

Breast Cancer

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SURGERY and RADIOTHERAPY Ideas for clinical research in breast cancer derive mainly from the Task Force for breast cancer, the first multidisciplinary group set up at the EIO. Main areas of clinical study are

1) Breast Conservation. The quest for more conservative and effective treatments for breast cancer continues with studies on the feasibility of a second conservative approach in women with local disease recurrence. The increasing number of patients who underwent breast conserving surgery and radiotherapy (BCT) resulted in a corresponding increase of ipsilateral breast recurrence (IBTR) that is the intrinsic risk in the preserved breast. The 10-years local recurrence rate is reported in the range of 10-20% in various series. Since long time salvage mastectomy has been considered the standard local therapy after IBTR. Nonetheless patients are increasingly requesting a second chance to preserve the breast. Few experiences regard further conservative surgery with or without irradiation mostly performed using the brachitherapy technique. We study the feasibility of partial breast reirradiation by using intraoperative radiotherapy (ELIOT). From 2000, we enrolled 37 patients with small recurrent breast carcinoma after initial BCT and RT, who underwent a further quadrantectomy followed by partial breast irradiation using ELIOT. 12 of the 37 were treated in 2007. The median interval from initial diagnosis and IBTR was 122 months (range 23-312); the histology was invasive in all cases. In 12 of the 37 patients, tumor recurred in the same region of the breast. All patients underwent a second excision of the tumor and were treated with ELIOT directed to the tumor bed and the surrounding tissue with a margin according to tumor presentation. Different applicators with diameters ranging from 3 to 6 cm had been used and the level of energy varies from 4 to 10 MV according to the thickness of the breast tissue. The single dose administered for retreatment varies from 16 Gy to 21 Gy. The first 12 patients were treated with lower doses ranging from 8 to 15 Gy. At a median follow up of 20 months (range 11-63), 4 patients developed a second local relapse (10.8%) and in 2 cases a contralateral breast cancer (5.4%) was registered. Late side effects were available for 34 patients who completed at least 6 months of follow up.

Fibrosis in the site of recurrence and reirradiation of grade 0/1 was reported in the majority of cases (21/37), while a grade 3 in 4 patients. The evaluation of cosmesis showed a poor result in 2 patients, fair in 13 and good/excellent results in the remaining cases.

These preliminary experiences are encouraging. Reirradiation with ELIOT showed no significant side effects, acceptable cosmesis and high level of satisfaction of patients and may be an acceptable alternative to the mastectomy in selected cases.

2) Intraoperative Radiotherapy with Electrons (ELIOT). The rationale for the use of partial breast radiation therapy in place of whole-breast irradiation is based on the finding that approximately 85% of breast relapses are confined to the same quadrant of the breast as the primary tumor. Tumor foci are usually located in close proximity to the primary tumor and residual microscopic disease occurring in the same quadrant as the resection is often the cause of local disease recurrence. Intraoperative Radiotherapy with Electrons (ELIOT) clinical trial started in 2000 randomizing patients to receive either 21 Gy intraoperatively in a single fraction or external beam radiotherapy with conventional fractionation. The trial completed the enrollment of patients in December 2007. Inclusion criteria were the following: age ≥ 48 and < 75 years, tumor diameter ≤ 2.5 cm, infiltrating histology, no mammographic evidence of multifocality. Of the 1306 patients that entered the trial 1187 were confirmed eligible. 655 in the external beam radiotherapy arm and 651 in the ELIOT arm. 189 patients were enrolled just during 2007. Patients are actively followed (median follow up is now 33.8 months) and will allow us to determine whether ELIOT can substitute traditional radiotherapy in a subgroup of women undergoing conservative treatment for breast cancer.

3) ELIOT boost plus external beam radiotherapy. Many studies significantly correlated young age with a worse local control and local-relapse free survival rate. The EORTC trial 22881/10882 demonstrated that a higher radiation dose to the primary tumor area significantly reduced the rate of local recurrence at 5-yrs from 7.3 to 4.3% and the largest

clinical benefit is achievable in premenopausal women. These data suggest the need of a supplemental dose of irradiation to the surgical bed, in excess to the amount delivered to the rest of the breast, especially in young women. Based on these data in June 2004, at the European Institute of Oncology, started a trial for premenopausal women after breast conserving surgery where hypofractionated external beam radiotherapy course of 37.05 Gy delivered in 13 daily fractions of 2.85 Gy follows after 3 weeks an electron intra operative radiotherapy boost of 12 Gy. More than 230 patients have been treated with this scheme, 99 of them just in 2007. Acute toxicity has been acceptable and comparable with external beam radiotherapy with conventional fractionation. Patient's compliance is high. This treatment approach shortening the standard course of whole breast RT to less than 3 weeks avoid controversies regarding the sequence of local and systemic treatments. This procedure allow to deliver a high dose to the tumor bed and an adequate dose to the whole breast in a short overall treatment time with a potential gain in the radiobiological effect. Our institution is therefore currently treating premenopausal breast cancer patient with this innovative and very promising approach.

4) Shortening radiotherapy in breast cancer treatment. The most commonly used fractionation schedule in the post conserving surgery setting is 2 Gy per fraction to 45-50 Gy to the whole breast followed by a supplementary dose to the primary tumor area of 10-16 Gy boost. The five/ seven week's long treatment represents an inconvenience particularly for patients who live distantly from the cancer center. A shorter treatment fractionation schedule would not only reduce the burden and cost tolerated by the patient but also minimize the time women spend away from their families and work. Furthermore has been shown that the longer is the adjuvant treatment, the later the patients feel healed. Between March 2003 and October 2005, 270 women with early stage breast cancer were enrolled in an IEO pilot study of hypofractionated radiotherapy. Eligible patients had to have a confirmed diagnosis of breast cancer after breast conserving surgery (quadrantectomy + LS biopsy +/- axillary dissection) with a planned radiotherapy treatment. Radiotherapy had to be delivered with opposing tangential ports of 6 MV photons to the whole breast with a hypofractionated scheme of 2.25 Gy plus a concomitant boost of 0.25 Gy to the surgical bed for 20 daily fractions. The total biological equivalent dose calculated for the fractionation used was of 81.2 Gy assuming a α/β ratio of 4 for breast carcinoma. Follow-up policy consisted of a structured interview with a physical examination and a visual record (photograph) performed six months after the end of radiotherapy and every twelve months afterwards. We assessed objective toxicity using standardized assessment sheets of the SOMA-LENT breast criteria. Subjective

toxicities was recorded using the Visual Analogue Scale (VAS) for itch, pain, and burning with a score of 0 defined as "no symptoms" and a score of 10 defined as "the worst severity ever experienced". Cosmetic results collected using patient's opinion was evaluated also using a 0 to 10 VAS scale.

Objective late toxicity data is now available for more than 100 patients with a median follow-up time of 17.6 months (range 8.9 – 35.8). High grade (G3-4) objective late toxicity of any kind was observed in less than 5% of the patients. No patients had any grade of ulceration of the skin at the time of observation. 98 patients were evaluable for subjective symptoms. Patients were generally satisfied for the cosmetic result that was rated good in 51% and excellent in 33% of the patients. Overall 60 (60%) patients did not develop any long term radiation sequelae and 61 (62%) did not complain of any symptom to the breast. Our data suggest that a modest increase in the daily fraction size does not result in an increased objective and subjective late toxicity. Our hypofractionated scheme, shortening the overall treatment duration, appear to be a viable alternative to conventional fractionation helping to lessen the burden of the treatment. Following the pilot study the Radiotherapy Department started in April 2007 a randomized trial comparing a accelerated hypofractionated radiotherapy scheme of 2.25 Gy to the whole breast for 20 daily fractions plus a concomitant weekly boost of 1.25 Gy to the surgical bed given with electrons with a conventional fractionation after breast conserving surgery. The trial recruited 81 patients in the first 8 months and will allow us to determine whether the two weeks shorter external beam radiotherapy schedule is a viable alternative to the conventional treatment .

5) Sentinel Node Biopsy. A multicentric trial continues whose aim is to determine whether axillary dissection can be avoided when the sentinel node contains only micrometastasis.

6) SNOLL. This is a technique that combines radio-guided occult lesion localization (ROLL) with radio-guided sentinel node biopsy (SNB). It is used in patients with non-palpable lesions shown to be malignant by pre-operative biopsy or intraoperative histological analysis. The radio-tracer for ROLL and SNB are injected separately, but during surgery a gamma-probe is used to guide removal of both the occult lesion and the sentinel node. The advantage of SNOLL is that it allows complete treatment of non-palpable lesions (tumor removal, SNB and axillary dissection) in a single surgical session.

7) Intraoperative Avidination for Radionuclide Therapy (IART). This technique is being developed in conjunction with Nuclear Medicine. Avidin is injected intraoperatively in

and around the tumour bed after cancer removal (quadrantectomy) and the wound is closed as normal. Later, radio-labelled biotin is injected intravenously. The biotin binds to the avidin in the tumour bed, bringing with it radionuclide whose decay kills residual tumour cells. Preliminary studies indicate fast and stable uptake of radiolabelled biotin at the operated site, and the radiation dose released to the breast is over 5 Gy/GBq. These encouraging results have stimulated a feasibility study on IART.

The *IEO Breast Cancer Data Base* was started in 1999, to prospectively collect data on all patients operated on for invasive breast cancer. Later, the database was extended to non invasive breast lesions. By the end of 2007, almost 20,000 cases of invasive and 2,000 non-invasive cases had been collected providing epidemiological, clinical and pathological data for research and publication. The database is updated continuously particularly with follow-up data.

Studies to improve and refine treatment: Is avoiding post-mastectomy radiotherapy justified for patients with four or more involved axillary nodes and endocrine-responsive tumours? Lessons from a series in a single institution. (*Gentilini O, et al. Ann Oncol. 2007;18:1342-7*) There is still considerable controversy as to whether radiotherapy is useful after mastectomy for breast cancer. At the European Institute of Oncology, radiotherapy is not usually given after mastectomy and complete axillary dissection, even if four or more axillary lymph nodes are found to be metastatic. This policy, which is different to that recommended by the American Society of Oncology and National Institutes of Health, is justified by the consideration that we perform complete axillary dissection (up to the apex of the axilla) – a treatment not given by most USA centers. We recently published a study describing our experience in this area and analyzing loco-regional recurrence rates in 650 women who received mastectomy, complete axillary dissection and no radiotherapy. The series excluded those with metastatic disease, those with very large cancers (T₄) and those who received pre-operative chemotherapy. The five-year cumulative incidence of loco-regional recurrence was 1.8%. However in patients with four or more metastatic lymph nodes the rate was still only 4.5%. The most important predictive factor in the series was hormone receptor status, so that those with positive estrogen and progesterone receptors still had an acceptable low recurrence rate even with four or more metastatic lymph nodes. In this group of patients the possible benefit of radiotherapy in a few cases would be counterbalanced by more widespread adverse effects in the series. It was concluded that the present policy is justified but that more prognostic analysis is desirable to identify the small subset of patients who will benefit from more severe treatments like post mastectomy radiotherapy.

A comparative study on the value of FDG-PET and sentinel node biopsy to identify occult axillary metastases. (*Veronesi U, et al. Ann Oncol. 2007;18:473-8*). We use minimally-invasive sentinel node biopsy (SNB) to determine whether breast cancer has spread to the axillary lymph nodes. However, it would be better to have a completely non-invasive method to determine axillary node status, so that those with no involvement are spared even the minor surgery required to remove the sentinel node. Furthermore, although SNB is generally well tolerated by patients, it is a time-consuming surgical procedure and also requires pre-surgical lymphoscintigraphy. We performed a study to evaluate whether positron emission tomography (PET) was able to reliably identify axillary metastases. We studied 236 breast cancer patients with no overt axillary involvement, and evaluated the ability of PET to identify occult metastatic axillary nodes in comparison with the established methodology of SNB. By SNB, 44% of patients had a metastatic axilla. The specificity and positive predictive value of the PET technique were found to be acceptable (96% and 88% respectively). In particular, in 38 of 43 cases with a positive PET finding the axilla harbored metastases, suggesting that whenever PET is positive, axillary dissection should be performed directly. However the sensitivity of the PET technique was low (37%), and this finding indicates that a negative PET result does not reliably indicate that the axilla is free of metastases, so in these cases SNB should be performed as a more reliable way of determining axillary status. In conclusion PET is useful for identifying an involved axilla, but it is an expensive procedure and should probably be used only in selected patients: for example those with a clinically suspicious axilla.

When can a second conservative approach be considered for ipsilateral breast tumour recurrence? (*Gentilini O et al., Ann Oncol. 2007;18:468-72*). A minority of breast cancer patients treated by conservative breast surgery experience a local recurrence of their disease. The standard approach to such patients is complete breast removal (mastectomy). Recurrence therefore represents a failure of the conservative approach. However in many cases, thanks to regular follow-up, the recurrent lesion is detected when it is small and at an early stage. The possibility arises that some of these women might be better-served by a further conservative treatment. We performed a retrospective study assess outcomes in 161 women treated by second conservative surgery following cancer recurrence. These women formed 55.9% of the 288 patients with local failure over the study period. Conservative re-treatment was only given after careful pre-surgical evaluation to determine the feasibility of conservative treatment. In all cases the resection margins were free of disease. Overall survival in the series was 82.2% at five years, however the cumulative incidence

of local relapses at five years was quite high at 31.4%. By multivariate analysis, large recurrent tumor size was the most important predictor of second treatment failure, suggesting that second conservative surgery should generally be offered to women with recurrence size <2cm. It is stressed however that patient preference is important, and that the desire for breast conservation should be respected whenever feasible.

Tamoxifen for the Prevention of Breast Cancer: Late Results of the Italian Randomized Tamoxifen Prevention Trial Among Women With Hysterectomy. (Veronesi U. et al., *J Natl Cancer Inst*, 2007 2;99:727-37). Results after 11 years of follow up demonstrate that the risk-lowering effect of tamoxifen is maintained long stopping taking the drug. However it is important to select the right women for tamoxifen since the beneficial effect was evident only in the group at high risk (24 breast cancers in the placebo arm vs. 6 breast cancers in the tamoxifen arm; RR 0.23 95% CI 0.09-0.58). In the average and low risk groups tamoxifen had no effect compared to placebo. This finding is in line with other tamoxifen chemoprevention trials. Tamoxifen was also found to decrease the incidence of headache and to slightly increase the risk of developing menopausal symptoms, hyperlipidemia, superficial thromboembolic events, arrhythmias, and cerebrovascular disease.

Randomized Dose-Ranging Trial of Tamoxifen at Low Doses in Hormone Replacement Therapy Users (A. Decensi et al, *J Clin Oncol*, 2007) The aim of this study was to determine whether the combination of hormone replacement therapy (HRT) and low-dose tamoxifen might retain the benefits while reducing the risks of either agent. We assessed the optimal biologic dose and schedule of tamoxifen in HRT users using surrogate end point biomarkers and menopausal symptoms. HRT users, which were randomized to tamoxifen, had a positive modulation on a variety of circulating biomarkers related to breast cancer risk and cardio-vascular diseases. Relative to placebo, tamoxifen significantly reduced IGF-I levels after 12 months ($p .005$), and on average, tamoxifen increased IGFBP-3 relative to placebo. Mammographic density was reduced by tamoxifen, with a greater effect with at 5 mg/day than the two lower doses ($p .044$). Finally Tamoxifen significantly modulated antithrombin III and C reactive protein, with a greater reduction of 5 mg/day versus the lower doses. There was no increased endometrial proliferation and worsening of menopausal symptoms due to tamoxifen. Five mg per day was the most favorable dose and we chose this dose to set a larger phase III trial with tamoxifen in HRT users, the "HOT Study".

Germline mutations of TP53 and BRCA2 genes in breast cancer/sarcoma families (S. Manoukian et al, *Eur J Cancer*,

2007) BRCA1 and BRCA2 are the main genetic risk factors for breast cancer known to date and are found to be mutated in families with the hereditary breast/ovarian cancer (HBOC) syndrome. Their involvement in breast cancer/sarcoma families had not been investigated. We have analyzed 23 families, with one case of sarcoma and at least one case of breast carcinoma, for mutations in TP53, BRCA1 and BRCA2, in order to assess the relative contribution of each gene. Families were classified according to their conformity to the criteria defining the Li-Fraumeni syndrome (LFS), Li-Fraumeni-like (LFL) syndrome and HBOC. Our analysis showed that in breast cancer/sarcoma families, failing to meet the LFS classical definition, TP53 mutations are infrequent ($2/21 = 9.5\%$). We have also found that a fraction of breast cancer/sarcoma families, both LFL and non-LFS/non-LFL, which were consistent with the criteria that define HBOC, carried germline BRCA2 mutations ($3/20 = 15\%$). Although in our study TP53 and BRCA2 mutations accounted for only a minority of non-LFS breast cancer/sarcoma families, if confirmed, the observed mutation rates would provide justification for genetic testing of both genes for clinical purposes in these families. Moreover, testing for BRCA genes could contribute to a better understanding of the possible role of these genes in sarcoma risk.

Methylenetetrahydrofolate reductase (MTHFR) and breast cancer risk: a nested-case-control study and a pooled meta-analysis (D. Macis et al, *Breast Cancer Res Treat*).

A reduced activity of methylenetetrahydrofolate reductase (MTHFR) due to frequent C677T polymorphism affects DNA synthesis, repair and methylation and may be implicated in breast cancer risk. We conducted a nested case-control study within a phase III prevention trial of tamoxifen. A total of 46 breast cancer cases and 80 unaffected controls matched to treatment allocation, years from randomization (± 2 years) and age at randomization (± 5 years), underwent genotyping for MTHFR C677T polymorphism using real time PCR. We observed a borderline significant odds ratio of 2.51 (95% CI, 0.96–6.55) of breast cancer in subjects with 677TT genotype, with no further association after stratifying for age and treatment group. A meta-analysis of 18 studies, including our own, showed that the MTHFR gene was not associated with breast cancer risk of breast cancer. In seven studies, which reported the menopausal status, the polymorphism 677TT, in the premenopausal women, was significantly associated with breast cancer risk, odds ratio 1.42 (95% CI, 1.02–1.98). In conclusion, results from the Italian Chemoprevention Trial of Tamoxifen and our meta-analysis indicate MTHFR C677T SNP as a possible modifier of breast cancer risk, especially in association with other risk factors such as estrogen exposure in premenopausal women.

Medical Oncology

The scientific production on Breast Cancer addressed issues encountered commonly by the practicing oncologist. The research themes varied, including:

1. Biological features of disease as target to adjuvant systemic therapy of early breast cancer.
2. Identification of biological features predictive of response to neo-adjuvant chemotherapy.
3. Analysis of the addition of trastuzumab to chemotherapy in HER2 positive breast cancer
4. Role of aromatase inhibitors (AIs) and switching to AIs from Tamoxifen in the adjuvant setting for the treatment of early breast cancer;
5. Prevention of venous thromboembolism in metastatic breast cancer patients
6. Identification of surrogate markers predictive of response to treatment and predictive of prognosis in metastatic setting of disease (circulating tumor cells)
7. Identification of new strategies (metronomic chemotherapy or combination of metronomics with biological agents) in the treatment of metastatic breast cancer.
8. Development of new strategies to target HER2 positive breast cancer.
9. Identification and development of individualized treatments of specific conditions (pregnancy, very young patients, elderly patients).

One of the most cited reference in breast cancer treatment field for 2007 is related to the 10th St Gallen expert consensus meeting on adjuvant treatment of early breast cancer, that refined and extended a target-oriented approach to adjuvant systemic therapy of early breast cancer. Target definition is inextricably intertwined with the availability of target-specific therapeutic agents. Since 2005, the presence of HER2 on the cell surface has been used as an effective target for trastuzumab much as steroid hormone receptors are targets for endocrine therapies. An expert Panel reaffirmed the primary importance of determining endocrine responsiveness of the cancer as a first approach to selecting systemic therapy. Three categories were acknowledged: highly endocrine responsive, incompletely endocrine responsive and endocrine non-responsive. The Panel accepted HER2-positivity to assign trastuzumab, and noted that adjuvant trastuzumab has only been assessed together with chemotherapy. They largely endorsed previous definitions of risk categories. While recognizing the existence of several molecularly-based tools for risk stratification, the Panel preferred to recommend the use of high-quality standard histopathological assessment for both risk allocation and target identification. Chemotherapy, although largely lacking specific target information, is the only option in cases which are both endocrine receptor-negative and HER2-negative. Chemotherapy is convention-

ally given with or preceding trastuzumab for patients with HER2-positive disease, and may be used for patients with endocrine responsive disease in cases where the sufficiency of endocrine therapy alone is uncertain. Recommendations are provided not as specific therapy guidelines but rather as a general guidance emphasizing main principles for tailoring therapeutic choice (*Goldhirsch A. Progress and promise: highlights of the international expert consensus on the primary therapy of early breast cancer 2007. 1: Ann Oncol. 2007 Jul;18(7):1133-44.*)

Biological features of disease have been a field of investigation to identify the role of estrogen (ER), progesterone (PgR), epidermal growth factor 1 (HER1), and HER2 receptors in predicting response to preoperative chemotherapy. We reviewed the pretreatment biopsies of 485 patients with locally advanced breast cancer (cT₄, No-2, Mo) treated with preoperative chemotherapy. The incidence of pathological complete remission (pCR) and outcome were assessed with respect to clinical and pathological findings including ER/PgR status (absent versus expressed), HER1 (absent versus expressed) and HER2 (overexpressed versus none) expression. Patients with ER/PgR-absent tumors were 12.0 times more likely to achieve a pCR ($P < 0.0001$). The pCR rate is higher and outcome worse for patients with ER/PgR-absent tumors. HER1 and HER2 expression may have a prognostic role in locally advanced breast cancer and warrant further studies (*Colleoni M et al. Expression of ER, PgR, HER1, HER2, and response: a study of preoperative chemotherapy. Ann Oncol. 2007 Nov 6*)

Optimizing integration of aromatase inhibitors in the treatment of early breast cancer has been reported in the update of the Breast International Group (BIG) 1-98 four-arm study that compared initial therapy with letrozole or tamoxifen including patients randomly assigned to sequential treatment whose information was censored at the time of therapy change. At a median follow-up time of 51 months this study reflected an 18% reduction in the risk of an event. No predefined subsets showed differential benefit (*Coates A. et al. Five years of letrozole compared with tamoxifen as initial adjuvant therapy for postmenopausal women with endocrine-responsive early breast cancer: update of study BIG 1-98 J Clin Oncol. 2007 Feb 10;25(5):486-92*)

The role of trastuzumab in the adjuvant treatment of breast cancer has been investigated in the report of the 2-years follow up of HERA trial. Our results show that 1 year of treatment with trastuzumab after adjuvant chemotherapy has a significant overall survival benefit after a median follow-up of 2 years. The emergence

of this benefit after only 2 years reinforces the importance of trastuzumab in the treatment of women with HER2-positive early breast cancer (Smith I et al. 2-year follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer: a randomised controlled trial. *Lancet*. 2007 Jan 6;369(9555):29-36)

Another study aimed to evaluate the prognostic significance of circulating tumor cells (CTCs) detection in advanced breast cancer patients. We tested 80 patients for CTC levels before starting a new treatment and after 4, 8 weeks, at the first clinical evaluation and every 2 months thereafter. CTCs were detected using the CellSearch System trade mark. Results: Forty-nine patients had ≥ 5 CTCs at baseline. At the multivariate analysis, baseline number of CTCs was significantly associated with progression-free survival [hazard ratio (HR) 2.5; 95% confidence interval (CI) 1.2-5.4]. The risk of progression for patients with CTCs ≥ 5 at last available blood draw was five times the risk of patients with 0-4 CTCs at the same time point (HR 5.3; 95% CI 2.8-10.4). Patients with rising or persistent ≥ 5 CTCs at last available blood draw showed a statistically significant higher risk of progression with respect to patients with < 5 CTCs at both blood draws (HR 6.4; 95% CI 2.8-14.6). CTCs basal value is a predictive indicator of prognosis and changes in CTC levels during therapy may indicate a clinical response. Testing CTC levels during targeted treatments might substitute other measurement parameters for response evaluation. (Nolè F. et al. *Variation of circulating tumor cell levels during treatment of metastatic breast cancer: prognostic and therapeutic implications*. *Ann Oncol*. 2007 Dec 4 Epub ahead of print)

We previously demonstrated a high incidence (7.7%) of venous thromboembolism (VTE) in breast cancer patients treated with infusional chemotherapy after insertion of central vein catheters (CVC). We evaluated the efficacy and safety of low-dose aspirin for the prevention of VTE. In a monocentric prospective study, patients with stage II-IV breast cancer, who underwent CVC insertion for continuous infusional chemotherapy, were assigned to receive low-dose aspirin (100 mg daily). Treatment was started after CVC implantation and continued until the last day of chemotherapy. Patients were assessed for safety and for the incidence of symptomatic deep venous thrombosis (DVT) confirmed by color-Doppler ultrasonography. Administration of low-dose aspirin is safe and seems to correlate with a low risk of DVT in breast cancer patients treated with infusional chemotherapy. Further randomized studies comparing low-dose aspirin with other anticoagulative agents are warranted (G. Curigliano et al. *Low-dose aspirin for the prevention*

of venous thromboembolism in breast cancer patients treated with infusional chemotherapy after insertion of central vein catheter. *Support Care Cancer*. 2007 Oct;15(10):1213-7)

INTRAOPERATIVE AVIDINATION FOR RADIONUCLIDE THERAPY

Breast conservative surgery followed by sentinel node biopsy and postoperative regional radiotherapy represents the treatment of choice in patients with early breast cancer. A standard course of whole-breast external-beam radiation therapy (EBRT) accompanied with a boost to the tumour bed generally requires 6–8 weeks to be completed. This can represent a logistical problem for many patients, particularly the elderly and those who live far from a radiation treatment facility. As an alternative, following our previous experience in locoregional treatment of peritoneal carcinomatosis and recurrent high-grade glioma using the avidin–biotin pretargeting technique, we developed a new application. The Intraoperative Avidination for Radionuclide Therapy (IART) is the “avidination” of the anatomical area of the tumour with native avidin, directly injected by the surgeon into and around the tumour bed, which constitutes a target for the ^{90}Y -radiolabelled biotin injected intravenously 1 day later.

To prove the feasibility of this approach, a pilot study with ^{111}In -DOTA-biotin simulation was carried out in 10 patients. This simulation study predicted a favourable dosimetry with ^{90}Y -DOTA-biotin to nontarget organs (< 0.15 Gy/GBq), with kidneys and urinary bladder receiving slightly higher doses (1.20 and 1.39 Gy/GBq, respectively). Interestingly, the absorbed dose to the target area reached 5.5 Gy/GBq (50% ISOROI) and 4.8 Gy/GBq (30% ISOROI). Images showed early and long-lasting radioactive biotin uptake in the operated breast.

Such findings led to apply IART with ^{90}Y -DOTA-biotin in a phase I-II therapy trial, where IART was intended as anticipated boost to EBRT.

After the primary tumor excision, the surgeon injected native avidin diluted into 30 ml of saline solution into and around the tumor bed. Patients had 3 avidin levels of dose: 50 mg (10 pts), 100 mg (15 pts) and 150 mg (7pts). From 12 to 16 hours after surgery, injection of 3.7 GBq ^{90}Y -biotin and 185 MBq ^{111}In -biotin were delivered intravenously through a slow infusion. Whole body scans and SPECT images were performed up to 36 hours post injection for dosimetric purpose. Local toxicity and quality of life were evaluated by RTOG scale EORTC QoL questionnaire, respectively.

32 patients (mean age 62 y; range 42-74) have been evaluated. No side effects were observed after avidin administration and ^{90}Y -biotin infusion. The dose of 100

mg of avidin resulted to be the most appropriate in order to deliver 21.2±4.3 Gy to the surgical bed. The absorbed dose to the kidney was 0.9±0.3 Gy/GBq and no hematological toxicity was observed.

At the end of IART treatment no local toxicity occurred, and the overall cosmetic result was good.

Twenty-five patients completed EBRT so far. The tolerance to EBRT was good. The greatest grade of transient local toxicity was G3, occurred in 3/25 pts after 30Gy.

IART was well accepted by the patients, without any changes in their quality of life.

These results support the hypothesis that IART may represent a valid approach to partial breast irradiation after BCS combined to reduced EBRT.

Paganelli G, et al. Intraoperative avidination for radionuclide therapy: a prospective new development to accelerate radiotherapy in breast cancer. *Clin Cancer Res.* 2007 Sep 15;13(18 Pt 2):5646s-5651s

Sentinel lymph node investigation: The ever-increasing adoption of axillary sentinel lymph node (SLN) biopsy for staging breast carcinoma patients with minimal morbidity has raised the question about the best way for examining SLN biopsies, ensuring standardization of the techniques and reproducibility of the results and minimizing the risk of false-negative results. Owing to the lack of standardized and widely accepted protocols for a truly accurate histopathological examination of SLN, the relative merits of alternative assays based on the identification of tumor specific mRNA markers have been exploited.

The current investigation aimed at assessing the accuracy of a commercially-available first FDA-approved real-time RT-PCR assay for mammapoglobin and cytokeratin 19 mRNAs (GeneSearch™ Breast Lymph Node [BLN] Assay, Veridex LLC, Warren, NJ) in the detection of axillary SLN metastases in patients with breast carcinoma. The assay may be performed in a very short time (approximately 40 minutes) so that it was also suitable for the intraoperative detection of SLN metastases. A prospective series of 293 SLNs from breast cancer patients operated consecutively at EIO were snap frozen and examined completely by serial sectioning at 40-50 μm intervals. While frozen sections were examined by histology (including immunohistochemical testing), the interval tissue was subjected to RT-PCR assays. The BLN assay correctly identified 51/52 macrometastatic and 5/20 micrometastatic SLNs, with a sensitivity of 98.1% to detect metastases larger than 2 mm, 94.7% for metastases larger than 1 mm, and 77.8% for metastases larger than 0.2 mm. The overall concordance with histopathology was 90.8%, with specificity of 95.0%, positive predictive value (PPV) of 83.6% and negative predictive value (NPV) of 92.9%.

When the results were evaluated according to the occurrence of additional metastases to non-sentinel axillary lymph nodes in patients with histologically positive SLNs, the assay was positive in 33 (91.7%) of the 36 patients with additional metastases and in 22 (66.6%) of the 33 patients without further involvement. The current study showed that sensitivity of the RT-PCR assay is comparable to that of the histopathological examination of the entire SLN by serial sectioning at 1.5-2 mm.

Vascular invasion assessment. Assessing new and reliable predictive parameters is pivotal for offering breast cancer patients the most effective therapeutic modalities. Although there is evidence that the molecular characterization may provide useful prognostic information, the traditional pathological analysis still remains the most reliable tools on which rely the therapeutic decisions. Along this line, our group recently focused on the clinical relevance of peritumoral vascular invasion (PVI) in breast cancer patients with no or limited (pNo and pNia) axillary lymph node involvement. We investigated a mono-institutional series of 2606 consecutive breast cancer patients with pT1-3, pNo-Nia operated and counselled for medical therapy. The occurrence of PVI was carefully evaluate in routinely haematoxylin and eosin stained slides from at least two blocks in tumors less than 1 cm, and three to four blocks in larger tumors. PVI was prospectively scored as absent (no evidence of PVI), focal (one focus of PVI in one tumor block only), moderate (more than one focus of PVI in one tumor block only), and extensive (one or more foci of PVI in more than one tumor block). Interestingly, we document that patients with an extensive PVI experience a significantly higher prevalence of distant metastases and a reduced overall survival, thus being candidate to more aggressive therapies.

Pre-operative therapy combining letrozole with GnRH analogue. Endocrine therapy is a treatment tailored for patients with breast cancer expressing oestrogen (ER) receptor, that is highly effective in the adjuvant setting, while its activity as a neoadjuvant treatment has not yet been fully elucidated, especially in pre-menopausal patients. We therefore investigated the efficacy of a pre-operative therapy combining letrozole with GnRH analogue in patients with ER and progesterone (PgR)-expressing breast cancer. Since ER and PgR participate in a complex regulatory pathway together with a variety of players, including ER-alpha, EGFR, HER-2, MAP kinases (and their phosphorylated forms), we analyzed their expression by immunohistochemistry in the diagnostic biopsies and in post-treatment surgical samples. Complete or partial response was reached after treatment in up to 50% of the patients,

and clinical response was positively associated with duration of letrozole treatment, lasting 4.2 months for clinical responders and 3.4 months for patients obtaining stable disease ($p < 0.05$). The levels of oestradiol did not significantly change upon treatment. Univariate analysis failed to show any association between baseline values of any molecular parameter and clinical response. Likewise, no significant difference between baseline and post-treatment values were observed for any parameter between responding and not responding patients.