bevacizumab (BV) for second-line treatment of HER2-negative, locally recurrent or metastatic breast cancer (MBC). J Clin Oncol 2010;28(Suppl.):15s [Abstr 1021].
69.Bear HD, Tang G, Rastogi P, et al. The effect on pCR of bevacizumab and/or antimetabolites added to standard neoadjuvant chemotherapy: NSABP protocol B-40. J Clin Oncol 2011;29(Suppl.) [Abstr LBA1005].

70.von Minckwitz G, Eidtmann H, Rezai M, et al. Neoadjuvant Chemotherapy with or without Bevacizumab: Primary Efficacy Endpoint Analysis of the GEPARQUINTO Study (GBG 44). Cancer Res 2010;70(Suppl.):24 [Abstr S4-6].
71. von Minckwitz G, Eidtmann H, Loibl S, et al. Integrating bevacizumab, everolimus, and lapatinib into current neoadjuvant chemotherapy regimen for primary breast cancer. Safety results of the GeparQuinto trial. Ann Oncol 2011;22:301–6.
72. Gerber B, Eidtmann H, Rezai M, et al. Neoadjuvant bevacizumab and anthracycline–taxane-based chemotherapry in 686 triple-negative primary breast cancers: Seconday endpoint analysis of the GeparQuinto study (GBG 44). J Clin Oncol. 2011;29(Suppl.) [Abstr 1006].
73. Miller KD, O'Neill A, Perez EA, Seidman AD, Sledge GW. Phase II feasibility trial incorporating bevacizumab into dose-dense doxorubicin and cyclophosphamide followed by paclitaxel in patients with lymph node-positive breast cancer: A trial of the Eastern Cooperative Oncology Group (E2104). J Clin Oncol 2008;26:520.
74. Morris PG, Dickler M, McArthur HL, et al. Dose-dense adjuvant Doxorubicin and cyclophosphamide is not associated with frequent short-term changes in left ventricular ejection fraction. J Clin Oncol 2009;27:6117–23.
75. Miller KD, O'Neill A, Perez EA, Seidman AD, Sledge GW. A phase II pilot trial incorporating bevacizumab into dose-dense doxorubicin and cyclophosphamide followed by paclitaxel in patients with lymph node positive breast cancer: a trial coordinated by the Eastern Cooperative Oncology Group. Ann Oncol. 2011.
76. Cameron D, Brown J, Dent R et al., Adjuvant bevacizumab-containing therapy in triple-negative breast cancer (BEATRICE): primary results of a randomised, phase 3 trial, Lancet Oncol 2013, 14, 933-42
77. Wildiers H, Fontaine C, Vuylsteke P, et al. Multicenter phase II randomized trial evaluating antiangiogenic therapy with sunitinib as consolidation after objective response to taxane chemotherapy in women with HER2-negative metastatic breast cancer. Breast Cancer Res Treat 2010;123:463–9.
78. Barrios CH, Liu MC, Lee SC, et al. Phase III randomized trial of sunitinib versus capecitabine in patients with previously treated HER2-negative advanced breast cancer. Breast Cancer Res Treat 2010;121:121–31.
79. Mayer EL, Dhakil S, Patel T, et al. SABRE-B: an evaluation of paclitaxel and bevacizumab with or without sunitinib as first-line treatment of metastatic breast cancer. Ann Oncol 2010;21:2370–6.
80. Crown J, Dieras V, Staroslawska E, et al. Phase III trial of sunitinib (SU) in combination with capecitabine (C) versus C in previously treated advanced breast cancer (ABC). J Clin Oncol 2010;28(Suppl.):18 [Abstr LBA1011].
81. Bergh J, Bondarenko I, Lichinitser M, et al. First-Line Treatment of Advanced Breast Cancer With Sunitinib in Combination With Docetaxel Versus Docetaxel Alone: Results of a Prospective, Randomized Phase III Study. J Clin Oncol 2012; 30, 1-9
82. Robert NJ, Saleh MN, Paul D, et al. Sunitinib plus paclitaxel versus bevacizumab plus paclitaxel for first-line treatment of patients with advanced breast cancer: a phase III, randomized, open-label trial. Clin breast cancer 2011;11:82–92.
83. Wilhelm SM, Adnane L, Newell P, Villanueva A, Llovet JM, Lynch M. Preclinical overview of sorafenib, a multikinase inhibitor that targets both Raf and VEGF and PDGF receptor tyrosine kinase signaling. Mol Cancer Ther 2008;7: 3129–40.
84. Moreno-Aspitia A, Morton RF, Hillman DW, et al. Phase II trial of sorafenib in patients with metastatic breast cancer previously exposed to anthracyclines or taxanes: North Central Cancer Treatment Group and Mayo Clinic Trial N0336. J Clin Oncol 2009;27:11–5.
85. Bianchi G, Loibl S, Zamagni C, et al. Phase II multicenter, uncontrolled trial of sorafenib in patients with metastatic breast cancer. Anticancer Drugs 2009;20:616–24.
86. Gomez P, Roche H, Costa F, et al. Overall Survival Data from SOLTI-0701: A Multinational, Double-Blind, Placebo-Controlled, Randomized Phase 2b Study Evaluating the Oral Combination of Sorafenib and Capecitabine in Patients with Locally Advanced or Metastatic HER2-Negative Breast Cancer. Cancer Res 2010;70(Suppl.):24 [Abstr P2-16-01].
87. Hudis C, Tauer KW, Hermann RC, et al. Sorafenib (SOR) plus chemotherapy (CRx) for patients (pts) with advanced (adv) breast cancer (BC) previously treated with bevacizumab (BEV). J Clin Oncol 2011;29(Suppl.) [Abstr 1009].
88. Bondarde S, Kaklamani V, Prasad Sahoo T, et al. Sorafenib in Combination with Paclitaxel as a First-Line Therapy in Patients with Locally Recurrent or Metastatic Breast Cancer: Overall Survival Results from a Double-Blind, Randomized, Placebo-Controlled, Phase 2b Trial. Cancer Res 2011;70(Suppl.):24 [Abstr P2-16-03].
89. Gradishar WJ, Dalenc F, Kaklamani VG, et al. Multivariate analysis (MVA) of progression-free survival (PFS) in two phase IIb, multinational, double-blind, randomized, placebo (PL)-controlled trials evaluating sorafenib (SOR) plus standard chemotherapy in patients (pts) with HER2-negative locally advanced or metastatic breast cancer (BC). J Clin Oncol 2010;28(Suppl.):15s [Abstr 1073].

90. Boer K, Lang I, Llombart-Cussac A, et al. Vandetanib with docetaxel as second-line treatment for advanced breast cancer: a double-blind, placebo-controlled,randomized Phase II study. Invest New Drugs 2010.
91. Rugo HS, Stopeck AT, Joy AA, et al. Randomized, placebo-controlled, double-blind, phase II study of axitinib plus docetaxel versus docetaxel plus placebo in patients with metastatic breast cancer. J Clin Oncol 2011;29: 2459–65.
92. Otrock ZK, Mahfouz RA, Makarem JA, Shamseddine AI. Understanding the biology of angiogenesis: review of the most important molecular mechanisms. Blood Cells Mol Dis 2007;39:212–20.
93. Cao Y, Liu Q. Therapeutic targets of multiple angiogenic factors for the treatment of cancer and metastasis. Adv Cancer Res 2007;97:203–24. Shibuya M. Vascular endothelial growth factor-dependent and -independent regulation of angiogenesis. BMB Rep 2008;41:278–86.
94. Angelo LS, Kurzrock R. Vascular endothelial growth factor and its relationship to inflammatory mediators. Clin Cancer Res 2007;13:2825–30.
95. Miller VM, Duckles SP. Vascular actions of estrogens: functional implications.Pharmacol Rev 2008;60:210–41.
96. Relf M, LeJeune S, Scott PA, et al. Expression of the angiogenic factors vascular endothelial cell growth factor, acidic and basic fibroblast growth factor, tumor growth factor beta-1, platelet-derived endothelial cell growth factor, placenta growth factor, and pleiotrophin in human primary breast cancer and its relation to angiogenesis. Cancer Res 1997;57:963–9.
97. Shibuya M. Vascular endothelial growth factor-dependent and -independent regulation of angiogenesis. BMB Rep 2008;41:278–86.
98. Braga et al. Does hypoxic response mediate primary resistance to sunitinib in untreated locally advanced breast cancer? Submitted
99. Kerbel RS. Tumor angiogenesis. N Engl J Med 2008;358:2039–49.
100. Kerbel RS. Antiangiogenic therapy: a universal chemosensitization strategy for cancer? Science 2006;312:1171–5.
101. Lee K, Qian DZ, Rey S, Wei H, Liu JO, Semenza GL. Anthracycline chemotherapy inhibits HIF-1 transcriptional activity and tumor-induced mobilization of circulating angiogenic cells. Proc Natl Acad Sci U S A 2009;106:2353–8.
102. Rapisarda A, Hollingshead M, Uranchimeg B, et al. Increased antitumor activity of bevacizumab in combination with hypoxia inducible factor-1 inhibition. Mol Cancer Ther 2009;8:1867–77.
103. Dellapasqua S, Bertolini F, Bagnardi V, et al. Metronomic cyclophosphamide and capecitabine combined with bevacizumab in advanced breast cancer. J
Clin Oncol 2008;26:4899–905.

Chapter 7: Does hypoxic response mediate primary resistance to sunitinib in untreated locally advanced breast cancer?

Conception and design: Sofia Braga, José-Luis Passos-Coelho

Provision of study materials or patients: Sofia Braga, Margarida Brito, Pedro Sanchez, Lurdes Orvalho, Lucilia Salgado, Sérgio Dias, José-Luis Passos-Coelho

Collection and assembly of data: Sofia Braga, Joana Vaz

Data analysis and interpretation: Sofia Braga, Joana Vaz

Manuscript writing: Sofia Braga, Joana Vaz, José Pereira-Leal, José Luis Passos-Coelho

Final approval of manuscript: All authors

Funding: Sofia Braga was funded during three years (from October 2008 until October 2011) by the Gulbenkian Foundation and worked at the Computational Genomics Laboratoty (CGL) at Instituto Gulbenkian de Ciência (IGC).
The Clinical Trial was funded by Pfizer

Ethical Committee approval: Comissão de Ética para a Investigação Clínica (Portuguese National Ethics Committee)

Does hypoxic response mediate primary resistance to sunitinib in untreated locally advanced breast cancer?

Sofia Braga, MD [1 2 7], Joana Cardoso, PhD [2], Saudade André, MD [3], Margarida Brito, MD [1], Pedro Sánchez MD [1], Lurdes Orvalho, MD [4 8], Lucilia Salgado, MD [5], Sérgio Dias, PhD [6], José Pereira-Leal, PhD [3], José Luis Passos-Coelho, MD [1 9]

[1] Medical Oncology Department, Instituto Português de Oncologia, Lisboa, Portugal
[2] Computational Genomics Laboratory, Instituto Gulbenkian Ciencia, Oeiras, Portugal
[3] Pathology Department, Instituto Português de Oncologia, Lisboa, Portugal
[4] Radiology Department, Instituto Português de Oncologia, Lisboa, Portugal
[5] Serviço Medicina Nuclear Medicine Department, Instituto Português de Oncologia, Lisboa, Portugal
Oncology Department, Hospital Universitario Reina Sofía, Córdoba, España
[6] Instituto de Medicina Molecular, Centro Académico de Lisboa, Lisboa, Portugal
[7] Currently at José de Mello Saúde, Lisboa, Portugal (Corresponding author)
[8] Currently at Radiology Department, Hospital da Luz, Lisboa, Portugal
[9] Currently at Oncology Department, Hospital Beatriz Angelo, Lisboa, Portugal

Statement of translational relevance:

We have witnessed the rise and fall of antiangiogenic therapy in breast cancer (BC). Nevertheless, clinical remissions were observed in patients and we were interested in studying the activity of antiangiogenic drugs in BC. Inefficacy of sunitinib was observed in mouse models of metastatic BC, where evidence of enhanced metastasis was reported, and lack of efficacy of sunitinib-docetaxel combination was recently reported in a phase III clinical trial. Our aim was to understand the mechanisms and predictors of response to sunitinib in BC in a cohort of patients with untreated locally advanced or operable BC treated with an upfront window of single agent sunitinib, followed by the combination of sunitinib and docetaxel. We observed primary resistance to angiogenic therapy in 4 of 12 patients likely mediated by an adaptive transcriptional response to hypoxia in these resistant tumors. In BC patients this is the first demonstration of primary resistance to antiangiogenic therapy.

Abstract

Purpose

The antiangiogenic drug sunitinib has never been evaluated as single agent in untreated BC patients. We aimed to characterize the activity of sunitinib, alone and with docetaxel, in untreated locally advanced or operable BC and to uncover the mechanisms of response.

Experimental design

Twelve patients were treated with an upfront window of sunitinib (50 mg/day, 14 days) followed by four cycles of sunitinib (37,5mg/day, 14 days, q3wk) plus docetaxel (75mg/m2 q3wk). Response, resistance and toxicity were evaluated according to standard clinical parameters, magnetic resonance imaging, positron emission tomography, pathology characterization and gene expression profiling.

Results

We detected primary resistance to sunitinib upfront window in untreated BC, as evidenced by four non-responding patients. At surgery, five patients had viable disease in the breast and axilla, four had viable tumor cells in the breast alone and three were taken off study and thus not evaluated, due to unacceptable toxicity. Early functional imaging was useful in predicting response. There were no pathologic complete responses (pCR). Comparison of gene expression profiling tumor data between early responders and non-responders allowed us to identify the up-regulation of VEGF and angiogenic pathways in non responders. Specifically, in tumors resistant to the single-agent sunitinib we detected a transcriptional response to hypoxia characterized by over-expression of several HIF1α target genes.

Conclusion

In this report of single-agent sunitinib treatment of untreated localized BC patients, we found molecular evidence of primary resistance to sunitinib likely mediated by up-regulation of hypoxia responsive genes.

Introduction

Neoadjuvant chemotherapy is standard treatment for inflammatory, locally advanced and large operable BC. Randomized trials comparing neoadjuvant to adjuvant chemotherapy (CT) demonstrated similar survival outcome for large operable lesions [1 2, 3]. Neoadjuvant therapy has three advantages: 1) 10–

20% increased rate of breast preservation, 2) in-vivo assessment of chemosensitivity and 3) early indication of overall survival because survival correlates with pathologic complete response (pCR) rate.

Angiogenesis is known to play a role in BC growth and metastatic spread. In primary BC the expression of vascular endothelial growth factor receptor (VEGFR) and platelet-derived growth factor receptor beta (PDGFR) are associated with worse prognosis [4, 5]. This led to the concept of early antiangiogenic therapy before the development of new cancer-induced vessels..

Sunitinib malate (Sutent; Pfizer, New York, NY) is an oral small molecule inhibitor of tyrosine kinases coupled to VEGFR, PDGFR, stem cell factor receptor and colony-stimulating factor-1 receptor. Sunitinib is approved as first-line therapy of metastatic renal cancer, where it targets the constitutively activated angiogenic pathway, as second-line therapy after imatinib, for inoperable gastrointestinal stromal tumors (GIST), to inactivate the mutant c-KIT receptor and for unresectable locally advanced or metastatic pancreatic neuroendocrine tumors.

In preclinical studies using the human BC MX-1 xenograft model, sunitinib in combination with docetaxel, doxorubicin or fluorouracil enhanced the activity of chemotherapy and increased survival [6]. In a phase II trial in metastatic BC, sunitinib showed activity as a single-agent in heavily pretreated patients (N=64; objective response rate (ORR), 11%; median time to progression (TTP), 10 weeks) [7].

The effect of sunitinib in combination with docetaxel in patients with human epidermal growth factor receptor 2 (HER2)/*neu*-negative metastatic BC was studied in a phase Ib and a phase III trial [8, 9]. In both trials, the therapeutic regimen was sunitinib (37.5 mg/d 2 weeks starting on day 2 every 3 weeks) in combination with docetaxel (75 mg/m² day 1 every 3 weeks). The exploratory study (Phase Ib) gave encouraging results (N=22; ORR, 74%; median progression-free survival (PFS), 8.7 months). In the randomized phase III trial 296 patients were assigned to combination therapy and 297 patients were assigned to docetaxel monotherapy. Median PFS times were 8.6 and 8.3 months with combination therapy and monotherapy, respectively (hazard ratio, 0.92; one-sided P = .265). The ORR was significantly higher with the combination (55%) than with monotherapy (42%; one-sided P = .001). Duration of response (7.5 *vs* 7.2 months) and overall survival (OS, 24.8 *vs* 25.5 months, one-sided P = .904) were similar in both arms. This clinical trial was negative for the primary endpoint [8, 9]

We report the results of a single center phase II neoadjuvant clinical trial where an upfront window of single-agent sunitinib (50 mg/d, 14 days) was used in untreated locally advanced or operable BC patients, followed by a combination of sunitinib and docetaxel, similar to the metastatic BC trials combination. To evaluate drug response, we used a combination of clinical, radiological, pathological, molecular and genomic techniques. This trial represents a unique opportunity, since it is, to our knowledge, the only one conducted with single agent sunitinib in non-metastatic BC.

Patients and methods

Study design and clinical evaluation

The trial was approved by the institutional review board, the National Ethics Committee and was registered under EudraCT number 2007-007257-31.

Patients signed an informed consent stating all aspects of the trial, including extra biopsies and breast surgery. This is an investigator designed trial supported by a research grant from Pfizer. The trial design included the administration of sunitinib in an upfront window of 14 days, followed by 4 cycles of combination therapy with docetaxel and sunitinib in patients with previously untreated locally advanced or large operable BC.

Patients underwent a baseline tumor biopsy (t1, day 1), a second biopsy, after the sunitinib upfront window (t2, day 15), and a final tumour evaluation at surgery (t3, day 112±2). The biopsy was performed at day 15, the day after the last dose of the sunitinib upfront window, after reports [10] of noticeable reduction in tumour size during the treatment period and tumour regrowth during the off period. Similarly, patients underwent breast Gadolinium-enhanced magnetic resonance imaging (MRI) to evaluate tumour vascularization and 18F-fluorodeoxyglucose positron emission tomography (FDG-PET) scan to evaluate BC cell survival at t1 (baseline) and at t2 (day 15), before the second biopsy.

Pre-treatment evaluation included electrocardiography and left ventricular ejection fraction (LVEF) assessment by radionuclide angiography, thyroid function tests (fT4, fT3 and TSH) and BC staging. During chemotherapy, besides routine blood biochemistry, thyroid function was also evaluated. Similarly, at end of study (or at patient withdrawal) evaluation of LVEF and thyroid function were performed. Toxicities were assessed using the CTCAE v3.0 [11].

Patient Eligibility

Patients were eligible if: 1) they had histologically or cytologically proven newly diagnosed untreated invasive BC (independent of estrogen receptor (ER), progesterone receptor (PgR) and Human Epidermal

Growth Factor Receptor 2 (HER2) status); 2) a breast tumor diameter > 3 cm by caliper measurement and/or cN2-3 disease plus a palpable breast tumor; 3) recommendation of neoadjuvant CT by the institutional multidisciplinary conference. Multifocal and multicentric breast tumours were allowed as long as only two tumour foci were identified, since two sequential tumour samples were to be collected from each tumour focus.

Other eligibility criteria were age ≥18 years, no pregnancy or lactation, Eastern Cooperative Oncology Group performance status of 0, 1 or 2 and adequate liver, kidney, cardiac and hematopoietic organ function as defined by blood tests and cardiac examinations.

Treatment protocol

The treatment protocol timeline is shown in Figure 1. The initial upfront window consisted in oral administration of single agent sunitinib 50 mg/d for 14 days, followed by 7 days off drug. On day 22, pre-operative docetaxel (75 mg/m² every 3 weeks for 4 cycles), combined with sunitinib (37.5 mg/day for 14 days starting, 7 days off) starting one day after docetaxel for 4 cycles, were given. Both treatments were stopped on study day 100, 11 weeks after beginning taxane preoperative chemotherapy. Two additional drug-free weeks were allowed before surgery. After surgery, patients were treated with four cycles of adjuvant FEC100 CT (5-Fluouracil 500mg/m², Epirubicin 100 mg/m² and Cyclophosphamide 500 mg/m² every 3 weeks). Locoregional radiation therapy was administered according to institutional guidelines. Patients with hormone receptor positive tumors started tamoxifen or an aromatase inhibitor. Patients with Her2-positive tumors received trastuzumab for a year.

Clinical evaluation

Patients were evaluated for clinical response and toxicity after upfront sunitinib and before each CT cycle at days 15 (t2), 22, 43, 64 and 85. Tumours were measured by caliper in two perpendicular diameters and the greatest diameter was considered for evaluation. A 10%-30% reduction in the greatest diameter of the primary tumor at 22 days was defined as minor response (MR). A clinical partial response (cPR) at t2 was considered when the decrease in greatest diameter of the primary tumor was ≥ 30%. Progression (PD) was established if the primary tumor increased in size or new tumor lesions were observed. Otherwise, was considered stable disease (SD).

Pathology and other molecular studies

Evaluation of tumor biopsies and surgical specimen evaluation were performed by an investigator blinded to clinical data (S.A.). Three biological samples from each patient (t1, t2 and t3) were studied by histopathology. Surgical samples (t3) were evaluated according to ypTNM. Tumor samples were classified according to standard pathology criteria for histological subtype, grade of differentiation, Ki67 staining, hormonal receptor expression and HER2 expression and/or amplification. Primary antibodies: anti-Ki67 (clone MIB-1; Dako cat. 7240, 1:300), estrogen receptor (clone SPI; Ventana Roche cat. 790-4324), progesterone receptor (clone 1E2; Ventana Roche cat. 790-2223) and HER2 expression and/or amplification was evaluated by pathway HER-2/neu (clone 4B5; Ventana Roche cat. 780-001) in Ventana BENCHAMRK ULTRA instrument.

Sample collection for molecular studies

Tumor tissue was obtained by image guided biopsy prior to treatment (t1), after 14 days of sunitinib (t2), and from the definitive surgical specimen (t3). Tissue was flash-frozen in liquid nitrogen and stored at –80°C. Hematoxylin and eosin stained sections of formalin-fixed paraffin embedded specimen were used to evaluate histological characteristics.

Total RNA was extracted from frozen material with RNeasy Mini Kit with RNase-Free DNase Set “on column” DNA digestion (Qiagen), according to manufacturer’s instructions. RNA concentration was determined on a RNA quantity and quality was respectively examined with a Nano Drop ND-1000 UV-Vis Spectrophotometer (Nano- Drop Technologies) and a RNA 6000 Nano Assay Kit (Agilent Technologies) in a Bioanalyser 2100 (Agilent Technologies). Only high-quality total RNA samples with a 260/280 ratio > 1.8 and containing at least 100 ng were further processed.

Expression profiling

Sample labeling and GeneChip processing was performed at the Affymetrix Core Facility 144 (Instituto Gulbenkian de Ciência, Oeiras, Portugal; http://www.igc.gulbenkian.pt/node/view/131). Total RNA (100 ng) was used to generate cDNA with the Ambion WT Expression Kit for Affymetrix GeneChip Whole Transcript WT Expression Arrays [12], (Applied Biosystems, Carlsbad, CA). Biotin labeled cRNAs, produced with the Affymetrix GeneChip WT Terminal Labeling kit according to Hybridization User Manual [13] were hybridized to Human Gene 1.0 ST arrays (Affymetrix, Santa Clara, CA). Liquid handling steps were performed with GeneChip Fluidics Station 450 and arrays scanned with GeneChip Scanner 3000 7G (Affymetrix, Santa Clara, CA) using Command Console v1.1. Raw data is publicly available at GEO [14] under the series number number GSE xxxxxxxx.

Microarray data analysis

Data analysis was performed with R Statistical Computing software complemented with Bioconductor packages**.** Microarrays pre-processed using aroma.affymetrix v2.4.0 package (http://aroma-project.org/). Heatmaps were plotted using "gplots" [15].

Differential expression analysis was obtained with limma package [16] for comparison 1, diagnosis (t1) versus single agent sunitinib (t2) collected samples; comparison 2, non responding versus responding t2 tumor samples and comparison 3, t2 versus surgery (t3) collected samples. Selection of differentially expressed genes (DEGs) were based on limma output parameters LODS >0 (log-odds) and log2-ratio ≥ +0.58 or ≤ 0.58.

Gene set enrichment analysis (GSEA) of GO biological processes (GO-BP) among filtered DEGs was done with GSEA tools from InnateDB (http://www.innatedb.ca/).

DCE-MRI

Two MRIs were performed, at days 1 and 15, on a 1.5 T system (Siemens, Medical Solutions, USA, IC) using a double-breast surface coil. The protocol corresponds to a 3D gradient-echo, dynamic imaging before and after intravenous contrast administration (gadolinium dimeglumine pentatate) and after a subtraction technique. Regions of interest (ROIs) were drawn around tumour areas. Wash in and wash out in the same point of the tumour was measured to quantify vascular permeability. Tumors were measured in their two longest perpendicular axes and were reassessed at day 15 reported using RECIST [11].

FDG-PET

Two FDG-PET examinations were performed, on days 1 and 15. Imaging was performed on an ECAT-ACCEL-LSO PET (Siemens, Medical Solutions, USA, IC) one hour after iv injection of 370 MBq 18F-fluorodeoxyglucose. The PET Scan is equipped with a lutetium oxyorthosilicate crystal (64 crystals/block), with an axial extent of 162 mm and a spatial resolution of 6 mm at 1 cm. Standardized Uptake Value (SUV) measurement of glucose metabolism was analyzed. Response was defined as a reduction of 20% or more (maximum values of SUV) in all lesions present in the baseline timepoint (t1).

Results

Patient and tumor characteristics

Patients and tumor characteristics are summarized in Table 1. Twelve women (aged 33 to 60 years with a median 42.5 years) were included; all but one (african) were caucasians. All tumors were diagnosed by self examination. All tumors measured more than 5 cm (≥T3) and four of them were T4 tumors. Four candidates had inoperable lesions, the remaining were candidates for mastectomy at presentation.

Toxicity

The therapeutic regimen used in this trial was considerably toxic. Every patient in the trial experienced at least one grade 2 adverse event (Supplementary table S1).

During the upfront window of single agent sunitinib the most frequent toxicities were asthenia (10 patients, grade 2 in two) and disgeusia (9 patients). There were no grade 3 ou 4 toxicities during the upfront window.

During the administration of sunitinib and docetaxel there were nine episodes of grade 3 adverse events, with 75% of the patients experiencing at least one grade 3 toxic event. The most frequent grade 3 adverse events were asthenia (two events), hypersensitivity reactions (two events) and gastrointestinal toxicity (three events: two mucositis and one diarrhea). Two patients were admitted for grade 3 mucositis with dehydration and hypotension.

Three patients were taken off study during combined treatment; one per patient request (patient number 2), and two due to serious adverse events (patients number 11 and 12). Patient number 2 withdrew consent before the second docetaxel cycle (at cycle 1 day 21), due to grade 3 asthenia. Patients 11 and 12 presented in the same day with grade III anaphylactic reactions during the second administration of docetaxel. Both had received premedication with prednisolone 50 mg po in the evening prior and in the morning of treatment and dexametasone 20 mg iv just before docetaxel. No other unexpected hypersensitivity reactions in other patients treated with docetaxel the same day or with the same batch in other days were reported. These patients were withdrawn from the trial and further treated outside protocol. The funder, Pfizer, was informed of the anaphylactic events with the request for further information on similar events reported in the, at the time, ongoing phase III study (with a docetaxel-sunitinib arm). Pfizer recommended temporary closure of recruitment to the trial, which became definitive based on the efficacy analysis of the phase III study data and closure of the sunitinib development program in BC.

Efficacy and Outcome

Clinical efficacy

The clinical assessment after upfront 14 days course of sunitinib treatment is shown in Table 1, Figure 2 and Supplementary S2. Eight tumors regressed after the short course of single agent sunitinib (1 cPR, 7 MR). The largest tumor size decreased 1 cm in three patients, 2 cm in three patients, 3 cm in one patient and 4 cm in one patient. Four tumors remained unchanged and no cases of disease progression were observed. The median decrease in largest diameter was 20% (range, 0%-60%). In the five patients with clinically detectable axillary nodes, the largest diameter reduced more than 30% (cPR) in two patients and in three remained unchanged.

The three patients that were taken off protocol could not be assessed for response at surgery. However they were assessed for response at study withdrawal and all had evidence of reduction in tumor size. The patient that withdrew consent after the first cycle of docetaxel-sunitinib had a MR and the other two had cPR at withdrawal which was after the third cycle of chemotherapy.

In the evaluation performed before surgery, the remaining nine patients that completed the study protocol, all had clinical evidence of response. Four patients had no palpable tumor in the breast or axilla (cCR) and five had cPR.

DCE-MRI assessment

The evaluation of breast tumor size by MRI after single agent sunitinib (t1 versus t2) showed reduction of the breast tumor lesion in all but one patient, as shown in figure 2B. However, the reductions in size were all within the one cm range except for one patient with a 3.5 cm reduction in the largest tumor diameter.

FDG-PET assessment

In the initial PET scan all patients had primary breast tumor 18F-FDG uptake, ten patients had axillary uptake and four patients had thoracic uptake. The later was either in internal mammary nodes or mediastinal nodes, with an SUV ranging from 1.8 to 3.4. The protocol response criteria of at least 20% decrease in SUV uptake in all lesions was documented in five patients alone (patients number 3, 4, 9, 10 and 11). Graphical changes in SUV are shown in Figure 2C and D and Supplementary Table 2.

Overall assessment at 15 days (t2)

Activity of single agent sunitinib was defined as a clinical decrease of ≥1 cm in tumor largest diameter size and either a decrease in tumor size by MRI or a decrease in ≥ 20% metabolic activity with PET. Using such criteria there were eight responders (patients 1, 3, 4, 6, 9, 10, 11 and 12) and four non-responders (patients 2, 5, 7 and 8). These last four patients were considered to have primary resistance to sunitinib.

Surgical assessment and follow-up

All evaluable patients underwent local treatment by surgery, six with breast conservation and three with mastectomy. Axillary dissection was performed in all patients. The three patients that were treated off protocol (during sunitinib docetaxel combination therapy) eventually underwent mastectomy. All patients enrolled in this trial are followed at the recruitment institution according to guidelines. Patients are currently alive without evidence of BC or major toxicity, with a median follow-up of four years.

Pathology

All BCs were invasive carcinomas of no special type (NST), intermediate or high grade; five were HER2-positive (three were also ER positive), five ER-positive HER2-negative and two were triple negative. There was one inflammatory BC.

All nine patients underwent breast surgery. Only two patients had residual tumor cells in the breast. One had a 22 mm lesion (patient 4) and another a 12 mm lesion with less than 10% viable cells (patient 6), respectively. Seven patients had involved axillary lymph nodes in the axillary dissection specimen, these were scored at t3 as non responders.

Serial scoring of nuclear Ki67 staining is shown in Figure 3. In the initial biopsy, at diagnosis (t1), eight tumors had more than 35% Ki67 positive tumor cells (“Ki67 high” tumors); the other four had Ki67 staining below 4% (“Ki67 low” tumors). In all patients but one, Ki67 staining decreased at day 15 (t2), after single agent sunitinib. In six of the eight “Ki67-high” tumors, Ki67 expression decreased by ≥ 40%. Patient 5, a non reponder, increased from 60 to 90% Ki67 positivity. At surgery, all tumors further decreased Ki67 nuclear staining with all but one tumor with Ki67 staining < 10%.

Gene expression

Expression profiling on tumor material collected at the three timepoints was used to obtain an unbiased view of sunitinib response. Several tumor biopsies and specimens had no frozen sample available or RNA with insufficient quality for expression analysis. Were include in the analysis, at diagnosis (t1), replicated material from patients 4, 5, 6, 7 and 11 (patient 10 had no replica), at day 15 (t2) replicated material from patients 5, 7, 11 and 12 (patient 4 had no replica) and at surgery (t3) replicated material from patients 7, 9, 10, 11 and 12 (patient 6 had no replica).

We performed differential expression analysis between t1 and t2 samples, between “responders” and “non-responders” to single agent sunitinib (t2 samples) and before and after combination treatment (t2 versus t3 samples).

Between t1 and t2, after 14 days of treatment with single agent sunitinib, DEGA results highlighted 85 significant DEGs (Figure 4) - 59 genes with lower expression and 26 genes with increased expression (Figure 4A). Among the downregulated genes, we have found TIMP3 (tissue inhibitor of metalloproteinase 3), the metalloproteinase ADAMSTS12, DLL4 (delta-like 4), a component of the Notch pathway, that is known to be affected by antiangiogenic drugs [17] and FLT1 (vascular endothelial growth factor receptor 1), one of the transmembrane receptors coupled to a tirosine kinase and a target of sunitinib[18]. The overexpressed set of genes contained mainly immunoglobulins and inflammatory mediators.

Interestingly, gene set enrichment analysis (GSEA) with GO biological processes (GOBP) using the set of underexpressed genes, revealed among the top 20 GOBP significant categories the negative regulation of blood vessel endothelial cell migration, cellular response to bone morphogenic protein stimulus, regulation of notch signaling pathway, positive regulation of mitogen activated protein (MAP) kinase activity previously [17] associated with anti angiogenic treatment (Figure 4B). Also significant among selected GOBP were processes that can be linked directly to manipulation of vascularization such as angiogenesis, regulation of vascular endothelial growth factor receptor signaling pathway, cell adhesion, notch signaling pathway and negative regulation of epidermal growth factor-activated receptor activity (Figure 4B). Among the processes enriched in the set of overexpressed genes we observed several GOBP mainly linked to immunological pathways and inflammatory processes (Figure 4B).

The most relevant expression analysis is the comparison between responding (patients 4, 11 and 12) and non-responding tumors (patients 5 and 7) to sunitinib (t2). In total, 147 genes were selected by the statistical significance cutoff, of which 110 genes lowered expression and 37 genes increased expression (Figure 5A). GSEA highlighted the energy metabolism as top GOBP categories among upregulated genes (Figure 5B). Overexpression of the glycolytic enzymes (ENO2, enolase 2; GPI, glucose 6 phophate isomerase, and HK2, hexokinase 2) and the glucose transporter 1 gene (SLC2A1) which are indicative of a shift towards anaerobic metabolism in the tumours resistant to the anti-angiogenic drug. In such tumors, the upregulation of VEGF and IL8 is also indicative of rebound angiogenic signaling. We also detected an increased expression of the anti adhesive transmembrane protein podocalyxin-like 1 (PODXL), a known independent predictor of worse outcome in breast cancer [19].

The t2 versus t3 comparison (response to combined sunitinib-docetaxel therapy), had more pronounced effects in terms of DEGs. In total 1569 genes significantly changed their expression, with 337 and 1232 genes being respectively less and more transcribed (Figure 6A). We observed a profound downregulation in genes and GOBPs induced by CT, similar to prior reports in BC literature[20] namely a profound downregulation of proliferation, cell division and mitosis (Figure 6B). Upregulation of genes and associated GOBPs related with cell adhesion, angiogenesis and wound healing (Figure 6B) were observed.

Discussion

Neoadjuvant chemotherapy is the ideal clinical experiment to test new systemic treatments. This is, to the best of our knowledge, the first neoadjuvant trial of sunitinib in BC. The upfront window of 14 days of sunitinib provides the only existing data of administration of sunitinib as a single-agent in newly-diagnosed untreated BC. Unfortunately, due to premature study closure only a limited number of paired tumor samples were available for several analyses (e.g. gene expression). The closure of the clinical research program of sunitinib in BC means that the herein presented data is a unique opportunity to understand resistance mechanisms to sunitinib in BC.

The patients enrolled in the trial are young women with advanced stage non-metastatic BC with tumors larger than 5 cm, reflecting the institution recruitment profile (table 1). The treatment was toxic with 8 of 12 patients experiencing grade 3 toxicity events (Supplementary Table S1). We were particularly struck by the severe hypersensitivity reactions to docetaxel administration which we postulate may be exacerbated by sunitinib co-treatment, although not reported in the phase III metastatic breast cancer trial [8]. Regarding the constitutional, gastroenterological and hematological toxicity, while reported with both single agent docetaxel and sunitinib, they likely represent an additive toxicity effect.

The clinical evaluation performed at t2 showed the expected results when compared to other trials evaluating targeted agents with a short therapeutic course in untreated locally advanced BC [21]. It has been postulated that anti-angiogenic agents such as sunitinib might be more efficacious in early stage disease, which is likely more dependent on neo-vessel formation, compared to advanced stage disease. In the phase III metastatic BC trial by Bergh *et al.* the reported ORR was 52% [8]. In our trial all patients had some evidence of tumor shrinkage, there were only 4 cPR out of 9 evaluable patients and no pathological complete response (pCR) were observed. However, all patients had BC over 5 cm in diameter. This may also explain

the contrast with the reported 20 to 30% pCR in trials of more prolonged neoadjuvant anthracycline-taxane combination chemotherapy [1, 22-24].

A decrease in Ki67 tumor cell nuclear staining, a marker of cell proliferation, is an established early predictor of response to neoadjuvant systemic therapy [25, 26]. Indeed, the only patient (P 5) with an increase in Ki67 staining at t2 (Figure 3) was a "non responder". At time t2, MRI results (Figure 2B) do not add information to the clinical assessment while PET (Figure 2C and D) identified two early "non-responders" (P2 and P5) by increased axillary uptake.

The gene expression results suggest potential mechanisms involved in BC sunitinib activity. The downregulated genes in the sunitinib upfront window are representative of the biological processes anticipated to be affected by tirosine kinase inhibition with sunitinib - angiogenesis, endothelial cell migration, negative regulation of blood vessels, Notch pathway and MAP kinase activity - which have been described in similar clinical experimental contexts [18]. The t2 comparison between responders and non responders to single agent sunitinib, although limited by a small number of samples, suggests that resistance may be related to an hypoxia generated transcriptional response. Thus the hypoxia responsive elements: the glycolytic enzymes, pointing to a shift to anaerobic metabolism and the glucose transporter that enables glucose to enter cells as well as to VEGF. It has been shown that 1 to 1.5% of the genome is transcriptionally responsive to hypoxia [27]. In a large meta-analysis of BC, the hypoxia metagene could be replaced by three genes: (hypoxia inducing factor 1 alpha HIF1α, glucose transporter type 1 GLUT1 and vascular endothelial growth factor VEGF), considered most informative [28]. Two of these genes are overexpressed in sunitinib-resistant tumors: the glucose transporter (GLUT1) and VEGF. Regarding other genes, not previously linked to hypoxia, podocalyxin and IL8 merit attention. Podocalyxin (PODXL), previously associated with worse prognosis in breast cancer is an anti-adhesion molecule [19]. Low oxygen tension stimulates angiogenesis which requires tissue remodeling that could provide a link between this molecule and angiogenesis. As reported in renal cell carcinoma cell lines [18], upregulation of IL8 may be one of the mechanisms of resistance to sunitinib. IL8 enhances chemotaxis and infiltration or activation of resident inflammatory cells which has been associated with anti angiogenic therapy resistance [18]. In mice xenografts of lung cancer, circulating bone marrow-derived cells are massively recruited to tumor beds after angiogenic treatments due to vessel disruption and hypoxia [29]. In patients with glioblastoma multiforme that progressed after an anti angiogenic drug, the serum levels of inflammatory mediators and circulating endothelial cells (CEC), precursors of new vessels, are increased [30]. A similar increase in CECs has been reported in patients with renal cell cancer progressing on sunitinib [31].

Lastly, when the effect of the combination of sunitinib and docetaxel, was studied (t2 vs. t3), we found similar effect on gene expression and cellular pathways to those reported by Lindstrom *et al.* [20]: downregulation of cell division and upregulation of angiogenesis and survival pathways. Rebound angiogenesis through alternative pathways has been reported [32], albeit not in BC, upon anti-angiogenic treatment. Since one would not expect nor has it been reported, angiogenic stimulation induced by taxane chemotherapy treatment [33] may be a consequence of sunitinib.

We found evidence, in some patients, of primary resistance of BC to the sunitinib upfront window. After the first two weeks, docetaxel was added to sunitinib. Nevertheless, the combination was less efficacious than might be expected, possibly because docetaxel was administered at 75mg/m2, instead of 100mg/m2.

The lower efficacy is concordant with the Phase III data [8]. In mouse models, Kerbel and collaborators, were able to demonstrate, in several BC settings, resistance and increased metastasis in sunitinib treated animals [34, 35]. However, with a median follow-up of four years, we have no evidence of this in our patients, possibly because patients were treated with taxanes and anthracyclines, either before or after surgery.

While this series supports the inefficacy of sunitinib in BC, it has the novelty of including treatment-naive patients and suggesting a mechanism of resistance. BC resistance to sunitinib can be shown upon two weeks of administration in untreated patients, this suggests a rapid adaptation with an impact on gene expression. We found evidence that the mechanisms of resistance to sunitinib include transcription of glycolytic enzymes, a glucose transporter, and VEGF production, which are known transcriptomic adaptations to the hypoxic environment, here created by sunitinib. This trial was prematurely closed, nevertheless, several hypotheses can be generated by this work. Is the shift to the glycolytic phenotype, the hypoxic adaptation generated by sunitinib, a harbinger of non response to other drugs, namely to chemotherapy, or will combination treatment overcome the deleterious selection pressure? Would these alterations translate into unfavorable prognoses if sunitinib therapy was given as a single drug and for longer periods, similar to Kerbel's mouse model? Is our finding of adaptation to hypoxia be the mechanism by which the sunitinib treated mice develop resistance and increase metastasis[34, 35]? This hypothesis was tested in-vitro using a combination of bevacizumab and topotecan, an hypoxia inducing factor 1 alpha (HIF1α) inhibitor, and is the rationale for the treatment with bevacizumab plus irinotecan in recurrent high grade

glioma, leading to a 70% parcial and 15% complete radiological response[36-40]. These data provide encouragement that, also in BC, the combination of antiangiogenic therapy with antihypoxic drugs may synergize in cancer treatment.

References

1. Bear HD, Anderson S, Brown A et al. The effect on tumor response of adding sequential preoperative docetaxel to preoperative doxorubicin and cyclophosphamide: preliminary results from National Surgical Adjuvant Breast and Bowel Project Protocol B-27. J Clin Oncol 2003; 21, 4165-4174.
2. Bear HD, Anderson S, Smith RE et al. Sequential preoperative or postoperative docetaxel added to preoperative doxorubicin plus cyclophosphamide for operable breast cancer:National Surgical Adjuvant Breast and Bowel Project Protocol B-27. J Clin Oncol 2006; 24, 2019-2027.
3. Mieog JS, van der Hage JA, van de Velde CJ. Neoadjuvant chemotherapy for operable breast cancer. Br J Surg 2007; 94, 1189-1200.
4. Banerjee S, Dowsett M, Ashworth A, Martin LA. Mechanisms of disease: angiogenesis and the management of breast cancer. Nature clinical practice Oncology 2007; 4, 536-550.
5. Paulsson J, Sjöblom T, Micke P et al. Prognostic significance of stromal platelet-derived growth factor beta-receptor expression in human breast cancer. Am J Pathol 2009; 175, 334-341.
6. Abrams TJ, Murray LJ, Pesenti E et al. Preclinical evaluation of the tyrosine kinase inhibitor SU11248 as a single agent and in combination with "standard of care" therapeutic agents for the treatment of breast cancer. Mol Cancer Ther 2003; 2, 1011-1021.
7. Burstein HJ, Elias AD, Rugo HS et al. Phase II study of sunitinib malate, an oral multitargeted tyrosine kinase inhibitor, in patients with metastatic breast cancer previously treated with an anthracycline and a taxane. J Clin Oncol 2008; 26, 1810-1816.
8. Bergh J, Bondarenko IM, Lichinitser MR et al. First-Line Treatment of Advanced Breast Cancer With Sunitinib in Combination With Docetaxel Versus Docetaxel Alone: Results of a Prospective, Randomized Phase III Study. Journal of Clinical Oncology 2012; .
9. Liljegren A, Bergh J, Castany R. Early experience with sunitinib, combined with docetaxel, in patients with metastatic breast cancer. Breast (Edinburgh, Scotland) 2009; 18, 259-262.
10. Griffioen A, Mans L, De Graaf A et al. Rapid Angiogenesis Onset after Discontinuation of Sunitinib Treatment of Renal Cell Carcinoma Patients. Clinical Cancer Research 2012; 18, 3961-3971.
11. CTEP. Common Terminology Criteria for Adverse Events v3.0 (CTCAE). 2006;
12. technologies L. Ambion® WT Expression Kit for use with: Affymetrix® GeneChip® Whole Transcript (WT) Expression Arrays. 2011;
13. Affymetrix. GeneChip® WT Terminal Labeling and Hybridization User Manual for use with the Ambion® WT Expression Kit. 2012;
14. Barrett T, Troup DB, Wilhite SE et al. NCBI GEO: archive for high-throughput functional genomic data. Nucleic Acids Research 2009; 37, D885-90.
15. Warnes GR, Bolker B, Lumley T. gplots: Various R programming tools for plotting data. R package version 2.6.0. 2004;
16. Smyth G. Linear Models and Empirical Bayes Methods for Assessing Differential Expression in Microarray Experiments. Statistical Applications in Genetics and Molecular Biology 2004; 3,
17. Conley SJ, Gheordunescu E, Kakarala P et al. Antiangiogenic agents increase breast cancer stem cells via the generation of tumor hypoxia. Proceedings of the National Academy of Sciences of the United States of America 2012; 109, 2784-2789.
18. Huang D, Ding Y, Zhou M et al. Interleukin-8 mediates resistance to antiangiogenic agent sunitinib in renal cell carcinoma. Cancer Research 2010; 70, 1063-1071.
19. Somasiri A, Nielsen JS, Makretsov N et al. Overexpression of the anti-adhesin podocalyxin is an independent predictor of breast cancer progression. Cancer Research 2004; 64, 5068-5073.
20. Lindstrom L, Giorgetti C, Smeds J et al. Abstract P6-12-05: Ways To Understand Mechanism of Action for Targeted Drugs? Gene Expression Analyses from Paired Biopsies in Patients with Advanced Breast Cancer in a Substudy from the Randomized Study Docetaxel +/- Sunitinib A6181064. Cancer Research 2010; 70, P6-12-05-P6-12-05.
21. Mohsin SK, Weiss HL, Gutierrez MC et al. Neoadjuvant trastuzumab induces apoptosis in primary breast cancers. J Clin Oncol 2005; 23, 2460-2468.
22. Smith IC, Heys SD, Hutcheon AW et al. Neoadjuvant chemotherapy in breast cancer: significantly enhanced response with docetaxel. J Clin Oncol 2002; 20, 1456-1466.
23. von Minckwitz G, Kümmel S, Vogel P et al. Intensified neoadjuvant chemotherapy in early-responding breast cancer: phase III randomized GeparTrio study. J Natl Cancer Inst 2008; 100, 552-562.
24. von Minckwitz G, Kümmel S, Vogel P et al. Neoadjuvant vinorelbine-capecitabine versus docetaxel-doxorubicin-cyclophosphamide in early nonresponsive breast cancer: phase III randomized GeparTrio trial. J Natl Cancer Inst 2008; 100, 542-551.
25. Smith IE, Dowsett M, Ebbs SR et al. Neoadjuvant treatment of postmenopausal breast cancer with anastrozole, tamoxifen, or both in combination: the Immediate Preoperative Anastrozole, Tamoxifen, or

Combined with Tamoxifen (IMPACT) multicenter double-blind randomized trial. J Clin Oncol 2005; 23, 5108-5116.
26. Dowsett M, Ebbs SR, Dixon JM et al. Biomarker changes during neoadjuvant anastrozole, tamoxifen, or the combination: influence of hormonal status and HER-2 in breast cancer--a study from the IMPACT trialists. J Clin Oncol 2005; 23, 2477-2492.
27. Denko NC, Fontana LA, Hudson KM et al. Investigating hypoxic tumor physiology through gene expression patterns. Oncogene 2003; 22, 5907-5914.
28. Buffa FM, Harris AL, West CM, Miller CJ. Large meta-analysis of multiple cancers reveals a common, compact and highly prognostic hypoxia metagene. Br J Cancer 2010; 102, 428-435.
29. Kerbel RS, Benezra R, Lyden DC et al. Endothelial progenitor cells are cellular hubs essential for neoangiogenesis of certain aggressive adenocarcinomas and metastatic transition but not adenomas. Proceedings of the National Academy of Sciences of the United States of America 2008; 105, E54; author reply E55.
30. Gerstner ER, Duda DG, di Tomaso E et al. VEGF inhibitors in the treatment of cerebral edema in patients with brain cancer. Nature reviews Clinical oncology 2009; 6, 229-236.
31. Vroling L, van der Veldt AA, de Haas RR et al. Increased numbers of small circulating endothelial cells in renal cell cancer patients treated with sunitinib. Angiogenesis 2009; 12, 69-79.
32. Lucio-Eterovic AK, Piao Y, de Groot JF. Mediators of glioblastoma resistance and invasion during antivascular endothelial growth factor therapy. Clinical cancer research : an official journal of the American Association for Cancer Research 2009; 15, 4589-4599.
33. Creighton CJ, Li X, Landis M et al. Residual breast cancers after conventional therapy display mesenchymal as well as tumor-initiating features. Proceedings of the National Academy of Sciences of the United States of America 2009; 106, 13820-13825.
34. Ebos JM, Lee CR, Cruz-Munoz W, Bjarnason GA, Christensen JG, Kerbel RS. Accelerated metastasis after short-term treatment with a potent inhibitor of tumor angiogenesis. Cancer Cell 2009; 15, 232-239.
35. Francia G, Emmenegger U, Kerbel RS. Tumor-associated fibroblasts as "Trojan Horse" mediators of resistance to anti-VEGF therapy. Cancer Cell 2009; 15, 3-5.
36. Rapisarda A, Hollingshead M, Uranchimeg B et al. Increased antitumor activity of bevacizumab in combination with hypoxia inducible factor-1 inhibition. Mol Cancer Ther 2009; 8, 1867-1877.
37. Rapisarda A, Melillo G. Role of the hypoxic tumor microenvironment in the resistance to anti-angiogenic therapies. Drug Resist Updat 2009; 12, 74-80.
38. Rapisarda A, Shoemaker RH, Melillo G. Antiangiogenic agents and HIF-1 inhibitors meet at the crossroads. Cell Cycle 2009; 8, 4040-4043.
39. Sathornsumetee S, Cao Y, Marcello JE et al. Tumor angiogenic and hypoxic profiles predict radiographic response and survival in malignant astrocytoma patients treated with bevacizumab and irinotecan. J Clin Oncol 2008; 26, 271-278.
40. Zuniga RM, Torcuator R, Jain R et al. Efficacy, safety and patterns of response and recurrence in patients with recurrent high-grade gliomas treated with bevacizumab plus irinotecan. J Neurooncol 2009; 91, 329-336.

Table 1. Patient and tumor characteristics at evaluated timepoints.

		(diagnosis)										(upfront sunitinib)		(surgery)	
Patient		TNM classification			Caliper measurements (cm)		Pathology					Caliper measurements (cm)		Caliper measurements (cm)	
								IHC							
ID	Age	T	N	M	Tumor	Node	Elston Grade	ER	PR	HER2	HER2 FISH	Tumor	Node	Tumor	Node
P1	44	3	0	0	*8 x 6*	*0*	3	0	0	3+	na	*5x4*	*0*	*0*	*0*
P2	50	3	1	0	*6 x 6*	*2*	3	0	0	0	na	*6x6*	*1.7*	*4.5x4*	*0.5* (1)
P3	39	4b	1	0	*9 x 9 f*	*2*	2	100	80	2+	2	*7x7*	*2*	*6x6*	*1*
P4	60	3	0	0	**5 x 4**	**0**	2	90	40	2+	2	**2x2**	**0 (*)**	*0*	*0*
P5	56	4d	0	0	**7 x 5**	**0**	2	0	0	2+	2	**7x6**	**0**	*0*	*0*
P6	38	3	0	0	**6 x 6**	**0**	2	0	0	3+	na	*5x4*	*0*	**3x3**	**0 (*)**
P7	40	3	0	0	**6 x 4**	**0**	2	100	100	2+	2	**5.5x5.5**	**0**	**0.5x0.5**	**0**
P8	41	3	0	0	*7 x 6*	*0*	2	75	75	3+	20	*7 x 6*	*0*	*3x3*	*0*
P9	52	3	1	0	*7 x 6*	*2*	3	10	0	3+	na	*5x4*	*2*	**2x2**	**0**
P10	33	4b	1	0	**16 x 14**	**1 (*)**	2	95	0	2+	2	*12x12*	*0.5*	**0**	**0**
P11	36	4c	0	0	**6 x 5**	**0**	2	100	25	2+	2	**5x4**	**0**	**2.5x2.5**	**0** (1)
P12	45	3	1	0	*5 x 4*	*2*	2	100	0	3+	na	**4x4**	**0.5**	**3x3**	**0** (1)

For the distinct timepoints, samples with available RNA for gene expression are represented in bold whereas samples without enough RNA due to distinct reasons are represented in italic. Samples without enough RNA for replicated measurements are marked with (*). (1) identifies surgical specimens (t3) from patients that withdrew from study.

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; FISH, fluorescence *in situ* hybridization.

Figure 1 - Braga S. *et al.*

Figure 1. Timeline scheme of sunitinib and docetaxel administration, tumor collection and evaluation are represented in days. Cycles of sunitinib administration (14 days) are highlighted in white characters, with the window of single agent sunitinib in intermediate grey and off treatment periods (7 days) represented in white. The four cycles of docetaxel administration (days 22, 43, 64 and 85) correspond to the dark grey. Timepoints of tumor collection and evaluation by physical examination, MRI and PET (t1, t2 and t3) are represented with black arrows. Breast cancer surgery was performed on t3 (day 112 ±2 days).

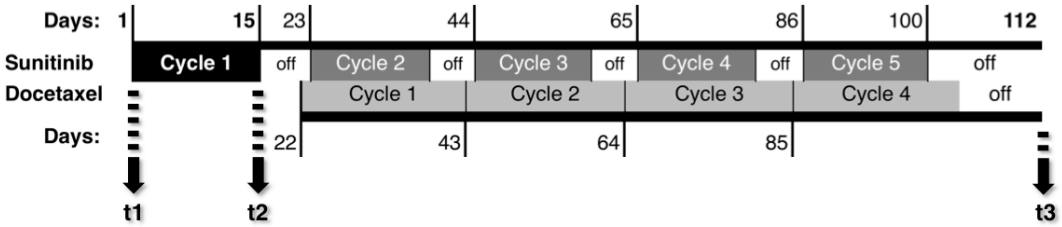


Figure 2 - Braga S. *et al.*

Figure 2. Evaluation of response to single-agent upfront Sunitinib, by comparison of measurements performed at diagnosis (Pre, day 1) and after Sunitinib treatment (Post, day 15), by measuring the greatest diameter of the primary lesion on physical examination (A, caliper measurement) and by breast MRI (B). The largest lesion was assessed when multicentric tumors were examined. Standardized Uptake Value (SUV) of glucose on PET was used to assess the metabolic response changes in the primary lesions (C) and homolateral axillary lymph nodes (D). Individual patients are identified by distinct lines and colors as indicated on the legend (lower right, panel D).

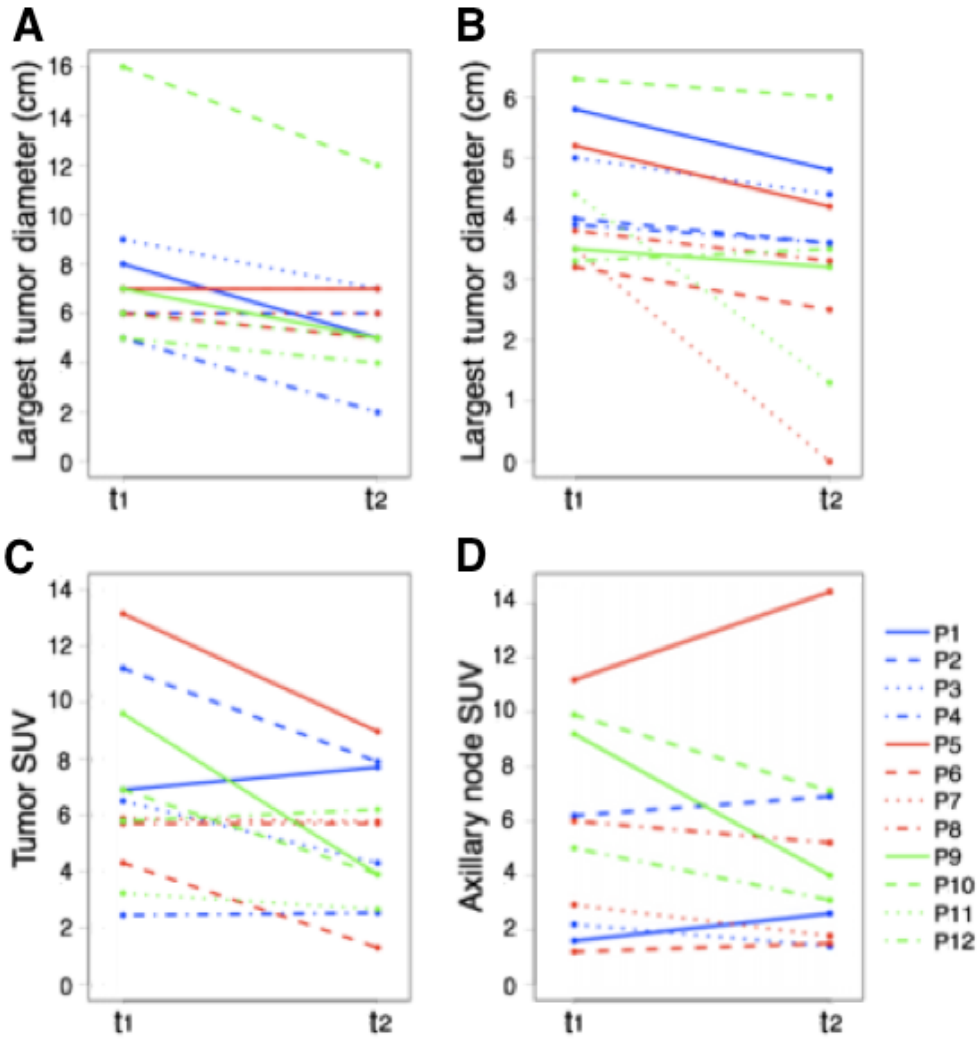


Figure 3 - Braga S. *et al.*

Figure 3. KI67 antigen nuclear staining in each sample was quantified by immunohistochemistry at diagnosis (t1, pre-sunitinib cycles), after the first cycle of single-agent sunitinib (t2, post-sunitinib) and after 4 cycles of docetaxel-sunitinib (t3, day 112 ±2 days, surgery). Individual patients are identified by distinct lines and colors as indicated on the legend at the right.

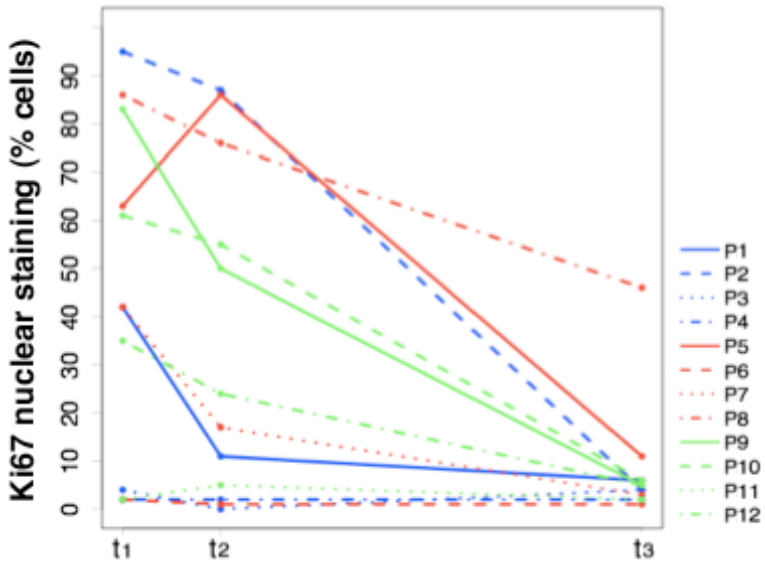


Figure 4 - Braga S. *et al.*

Figure 4. (A) Differentially expressed genes (85 genes) between samples t1 (diagnosis) and t2 (single agent-sunitinib) correctly assigned samples from patients' distinct timepoints (outlier sample P4_2) by two dimensional hierarchical clustering. Identical sample labels represent replicated samples. (B) The same gene set was also used to detect top functional categories enriched in the breast cancer after single agent neoadjuvant sunitinib here illustrated with the top 20 GO Biological pathways associated with up and down-regulated genes (red and blue bar plots, respectively). When no gene symbols were not available, were replaced by transcript cluster IDs.

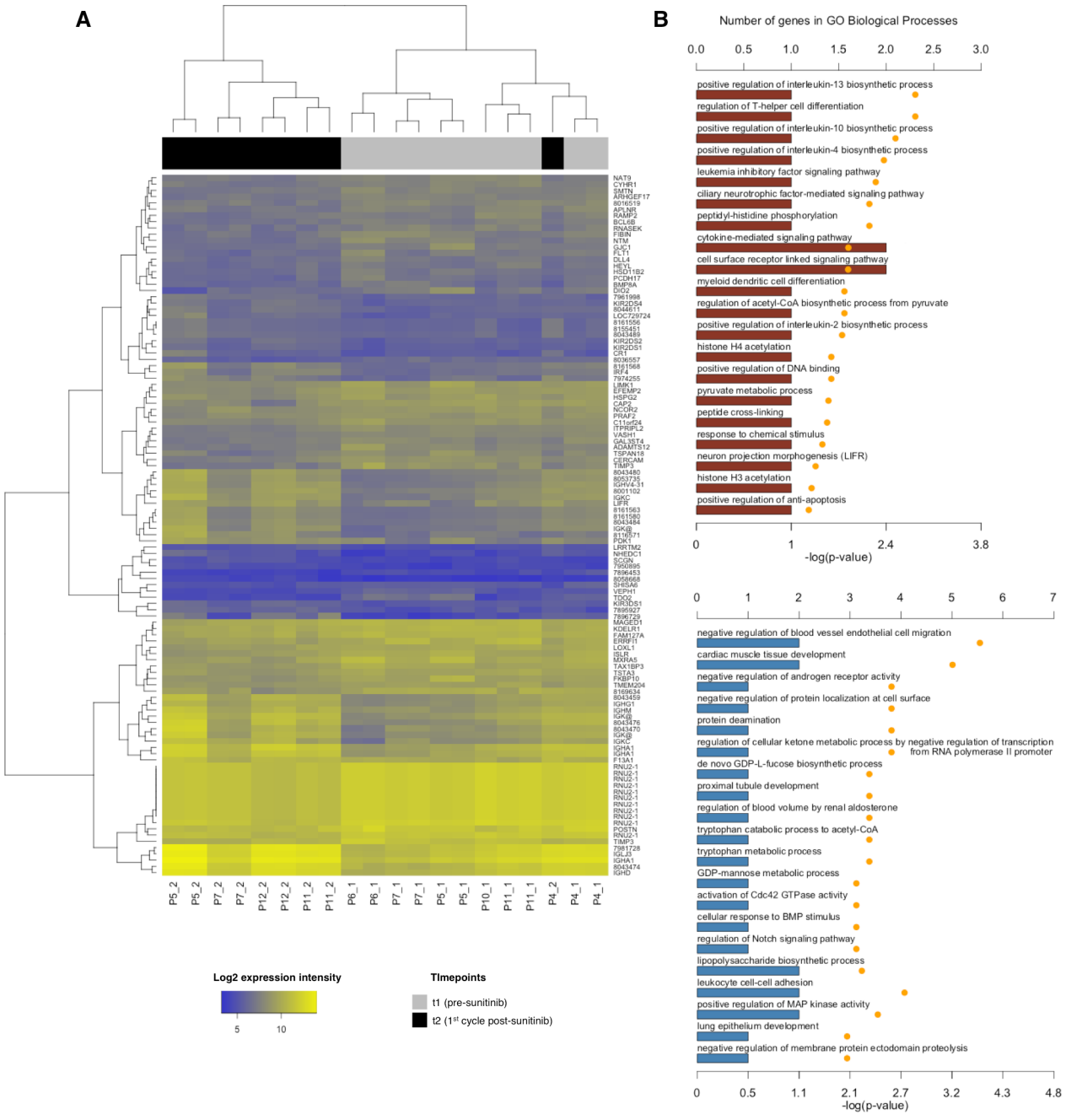
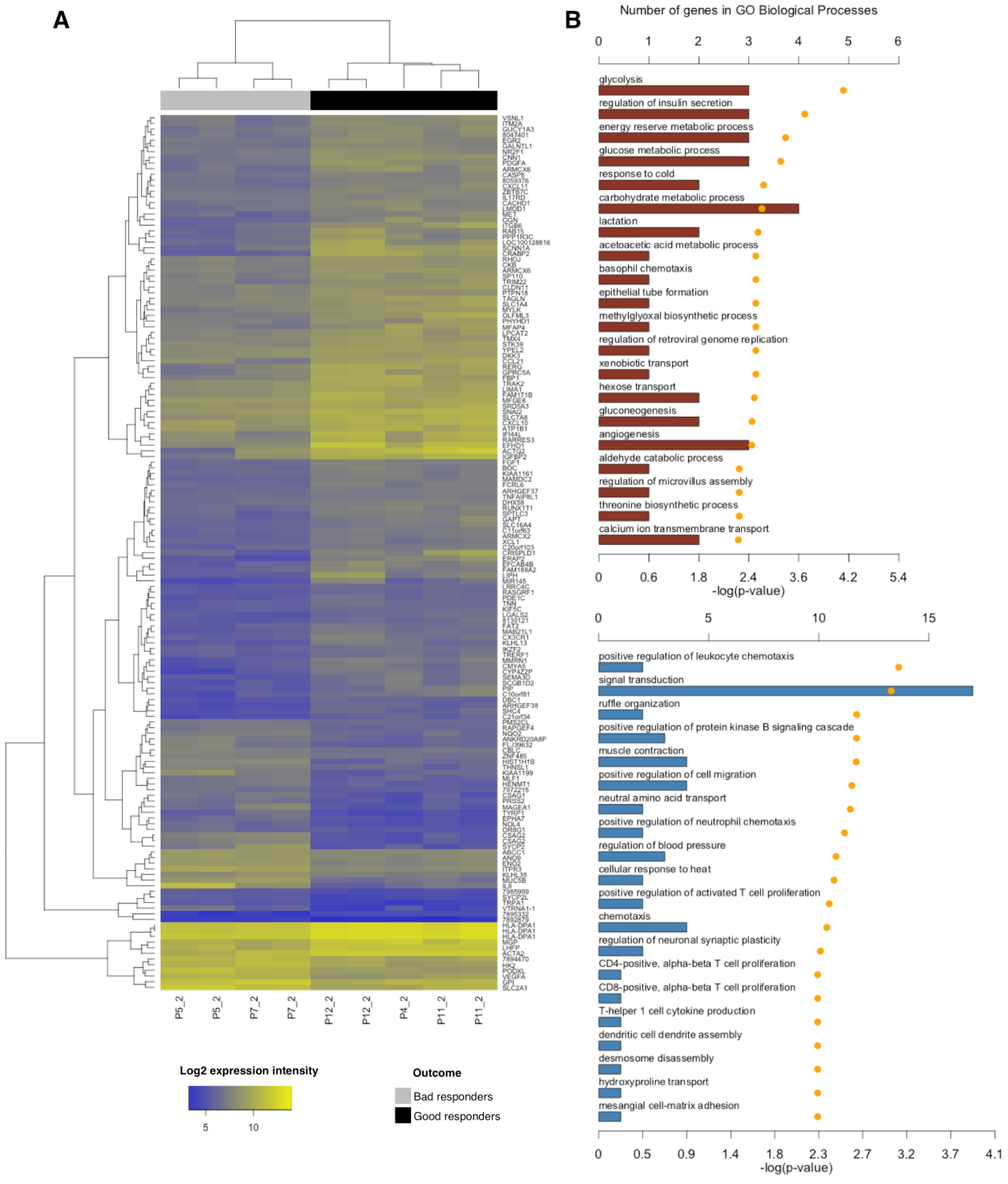


Figure 5 - Braga S. *et al.*

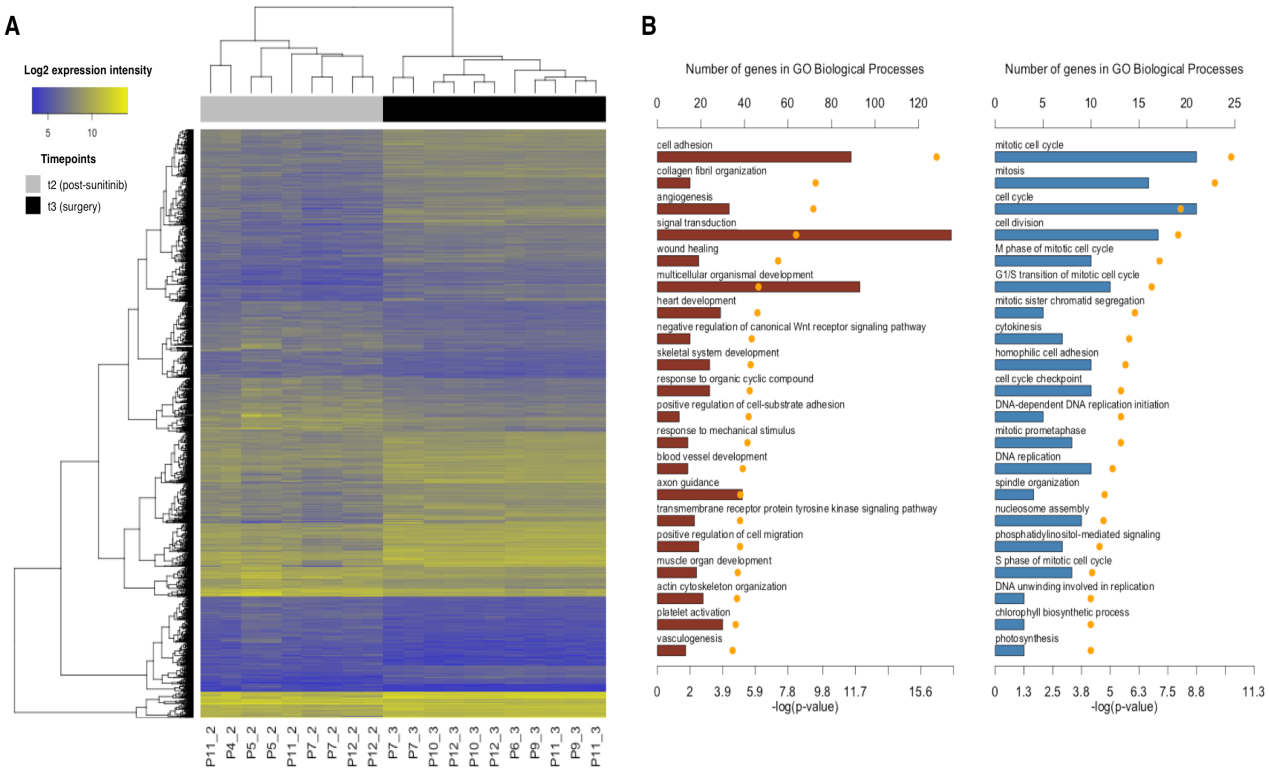
Figure 5. (A) Response outcome to single agent neoadjuvant sunitinib was assessed at molecular level by gene expression profiling for patients P5 and P7 (non-responders) and for P4, P11 and P12 (responders). The 147 selected differentially expressed unique genes correctly segregate responders from non-responders. Identical sample labels represent replicated samples. (B) Gene set enrichment analysis of response-associated genes highlighted GO Biological pathways enriched among up (red bar plot) and down-regulated (blue bar plot) genes of which the top 20 GO categories are illustrated. When no gene symbols were available, these were replaced by transcript cluster IDs



.

Figure 6 - Braga S. *et al.*

Figure 6. (A) Differential expression analysis between t2 (after single agent sunitinib) and t3 (surgery) allowed us to select the 1569 most significant genes whose expression changed with sunitinib/docetaxel combination treatment as illustrated by two dimensional hierarchical clustering. Identical sample labels represent replicated samples. (B) The functional context of this large set of genes significantly enriched GO Biological Pathways of which the top 20 categories are illustrated. Categories associated with up and down-regulated genes are represented in red and blue bar plots, respectively.



Supplementary Materials
Supplementary Table S1 – Braga S. *et al.*
Table S1 – Adverse events according to NCI Common Terminology Criteria v3.0 grading.
(1) One patient withdrew consent due to event;
(2) Patient enrollment was stopped after these two anaphylactic events;
(3) Both patients had mucositis accompanied with dehydration, hypotension leading to hospital admission;
(4) An isolated asymptomatic episode in an otherwise well controlled diabetic patient.

Events	# patients affected by	
	Grade 2	Grade 3
Alopecia	12	---
Asthenia	5	2 (1)
Hypersensitivity	3	2 (2)
Myalgia	1	---
PPE	2	---
Diarrhea	1	1
Mucositis	2	2 (3)
Dysgeusia	1	---
Hyperglicemia	---	1 (4)
Neutropenia	2	1

Supplementary Table S2 – Braga S. *et al.*

Table S2 – Overview of response outcome scoring at distinct timepoints.

The values are for absolute differences in cm of breast tumor size for clinical and MRI assessment and in SUV uptake for PET. Patients are scored as responders at t2 if they responded clinically and by either MRI or PET. Column t3 shows the number of involved axillary nodes at surgery.

	t2 vs. t1			t2 response score (15 days)	t3
Patient ID	Clinical	MRI	PET		# axillary nodes involved
P1	-3	-1	0.8	+	4
P2	0	-0.4	0.7	-	0
P3	-2	-0.6	-1.8	+	7
P4	-3	-0.3	-0.9	+	0
P5	0	-1	3.2	-	1
P6	-1	-0.7	0.3	+	0
P7	0	-3.5	0	-	1
P8	0	-0.5	0	-	6
P9	-2	-0.3	-5.7	+	1
P10	-4	-0.3	-3	+	4
P11	-1	-0.9	-0.6	+	0
P12	-1	0.2	0.4	+	0

Part 3: When to stop systemic treatment of breast cancer patients?

Chapter 8: The aggressiveness of cancer care in the last three months of life: a retrospective single centre analysis

Conception and design: Sofia Braga, António Moreira

Provision of study materials or patients: Sofia Braga, José Luis Passos-Coelho, Aires Fernandes, José Duro da Costa, António Moreira

Collection and assembly of data: Sofia Braga

Data analysis and interpretation: Sofia Braga, Ana Miranda, Rute Fonseca

Manuscript writing: Sofia Braga, José Luis Passos-Coelho

Final approval of manuscript: All authors

Funding: Not applicable

Ethical Committee approval: Ethical committee of Instituto Português de Oncologia, Lisboa

The aggressiveness of cancer care in the last three months of life: A retrospective single centre analysis

Sofia Braga*, Ana Miranda, Rute Fonseca, J. L. Passos-Coelho, Aires Fernandes, J. D. Costa and António Moreira
Instituto Português de Oncologia, Lisboa, Portugal

**Correspondence to: Serviço de Oncologia Médica, Instituto Português de Oncologia, Rua Prof Lima Basto 1099-023 Lisboa, Portugal. E-mail: duarte_braga@hotmail.com*

Received: 27 March 2006
Revised: 15 June 2006
Accepted: 9 November 2006

Abstract

***Background*: There is concern that terminally ill cancer patients are over treated with chemotherapy, even when such treatment is unlikely to palliate symptoms. The study objective was to evaluate the use of chemotherapy in the last three months of life in a cohort of adult patients with advanced solid tumours.**

***Methods*: All adult patients with solid tumours who died in our hospital in 2003 and received chemotherapy for advanced cancer, were included. Detailed data concerning chemotherapy and toxicity, in the last three months of life, were collected from patients' clinical charts.**

***Results*: A total of 319 patients were included. Median age was 61 years. Median time from diagnosis of metastatic disease to death was 11 months. The proportion of patients who received chemotherapy in the last three months of life was 66% ($n = 211$), in the last month 37% and in the last two weeks 21%. Among patients who received chemotherapy in the last three months of life, 50% started a new chemotherapy regimen in this period and 14% in the last month. There was an increased probability of receiving chemotherapy in the last three months of life in younger patients and in patients with breast, ovarian and pancreatic carcinomas.**

***Conclusion*: There was a large proportion of patients who received chemotherapy in the last three months of life, including initiation of a new regimen within the last 30 days. Thus, further study is needed to evaluate if such aggressive attitude results in better palliation of symptoms at the end of life.**

Keywords: cancer; oncology; palliative chemotherapy; aggressiveness; end-of-life care

Background

There is growing concern that the treatment of patients with advanced cancer is becoming increasingly aggressive near the end-of-life but there are few published data on this topic [1,2]. One of the reasons is the difficulty in designing, implementing, conducting and analyzing studies in these terminally ill patients [3]. While we acknowledge the necessity of symptom and quality of life evaluation in clinical practice, we are confronted with the shortcomings of these endpoints when used in research [4,5].

The possible aggressiveness of cancer care near the end of life is mostly documented by an increasing use of palliative chemotherapy [1]. Earle *et al*. analyzed the administration of systemic chemotherapy in terminally ill patients over a four year period, from 1993 to 1996, and concluded that there was increasing propensity to use aggressive interventions over time, although the absolute change from one year to the next were small. While the use of palliative chemotherapy in this setting has limited impact on survival, clinical trials have shown that better quality of life and palliation of symptoms can be achieved in the treatment of the most frequent solid tumours of adults [4–14]. Prescription of palliative chemotherapy represents a very delicate equilibrium between toxicity and potential clinical benefit that is even more complex when treating patients with a short life expectancy, therefore there should be a clear definition of treatment goals. Simultaneously, the importance of high quality of end-of-life care, including symptom control, end-of-life decision making, choice of place of death, need for psychological and social support is increasingly valued by health care providers.

Other indicators of aggressiveness of care in patients with advanced cancer include the number and frequency of emergency room visits, hospital and intensive care unit admissions and treatment with surgery or radiotherapy [2,15–20]. These may reflect the expansion of therapeutic possibilities for patients with advanced cancer and there are data suggesting that terminally ill patients are frequent users of health care system resources, irrespective of treatment modalities [3,21]. There is striking regional and national variation in such use reflecting cultural differences, including the acceptance of death, and difference in health care systems [22–24].

This study's objective was to measure indicators of potential aggressiveness of end-of-life care in a cohort of adult patients with advanced solid tumours. It is a retrospective single institution study on the administration of chemotherapy and other potential indicators of aggressive care in the last three months of life.

Patients and Methods

Eligibility criteria

All adult patients with solid tumours that were treated in the medical oncology department of our institution with palliative systemic chemotherapy, that is systemic chemotherapy for non-curable locally advanced or metastatic cancer and had died in 2003 were included. Primary central nervous system tumours were not included.

In an attempt to quantify the proportion of patients with advanced solid tumours that were treated with systemic chemotherapy in the medical oncology department, we also collected data on cancer diagnosis in all patients treated in the cancer centre who died in 2003. This review was conducted with the approval of the institutional ethics committee.

Collected data

Demographic data included sex, age and residence. Cancer-related data included date of diagnosis and death, cancer diagnosis and date of documentation of advanced/metastatic disease. Regarding administration of chemotherapy, data were collected on its use in the treatment of advanced disease and during the last three months of life. Due to incomplete documentation in patients' clinical charts, data were not collected on the purpose of chemotherapy administration.

For those patients that were treated with chemotherapy in the last three months of life further data were collected on potential indicators of aggressiveness of treatment. These included: (i) the number of chemotherapy regimens as treatment for advanced disease, with date of initiation and ending; (ii) the number of emergency room visits and hospital admissions for all causes in the last three months of life, with identification of toxicity related events; (iii) treatment with surgery or radiation therapy in the last three months of life with identification of its purpose, date and hospitalization requirements. The duration of a chemotherapy regimen was defined as the time between the first and the last day of administration of the drug(s) that were part of each regimen.

Data source

Data were collected through the institutional database and from patient clinical charts. Population-based death certificates were used to complement clinical chart data, assuring that no deaths were missed, although only rarely are patients referred from our cancer centre to other institutions for end-of-life care.

Statistical Methods

Median and ranges were used as descriptive statistics. Chi-square statistic was used to compare categorical data. Logistic regression analysis was used to test the association between administration of palliative chemotherapy and patient or cancer related characteristics. The statistical software Stata 6.0 was used.

Results

Demographic data

Three hundred and nineteen adult patients with solid tumours other than primary central nervous system tumours (195 women and 124 men) who died in 2003 were treated in the medical oncology department with palliative chemotherapy. The median age was 61 (range 21–92). Their place of residence was less than 30 km away from the hospital in 67% and more than 30 km in 33%. The total number of patients treated in the cancer centre who died in 2003 was 639.

Cancer-related data

For the 319 patients treated with palliative chemotherapy who died in 2003, the median survival from the time of documentation of metastatic disease was 11 months (range 18 days to 15 years). The most frequent diagnoses for such population were breast (31.7%), gynaecological (14.7%), lung (11.9%) and head and neck cancers (9.7%) (Table 1).

A total of 639 adult patients with solid tumours treated in the cancer centre died in the year of 2003. Not all of them were cared for in the medical oncology department. The proportion of such patients who were primarily treated with palliative systemic chemotherapy in the medical oncology department were 86% for breast cancer, 82% for melanoma, 53% for gynaecological cancer and for lung cancer, 44% for colorectal cancer, 48% for sarcomas and 50% for pancreatic cancer (Table 2).

Indicators of potential aggressiveness of treatment

Indicators were developed empirically to identify clinical interventions, which could be considered inappropriately aggressive for the treatment of patients with advanced cancer in the last three months of life. Such indicators were the administration of chemotherapy in the last three months of life, number of emergency room visits and hospital admissions, including to the intensive care unit, and treatment with surgery and radiation therapy.

Administration of chemotherapy

Two hundred and eleven of the 319 patients treated with palliative systemic chemotherapy (66%) received chemotherapy in the last three months of life. Of these 211 patients, 120 (37%) were treated with chemotherapy in the last month of life and 68 in the last two weeks (21%). One hundred and fifty nine of the 211 patients (75%) were treated with only one chemotherapy regimen in the last three months of life, 48 patients (23%) with two chemotherapy regimens and four patients (2%) with a total of three regimens. The median number of days between the last administration of chemotherapy and patient death was 27 (range 0–90). The median duration of administration of the last chemotherapy regimen was 31 days (range 0–319).

A new chemotherapy regimen was started in the last three months of life in 106 patients (50.2%). In the remaining 105 patients who received chemotherapy in the last three months of life, the chemotherapy regimen had been started earlier. A new chemotherapy regimen was started in the last month of life in 30 patients (14.2%). Of these 30 patients, 28 (93%) were treated with palliative chemotherapy for the first time.

In 67 patients the first chemotherapy regimen for treatment of advanced disease was initiated during the last three months of life and in 28 patients in the last month of life. The diagnoses for the 67 patients are shown in Table 3. The most frequent primary tumor was breast cancer. Of these 67 patients, 10 of them received a second line of chemotherapy (three patients with breast cancers, two with cervical cancer and with sarcoma and one each with ovarian cancer, non-small cell lung cancer and colorectal cancer). A patient with an alveolar rhabdomyossarcoma started palliative chemotherapy in the last three months of life and received three lines of treatment.

The cancer diagnoses of the 28 patients who were treated for the first time with palliative systemic chemotherapy in the last month of life was breast cancer (9), head and neck cancer (4), lung cancer (4), cervical cancer (3), colorectal cancer (2), pancreatic cancer (2), stomach cancer (2), ovarian cancer (1) and epitheliod sarcoma (1).

There was an association between the administration of palliative systemic chemotherapy in the last three months of life and decreasing patient age (OR = 0.96, for each additional year of life; 95% confidence interval 0.94–0.98) and diagnosis of breast, ovarian or pancreatic cancer (OR = 3.3,

Table 1. Cancer diagnoses of the overall population

Diagnosis	Number patients	%
Breast	101	31.7
Gynecological	47	14.7
Lung	38	11.9
Head and Neck (H & N)	31	9.7
Colorectal	27	8.5
Oesophagus and stomach	24	7.5
Melanoma	14	4.4
Urinary tract	13	4.1
Sarcoma	10	3.1
Pancreas	8	2.5
Other	6	1.9
Total	319	100

Cancer diagnoses of all 319 adult patients with solid tumours, other than primary CNS tumours, who died in 2003 and had received palliative chemotherapy.

Table 2. Proportion of patients who received palliative chemotherapy

Diagnosis	Number of patients treated with chemo therapy	Total number of patients[a]	Proportion
Breast	101	118	0.86
Gynaecological	47	88	0.53
Lung	38	71	0.53
Head and Neck (H&N)	31	119	0.26
Colorectal	27	62	0.44
Oesophagus and Stomach	24	76	0.32
Melanoma	14	17	0.82
Urinary tract	13	33	0.39
Sarcoma	10	21	0.48
Pâncreas	8	16	0.5
Other	6	18	0.3
All	319	639	0.5

Number of patients who received palliative chemotherapy and overall number of patients who died in the same year with the same diagnosis in the cancer centre.[a] The determination of overall cancer centre death data were obtained from patient medical records only, without supplementation with death certificate data.

Table 3. Cancer diagnosis of patients that started palliative chemotherapy in the last three months of life

Primary tumour	Number of patients
Breast	19
Gynaecological	10
Lung	8
Head and Neck	6
Colorectal	4
Oesophagus and Stomach	3
Melanoma	3
Urinary tract	2
Sarcoma	2
Pâncreas	6
Other	4
Total	67

95% confidence interval 2.0–5.5) but no relationship with duration of metastatic disease or distance between cancer centre and area of residence.

Emergency room visits and hospital admissions

Eighty percent of patients (169/211) had at least one emergency room visit in the last three months of life and the median number of visits during this period was 2 (range 1–10). Fifty patients had 67 emergency room visits (15% of all emergency room visits) due to treatment-related toxicity—32 for haematological toxicity and 35 for non-haematological toxicity.

Ninety-six percent of patients (201/211) had at least one hospital admission in the last three months of life and the median number of days spent in the hospital was 16 (range 2–90). Thirty-four patients had 40 hospital admissions (16% of the total number of admissions) due to treatment-related toxicity—29 for haematological toxicity and 11 for non-haematological toxicity. These hospital admissions resulted in 12 toxic deaths, all from neutropenia with sepsis. The median duration of hospital admissions due to treatment related toxicity was 7 days (range 2–31).

Five patients were admitted to the intensive care unit in the last three months of life. None were discharged from the hospital alive. Two patients were admitted in the post-operative period, and one each with aspiration pneumonia, cancer-related hemorrhagic shock and treatment-related septic shock.

Surgery and radiation therapy

Thirteen patients (6%) underwent surgical procedures in the last three months of life—10 abdominal and 3 of the central nervous system. All surgeries were due to cancer related complications and not due to treatment related toxicity.

Fifty patients (16%) received radiation therapy in the last three months of life (20 as outpatients, 30 as inpatients), mostly for palliation of cancer-related symptoms. The median number of days between the end of radiation and death was 39 (range 0–90).

Discussion

There are little objective data on the evaluation of aggressiveness of cancer care in the end of life. To our knowledge this is the first hospital-based study that characterizes the aggressiveness of cancer care in terminally ill adults with solid tumours. This study represents the experience of a single institution during one year. In contrast, other studies collect administrative data from the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) registries and from Centres for Medicare and Medicaid Services Medicare claims data [1,2]. These studies are population based and characterize the care given to thousands of patients. The study by Earle *et al.* [1] analysed claims of 28777 patients and the study by Emanuel *et al.* [2] analysed claims of 8875 patients.

The present study documents that palliative chemotherapy is administered until very late in patients' lives. A large proportion of patients ever treated with palliative chemotherapy are still on active anti-neoplastic treatment in the last three months of life and, similarly, a large proportion start a new chemotherapy regimen in that period. In this study 21% of the patients treated with chemotherapy in the last three months of life were still being treated within two weeks of death. In the large study by Earle *et al.* [1], with a population over 65 years-old and restricted to Medicare or Medicaid beneficiaries with lung, breast, colorectal or other gastrointestinal cancers, this proportion was 13.8% in 1993 and rose to 18.5% in 1996.

Patients who received the first chemotherapy regimen for advanced disease represent 32% (67/211) of the patients treated in the last three months of life. Of the 30 patients that started a new chemotherapy regimen in the last month of life, 28 (93%) were treated with palliative chemotherapy for the first time. The prescription of a new chemotherapy regimen within one month of death occurred in 13.3% (28/211) of this population. Thus, the second conclusion of this study is that a sizeable proportion of patients start palliative chemotherapy near the end of life. This same indicator was reported by Earle *et al.* [1] and, in their series, rose from 4.9% in 1993 to 5.7% in 1996. However, the characteristics of the population in their study are different from ours, as previously mentioned.

The proportion of patients who received chemotherapy for the treatment of advanced cancer differs widely dependent on cancer diagnosis. While 86% of patients with breast cancer received

chemotherapy for metastatic disease, only 26% of similar patients with head and neck cancers did. Likewise, only 32% of patients with upper digestive tract cancer (stomach and oesophagus) and 39% of patients with urothelial cancer received chemotherapy for advanced disease. These findings probably reflect not only the chemo sensitivity of each disease but also the performance status and age group of the patients. The fact that 82% of patients who died from metastatic melanoma were treated with systemic chemotherapy, despite its very limited sensitivity to such treatment, is likely due to the few systemic therapeutic options available to these patients.

Many reasons could justify the use of chemotherapy in terminally ill patients. One potential explanation for the protracted use of chemotherapy is that it may impact on quality of life and symptom control, even without any measurable change in tumour bulk and there are data supporting this in metastatic breast, lung, colon, pancreatic, stomach and ovarian cancers [8–16,25–27]. It is a good reason for the prescription of chemotherapy although, unfortunately, such conclusion cannot be drawn from our data. Similarly, the trials that showed that palliative chemotherapy is beneficial to patients with these common adult solid tumours do not adequately reflect the use of chemotherapy in terminally ill patients where the balance between efficacy and toxicity is less favourable. Patients in such trials have better performance status, less symptoms, shorter duration of advanced disease, better organ function to overcome toxicity and are not heavily pre-treated. Furthermore, there are studies showing the relative lack of efficacy of second and subsequent lines of chemotherapy in patients with metastatic breast cancer that, in our series, represents nearly half of the patients [22].

It is frequent in our daily practice to see anxious patients and families who are willing to endure toxicity in the hope of small prolongations of life. Sometimes oncologists start chemotherapy while patients and families adjust to cancer diagnosis and prognosis [6,7]. Obviously, these subtle interactions are not retrievable from these data. However, it shows that in our cancer centre half of the patients with cancer poorly responsive to chemotherapy (lung, melanoma and pancreas) received such treatment in the three last months of life.

Some publications have focused on physician-related causes of aggressiveness in treatment of terminally ill cancer patients. They raise the possibility that the professional's feelings of failure, helplessness and frustration as well as stresses in their private life might have impact the treatment of patients [23,24]. In a study where psychiatric morbidity was estimated with a questionnaire administered to 1133 English consultants in gastroenterology, radiology, surgery or oncology, its prevalence was 27% in the 882 questionnaires retrieved [28]. Albeit being a possible explanation for the observed results, we have no data to support that physician's emotional exhaustion played a role in the management of this cohort of patients. The evaluation of burnout in this group of oncologists is an interesting topic for further research.

One critical question in the care of patients with advanced cancer is the ability to estimate individual patient survival. Published data show how inaccurate oncologists are in predicting their patients' survival and how they overestimate it [29–31], leading to overly aggressive anti-cancer treatment. In our study, deaths due to toxicity represented 5.7% of all deaths and 16% of patients treated with chemotherapy in the last three months of life were hospitalized due to toxicity. This toxicity rate is higher than that of published clinical trials in advanced cancer, but the characteristics of the patients included are very different. In the study by Earle *et al.* [1] there are data on emergency room visits and ICU admissions but without separation of cancer-related complaints from toxicity. In our study the majority of emergency room visits, hospital admissions and ICU admissions were due to cancer related symptoms and not toxicity. Similarly, surgical procedures and radiation treatments were only used for the relief of cancer related complications.

Can predictors of the likelihood of chemotherapy administration be identified in this patient population? Similarly to other studies [17], the probability of chemotherapy administration varied inversely to patient's age. Some [6,7] but not all [17] studies, found an inverse relationship between distance from patient's home to the cancer centre and both the probability of administration of chemotherapy as well as increased likelihood of hospice care referral [6,7]. Such relationship was not seen in our study. Cancer diagnosis also influences the decision to treat advanced disease with chemotherapy in the end of life. Chemotherapy-sensitive cancers, such as breast and ovarian cancer, as well as cancers that are generally diagnosed in advanced stages, such as pancreatic cancer, are more likely to be treated with systemic chemotherapy late in patients' lives.

This study has important limitations. Firstly, we do not have data on the reasons for the protracted use of chemotherapy or on its palliative efficacy. Although this is a critical question, we did not collect these data because of heterogeneous documentation in patients' charts. Thus, we cannot draw conclusions on the appropriateness of the prescription of the palliative chemotherapy. Collection of data on treatment goals and symptom control are vital to characterize the aggressiveness and/or appropriateness of such treatment. Secondly, patients were included based on the occurrence of death and this might represent a bias since we have no data on patients that may have obtained sustained clinical benefit and

objective anti-tumour response with palliative chemotherapy. Further studies should evaluate prospectively the appropriateness of chemotherapy prescription in terminally ill cancer patients measuring clinically meaningful quality of life parameters, performance status, symptom description and control and use of concomitant medication.

Acknowledgements

The retrospective research conducted in this work has the full approval of the institutional ethics committee.

References

1. Earle CC, Neville BA, Landrum MB *et al*. Trends in the aggressiveness of cancer care near the end of life. *J Clin Oncol* 2004;**22**:315–321.
2. Emanuel EJ, Yinong YX, Levinsky N *et al*. Chemotherapy use among medicare beneficiaries at the end of life. *Ann Intern Med* 2003;**138**:639–644.
3. Browner I, Carducci M. Palliative chemotherapy: historical perspective, applications and controversies. *Semin Oncol* 2005;**32**(2):145–155.
4. Doyle C, Crump M, Pintilie M *et al*. Does palliative chemotherapy palliate? Evaluation of expectations, outcomes, and costs in women receiving chemotherapy for advanced ovarian cancer. *J Clin Oncol* 2001;**19**: 1266–1274.
5. Kornblith A. Does palliative care palliate? *J Clin Oncol* 2001;**19**:2111–2113.
6. Gough IR, Furnival CM, Burnett W. Patient attitudes to chemotherapy for advanced gastro-intestinal cancer. *J Clin Oncol* 1981;**7**:5–11.
7. Ellis PA, Smith IE, Hardy JR *et al*. Symptom relief with MVP (mitomycin C, vinblastine and cisplatin) chemotherapy in advanced non-small-cell lung cancer. *Br J Cancer* 1995;**71**:366–370.
8. Cullen MH, Billingham LJ, Woodroffe CM *et al*. Mitomycin, ifosfamide, and cisplatin in unresectable non-small-cell lung cancer: effects on survival and quality of life. *J Clin Oncol* 1999;**17**:3188–3194.
9. Cullen M. 'Best supportive care' has had its day. *Lancet Oncol* 2001;**2**:173–175.
10. Cunningham D, Pyrhönen S, James RD *et al*. Randomised trial of irinotecan plus supportive care versus supportive care alone after fluorouracil failure for patients with metastatic colorectal cancer. *Lancet* 1998;**352**:1413–1418.
11. Doyle C, Crump M, Pintilie M, Oza AM. Does palliative chemotherapy palliate? Evaluation of expectations, outcomes, and costs in women receiving chemotherapy for advanced ovarian cancer. *J Clin Oncol* 2001;**19**:1266–1274.
12. Burris HA 3rd, Moore MJ, Andersen J *et al*. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. *J Clin Oncol* 1997;**15**: 2403–2413.
13. Cormier Y, Eisenhauer E, Muldal A *et al*. Gemcitabine is an active new agent in previously untreated extensive small cell lung cancer (SCLC): A study of the National Cancer Institute of Canada Clinical Trials Group. *Ann Oncol* 1994;**5**:283–285.
14. Comis RL, Friedland DM. New chemotherapy agents in the treatment of advanced non-small cell lung cancer: An update including data from the Seventh World conference on Lung Cancer. *Lung Cancer* 1995; **12**(suppl 2):S63–S99.
15. Casarett D, Abrahm JL. Patients with cancer referred to hospice versus a bridge program: patient characteristics, needs for care, and survival. *J Clin Oncol* 2001;**19**: 2057–2063.
16. Du X, Goodwin JS. Patterns of use of chemotherapy for breast cancer in older women: findings from Medicare claims data. *J Clin Oncol* 2001;**19**:1455–1461.
17. Goodlin SJ, Winzelberg GS, Teno JM *et al*. Death in the hospital. *Arch Intern Med* 1998;**158**:1570–1572.
18. Emanuel EJ, Ash A, Yu W *et al*. Managed care, hospice use, site of death, and medical expenditures in the last year of life. *Arch Intern Med* 2002;**162**:1722–1728.
19. Barnato AE, McClellan MB, Kagay CR, Garber AM. Trends in inpatient treatment intensity among Medicare beneficiaries at the end of life. *Health Serv Res* 2004;**39**:363–375.
20. Wennberg JE, Fisher ES, Stuckel TA *et al*. Use of hospitals physician visits, and hospice care during the last six months of life among cohorts loyal to highly respected hospitals in the United States. *Br Med J* 2004;**328**:603–607.
21. Teno JM, Clarridge BR, Casey V *et al*. Family perspectives on end-of-life care at the last place of care. *J Am Med Assoc* 2004;**291**:88–93.
22. Porkka K, Blomqvist C, Rissanen P *et al*. Salvage therapies in woman who fail to respond to first-line treatment with fluorouracil, epirrubicin, and cyclophosphamide for advanced breast cancer. *J Clin Oncol* 1994;**12**:1639–1647.
23. Meier DE, Back Al, Morrison S. The inner life of physicians and care of the seriously ill. *J Am Med Assoc* 2001;**286**(23):3007–3014.
24. Turner J, Kelly B. The concept of debriefing and its application to staff dealing with life-threatening illness such as cancer, AIDS and other conditions. In Raphael B, Wilson JP. (eds). *Psychological Debriefing: Theory, Practice and Evidence*, UK: Cambridge University Press, 2000.
25. Burge F, Lawson B, Johnston G. Trends in the place of death of cancer patients, 1992–1997. *Can Med Assoc J* 2003;**168**:265–270.
26. Cintron A, Hamel MB, Davis RB *et al*. Hospitalization of hospice patients with cancer. *J Palliat Med* 2003; **6**:757–768.
27. Earle CC, Neumann PJ, Gelber RD *et al*. Impact of referral patterns on the use of chemotherapy for lung cancer. *J Clin Oncol* 2002;**20**:1786–1792.
28. Ramirez AJ, Graham J, Richardsa MA, Cull A, Gregory WM. Mental health of hospital consultants: the effects of stress and satisfaction at work. *Lancet* 1996;**347**:724–728.
29. Llobera J, Esteva M, Rifà J *et al*. Terminal cancer: duration and prediction of survival time. *Eur J Cancer* 2000;**36**:2036–2043.
30. Christakis N, Lamont E. Extent and determinants of error in doctors' prognoses in terminally ill patients: prospective cohort study. *Br Med J* 2000;**320**:469–473.
31. Glare P, Virik K, Jones M *et al*. A systematic review of physicians' survival predictions in terminally ill cancer patients. *Br Med J* 2003;**327**:195–198.

Chapter 9: Is breast cancer treatment in the end of life less aggressive?

Conception and design: Sofia Braga

Provision of study materials or patients: Sofia Braga, António Moreira, Carlos Carvalho

Collection and assembly of data: Sofia Braga, Nuno Guerra-Pereira, Fábio Cassiano, Patrícia Gandolfo, Patricia Cortes

Data analysis and interpretation: Sofia Braga, Nuno Guerra-Pereira

Manuscript writing: Sofia Braga

Final approval of manuscript: All authors

Funding: Not applicable

Ethical Committee aproval: Ethical committee of Instituto Português de Oncologia, Lisboa and of Hospital Fernando da Fonseca, Amadora

Is breast cancer treatment in the end of life less aggressive?

Nuno Guerra-Pereira[1], Fábio Cassiano[2], Patrícia Gandolfo[1], Patricia Cortes[1], António Moreira[3], Carlos Carvalho[2], Sofia Braga[4]
1- Departamento de Ciências Biomédicas e Medicina, Universidade do Algarve, Faro, Portugal
2- Unidade de Oncologia, Hospital Fernando Fonseca, Amadora, Portugal
3- Serviço de Oncologia Médica, Instituto Português de Oncologia, Lisboa, Portugal
4- José de Mello Saúde, Lisboa, Portugal (Corresponding author)
sofia.braga@jmellosaude.pt
Av. do Forte 3, Ed Suecia 3, piso 2, 2790-073 Lisboa

Abstract

Background
There is overuse of palliative systemic therapy in the end of life (EoL) of advanced breast cancer (ABC) patients but the growing awareness of this practice and the emphasis put in quality of care in the EoL might be shifting this practice. We consider three hypothesis concerning EoL care in ABC: (1) that there is more evidence of palliative strategies being implemented in this group of patients; (2) that there is less usage of palliative systemic anti-cancer therapy and (3) that other indicators of aggressiveness of care in this population show a decrease.

Patients and methods
We aimed to characterize the shifting trends in use of anti-cancer chemotherapy and palliative care approaches in the EoL of ABC patients in different institutions and times.
For this, we selected women that died of advanced BC during six years, from 2007 to 2012, and were treated in a central acute care general hospital and compared it with the BC patients that died in 2003 and were treated in a large cancer center. We analyzed a total of 232 patients: the more recent group has 114 women and the older cohort has 118. We used descriptive statistics to characterize the use of CT in the EoL and the use of palliative care resources.

Results
Both populations were similar in terms of BC characteristics. In the latter cohort there was more utilization of palliative care resources, pain clinic, palliative care team intervention and palliative radiotherapy involved in the care of advanced BC patients and there were also more deaths at the hospices. Systemic anti cancer treatments continue to be prolonged until very late in patients' lives, but in the last cohort there was a tendency for decrease. Other indicators of agressiveness, namely hospital admissions also show a decrease.

Conclusion
In the two distinct institutions analyzed and with the time interval considered, we confirmed our hypothesis that there is more integration of multidisciplinary palliative care and less aggressiveness in the treatment of ABC patients, namely, use of palliative anti-cancer treatment and hospital admissions. Nonetheless, in both cohorts patients still receive systemic therapy until too late and there is still a deficit or underutilisation of palliative medicine.

Introduction
Cancer remains a leading cause of death in developed countries. Breast cancer (BC) is a very significant public health problem since it is the most frequent cancer in incidence, prevalence and in mortalityit is second to lung cancer in women {Malvezzi et al., 2013, Ann Oncol}. Metastatic breast cancer (MBC) patients have a longer median survival than other metastatic solid tumors, making this population a challenge for the practicing oncologist {Chung and Carlson, 2003, Oncologist, 8, 514-20}. Overuse of anti-cancer therapy is an important quality-of-care issue as are other indicators of aggressiveness in EoL cancer care, like the use of acute care hospital facilities . An aggressive approach to treatment can have negative effects on quality of life, cost and possibly on survival.
In the last decades, more emphasis has been put on directing patients towards a more symptom and care oriented approach. In Portugal there are very little hospice care beds. Most cancer patients die in acute care beds, although increasing awareness is prompting the creation of chronic and palliative care beds. There are very few physician home care visits and specifically the interaction between acute care hospitals and primary care teams that provide home care visits is lacking.

We had previously presented data regarding aggressiveness of cancer care in the EoL using data from a comprehensive cancer center {Braga et al., 2007, Psychooncology, 16, 863-8}. At the time, a third of our sample size was composed of advanced (A) BC patients. We now analyzed another population of similar size from a central acute care hospital, circa 6 years later. With this data we aim to (1) characterize EoL care to ABC patients in different institutions and in different time points; (2) show trends in implementation of palliative care strategies; and in aggressiveness of cancer care through systemic anti-cancer treatments, emergency room, hospital and intensive care unit admissions.

Patients and methods

Eligibility criteria

We analyzed two cohorts of patients. The oldest cohort, from a comprehensive cancer center, included all patients with ABC that were treated in the medical oncology department with palliative systemic chemotherapy, that is systemic chemotherapy for non-curable locally advanced (LA) or metastatic (M) BC and died during the year 2003. This review was conducted with the approval of the institutional ethics committee.

The more recent cohort, from the general hospital, included all patients with ABC that were followed in the medical oncology unit and that died between 2006 and 2012. In this cohort we similarly selected BC patients that received palliative systemic chemotherapy, that is systemic chemotherapy for non-curable LA or MBC. This review was conducted with the approval of the institutional ethics committee. Because estrogen receptor (ER) positive BC is amenable to endocrine manipulations during long periods of metastatic disease we chose to define therapy as systemic anti-cancer therapy for ABC that includes chemotherapy and not endocrine therapy.

Collected data

Demographic data included sex and age. Cancer-related data included date of diagnosis and death and date of documentation of ABC. Regarding administration of systemic therapy, data were collected on its use in the treatment of ABC. For those patients that were treated with CT for ABC further data were collected on potential indicators of aggressiveness of treatment. These included the number, and duration of each CT regimens, CT toxicity and CT response. CT response was considered as clinical or radiological response. In the older cohort we only collected data on the systemic therapy regimens used in the last three months of life, in the recent cohort we collected data throughout the course of ABC. The duration of a chemotherapy regimen was defined as the time between the first and the last day of administration of the drug(s) that were part of each regimen. In this group of ABC we also recorded palliative care interventions, namely, consultations with other health care providers part of the cancer multidisciplinary team: pain clinic, palliative care specialists, psychiatry and use of palliative radiotherapy (RT) for ABC.

Data source

Data were collected through the institutional database and from patient clinical charts. Population-based death certificates were used to complement clinical chart data, assuring that no deaths were missed.

Statistical Methods

Median and ranges were used as descriptive statistics. Chi-square statistic was used to compare categorical data. All data were collected and treated in IBM SPSS Version 20.0.

Results

Patient related data

The whole cohort has 232 patients. The older cohort has 118 and the more recent one has 114. The median age of the population at diagnosis was 60 years, for both cohorts. The age range is between 18 and 95 years (Table 1). The oldest cohort had no men and the recent cohort had 2 men. There were 0.8% in the analyzed group, which is in accordance to the frequency of BC in men.

Cancer related data

The BC characteristics are similar between the two populations (Table 1). Regarding early disease, we recorded data on histologial subtypes, staging, ER status and treatment modalities. Concerning ABC, we recorded data on median survival and locoregional or distant organ relapse.

Palliative treatment related data
In the more recently treated patients the referral to the pain clinic increased significantly, as well as shared care by a multidisciplinary palliative team. The use of palliative RT has also increased. Only psychiatric referral remained constant through time and both institutions. The great majority of MBC patients are still dying in the hospitals that first treated their BC, in our case, the comprehensive cancer center and the general acute care hospital where they were initially treated. However, in this time interval, there are significantly more patients dying in hospices and there has been a shift towards dying more at home.

Chemotherapy in the end of life
In the initial sample, nearly 30% of patients were treated with CT in the last two weeks of life, nearly 40% of patients were treated in the last month, nearly 60% were treated in the last two months and nearly 70% in the last three months of life (Table 2). This trend is changing. In the recent cohort we show only 11% of patients are being treated with CT in the last two weeks of life, nearly 30% in the last month, 43% in the last two months and nearly 50% in the last 3 three months. These are the most relevant results of our study. There is a statistically significant change towards less systemic anti-cancer therapy administration in the last 15 days of life of ABC patients. There is less administration in the last month, two months and three months but the difference is not statistically significant.
We analyzed systemic therapy regimens in the last three months of life in greater detail (Table 2). The median amount of different regimens in both groups is 1. In the older cohort there is one patient undergoing a third regimen of chemotherapy but it was one administration on the day of death. In the more recent cohort, there is no patient with a third regimen.
Of interest is the fact that patients are starting new regimens of CT in the last three months of life. We observed this and still observe this trend. Thirty-eight patients in the older cohort and 28 in the recent one are starting new CT regimens in the last three months of life. Another indicator is if ABC patients are being treated for the first time with systemic CT in the last three months of life. This indicator had been observed in our previous report and continues to be observed in the recent cohort. Although this practice is decisively decreasing with the comparison of these two datasets. The older cohort has 16% patients in this condition and the recent one only has 4%. In those 19 (16%) patients that started palliative CT for the first time three months before death, there were three women that started a second regimen after the failure of the first.
As has been said, in the more recent cohort we collected data on the whole spectrum of systemic anti-cancer treatments for ABC. Without focusing only on the last three months like we did in the earlier cohort. As such, we have data on the number of regimens administered during the whole duration of ABC. The number of regimens most frequently used in ABC patients is six, this happened in 20% of the patients (Table 3). With reference to duration of each subsequent regimen we could show that the median duration decreases in a statistically significant way, as decreases the number of treated patients, as would be expected. We were able to show a trend towards less clinical response and more resistance to subsequent regimens as is empirically observed in ABC clinics, although not statistically significant.

Other indicators of aggressiveness:
For the patients treated in the last three months of life, we were able to show a significant decrease in the admissions to the medical oncology ward and a decrease in the duration of admission. We saw a trend towards more emergency room visits and less intensive care unit admissions. This data corroborates our hypothesis but it is not statistically significant (Table 2).

Discussion
The stage distribution and ER status is different in this population than in a population of BC patients because these women died of BC, therefore they tend to have a higher TNM stage at presentation and less ER positive tumors. The median survival of this cohort of ABC patients is two years, which is as reported in the literature {Chung and Carlson, 2003, Oncologist, 8, 514-20}.
Pain clinic referral is higher in the recent cohort than in the initial one, it took place in 50% of the patients. The very different percentages of pain clinic referral are not only due to a change in practice but due to the fact that we are analyzing two different institutions. The robust implementation of pain clinics in the multidisciplinary team that takes care of advanced cancer patients has increased and our data reflects this. There is a statistically significant difference between palliative care consultation in the older and recent cohorts the trend is not only explained by more awareness of these health care providers but also that the acute care hospital happens to have a more robust palliative care team. We found only 2.6% of women dying at home. This percentage is low. In Canada, for example, this percentage was already 6.9% 10 years ago {Gagnon et al., 2004, J Clin Oncol, 22, 3458-65}. There is still shortage of home care teams with ability to provide care for dying cancer patients.

Concerning palliative radiotherapy the difference is possibly due to change in awareness of radiotherapy, the referral to radiotherapy is greatly increased and it is not an issue of availability or logistics because both institutions have such facilities in place.

Mental health practicioner intervention, measured by psychiatric consultations, is unchanged between both cohorts, although with an increasing trend in the recent cohort. It is notable, that there is shortage of mental health practitioners working with metastatic cancer patients in both institutions, therefore these percentages might mitigate the true need of these professionals. The emergency room visits are higher in the acute care hospital cohort because there is less access to the medical oncologists in the acute care hospital where every non elective admission is centralized through the emergency department.

Despite the constant increase of therapeutic armamentarium available to treat ABC patients we were able to show a trend towards less use of systemic therapy in the end of life of ABC patients. Unlike other frequent solid tumors with high mortality, like lung, colon, gastric, head and neck and pancreatic carcinomas, where CT in the main systemic treatment, in ABC endocrine therapy represents a sizable proportion of systemic treatments. And once endocrine resistance supervenes there are numerous CT drugs available to treat ABC patients. In this dataset, 20% of the patients were treated with six different regimens, these are more regimens than there are available to treat other disseminated carcinomas. The median duration of different systemic chemotherapy regimens in MBC is also higher than in other disseminated carcinomas. In this cohort of ABC patients, the median duration of the different regimens is 3 months, higher than another of our datasets from the same institution of patients with lung carcinoma {Cassiano et al., 2013, Why do we treat lung cancer patients with chemotherapy until the end of life, World lung cancer congress}. In ABC patients, there is response to systemic CT until very late in the natural history of the disease. Confirming this, we were able to show 20% patients exhibiting response to most CT regimens. Opposed to our study, a North American study looking at trends in aggressiveness of cancer care in patients from the United States and Canada, shows more aggressiveness over time {Ho et al., 2011, Journal of Clinical Oncology, 29, 1587-1591}. The sample size is much greater and the years and intervals are not the same, but, in this study, the diagnosis of BC is an independent predictor of aggressiveness. Our work has an important weakness, we do not compare equivalent populations. The two institutions are unrelated and so are the majority of the physicians caring for the patients. The characteristics of the institutions are different, one is a comprehensive cancer center and the other an acute care hospital. Although they are both in the same geographical area of the country and have the same funding from the Ministry of Health. The comprehensive cancer center, as expected, treats more BC patients. To overcome the different numbers of patients, we joined the patients who died of BC during six years in the acute care hospital to have a similar sample size to our older cohort. Another important weakness is the small sample sizes, we are studying just over 200 patients and this population is not large enough for statistical testing.

It is vital to start conducting prospective research in end of life questions in cancer. Continuing to work retrospectively with patient clinical records that are not standardized, in different institutions, and, with different physicians, we might be comparing different realities and collecting incomplete data. Research with data collected in electronic medical records and in population based cancer registries will not have the fine granularity needed to understand decisions in the end of life of cancer patients.

Novel anti-cancer drugs appear daily and that is what patients, society, scientists, pharmaceutical companies and oncologists strive for. What about the end of life of cancer patients, are we providing the best possible care? There are no prospective data collection instruments to assess care in the end of life of cancer patients. We do not measure the delivered care routinely in a standardized approach. We are not changing the focus of health professionals, patients and families from the next new drug to prolong life to adress the issues of end of life. Are oncologists the best trained professional group for this task? {Braga, 2011, Ann Oncol, 22, 2345-8} It is time for oncologists to discuss terminal patients in a specific end of life multidisciplinary round, similar to the decision forum for early cancer cases. In this meeting, every new patient should start prospective data collection on the quality of end of life management in institutions with research groups interested in end of life in cancer. Institutional multidisciplinary decision is an established indicator of quality of cancer care. Cancer patients reaching the end of life should be discussed routinely in a meeting with attendance composed by medical oncology physicians and nurses, palliative medicine, pain, mental health, nutrition and radiotherapy health care providers: The end of life multidisciplinary round. When possible, every newly diagnosed patient with a metastatic solid tumor should be similarly discussed. Our data is telling us the time is ripe for the onset of such practice.

Comparative characteristics of BC		Older cohort (n=118)	Recent cohort (n=114)	p value
Age incidence	median (range)	60 (32-92)	60 (18-95)	1
Histology	Invasive carcinomas of no special type (NST)	110 (93%)	107 (94%)	0.8
	Lobular carcinoma	3 (2.5%)	3 (2.6%)	1
	Mucinous carcinoma	2 (1.7%)	2 (1.7%)	1
	Metaplastic carcinoma	2 (1.7%)	1 (0.9%)	0.5
	Medullary carcinoma	1 (0.9%)	1 (0.9%)	1
Disease stage at presentation	I (T1N0)	1 (0.9%)	2 (1.7%)	0.5
	II (T1-2 N1)	64 (54%)	68 (60%)	0.0002
	III	22 (19%)	25 (22%)	0.6
	IV	31 (26%)	19 (17%)	0.08
ER status	positive	63 (53%)	56 (49%)	0.5
	negative	37 (47%)	44 (51%)	0.5
Treatment at presentation	Surgery	93 (79%)	83 (73%)	0.5
	Chemotherapy	94 (80%)	83 (73%)	0.0002
	Radiotherapy	94 (80%)	90 (79%)	0.6
	Endocrine therapy	65 (55%)	56 (49%)	0.08
MBC	median survival (months)	24	20	
Location of organ metastasis	Bone	36 (32%)	46 (40%)	0.2
	Locoregional	37 (30%)	35 (31%)	0.8
	Lung	24 (20%)	15 (13%)	0.1
	Liver	10 (9%)	11 (10%)	0.8
	Brain	4 (3.5%)	4 (4%)	1
	Ovaries & peritoneum	3 (1.7%)	3 (2.6%)	1

Table 1 Comparison of the two populations: demographics, early BC and MBC variables. Chi-square test was used to show populations are comparable. P values lower than 0.05 are considered significant.

Palliation, location of death, systemic anti-cancer treatment and other aggressive care for MBC		Older cohort (n=118)	Recent cohort (n=114)	p value of chi-square test for difference
Palliative interventions	Pain clinic	12 (10%)	58 (51%)	$3.8x10^{-8}$
	Palliative care consultation	5 (4.2%)	26 (23%)	$1.6x10^{-4}$
	Psychiatry	10 (8.4%)	18 (16%)	0.1
	Palliative radiotherapy	8 (6.8%)	63 (55%)	$6.7x10^{-11}$
Location of death	Hospital where was treated	108 (91.5%)	80 (70%)	0.04
	Another hospital	0 (0%)	5 (4.4%)	0.02
	Hospice	0 (0%)	16 (14%)	$6.3x10^{-5}$
	Home	2 (1.7%)	3 (2.6%)	0.6
	Unknown	8 (6.8%)	10 (8.8%)	0.6
Patients treated systemic anti-cancer therapy	15 days	32 (27%)	13 (11%)	0.004
	last month	45 (38%)	31 (27%)	0.1
	last 2 months	67 (57%)	49 (43%)	0.09
	last 3 months	80 (68%)	58 (51%)	0.06
Other indicators of aggressiveness in the patients treated in the last three months	Patients admitted	74/80 (93%)	53/58 (91%)	0.06
	Hospital admissions	284	174	$2.7x10^{-7}$
	Days in hospital	1725	1002	$2.2x10^{-16}$
	Patients ER visits	66/80 (83%)	51/58 (88%)	0.16
	Emergency room admissions	166	201	0.06
	Intensive care unit admissions	2 (2.5%)	0	0.15
Patients starting treatments in the last three months	A new regimen	38 (32%)	28 (24%)	0.2
	A 2nd regimen	6 (5%)	2 (1.7%)	0.1
	First ever regimen for MBC	19 (16%)	5 (4.4%)	0.004

Table 2 Comparison of the two populations: MBC care variables. Chi-square test was used to show differences. P values lower than 0.05 are considered significant.

Recent cohort (n=114)	Systemic therapy data
No systemic therapy	28 (25%)
1 regimen	18 (16%)
2 regimens	16 (14%)
3 regimens	14 (12%)
4 regimens	10 (8.8%)
5 regimens	5 (4.4%)
6 regimens	23 (20%)
Median regimens	2

Table 3 Number of regimens of systemic anti cancer therapy used during the care for MBC patients in the recent cohort.

Systemic therapy regimens, recent cohort (n=114)	Median duration of regimens & range (days)	Number treated patients (% of population)	Number of patients w/ response or stable disease (%)	Number of patients w/ progression (%)
1st	87 (1-736)	86 (75%)	22 (26%)	64 (74%)
2nd	92 (1-562)	68 (60%)	20 (29%)	48 (70%)
3rd	96 (7-1360)	52 (46%)	13 (25%)	39 (75%)
4th	54 (1-511)	38 (33%)	10 (26%)	28 (74%)
5th	67 (1-790)	27 (24%)	4 (15%)	23 (85%)
6th	59 (1-331)	23 (20%)	3 (13%)	20 (87%)
p-value chi square test	0.001	$5.5x10^{-12}$	0.08	0.7

Table 4 Characteristics of each subsequent systemic anti cancer treatment regimen used to treat the MBC patients of the recent cohort. Median duration, numbers of patients and response or progression are recorded. Response is defined by objective response or symptomatic response. Objective response is assessed by observation or imaging.

Chapter 10: Why do our patients get chemotherapy until the end of life?

Conception and design: Sofia Braga

Provision of study materials or patients: Sofia Braga

Collection and assembly of data: Sofia Braga

Data analysis and interpretation: Sofia Braga

Manuscript writing: Sofia Braga

Final approval of manuscript: Not applicable

Funding: Sofia Braga was funded during three years (from October 2008 until October 2011) by the Gulbenkian Foundation and worked at the Computational Genomics Laboratoty (CGL) at Instituto Gulbenkian de Ciência (IGC).

Ethical Committee approval: Not applicable

Annals of Oncology
doi:10.1093/annonc/mdr416

Why do our patients get chemotherapy until the end of life?

the patient

Some years ago, I treated a 21-year-old woman. During her first pregnancy, an enlarging mass appeared in her right leg. Diagnostic procedures done after delivery indicated that she had alveolar rhabdomyosarcoma. She was referred to the cancer center for isolated limb perfusion, which was deemed impossible due to inguinal masses encountered during the attempt to canalize the femoral vessels. A computed tomography scan showed peripheral micronodules in both lungs, after which she was referred to the medical oncology department for systemic chemotherapy. By then, the primary tumor was a fungating mass requiring morphine for pain control. Treatment with cyclophosphamide, doxorubicin and vincristine led to a prolonged hospital admission for bacterial sepsis, during which she was visited by her husband daily late after work and on Sundays by her family and the baby. On the subsequent cycle, and despite reduced chemotherapy doses, she again experienced severe hematological toxicity and no antitumor effect or decreased requirement of narcotics. Next, single-agent doxorubicin was administered in the outpatient clinic as an attempt to preserve quality of life. Still, no antitumor or symptom response was achieved and multiple hospital admissions due to hematological toxicity ensued. Eventually, ifosfamide was prescribed in progressively lower doses due to increasing hematological toxicity but still without clinical benefit. She finally died of fungal sepsis, 3 months after being referred to the medical oncology department.

the problem

This case illustrates therapeutic futility at the end of life. Why are we not ceasing chemotherapy when it is useless, toxic, logistically complex and expensive? Are we prescribing chemotherapy until too late in solid tumor patients' lives? Medical oncologists have overly optimistic predictions and, sometimes excessive, treatment-prone attitude and they are criticized by other health care providers for this. Increasingly, patients, their families, advocacy groups, policy makers, journalists and society at large dwell on this topic, which is a perplexing conundrum, because sometimes they are the ones demanding not to stop aggressive systemic anticancer treatments. There is a growing culture of awareness toward preserving quality of life, palliative care, symptom-directed care, hospice referral and end-of-life issues regarding terminal cancer patients. Sadly, this issue is gaining momentum, not because oncologists are questioning their practice but because health care costs are soaring. Whatever the motive, the reasons for administering chemotherapy at the end of life should be known. Striking a balance is not easy. Hippocrates in 400 BC wrote, about medicine in general, an aphorism that illustrates this difficulty: Life is short, the art long; the occasion fleeting; experience fallacious and judgment difficult. Medical decision making on ceasing systemic chemotherapy remains a very complex, intimate and subjective process. There are few and conflicting scientific data to guide treatments in this delicate setting.

the available data

What data do we have that characterizes the situation? Most of available data are retrospective death-centered studies, population or institution based [1]. The institution-based studies have access to the complete medical records where eventually data can be retrieved regarding decisions and goals of interventions. On the other hand, population-based studies, such as the one by Näppä et al. (this issue), and the Medicare system-based studies claim to picture reality in a more unbiased way.

In the current issue, Näppä et al. [2] examine chemotherapy administration in the last month of life. They have chosen a population-based cohort from Northern Sweden in which they were able to characterize 374 adults affected by solid tumors that were treated with chemotherapy in the last month of life. Their results show that one-fourth of Swedish terminal cancer patients still receive chemotherapy, which is in agreement with reports from other geographies. In this study, patients that receive chemotherapy have a shorter duration of metastatic disease, more hospital admissions and often lack a documented decision to stop chemotherapy.

Regarding other similar reports, two studies based on Medicare claims, encompassing roughly 8000 patients each, reported that 15% of terminal cancer patients receive chemotherapy in their last 2 weeks of life [3, 4]. In an institutional report from England, only 8% of the patients received chemotherapy in the last month of life but 7.5% and 4.3% of these patients had a toxic death or died of neutropenic sepsis, respectively [5]. Two institution-based studies from Italy showed that 23% and 15% of advanced cancer patients are receiving chemotherapy in the last month of life [6, 7]. Two similar Portuguese studies showed 37% and 13% of the patients being treated with chemotherapy in the last month of life [8, 9]. Further examples include two Korean reports, one where 30% of the patients receive chemotherapy in the last month of life and another where 50% receive treatment in the last 2 months of life [7, 10], and an Australian publication showing that 18% of cancer patients are being treated in the last month of life [11].

In these studies, the parameters presented as being predictors of receiving chemotherapy were young age, short metastatic disease course, tumor type and chemosensitivity of the tumor. Tumor type data show that lung cancer patients are overrepresented possibly because of frequent metastatic disease at diagnosis and short life span. In fact, 43% of non-small-cell lung cancer patients treated by community oncology clinics across the United States receive chemotherapy in the last month of life and 20% in the last 2 weeks [12].

In summary, up to a fifth of cancer patients are treated with chemotherapy in the last month of life without clear benefits (e.g. no prolongation of life) and sometimes even with visible negative consequences (increased toxicity, costs and decreased quality of life). The need to critically evaluate chemotherapy prescription in this context evokes four questions discussed below.

will the patient benefit?

Can doctors estimate patient survival in an accurate way? Most likely no; all physicians, oncologists in particular, tend to overestimate survival due to multiple reasons: strong emotional bonding, underestimating catastrophic complications and relative or forced stability during the doctor visit. A strategy to overcome this is to make frequent reassessments and ask experienced colleagues who have been shown to make more accurate predictions. It may also be beneficial to use evaluators like the Karnofsky performance score, the World Health Organization performance status, specific palliative scores or the assessment of specific symptoms. In the case of symptoms, the most informative are anorexia, weight loss, xerostomia, dysphagia and dyspnea. Among other validated variables are blood biochemical tests (e.g. low albumin, high lactate dehydrogenase, high interleukin-6) and cell counts (e.g. high white blood cell counts, low lymphocyte counts) [13]. Several attempts have been made to use algorithms for death prediction in terminal cancer patients but these have not met with general acceptance because of inefficacy, difficult implementation and ultimately because in terminal care the patient is viewed globally and thus such scoring systems are viewed as an oversimplification.

Is it appropriate to start or to continue chemotherapy? What are the symptoms? Are they cancer or toxicity related? If the main problems are pain, asthenia and cachexia, chemotherapy may not be the only and sometimes not even the most appropriate solution for them since it may exacerbate them. Performance status; asthenia; weight loss; marrow, cardiac and lung reserve as well as kidney and liver function help guiding the evaluation of the relative risks and benefits of using chemotherapy. If chemotherapy is agreed upon, this decision should be reevaluated frequently. The careful weighing of clinical benefits and risks is the core of this issue: *Primum non nocere*. In the majority of solid tumors of adults, a classic rule still stands that after three failed lines of chemotherapy the possibility of benefit with a fourth line is minute. However, there are exceptions, for example, in breast cancer, especially Her2-positive disease [14].

In short, chemotherapy should be limited to ambulatory outpatients with good performance status, except in untreated chemosensitive solid tumors or malignancies that are specifically affecting the ability to walk. The goals of palliative chemotherapy differ from those of curative chemotherapy because metastatic solid tumors are generally incurable; the aim is to increase survival. Furthermore, instead of focusing on lesion diameter shrinkage, a clinical trial end point, clinically relevant outcome measures like symptoms are possibly more adequate.

what does the patient want?

There are important cultural and religious variations in the acceptance of death. Data show that patients with high levels of positive religious coping tend to receive intensive life-prolonging care possibly because they believe in miracles and divine interventions [15]. However, if a religious counselor is provided from within the oncology staff, it reduces aggressive end-of-life care and increases hospice use [16]. Does the acceptance of death mean that all hope is lost? Hope is an important defensive mechanism. Somewhat paradoxically, there are data showing that giving honest information, even bad, maintains hope [17]. Some patients want to live a specific event before feeling prepared to die. If not possible, patients can find other ways to get a sense of purpose out of the event, such as writing a letter or recording a legacy. Many patients and families get great satisfaction from this.

There are tools and checklists for communicating bad news. During consultation, one can follow the stepwise approach of the SPIKES acronym: choose a setting (i), assess perception of the disease (ii), invite the patient to hear (iii), transmit knowledge (iv), assess emotional reaction with empathy (v) and summarize the care plan (vi) [18]. Why is not communication more effective? It is not because patients and families cannot bear to be informed on prognosis, it is because oncologists are insufficiently trained and, even for experienced physicians, giving bad news is just too hard. A simulated consultation study reported that poor performance was correlated with emotional burnout and fatigue but not inexperience [19]. In a study that recorded hematological oncology consultations in tertiary centers, cure was not discussed quantitatively or at all in half of them [20]. A prospective study on palliative chemotherapy versus watchful waiting in advanced cancer patients showed that only 39% of patients reported discussing prognosis with the attending oncologist. In a longitudinal study, in admitted terminally ill cancer patients, 39% of the patients and 62% of the families said that the possibility of death had not been discussed [21]. Research shows that patients know more about their disease and their treatments at the time of diagnosis than at the time of relapse, progression and near death. One of the reasons for this discrepancy is that the established closer proximity between doctor and patient interferes with the phycisians' capacity to communicate unpleasant news [22]. Data show that when information is given to patients, it is provided with a range of values and patients cope with it by hoping to belong to the favorable tail of the distribution [21].

Information pays off. If physicians have discussed care at the end of life with their patients, patients are more likely to receive care according to their needs and preferences [23].

Additionally, when informed about their terminal illness, patients more often choose symptom-directed care [23]. Regarding biased or lack of information, a randomized trial of the use of the decision aid Adjuvant! for adjuvant breast cancer chemotherapy prescription concluded that only 58% (35 of 60) of the women who used the tool chose chemotherapy, while 87% (33 of 38) of the women that were informed by physicians chose it [24]. This is an indication that patients have unrealistically optimistic expectations on the benefits of chemotherapy.

In summary, fully understanding terminal patients' wishes and goals, realistically addressing the potential and limitations of palliative chemotherapy and discussing end-of-life logistics are items of successful communication that might help spare useless treatments.

can the patient get better care?

It is harder to provide a good death than to cure a patient. Research has shown that terminal patients want to die at home, with loved ones, with symptom control, feeling independent and as conscious as possible [25]. For most cancer patients, this is difficult but achievable with the aid of specific skills that unfortunately are not widespread. For example, in a survey to second-year oncology fellows, only 23% carried out correctly an opioid conversion [26]. Cancer death is predictable, i.e. bedridden, pain, dyspnea, cachexia, anorexia, constipation, dehydration, fleeting consciousness and coma; therefore, it is easy to prepare families for it. After death, there might be an urge to move on, but, families in bereavement need follow-up, provide feedback and studies show it to be insufficient [27].

In the last decade, there was an expansion of palliative care units with doctors, nurses and supportive staff, dedicated full time to the terminally ill, that have shifted gear from a cancer-centric approach to a patient-centered approach. Palliative care should be gradually integrated so that the patient, family and medical oncology team do not feel as they are getting rid of the patient to die under the care of another team, away from the environment they lived in during the most extensive and easier part of the disease. The intervention of a palliative care team should start at the time of distant dissemination because the majority of metastatic patients are incurable. As the disease progresses, the emphasis slowly shifts from one of aggressive antitumor treatment to more focus on palliation. Near death, the only treatment is palliation with no blood tests, artificial feeding, emergency room or intensive care unit admissions because families communicate by phone with the staff that visits at home. Why, then, are patients and families sometimes reluctant to accept this? Apparently due to lack of information. Studies have shown higher use of hospice care by informed patients [21]. Additionally, palliative care should not be a one-way road. Different reimbursement systems in some countries might preclude the utilization of hospice care because patients transferred to hospice loose the rights to cancer center care. It would be an improvement if in this setting patients gained rights instead of loosing rights.

Is chemotherapy more effective than best supportive care as treatment of metastatic cancer? This depends on how one defines 'effective'. If it is survival, then, yes, chemotherapy prolongs survival in the majority of metastatic solid tumors of adults. So the question is not if it should be administered but rather until when should it be administered. On the other hand, if effective means achieving a 'good death', with symptom control and quality of life, chemotherapy is not as good. But, do hospice patients die sooner? A retrospective study designed to answer this question actually found either prolongation of life, in case of lung cancer, or no difference, in case of three other common solid tumors (breast, colon and prostate carcinomas). This study is based on Medicare records and the inclusion criteria for the hospice care group is one Medicare claim. Therefore, it might reflect better care in general and not necessarily capture the dichotomy between chemotherapy administration and symptom control [28].

Finally, research must be conducted regarding end-of-life care to identify which patients are best managed with etiological versus symptomatic approach. Qualitative outcomes and health services research increased through the 90s and peaked in 2000 [29]. Futility, toxicity and aggressiveness are measured by following patients with a predicted reduced life span prospectively and collecting data on the justifications, decisions and goals of terminal care interventions and recording indicators of aggressiveness, like emergency room, intensive care unit admissions and surgeries.

are there conflicts?

Oncologists are frequently subject to pressure sometimes from patients but more often from families to continue therapies of doubtful efficacy [30]. Conflict often starts with members of the family that are absent or health illiterate [31].

There are health care systems in which the physician and the institution are better reimbursed for chemotherapy administration and by requesting radiological examinations than for carrying out a complex cognitive discussion. In fact, there are systems where burdensome family conferences are not reimbursed. This creates a perverse incentive because the hardest actions are poorly compensated, while the easier ones are more lucrative. This would be avoided if reimbursement was done on the basis of consultation with the physician with no link to drug administration. Additionally, admitting that there are better alternatives for symptom control and quality of life preservation might include the referral to another team of physicians and the potential loss of the patient as client of the clinic.

Lastly, why is not there more research on terminal cancer care, as has been discussed? Clinical cancer research mainly asks drug-oriented questions by doing clinical trials. But even academic trials led by institutions and collaborative groups are exquisitely dependent on pharmaceutical industry funding to happen. It is extremely difficult to fund applied clinical research questions that do not involve drugs; this might be one of the reasons it is less attractive.

the patient, again

At this point, I return to the initial story and imagine how I could have done better. Regarding the first question, I should have assumed that widely metastatic alveolar

rhabdomyosarcoma was likely to be chemoresistant. The specific disease had taken an aggressive biological behavior, the lesion was increasing daily and the natural history of the untreated primary had only 4 months. I should have set my goals accordingly. The patient, who was supposedly a fit young woman, had feeble marrow reserve, possibly because of infiltration, which I should have diagnosed. Her performance status was three, i.e. she was partially bedridden, with lung metastases, so the potential for infectious complications, with the regimens used, was high. Her symptoms were pain in the primary lesion, which was well controlled with morphine, and she was not dyspneic.

Regarding the three other questions: What did she want? She wanted to be with her baby daughter, husband and parents, at home. Was there a better team to care for her? Possibly, yes. I donot think that she would have objected to a discussion about therapeutic futility and end-of-life care by different staff, provided the medical oncology team could remain available. Were there conflicts? Not at all. They had accepted distressingly peacefully the catastrophe of incurable cancer at a young age.

What went wrong? Why did this young woman get chemotherapy until the end of life? Clearly because I failed. I hope I have learned the lesson.

funding

The Programme for Advanced Medical Education is sponsored by Fundação Calouste Gulbenkian, Fundação Champalimaud, Ministério da Saúde e Fundação para a Ciência e Tecnologia, Portugal.

S. Braga*

Instituto Português de Oncologia and Instituto Gulbenkian de Ciência (Gulbenkian Program for Advanced Medical Education), Lisboa, Portugal
*(*E-mail: sbraga@igc.gulbenkian.pt)*

disclosure

The author declares no conflict of interest.

references

1. Earle CC, Ayanian JZ. Looking back from death: the value of retrospective studies of end-of-life care. J Clin Oncol 2006; 24: 838–840.
2. Näppä U, Lindqvist O, Rasmussen BH, Axelsson B. Palliative chemotherapy during the last month of life. Ann Oncol 2011; 11: in press.
3. Emanuel EJ, Young-Xu Y, Levinsky NG et al. Chemotherapy use among Medicare beneficiaries at the end of life. Ann Intern Med 2003; 138: 639–643.
4. Earle CC, Neville BA, Landrum MB et al. Trends in the aggressiveness of cancer care near the end of life. J Clin Oncol 2004; 22: 315–321.
5. O'Brien ME, Borthwick A, Rigg A et al. Mortality within 30 days of chemotherapy: a clinical governance benchmarking issue for oncology patients. Br J Cancer 2006; 95: 1632–1636.
6. Andreis F, Rizzi A, Rota L et al. Chemotherapy use at the end of life. A retrospective single centre experience analysis. Tumori 2011; 97: 30–34.
7. Matsuyama R, Reddy S, Smith TJ. Why do patients choose chemotherapy near the end of life? A review of the perspective of those facing death from cancer. J Clin Oncol 2006; 24: 3490–3496.
8. Braga S, Miranda A, Fonseca R et al. The aggressiveness of cancer care in the last three months of life: a retrospective single centre analysis. Psychooncology 2007; 16: 863–868.
9. Gonçalves J, Goyanes C. Use of chemotherapy at the end of life in a Portuguese oncology center. Support Care Cancer 2008; 16: 321–327.
10. Keam B, Oh DY, Lee SH et al. Aggressiveness of cancer-care near the end-of-life in Korea. Jpn J Clin Oncol 2008; 38: 381–386.
11. Kao S, Shafiq J, Vardy J, Adams D. Use of chemotherapy at end of life in oncology patients. Ann Oncol 2009; 20: 1555–1559.
12. Murillo JR, Koeller J. Chemotherapy given near the end of life by community oncologists for advanced non-small cell lung cancer. Oncologist 2006; 11: 1095–1099.
13. Christakis NA, Lamont EB. Extent and determinants of error in doctors' prognoses in terminally ill patients: prospective cohort study. BMJ 2000; 320: 469–472.
14. Smith TJ, Hillner BE. Bending the cost curve in cancer care. N Engl J Med 2011; 364: 2060–2065.
15. Phelps AC, Maciejewski PK, Nilsson M et al. Religious coping and use of intensive life-prolonging care near death in patients with advanced cancer. JAMA 2009; 301: 1140–1147.
16. Balboni TA, Paulk ME, Balboni MJ et al. Provision of spiritual care to patients with advanced cancer: associations with medical care and quality of life near death. J Clin Oncol 2010; 28: 445–452.
17. Smith TJ, Dow LA, Virago E et al. Giving honest information to patients with advanced cancer maintains hope. Oncology (Williston Park) 2010; 24: 521–525.
18. Back AL, Arnold RM, Baile WF et al. Efficacy of communication skills training for giving bad news and discussing transitions to palliative care. Arch Intern Med 2007; 167: 453–460.
19. Brown R, Dunn S, Byrnes K et al. Doctors' stress responses and poor communication performance in simulated bad-news consultations. Acad Med 2009; 84: 1595–1602.
20. Alexander SC, Sullivan AM, Back AL et al. Information giving and receiving in hematological malignancy consultations. Psychooncology 2011. Published online in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/pon.1891.
21. Harrington SE, Smith TJ. The role of chemotherapy at the end of life: ''when is enough, enough?'' JAMA 2008; 299: 2667–2678.
22. Hoff L, Tidefelt U, Thaning L, Hermerén G. In the shadow of bad news—views of patients with acute leukaemia, myeloma or lung cancer about information, from diagnosis to cure or death. BMC Palliat Care 2007; 6: 1.
23. Mack JW, Weeks JC, Wright AA et al. End-of-life discussions, goal attainment, and distress at the end of life: predictors and outcomes of receipt of care consistent with preferences. J Clin Oncol 2010; 28: 1203–1208.
24. Peele PB, Siminoff LA, Xu Y, Ravdin PM. Decreased use of adjuvant breast cancer therapy in a randomized controlled trial of a decision aid with individualized risk information. Med Decis Making 2005; 25: 301–307.
25. Goodman D. End-of-life cancer care in Ontario and the United States: quality by accident or quality by design? J Natl Cancer Inst 2011; 103: 840–841.
26. Buss MK, Lessen DS, Sullivan AM et al. Hematology/oncology fellows' training in palliative care: results of a national survey. Cancer 2011. Published online in Wiley Online Library (wileyonlinelibrary.com) DOI: 10.1002/cncr.25952.
27. Chau NG, Zimmermann C, Ma C et al. Bereavement practices of physicians in oncology and palliative care. Arch Intern Med 2009; 169: 963–971.
28. Connor SR, Pyenson B, Fitch K et al. Comparing hospice and nonhospice patient survival among patients who die within a three-year window. J Pain Symptom Manage 2007; 33: 238–246.
29. Borreani C, Miccinesi G, Brunelli C, Lina M. An increasing number of qualitative research papers in oncology and palliative care: does it mean a thorough development of the methodology of research? Health Qual Life Outcomes 2004; 2: 7–7.
30. Morita T, Akechi T, Ikenaga M et al. Communication about the ending of anticancer treatment and transition to palliative care. Ann Oncol 2004; 15: 1551–1557.
31. Smith AK, Sudore RL, Pérez-Stable EJ. Palliative care for Latino patients and their families: whenever we prayed, she wept. JAMA 2009; 301: 1047–1057, E1.

Conclusion: What is the future of breast cancer care?

Diagnosis: mammography wars

Screening mammography has changed breast cancer. All of us, breast cancer clinicians, see that every day in the clinic. Growing numbers of patients are diagnosed with non palpable lesions that lead to less mutilating surgery, usually enabling breast conservation, adjuvant radiotherapy and hormonal therapy, avoiding chemotherapy. Screening mammography has lowered mortality. But at what price? Critics say that the radiation received by 17 mammographies is enough to cause a cancer. Others say we are creating a breast cancer pandemic and are treating in situ lesions that would never become clinically relevant, with aggressive modalities like surgery and radiotherapy and, on a more subtle level, creating phobic behavior in patients. In other words, breast cancer physicians are behaving like prostate cancer physicians and the doubts cast on PSA evaluation can eventually be shared by mammography. The impact of mammography in the group aged 40 to 50 has specially been challenged because, critics say, we are diagnosing only the slower growing lesions and those that will kill these women will not be effectively diagnosed in an early stage with such annual screening. Unfortunately, we have witnessed such examples. Also, the relative rarity of breast cancer before age 50 makes the number of mammographies necessary to save one life close to a thousand. Proponents of screening mammography before 50, say that these are the women that die of breast cancer. Half a century after screening mammography, physicians and epidemiologists have not reached consensus if mammography should be done routinely and since what age.

Risk: The rise and fall of hormone replacement therapy, lifestyle and the search for "BRCA3"

The Women's Health Initiative (WHI) trial of combined estrogen plus progestin was stopped early and published in 2003, when overall health risks, including invasive breast cancer, exceeded benefits. In this trial, 16608 postmenopausal women aged 50 to 79 years, with an intact uterus, were randomly assigned to receive combined conjugated equine estrogens (0.625 mg/d) plus medroxyprogesterone acetate (2.5 mg/d), or placebo, for five years. Screening mammography and clinical breast examinations were performed at baseline and yearly thereafter. Estrogen plus progestin increased total and invasive breast cancers compared with placebo. The invasive breast cancers diagnosed in the estrogen plus progestin group were similar in histology and grade but were larger and at more advanced stage compared to with those diagnosed in the placebo group. The WHI results had strong impact in the field of women's health and since 2003 breast cancer incidence has dropped in the United States. This important result was published in 2007. The SEER registries showed that the age-adjusted incidence rate of breast cancer in women in the United States fell sharply (by 6.7%) in 2003, as compared with the rate in 2002. Data from 2004 showed a leveling off relative to the 2003 rate, with little additional decrease. The decrease began in mid-2002 and had begun to level off by mid-2003. A comparison of incidence rates in 2001 with those in 2004 showed that the decrease in annual age-adjusted incidence was 8.6%. The decrease was evident only in women who were 50 years of age or older and was more evident in cancers that were estrogen-receptor-positive than in those that were estrogen-receptor-negative. This decrease seems to be coincident to the first report of the Women's Health Initiative and the ensuing drop in the use of hormone-replacement therapy among postmenopausal women in the United States. These data were practice changing in women's health management and currently no post menopausal women should take hormone replacement for more than two years and that is if she has severe menopausal symptoms.

Besides estrogen, undoubtedly the most powerful modifier risk factor for breast cancer, obesity is a powerful risk factor in raising the risk of breast cancer. Several mechanisms might be at play: fat tissue pumps out tumor-nurturing molecules such as estrogen. Many obese people have insulin resistance or overt diabetes, and insulin and IGF may spur cancer cell growth as well. Cholesterol that accumulates in our arteries and contributes to cerebrovascular and ischemic heart disease, has other deleterious health effects. When metabolized by the body, it turns into a potent estrogen-like molecule that spurs the growth of breast cancer in mice, and in women. Besides cholesterol, and possibly through cholesterol, there are several epidemiological studies showing the influence of diet and physical activity on breast cancer risk. These studies are seldom performed in healthy women, they recruit breast cancer survivors, so the effect seen is mostly on breast cancer recurrence.

Regarding genes, the discovery of BRCA1 and BRCA2 were immensely helpful, but questions regarding familial risk of breast cancer persist. BRCA mutations are rare high penetrance mutations but moderate and low-penetrance genetic variants implicated in breast cancer etiology are known to exist. Other high penetrance alleles have been identified as part of inherited cancer syndromes. These include germ-line TP53 mutations found in Li-Fraumeni cancer syndrome, PTEN germ-line mutations in Cowden syndrome, and STK11/LKB1 mutations in Peutz-Jegher syndrome. In spite of the high risks conferred by high penetrance mutations, these mutations are rare in the population, and are estimated to account for only 20 to

25% of the familial risk. Another group of genetic variants associated with breast cancer risk are uncommon variants minor allele frequency with moderate effects on risk. Four such genes are ATM, CHECK2, BRIP1 and PALB2. Lastly, most of the unexplained familial risk is accountable by a polygenic model involving a combination of many individual variants with weak associations with risk, the so called low-penetrance polymorphisms. Whole genome sequencing and the mapping of single nucleotide polymorphisms (SNP) have advanced discovery of common genetic susceptibility factors. The current approach is to evaluate genetic variation in candidate cancer pathways and perform genome-wide association studies (GWAS) in very large study populations. With GWAS, in the last 6 years, several common variants that contribute polygenically to breast cancer risk have been discovered in specific genes: FGFR2, TOX3 MAP3K1, c-MYC, LSP1, NEK10, COX11, CASP8, IGFBP5, NOTCH2, RAD51L1, FGFR10 and ESR1d. Genome-wide linkage studies have failed to map cancer susceptibility genes with high penetrance, suggesting that no further genes of comparable importance to BRCA1 and BRCA2 exist. So, the search for BRCA3 has been elusive.

Local treatment: Less and less

After having established that there is no extra benefit of mastectomy, the need for axillary clearance was questioned. The widespread adoption of sentinel lymph node (SLN) biopsy as standard care for axillary staging in cN0 breast cancer is supported by the results of at least 69 observational studies, seven randomized trials, 3 meta-analyses, international societal guidelines, and an extensive literature covering all aspects of the procedure. These studies established that patients with negative SLN do not require axillary dissection (ALND), that axillary local recurrence after a negative SLN biopsy is rare (0.3%), that disease-free (DFS) and overall survival (OS) are unaffected by the addition of ALND to SLN biopsy, and that the morbidity of SLN biopsy is less than that of ALND. The next logical question in the evolution of axillary staging is to ask whether there are SLN-positive patients who can avoid ALND, and it is clear that there are 50% of SLN-positive patients have disease limited to the SLN.

With the widespread use SLN procedure, patients who did not require ALND were increasingly frequent. To answer the question if we could omit ALND, ACOSOG Z0011 was designed. In Z11, 6-year locoregional control and survival were equivalent with or without ALND in cT1–2N0 patients with 2 positive SLN treated by breast conservation with whole breast radiation therapy. A small but growing body of data now suggest that ALND may not be required for selected patients outside the Z0011. Specifically those treated by mastectomy without post-mastectomy radiation therapy, by partial breast irradiation, and by neoadjuvant chemotherapy. Looking ahead, the principal goals of axillary staging, prognostication, and local control will be accomplished by SLN biopsy for a substantial majority of patients, and the role of ALND will continue to diminish.

After breast-conserving surgery, 90% of local recurrences occur within the index quadrant despite the presence of multicentric cancers elsewhere in the breast. Thus, restriction of radiation therapy to the tumor bed during surgery might be adequate for selected patients. To answer this question, the intraoperative radiotherapy trial was conceived with a non inferiority design. The trial compared targeted intraoperative radiotherapy with whole breast external beam radiotherapy in 2232 women aged 45 years or older with invasive ductal breast carcinoma undergoing breast-conserving surgery. At 4 years, there were six local recurrences in the intraoperative radiotherapy group and five in the external beam radiotherapy group. The Kaplan-Meier estimate of local recurrence in the conserved breast at 4 years was 1.20% in the targeted intraoperative radiotherapy and 0.95% in the external beam radiotherapy group. The frequency of any complications and major toxicity was similar in the two groups. Radiotherapy toxicity was lower in intraoperative radiotherapy group than in the external beam radiotherapy group. The majority of professional societies of radiotherapists, surgeons and medical oncologists have endorsed partial breast irradiation (PBI) for limited situations. Nowadays, for patients who are over 45 years, have non lobular histology in a lesion up to 3 cm in size with negative microscopic surgical margins and where the sentinel lymph node is negative we can propose PBI with obvious logistic benefir.

We are well aware that the most frequent neoplastic disease early breast cancer survivors may develop is another breast cancer, homolateral or contralateral. The reasons are not clear but include genetic makeup, lifestyle and the effect of adjuvant chemotherapy used to treat the first carcinoma. The role of RT to the remaining breast tissue after breast conserving surgery might also be relevant. Therefore, the continuos follow-up of breast cancer survivors by trained breast cancer physicians, if possible, is strongly advocated.

The future of breast cancer surgery, because, no matter how non invasive we strive to make it, it is still a highly mutilating intervention, is minimalism. We are eager with the possibly of safely proposing no surgery in the near future to some patients.

Systemic treatment: Designer drugs

Since 2000, the endocrine therapy of breast cancer completely changed. Tamoxifen is still the backbone of therapy in premenopausal patients and the ever lasting question of chemical castration with LHRH analogs has not yet been solved. Surgical castration remains in itself a secure and very cost effective strategy.

Specifically, we have not established if some premenopausal patients can securely be treated just with castration and endocrine therapy omitting chemotherapy. That question is trying to be answered by a group of academic trials called the SOFT (Suppression Ovarian Function Trial), TEXT and PERCHE, where questions are mainly addressing endocrine therapy of premenopausal patients. Because physicians are not comfortable randomizing for omission of chemotherapy, PERCHE trial, that asked that question, was closed for inadequate accrual.
The tamoxifen duration of treatment question has been answered by another contribution of Richard Peto, the ATLAS trial. It has been established as useful beyond 5 years, currently for 10 years. Side effects of tamoxifen should be carefully taught to women and cautiously monitored, but, relapse, breast cancer survival, and bone health were documented to be as predicted.
An alternative to tamoxifen in post menopausal women are aromatase inhibitors, these have showed impact on relapse, never on survival, with different toxicity profiles. Aromatase inhibitors have frequent cumbersome side effects, while tamoxifen has rare serious side effects. The view of breast cancer physicians that treat hundreds of such women, and help them navigate through side effects, might still favor tamoxifen. Plus, for health economic reasons, absolutely vital nowadays, tamoxifen is still cheaper.
A new group of cytotoxic chemotherapy has also changed the landscape of breast cancer treatment: the taxanes. Since 2000, these drugs are used for early breast cancer treatment and there are adjuvant clinical trials showing we can replace alquilating agents and anthracyclines for taxanes, postulated to have a more favorable long term toxicity profile with no cardiotoxicity and no increased risk of acute leukemias. However, the most appealing strategies have been to increase the power of combination chemotherapy in high risk patients, adding taxanes to an alquilating agent and anthracycline backbone.
Since the 1970s, two types of breast cancer have been established: those that express ER and those that don't. Since 2000, two other subtypes of breast cancer were characterized. Tumors with amplification of the Her2 oncogene and tumors without overexpression of ER, PgR or Her2, triple negative tumors. The story of targeting Her2 has epitomized the "tell me the target, I'll tell you the drug" strategy. The adjuvant trials, that reported in 2005, were criticized for being unethical because it was so clear that they would be positive. Indeed, in the era of evidence-based medicine, and, with the current regulatory landscape, this is an extremely controversial opinion. Other drugs are being used today after trastuzumab failure or as double blockade: lapatinib, pertuzumab and TDM1. It has actually been said and written that what was bad news in the preceding decades, to have Her2 amplified breast cancer, is nowadays regarded as good news. Triple negative breast cancers remain without any targeted therapy available, despite vast efforts.

Metastatic breast cancer: Diversity generation
Inspirational scientists like Melvin Greaves and Mike Stratton have taught us, that cancer is "just" a problem of evolution. Greaves wrote an amazing book called "Cancer, the evolutionary legacy" where he elegantly shows that systemic therapy is fueling diversity and selecting the most aggressive clones. In the recent years, several large scale sequencing projects have shown what Greaves predicted in his book. The evolving landscape of cancer mutations after recurrence, metastasis and upon therapy is towering. As Darwin would view it, when we fail our patients the cells have acquired increased fitness. BC cells surviving through years of endocrine manipulations and chemotherapy are truly aggressive. We have circumstantial evidence of this in our patients.
My final concerns go to metastatic breast cancer (MBC) patients, our daily challenge.
The directions for MBC are to biopsy each and every new metastatic breast cancer patient, if possible, even in bone lesions. The rate of change of the four actionable items, ER, PgR, Her2 and proliferation indicators, is between 10% to 20%. The tendency is mostly to loose the targetable molecules. Metastatic lesions tend to loose ER expression in 20% and Her2 in 10% of the cases. Loosing differentiation is most frequent. If possible, MBC patients should be enrolled in gene mutation directed clinical trials. There is interest in circulating tumor DNA to monitor metastatic disease. In the preceding years, circulating tumor cells seemed to be promising. Nowadays we know these are rare, they are difficult to study and specially difficult to standardize between labs. Today, circulating DNA is analyzed for copy number profiles and mutations, for exome sequencing and low coverage full genome sequencing. The quantification of circulating tumor material will be an interesting way to monitor therapy and the qualitative analysis of predominant clones will reflect the selection pressure.
We know that breast cancers generally harbor a few hundreds of somatic mutations. In the phylogenetic tree context, we consider trunk mutations as essential or drivers, actionable or targetable genomic alterations and branches or even leaf mutations as non essential or passengers. We test for their presence in primaries and metastases, we test for intralesional consistency. In oncogene directed disease, we must find the target. These patients are for oncogene de-addiction therapy. Anti angiogenesis, immune boosting and epithelial growth factor inhibition are examples of this. The goal is for de-addiction for the most relevant cancer hallmark. But, in patients with tumors with high genomic instability that have profound intratumor

heterogeneity this might be an indication that no specific target is actionable and that we should treat with cytotoxic chemotherapy. Unstable tumors should correlate with high grade and high Ki67 staining.
The treatment of each MBC patient should be turned into an experiment in itself, it is a memorable task but that is the way forward.

Final comment

It is impossible to precisely enumerate the impact of these interventions on the survival of each individual patient. The shifting landscape of knowledge, diagnosis, screening and therapeutic trials of techniques and drugs does not allow the comparison of breast cancer treatments yesterday and today. But screening, surgery, radiotherapy, chemotherapy, endocrine and targeted therapy have likely added anywhere between 17 and 30 years to the survival of each women with breast cancer. In the beginning of last century a breast cancer diagnosis meant the patient would die; today, a breast cancer diagnosis is possibly the diagnosis of the most curable carcinoma.
I recall a story that illustrates dynamism and change. I met, in the medical oncology ward, a young, albeit postmenopausal, woman with locally advanced breast cancer. She was being treated by another oncologist and I was attending her in the ward because of grade 4 febrile neutropenia during neoadjuvant chemotherapy. She had a large inoperable breast tumor with palpable axillary nodes. After minimal response to full dose anthracyclines she was being treated with taxanes. She was oblivious of her ominous stage III breast cancer. She was a happy, energetic, small, slim woman, always accompanied by her daughter, taller, and trying not to be anxious. After 5 days with no neuthrophiles and 5 more to get counts up, she was discharged home, to continue chemotherapy with her oncologist. I remember the two, the patient with her optimistic, loose, demeanor and the daughter with a serene but conscious look. At the time, the adjuvant trastuzumab trial was enrolling patients. One day, a data manager calls me saying there was a possible new patient that had undergone surgery and had Her2 amplified BC. To my surprise, this lady walks into the consultation room, her constant daughter following her. She had removed a ypT3 tumor with 8 positive nodes in her axilla and surprisingly exhibited renewed strength for her next journey. She greeted the idea of an adventure with a promising drug and was randomized in the trial to one of the therapeutic arms. I was amazed at how courageous this woman was, every step of the way, accepting, almost with pride, each new therapeutic modality. She reached milestones and fought battles, in a concrete way. Nearly ten years have gone by, this woman contacts me occasionally, and she remains without evidence of breast cancer. I believe that if she had had this diagnosis in 1980, just 25 years before, she would have died. No demonstration of neoadjuvant chemotherapy for achieving resectability, no anthracyclines, no taxanes, no trastuzumab, different radiotherapy techniques and less powerful endocrine therapy.
There is a subtler reason to remember this story. While the content of medicine is constantly changing, its form, will remain astonishingly similar. It is about the form, not only about the content. We must master the past because history and science repeat and echo and we clearly walk in the footsteps of others. By looking back, we can see the way forward. Craig Jordan, the inventor of tamoxifen, when he delivered his Karnofsky memorial lecture in 2008, started by citing Haddow when he delivered his Karnosfsky lecture in 1970. Haddow showed that natural and synthetic estrogens had strong effect on some breast cancer patients and said the finding was of “major theoretical importance” but expressed disappointment that “so much of the underlying mechanism continues to elude us”. The tools that we will use to treat breast cancer in the future will doubtlessly alter so dramatically, in the next 25 years, that the landscape of breast cancer therapy might be unrecognizable, like the clinical course of the women that were treated in 1980. Future physicians will laugh at our primitive recipes of poisons to kill these magisterial cells. They will laugh at hospital admissions for ten days for profound neutropenia. But this woman’s attitude, embodies our struggle against breast cancer, and this, will reverberate through history. To keep pace with this heterogenous disease we have to keep inventing and reinventing strategies, learning and unlearning truths, living and reliving our experiences. We should be canny, relentless, obsessive, fierce, tireless, brilliant, adventurous and even mad, in our pursuit of creative research ideas, diagnostic and therapeutic elegance, cure and quality of life. We should devote all our energy to this mission, mobilizing and remobilizing our imagination, for, as we unfortunately witness every day, the five thousand year war on breast cancer has not yet been won.