# Treatment of triple negative breast cancer

Triple negative breast cancers, as a subgroup, are associated with a poor prognosis. But different subtypes within triple negative disease are associated with different outcomes, and they also differ in the way they respond to different treatments. Here Angelo Di Leo provides an overview of what is currently known and the questions being addressed by ongoing clinical trials.

In treating triple negative breast cancer, the first thing is to ensure the diagnosis is correct – it is essential to correctly evaluate oestrogen receptor (ER), progesterone receptor (PgR) and HER2 in primary tumour samples to eliminate 'false' triple negative tumours. The ALTTO trial, which is testing lapatinib and trastuzumab in the adjuvant treatment of HER2-positive breast cancer patients, included central pathology review for ER, PgR and HER2 status. Results showed discordance in 4%–16% of cases between the evaluation of ER, PgR and HER2 in local laboratories and the central laboratory (personal communication, Giuseppe Viale, European Institute of Oncology, Milan).

This is a serious issue because adjuvant therapy decisions are largely guided by these biomarkers. Good communication between oncologists and pathologists is essential to try to reduce such discordance.

Another important consideration is that not all triple negative tumours have a bad prognosis. For example, a retrospective study of 13 International Breast Cancer Study Group adjuvant

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**e-grandround**

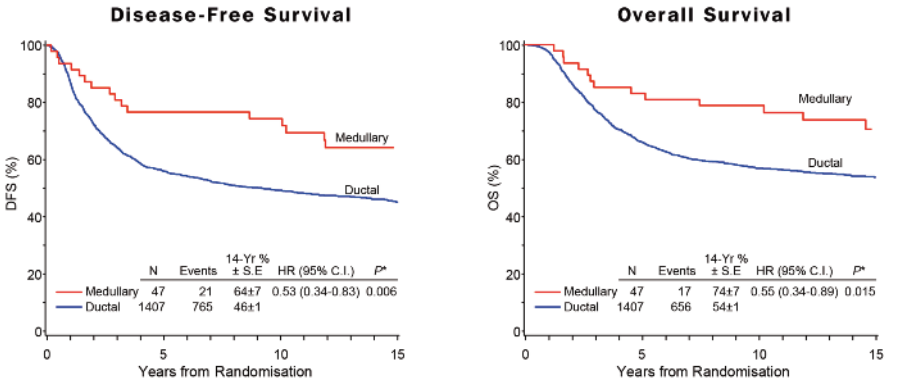
The European School of Oncology presents weekly e-grandrounds which offer participants the opportunity to discuss a range of cutting-edge issues, from controversial areas and the latest scientific developments to challenging clinical cases, with leading European experts in the field. One of these is selected for publication in each issue of *Cancer World*.

In this issue, Angelo Di Leo of Sandro Pitigliani Medical Oncology Unit, Prato Hospital, Italy, reviews the challenges of managing triple negative breast cancer, including the biological heterogeneity within the subgroup that can impact on clinical outcomes, the clinical trial evidence with cytotoxic agents and emerging data with PARP inhibitors.

Lisa Carey, of the Lineberger Comprehensive Cancer Center, University of North Carolina, United States, poses questions sent in by participants during the live presentation. The e-grandround was summarised by Susan Mayor.

**The recorded version of this and other e-grandrounds is available at www.e-eso.net**

**NOT ALL TRIPLE NEGATIVE TUMOURS ARE BAD**




**A pooled retrospective analysis of 13 adjuvant trials showed high-grade, ER-negative breast cancers vary markedly in outcome depending on whether they are of medullary or ductal subtype**

*Source:* Huober et al. (2010) *JCO* 28 (suppl 15): abstract 630

trials reported at ASCO 2010 identified patients with ER-negative, grade 3 early breast cancer and compared outcomes for central pathology laboratory confirmed medullary cancers ($n$=47) and ductal infiltrating cancers ($n$=1407). Despite high grade and ER negativity, patients with medullary tumours had less vascular invasion, better disease-free survival and better overall survival compared with those with the ductal subtype (see above).The better prognosis for the medullary subtype should be considered in treatment decisions.

## Sensitivity to cytotoxic agents

### Anthracyclines

Anthracyclines have been used to treat breast cancer for many years. Triple negative tumours may have proliferation-driven overexpression of the anthracycline drug target topoisomerase II (topoII) alpha and impaired DNA repair due to BRCA1/2 dysfunction. As such, this subgroup might be particularly sensitive to treatment with anthracyclines.

Preclinical data support the concept of increased activity for topoII inhibitors in tumours carrying BRCA1/2 dysfunction. In a cell line model, the topoII inhibitor etoposide was administered to BRCA1 wild type and BRCA1 deficient breast cancer cells, and BRCA2 wild type and BCRA2 deficient fibroblasts. Differential cytotoxicity was observed based on BRCA status with greater cytotoxicity in cells with BRCA loss (see opposite, *top graphs*). The same cell lines were pretreated with aclarubicin at low dose to block the topoII binding site without causing cell death, then re-treated with etoposide. This resulted in markedly reduced etoposide cytotoxicity and elimination of any differential BRCA effect (see opposite, *bottom graphs*). This highlighted that topoII-inhibitor-induced DNA damage is predominantly mediated by topoII binding rather than direct DNA binding.

In addition, an exploratory planned event-free survival analysis by four molecular subgroups (defined using ER, PgR, grade, HER2) reported at ASCO 2010 looked at five adjuvant clinical trials comparing anthracyclines with CMF (cyclophosphamide, methotrexate, 5-fluorouracil) in the adjuvant treatment of early breast cancer. Results showed no clear superiority of anthracyclines over CMF in the so-called highly hormone sensitive tumours, while anthracyclines appeared to be better than CMF in moderately hormone sensitive, HER2 amplified and triple negative subgroups. Notably, anthracyclines seemed to be more active than CMF in the triple negative subgroup (see p16).

These data have not been confirmed by other groups, but this is the largest dataset to date looking at these different subtypes. At ASCO 2009, contrasting results were presented in the exploratory subgroup overall survival analysis of the Canadian NCI-MA5 trial comparing anthracycline-based therapy versus CMF. In this analysis, CMF was favoured over anthracyclines in the core basal triple negative subgroup.

Retrospective and underpowered clinical data are interesting but conflicting, and as such cannot be translated to current clinical practice. More data are required from larger studies.

Summing up this section, cell lines and molecular pathology data suggest that triple negative ductal infiltrating carcinoma may have increased sensitivity to anthracyclines. Clinical data from retrospective studies with limited statistical power are controversial. While we wait for more data – ideally from prospective studies – anthracyclines should still be considered an important component of chemotherapy regimens for triple negative tumours.

**Question:** *You talked about medullary cancers, and what you called 'false' triple negative tumours. Can you expand on the different categories of prognosis separate from medullary and other triple negatives?*

**Answer:** *This is an important point. My impression is that we have slightly ignored the heterogeneity in the histology of triple negative tumours. Beyond ductal carcinoma, there are also medullary, apocrine and squamous cell carcinomas which are also triple negative. Based on retrospective data and not on prospectively designed studies, my impression is that these different subtypes may need a different treatment approach. Medullary tumours have better outcomes than infiltrating ductal triple negative carcinomas. So I think we should make an effort – ideally a collaborative effort – to gain more knowledge about the treatment and prognosis of the uncommon subtypes.*

**Question:** *One of the things that strikes me about patients with triple negative breast cancer when they come to the clinic is that they are convinced they have a poor prognosis, no matter what. It might be interesting to remind readers that disease-free survival at five years for triple negative breast cancer of conventional types is 85%, so many patients can do quite well.*

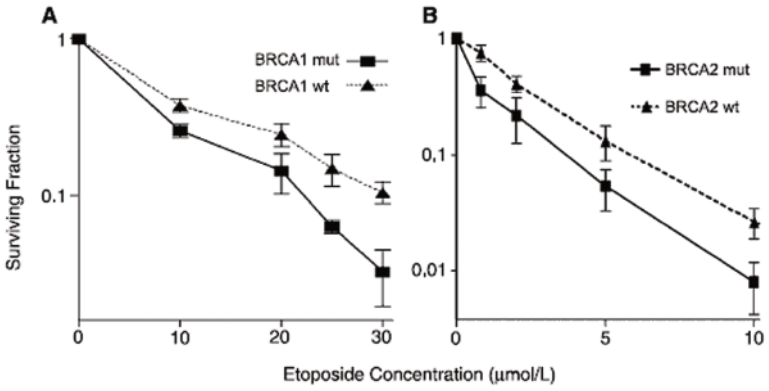
**Answer:** *When patients know they have triple negative status, they are often terrified, independently of whether they have medullary or ductal infiltrating carcinoma. I think it is our task to explain to patients that this is a disease in which there is heterogeneity in terms of outcome, even though these tumours are all characterised by triple negative status.*

## Platinum compounds

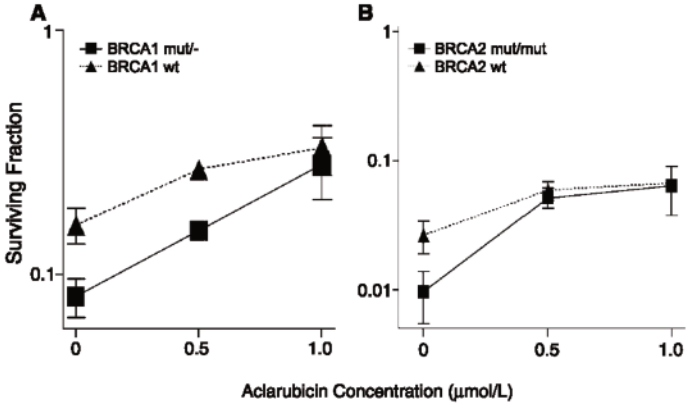
Four neoadjuvant, single-arm trials have been reported with platinum compounds in triple negative breast cancer, in which patients were treated with cisplatin alone or in combination. Results showed pathological complete response (pCR) rates ranging from 15% to 72%. It is particularly important to note results from a small study reported at ASCO 2009 (Gronwald 2009, abstract 502) of 25 patients showing a remarkably high, 72%, pCR rate with neoadjuvant cisplatin. Interestingly, this is the only study in which patients were selected according to the presence of BRCA1 mutation. This is an important signal, which seems to suggest there can be extraordinary activity with cisplatin if patients are selected according to the presence of BRCA1 dysfunction.

**BRCA1/2 DEFICIENT CELL LINES RESPOND MORE TO TOPOII α INHIBITORS**

BRCA1 deficient breast cancer cells and
BRCA2 deficient fibroblasts exposed to etoposide




Etoposide 25 µmol/L in presence of low dose
topoII α inhibitor (aclarubicin)




**Preclinical data show that DNA damage and cytotoxicity from topoII inhibitors is greater in BRCA deficient cells (top) and that cytotoxicity is mediated indirectly by topoisomerase binding, rather than direct DNA binding (bottom)**

*Source:* Treszezamsky et al. (2007) *Cancer Res* 67:7078–7081

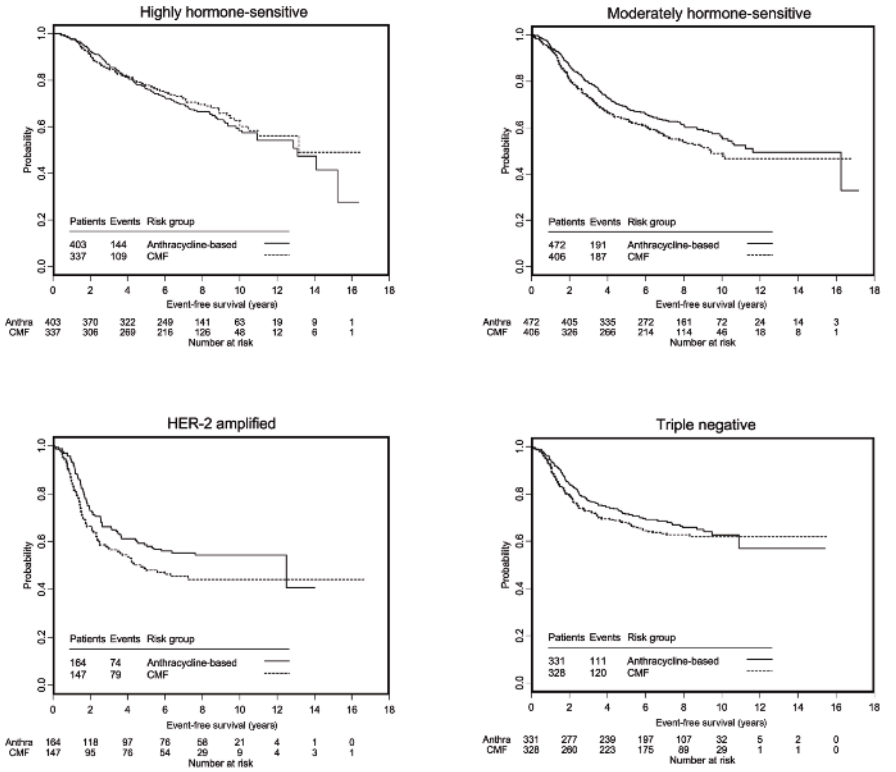
A single-institute phase II trial with cisplatin in triple negative advanced breast cancer was reported at ECCO/ESMO 2009, in which 126 patients pretreated with first-line chemotherapy were randomised to metronomic (repetitive, low doses) oral cyclophosphamide and methotrexate with or without cisplatin. Results showed better outcomes in patients treated with cisplatin: CR 8% vs 5%; PR 55% vs 28%; median time to progression 13 vs 7 months; median overall survival 16 vs 12 months (Bhattacharyya et al. *Eur J Cancer* 7 (3 Suppl):18). This gives further evidence for efficacy of cisplatin in triple negative breast cancer, but more data are needed.

Another study randomised patients with metastatic triple negative breast cancer to cetuximab followed by cetuximab plus carboplatin on progression, or to cetuximab–carboplatin (*JCO* 26: abstract 1009). Results showed higher overall response rate when the combination was used initially (17% vs 6% with cetuximab alone) and greater clinical benefit (31% vs 10% with cetuximab, and 25% for the two drugs used sequentially).

The first-line TNT trial, from King's College London, in patients with centrally confirmed triple negative advanced breast cancer is underway to compare four treatment arms: platinum, platinum plus PARP inhibitor, docetaxel, and docetaxel plus PARP inhibitor. Patients may cross over at progression (personal correspondence, Andrew Tutt, King's College London). I think this trial will fully address the issue of platinum compounds in triple negative advanced disease.

We are also starting a phase II randomised study in Italy comparing a non DNA damaging regimen (capecitabine plus oral vinorelbine) with a DNA damaging regimen (cisplatin plus cyclophosphamide). The triple negative status is

**ANTHRACYCLINES VS CMF IN DIFFERENT SUBTYPES**




**An exploratory (planned) analysis of four adjuvant clinical trials showed that certain subtypes of breast cancer, including triple negative tumours, are more sensitive to anthracyclines than to the CMF regimen**

*Source:* Di Leo, presented at ASCO 2010, abstract 519

defined not from the archived primary tumour sample but in real time from circulating tumour cells. Non-triple negative patients are also being treated in this trial, providing a control arm to demonstrate that the superiority of a DNA damaging regimen occurs predominantly in triple negative disease.

## Predicting sensitivity to DNA damaging regimens

Several studies have been conducted to identify molecular markers of sensitivity to DNA damaging regimens. A Japanese study in 60 patients with early disease treated with neoadjuvant epirubicin/cyclophosphamide followed by docetaxel assessed DNA repair proteins on tumour samples at baseline and 18–24 hours after chemotherapy. The panel of DNA repair proteins assessed by immunohistochemistry included BRCA1, Rad 51, γH2AX, and conjugated ubiquitin (all at baseline) and Rad 51 (post treatment). These proteins are all involved in homologous recombination, which is a key mechanism of repair of double-strand DNA breaks induced by anthracyclines. DNA damage response score was assessed from 0 to 4, with the highest score reflecting the greatest efficacy in DNA

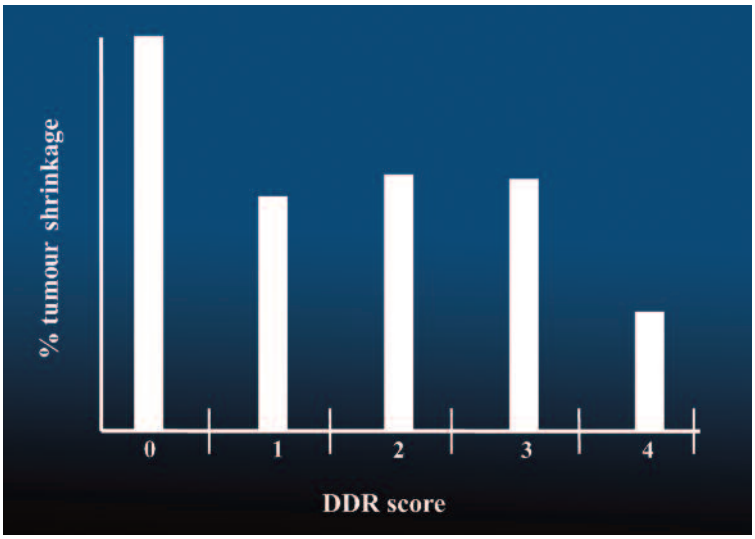
repair (Asakawa et al. *Breast Cancer Res* 12(2):R17). The DNA response score showed an inverse correlation with tumour shrinkage and response rate. The lowest score was associated with the highest efficacy (see below). These data are very interesting, but very preliminary.

The Comet assay is a potential tool for identification of DNA damage and prediction of sensitivity to DNA damaging therapy. Different chemotherapy agents can cause DNA strand breaks leading to DNA fragmentation. This fragmentation can be measured using Comet, a test commonly used in toxicology, in which cell scrapings are taken from fresh primary tumour and corresponding non-cancer tissue. Cells are layered on agarose pre-coated slides. Single gel embedded cells are lysed to isolate nucleoids containing supercoiled loops of DNA. Labile DNA at sites of damage is able to unwind and migrate differentially out of the cell during electrophoresis. When observed by fluorescence microscopy, cells with DNA damage resemble comets with a nuclear head and 'tails' of fragmentation (see p18). The comet tail in cancer cells are due to DNA fragmentation, while healthy cells show no DNA fragmentation.

Software linked to the microscope measures comet tail length and tail intensity for each cell, and calculates average values for each sample. The tumour sample Comet score is then adjusted by comparison to the non-tumour sample score (*Mol Biotechnol* 26:249–261). The non-tumour sample can have some DNA damage that is induced by tissue sampling, so it is very important to adjust for this.

In our pilot study of 91 patients with early breast cancer classified by molecular subtypes (highly endocrine-sensitive, moderately endocrine-sensitive, HER2 positive, triple negative), results showed no substantial differences in Comet scores by subtype. However, the triple negative subgroup had the largest inter-patient variation. There was also substantial intra-tumour heterogeneity in Comet scores, suggesting some areas of a tumour have substantial dysfunction in DNA repair while others do not. Intra-tumoural heterogeneity in DNA repair and presumed heterogeneity in sensitivity to DNA damaging agents may in part offer a biological rationale for resistance to DNA damaging therapy.

**TUMOUR SHRINKAGE BY DNA DAMAGE RESPONSE SCORE**




**Epirubicin–cyclophosphamide given neoadjuvantly led to greater shrinkage in tumours with a low DNA damage response score (DDR)**

*Source:* Asakawa et al. (2010) *Breast Cancer Res* 12(2):R17

Summing up this section, data from phase II non-randomised trials in the neo-adjuvant setting suggest that triple negative tumours carrying BRCA1/2 dysfunction might be highly sensitive to platinum compounds. Data from phase II and phase III randomised trials are still preliminary but are not against the hypothesis that triple negative tumours have increased sensitivity to platinum compounds. Ongoing studies are attempting to identify molecular profiles and biological features predicting response to DNA damaging cytotoxics.

## PARP INHIBITORS

PARP inhibitors potentially provide a targeted therapy approach for patients with triple negative disease. A tumour with dysfunction in BRCA and dysfunction in PARP may lack two important DNA repair mechanisms: the base excision repair mechanism, which is mainly dependent on PARP, and homologous recombination, which is mainly dependent on BRCA1/2. Inactivation of these two pathways may lead to endogenous cell damage and increased sensitivity to DNA damaging chemotherapy, radiotherapy and other agents that damage DNA.

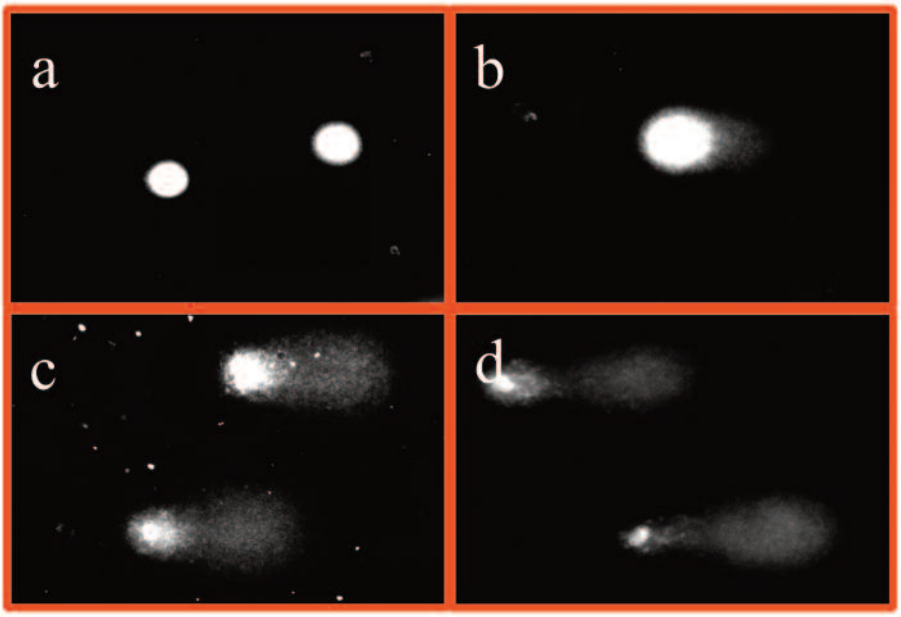
In triple negative breast cancer, homologous recombination may be inactivated due to BRCA1 dysfunction, with compensatory upregulation of PARP activity and the base excision repair pathway. If you then give a PARP inhibitor, you will also inactivate the base excision repair mechanism.

Cell death due to loss of these two pathways is called 'synthetic lethality'. This is the rationale for the use of PARP inhibitors in cancers carrying BRCA1 dysfunction, and also for the combination of DNA damaging chemotherapy with PARP inhibitors.

Several PARP inhibitors are being evaluated in clinical trials. The most advanced data on PARP inhibitors were reported recently in *The Lancet* by Andrew Tutt and colleagues (vol 376, pp 235–444). This international study was undertaken in BRCA-mutated patients with advanced, heavily pretreated breast cancer, in which all patients were treated with the oral PARP1 inhibitor, olaparib (400 mg twice daily or 100 mg twice daily). Both doses showed activity in this heavily pretreated population, with the 400 mg dose showing a higher overall response rate of 41% compared with 22% for the 100 mg dose. Some toxicity was reported, with the main grade 3–4 side-effects being fatigue, nausea, vomiting and anaemia. Olaparib appears to be tolerable, with manageable toxicity.

Other data come from a randomised phase II trial with the parenteral PARP inhibitor iniparib (*NEJM* 364:205–214). Patients had triple negative metastatic breast cancer, but dysfunction in BRCA1 was not considered in the eligibility criteria, as it was for the olaparib study. One hundred and twenty patients participated in this study. Most had received one or two prior lines of treatment for metastatic disease. The control arm was gemcitabine plus carboplatin and the experimental arm was the same chemotherapy plus iniparib. Results showed a higher rate of clinical benefit in the iniparib arm (55.6% vs 33.9%, $P$=0.015), with longer progression-free survival (5.9 months vs 3.6 months, $P$=0.012) and overall survival (12.3 vs 7.7 months, $P$=0.014). This phase II randomised study was the basis for moving to the phase III study, with the same therapy arms, that has recently completed accrual in the US. Results should become available in the coming year.

**PREDICTING SENSITIVITY TO DNA DAMAGING AGENTS**




**The Comet assay distinguishes healthy cells (a) from cancer cells, which have a comet-like tail indicating DNA fragmentation (b, c, d)**

*Source:* Galardi et al., presented at the IMPAKT Breast Cancer Conference, Brussels, 2010

## Remaining questions with PARP inhibitors

We have a lot of data on PARP inhibitors, but we also have many questions about their optimal use in breast cancer patients. It is important to understand whether there are differences in activity between continuously administered oral agents and intermittently administered IV agents. This might have implications in terms of the degree of inhibition of the PARP enzyme.

Other questions include: What are the best cytotoxic partners for these compounds? What is their activity in non-triple negative disease, particularly in those displaying some dysfunction in DNA repair? Are there molecular predictors of response to PARP inhibitors as well as to DNA damaging agents? What is the long-term safety of these compounds? This last question is extremely important, particularly because we are already looking at evaluating these compounds in early disease.

Three studies further evaluating PARP in triple negative disease are:

- The UK-led NeoBIG trial. This will test iniparib in combination with docetaxel or carboplatin/gemcitabine in the neoadjuvant setting.
- The BIG trial, co-ordinated by the International Breast Cancer Study Group. This will test iniparib in combination with epirubicin in the first-

**Lisa Carey, from the Lineberger Comprehensive Cancer Center, University of North Carolina, USA, hosted a question and answer session with Angelo Di Leo.**

**LC:** *The last section focusing on PARP inhibition is probably one of the most exciting areas. We have one phase II trial, but I think it is also worth noting that there were triple negative cancers that do not carry germline BRCA mutations, and there have been some phase II trials using various agents that have not been so exciting. This is obviously an area to be determined in a number of trials moving forward.*

*Could you expand on long-term safety? There is so much enthusiasm for these drugs that if they do turn out to be positive in the phase III setting, I think people may use them quite widely. You commented a little about your concerns in the adjuvant setting.*

**AD:** I think this is an interesting field of research. The concern is related to potential secondary tumours, leukaemia and myelodysplastic syndrome, particularly when these agents are used in combination with DNA damaging agents, specifically anthracyclines. This is an issue that needs to be clearly explored. Differential PARP inhibitor effect in tumour and healthy cells might suggest that we will not see any long-term safety concerns, but this is an issue that will have to be carefully evaluated in future-generation adjuvant therapy trials with PARP inhibitors.

**Q:** *At which point do you regard a patient triple negative in terms of ER and PR levels of negativity? The convention in the US has been to use ASCO/CAP guidelines, which set the threshold very low, so that essentially any ER or PR staining is called positive. We all recognise that this may misclassify some patients. The CALGB triple negative trial allows up to 10%. What has been your experience?*

**AD:** I think there is no definite answer. We can say that you and I are co-ordinating efforts across the Atlantic, in the context of the BIG and the US intergroup collaborative effort. We are trying to look at some neoadjuvant and adjuvant therapy trials in an attempt to understand if there is a threshold that can tell us when a tumour has to be considered triple negative versus non triple negative. For the time being, my impression is that if a patient is below 10% for ER and PgR, and has HER2-negative status, I would feel comfortable in considering the tumour triple negative, assuming there is ductal infiltrating histology and other characteristics suggesting triple negative status, such as high proliferation.

**Q:** *Could you comment on the role of bevacizumab in the treatment of triple negative breast cancer? Are there any data?*

**AD:** Some retrospective analyses have been done in the context of metastatic breast cancer trials where bevacizumab has been tested, but I do not think that these data were strong enough to make any definitive comment on its role in triple negative breast cancer. An ongoing trial is testing bevacizumab in the adjuvant setting after chemotherapy, specifically in triple negative tumours. My impression is there is no strong rationale for thinking that antiangiogenic treatment should be particularly active in triple negative disease.

**LC:** *I agree. I do not know of any reason to think that bevacizumab works any better or worse in triple negative than in any other subgroup of breast cancer. The forest plot seems to show that the extent to which it works is fairly uniform across all subsets of breast cancer. It is a real challenge for us as to how we select for these drugs as we go forward. The neo-adjuvant CALGB trial also has randomisation to receive or not receive bevacizumab, so that would also be directly tested there in the neoadjuvant setting.*

line treatment of metastatic disease, followed by cyclophosphamide, cisplatin plus iniparib. This study, as well as NeoBIG, has been designed with the aim of obtaining complementary data that can lead to a more rational design of an adjuvant therapy trial in the context of BIG.

- The US NSABP neoadjuvant trial. This is an interesting design proposal, addressing the role of PARP inhibition and the role of anthracyclines, with the primary endpoint of pCR.

The take home message on PARP inhibition is that this is a targeted approach, particularly in tumours carrying DNA repair dysfunction such as triple negative cancers. Several PARP inhibitors (oral and intravenous formulations) are currently being tested in clinical trials. Preliminary data from phase II trials suggest that this new class of agents may be particularly active in triple negative tumours without major safety concerns. Although current data are very provocative, treatment with PARP inhibitors is not yet a 'standard' for daily practice, and no PARP inhibitor has yet been approved. Further results are awaited and these will probably become available in the next year.