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REVIEW ARTICLE

Breast cancer subtypes: response to radiotherapy and potential radiosensitisation

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ABSTRACT

Radiotherapy (RT) is of critical importance in the locoregional management of early breast cancer. Over 50% of patients receive RT at some time during the treatment of their disease, equating to over 500 000 patients worldwide receiving RT each year. Unfortunately, not all patients derive therapeutic benefit and some breast cancers are resistant to treatment, as evidenced by distant metastatic spread and local recurrence. Prediction of individual responses to RT may allow a stratified approach to this treatment permitting those patients with radioresistant tumours to receive higher doses of RT (total and/or tumour cavity boost doses) and/or radiosensitising agents to optimise treatment. Also, for those patients unlikely to respond at all, it would prevent harmful side effects occurring for no therapeutic gain. More selective targeting would better direct National Health Service resources, ease the burden on heavily used treatment RT machines and reduce the economic cost of cancer treatment. Unfortunately, there are no robust and validated biomarkers for predicting RT outcome. We review the available literature to determine whether classification of breast cancers according to their molecular profile may be used to predict successful response to, or increased morbidity from, RT. Class-specific biomarkers for targeting by radiosensitising agents are also discussed.

Breast cancer is the most common cancer in females with just over 1 million new diagnoses and some 400 000 deaths recorded worldwide each year (World Health Organization). As such, this

disease represents one of the most serious and costly health issues. Locoregional treatment of breast cancer has evolved over the last two decades not only in the surgical techniques used but also in

the use and delivery of radiotherapy (RT). Surgical management has moved towards breast conserving surgery (BCS) and axillary sentinel lymph node biopsy wherever possible. Surgery aims to remove any disease that has been detected in the breast and regional lymph nodes. Surgery does not, however, remove undetected occult deposits of cancer that may remain within the breast, scar, chest wall or remaining lymph nodes. It is these undetected deposits that may lead to locoregional recurrence (LR) and furthermore to distant metastases. RT has therefore become a well-established adjuvant treatment modality to optimise local control following most BCS [1] and following mastectomy in those patients at high risk of recurrence [2]. According to a recently published National Health Service Breast Screening Programme audit of 17 013 screen-detected breast cancers collected from April 2009 to March 2010, 10 425 (61%) females received adjuvant RT. This includes those diagnosed with both invasive (9223, 88.5%) and non-invasive (1202, 11.5%) breast cancers. Of the 8739 patients who underwent BCS for invasive breast cancer, 8201 (94%) received RT. These data did not include females who presented to symptomatic breast clinics and indicate the considerable volume of patients requiring and receiving adjuvant RT each year in the UK alone. It is no surprise that there have been capacity issues leading to wide variations in the timelines of availability of RT from country to country, and even within the same country. RT achieves high cure rates where metastatic spread has not occurred [3–5] and offers improvements in overall survival (OS) and disease-free survival (DFS) in patients presenting with distant metastases at diagnosis [6]. A reduction in LR has tumour-related gains and also quality of life benefits, such as a reduction in the physical morbidity and psychological consequences of LR. However, as with all adjuvant therapies, there are recognised short-term (those which happen within ~3 months of completing treatment) and long-term side effects.

RADIOTHERAPY SIDE EFFECTS

By far, the most common short-term side effects subsequent to RT are those of skin erythema and fatigue, which can occur in up to 100% of patients [3, 7]. Following this, late side effects include telangiectasia (31.4%) and impaired cosmesis with fibrosis (6.7%) [3, 7–9]. Arm lymphoedema and shoulder stiffness are other long-term side effects that can impact on patients' everyday activities [3, 8]. Post-operative breast RT can

also damage the underlying chest organs, namely the lungs and heart. Pulmonary effects include radiation-induced pneumonitis and fibrosis [10]. Radiation pneumonitis is an acute exudative inflammatory process, typically occurring within 1–4 months after RT, which follows the initial damage of cells in the alveolar space (pneumocytes, fibroblasts, endothelial cells and macrophages; [11]). Pulmonary fibrosis is a late injury due to interstitial damage involving the parenchyma and pleura [12]. Radiation damage can also cause endothelial cell damage and atherosclerosis [13], myocardial ischemia and fibrosis [14]. Complications, such as acute pericarditis, pericardial effusion and arrhythmias, can also develop in patients and, in some cases, can occur up to 20 years post treatment. Over the last few decades, there has been an increased recognition of the late side effects of RT. Thus, the introduction of modern techniques (including image-based planning and directed therapies) has reduced irradiation doses to the heart and lungs. However, since some of the more severe late side effects of RT can occur many years following exposure, the full potential benefits of these changes is uncertain, and other possible significant adverse effects have yet to be reported in the literature.

CRITERIA FOR SELECTING PATIENTS FOR RADIOTHERAPY

Although RT is used routinely following BCS, many patients could be effectively treated with breast surgery alone without RT. Despite this, there is currently insufficient knowledge to enable the selection of patients who do not need RT. Studies have been unsuccessful in attempting to identify a low-risk group with a <5% chance of local relapse in whom RT can be reasonably omitted [15]. At the present time, it is therefore recommended that RT should be considered for all patients undergoing BCS, even those with low-grade node-negative disease. Likewise, not all patients derive therapeutic benefit since some breast cancers are refractory to this treatment, as evidenced by distant metastatic spread and LR. At present, decisions regarding who receives RT are based on clinical factors, stages, morphology-based pathological indicators and type of surgery rather than molecular profiles predictive of likely RT sensitivity. As a result, all breast cancer patients are currently treated with the same RT regimen, irrespective of whether their tumours are likely to respond or not. A more informed approach to identify individual responses would allow a stratified approach, allowing

those patients unlikely to respond to receive higher doses of RT (total dose and/or tumour cavity boost dose) and/or radiosensitising agents to increase the efficacy of their treatment. Those patients unlikely to respond at all could be spared from the associated iatrogenesis. This could have a positive impact on breast cancer mortality rates worldwide, ease burden on heavily used treatment machines and reduce the economic cost of cancer treatment.

BIOMARKERS FOR PREDICTING RESPONSE TO RADIOTHERAPY

Oncological therapies continue to be developed and are increasingly more specific and targeted towards cancer biomarkers. Prognostic and predictive biomarkers are the two major types of cancer biomarkers, and it is important to recognise that there is a clear distinction between these terms. Prognostic biomarkers are intrinsic factors that provide information about a patient's overall cancer outcome regardless of therapy. Such biomarkers may be used by physicians to select those high-risk patients who may benefit from more aggressive treatment. However, such biomarkers are unable to highlight those patients that will derive a clinical benefit from a given therapy. On the other hand, predictive biomarkers provide information regarding the probability of therapeutic benefit from a specific treatment [16]. Well-known examples of these include the oestrogen receptor (ER) and human epidermal growth factor receptor 2 (HER2), which are used to predict response to endocrine therapy and trastuzumab, respectively. However, it is also possible that biomarkers can be both prognostic and predictive.

Several potential predictive biomarkers that may correlate with RT response have been identified [17–19]. In breast cancers, expression levels of Holliday junction recognition protein (HJURP) mRNA can predict patient survival after RT [17] and high cytoplasmic expression of peroxiredoxin-I correlated with increased LRs after RT [18]. However, in most cases, predictive and prognostic information from potential biomarkers has not been separated. Unpublished work from our group has shown that high proteasome (prosome, macropain) 26S subunit, non-ATPase, 9 (PSMD9) expression is significantly associated with an increased incidence of LR in a cohort of breast cancer patients receiving adjuvant RT (log rank $p=0.02$) but not in those treated without RT, indicating that this protein might represent a predictive biomarker for RT response.

However, none of these biomarkers is currently used to guide treatment decisions within the clinic owing to a lack of large-scale randomised control trials in this area.

BREAST CANCER SUBTYPES AND RESPONSE TO RADIOTHERAPY

It is well known that breast cancer is not a single disease but rather a collection of diseases with diverse histopathologies and gene expression profiles. Gene expression profiles have enabled the classification of breast cancers into subtypes that were not apparent using more traditional histopathological criteria [19]. Correlating gene expression patterns in the different breast cancer subtypes to clinical, pathological and outcome data has supported the idea that breast cancer subtypes have strong differences in cell biology and tumour behaviour and can be treated as separate diseases. Although gene expression profiling remains the gold standard for classification, this is not currently feasible for large-scale clinical applications, and so, within the clinic, the system of defining subtypes still relies on the more traditional histopathological methods to assess expression of three common molecular markers; ER, progesterone receptor (PR) and HER2 [20–23]. The luminal subtypes (luminal A and luminal B) encompass the hormone receptor (ER and PR) positive breast cancers [22]. The HER2 subtype consists of those breast cancers expressing low levels of hormone receptors but overexpressing HER2. The basal-like subtype is typically categorised by routine immunohistochemistry as triple negative breast cancer owing to very low or negligible expression levels of ER, PR and HER2 [20–23]. Importantly, we acknowledge that, although most basal-like breast cancers have a triple negative phenotype and the vast majority of triple negative cancers comprise basal-like breast cancers, these are not synonymous [24]. However, in the absence of a more suitable marker for the classification of basal-like breast cancers for the purpose of this review, we will define this subtype in accordance with Schneider et al [25] by referring to basal-like breast cancers when gene expression profiling or more sophisticated immunophenotypes were used for identification and triple negative breast cancer when analyses were limited to clinical assays.

It is clear that the heterogeneous nature of breast cancer accounts for different outcomes among women diagnosed with this disease. For example, luminal subtypes are associated with a more favourable prognosis,

whereas HER2 and basal-like subtypes are associated with significantly worse recurrence rates and diminished OS [21–23, 26]. This was confirmed by Wang et al (2011) [27], who conducted a retrospective analysis of 2118 primary operable breast cancer patients and found that molecular subtype could robustly identify the risk of recurrence with luminal A tumours having the lowest rate of relapse (12.7%, $p < 0.001$), whereas luminal B, HER2 and basal-like subtypes were associated with higher rates of relapse (15.7%, 19.1%, 20.1%). It is also clear that such heterogeneity accounts for variations in response to therapy. However, the question remains as to whether these breast cancer subtypes may be used to predict response to RT or whether specific subtypes may benefit from radiosensitising agents to enhance the efficacy of this treatment. Data from our *in vitro* studies have shown that breast cancer cell lines representing the different subtypes exhibit differential inherent sensitivities to ionising radiation [28], which suggests that this may certainly be feasible. A clear advantage of being able to exploit these markers for selecting patients for RT is that their expression is already routinely analysed within the clinic for diagnostic and prognostic purposes and also for selecting patients for other breast cancer therapies.

LUMINAL SUBTYPES

In 2008, Nguyen et al [29] studied 793 consecutive patients with invasive breast cancer who received BCS followed by RT and reported LR incidences of 0.8% for luminal A, 1.5% for luminal B, 8.4% for HER2 and 7.1% for triple-negative cancers. Kyndi et al [30] also found a significantly improved OS rate after post-mastectomy RT (PMRT) only among patients characterised by good prognostic markers (ER/PR positivity and HER2 negativity), whereas no significant OS improvements after PMRT were found among ER/PR-negative and HER2-positive patients. Furthermore, a significantly improved survival rate was reported for patients with hormone-receptor-positive and HER2-negative tumours (resembling tumours of the luminal A subtype), who received PMRT when compared with those who received no RT [30]. This suggests that RT is particularly effective for breast cancers of the luminal phenotype, especially that of luminal A. This was confirmed by Wang et al [27] who found that, for breast tumours of the luminal A subtype, adjuvant RT reduces the risk of relapse ($p = 0.005$). It has been proposed that these tumours may be particularly sensitive to RT as a result of their dependence on oestrogen [30].

Oestrogen acts to accelerate G1 to S phase transition of the cell cycle which, hypothetically, could leave tumour cells with less time to repair DNA damage caused by RT, thereby inducing apoptosis [30].

HER2 SUBTYPE

The association between HER2 receptor status and patient response to RT remains unclear. Several groups have reported an increased risk of LR for breast tumours overexpressing HER2 following RT [29, 31]. In contrast, HER2 status alone failed to predict any significant survival benefits after PMRT in a cohort of 1000 breast cancer patients randomly assigned to receive this treatment [30]. However, when HER2 positivity was combined with ER/PR negativity, there was an increased probability of LR ($p = 0.01$) and distant metastasis ($p = 0.02$) after PMRT, indicating possible radioresistance associated with the HER2 positive subtype [30]. A functional role for HER2 in mediating response to RT has been shown in several *in vitro* studies. In 1993, Pirollo et al [32] transfected NIH 3T3 cells with genomic HER2 DNA isolated from an oesophageal cancer, which were insensitive to RT. Transfected NIH 3T3 cells exhibited a marked increase in their level of radioresistance, demonstrating that HER2 expression can influence response to RT. Likewise, a recombinant humanised monoclonal antibody raised against HER2 (4D5) was found to reverse the radioresistant phenotypes of HER2 overexpressing breast cancer cell lines by modulating the repair of radiation-induced DNA damage [33].

In vivo studies using HER2 overexpressing human breast cancer xenografts also demonstrated marked enhancement of radiation efficacy when given with this anti-HER2 receptor antibody. Mice treated with radiation or the anti-HER2 antibody alone showed no reduction in tumour size or remission. However, when combining radiation and anti-HER2 antibody therapies, there were marked reductions in tumour growth, and all animals receiving both treatments showed complete tumour remission [33]. Use of adjuvant trastuzumab for the treatment of HER2+ early breast cancers has been evaluated in several large multicentre, randomised clinical trials, including the HERceptin Adjuvant (HERA) study. Data show that adjuvant trastuzumab significantly extends both OS and DFS, and these patients are now routinely treated with adjuvant trastuzumab. Many of the published studies related to breast

cancer subtypes and response to RT did not include patients who received this treatment. This means that, in the future, we may see an even smaller number of LRs and distant disease events in this subtype. Nevertheless, the data above implicate the activation of this signal transduction pathway with radioresistance highlighting additional downstream targets which, when inhibited in combination with HER2, may further increase the efficacy of RT for this subtype.

The HER2 receptor is composed of an extracellular ligand binding domain, a single transmembrane domain and an intracellular domain with tyrosine kinase activity. Trastuzumab selectively binds to the ligand-binding domain, thereby suppressing HER2 activity. Inhibition of the tyrosine kinase domain represents another therapeutic strategy for suppression of HER2 activity, and several orally bio-available, low-molecular weight tyrosine kinase inhibitors (TKIs) are now also in clinical use. Examples include gefitinib, erlotinib and lapatinib. These TKIs show potent radiosensitising activity and are currently being studied in Phase I/II clinical trials in various primary solid tumours [34–36]. TKIs have also been shown to act synergistically with trastuzumab, enhancing cell death in response to treatment [37]. Thus, combining these agents may further increase the efficacy of RT for breast cancers of the HER2 subtype. Targeting additional downstream players within this signalling pathway may also act to increase RT efficacy for these breast cancers. Examples include the inhibition of Ras, Raf and/or MEK, and several molecules are currently in clinical trials as potential radiosensitisers [38–40].

The enzyme farnesyltransferase is involved in the initial post-translational modification of Ras that allows it to become associated with the plasma membrane and become active in signal transduction. Farnesyltransferase inhibition represents a promising strategy for radiosensitisation and was effective in Phase I studies in non-small cell lung cancer and cancers of the head and neck [38]. These agents may serve to further increase the efficacy of RT when used in combination with trastuzumab for the treatment of HER2 overexpressing breast cancers.

TRIPLE-NEGATIVE BREAST CANCER

A triple-negative phenotype is significantly associated with an increased risk of LR, distant metastasis and

increased overall mortality in patients treated with RT [29, 30]. This is quite surprising given that this is the type of breast cancer that *BRCA-1* and *-2* mutation carriers generally develop [26, 41]. Approximately 19.5% of triple-negative patients carry *BRCA* mutations [42]. *BRCA* mutation carriers are defective in DNA repair; therefore, it would be expected that these tumours might exhibit extreme sensitivity rather than insensitivity to RT. One possible explanation for this unexpected response to RT is that these tumours might possess compensatory DNA repair mechanisms that are more effective at dealing with radiation-induced DNA damage.

Triple-negative breast cancers represent the most problematic subtype with regard to effective management as, unlike luminal and HER2 positive breast cancers, there are no effective treatment targets. Since the majority of triple-negative cancers comprise basal-like breast cancers, then the identification of a basal-like specific marker would be invaluable both in terms of classification and identifying potential radiosensitising agents. Analyses performed by Nielsen et al [43] on tumours previously used in gene expression profiling studies [22] revealed that basal-like breast cancers express high levels of cytokeratin 5/6, epidermal growth factor receptor (EGFR) and c-kit. These results were found to correlate well with immunohistochemical patterns. It was therefore suggested that basal-like breast cancers might be better identified within the clinic by selecting cases that are ER negative, HER2 negative/low and cytokeratin 5/6 positive and/or EGFR positive [43].

The positive expression of these additional markers within this subtype also highlights potential therapeutic targets for the radiosensitisation of these breast cancers. In particular, EGFR is a member of the HER family of receptors, along with HER2. In combination with RT, basal-like breast cancers may therefore benefit from the concurrent administration of TKIs and/or the signal transduction inhibitors described previously. Furthermore, EGFR can also be selectively inhibited using monoclonal antibody therapy. Cetuximab is a monoclonal antibody that binds to the EGFR with high specificity and with a higher affinity than epidermal growth factors, thus blocking ligand-induced phosphorylation and activation of EGFR [44]. The radiosensitising properties of cetuximab have been well demonstrated in

solid tumours, and it appears to sensitise tumour cells to ionising radiation, either through increasing the proportion of cells in the radiosensitive G1 phase of the cell cycle while decreasing the proportion in the radioresistant S phase [45] or through restoration of apoptosis [46] or even anti-angiogenic mechanisms [47]. Bonner et al [48] found that the median duration of OS was 49.0 months among patients with locoregionally advanced head and neck cancers treated with RT plus cetuximab and 29.3 months among those treated with RT alone (hazard ratio 0.73, 95% CI 0.56–0.95; $p=0.018$). The 5-year OS rate was 45.6% in the combined treatment group and 36.4% in the RT alone group. This agent has therefore recently been approved for use in combination with RT for the treatment of locally advanced squamous cell carcinoma of the head and neck. Although cetuximab is not routinely considered for the treatment of breast cancer patients, it may well prove effective in the management of basal-like breast tumours.

The inhibition of poly[adenosine diphosphate (ADP)-ribose] polymerase (PARP) is a further novel radiosensitising strategy, which may increase the efficacy of RT in patients with triple-negative breast cancers. As previously mentioned, these cancers are more commonly found in individuals who have deleterious mutations in the *BRCA* gene [25, 41]. *BRCA* mutation carriers who lose the remaining wild-type allele exhibit inefficient homologous recombination DNA repair causing an accumulation of genetic aberrations, which drive carcinogenesis. The PARP enzymes play a key role in the repair of DNA single-strand breaks (SSBs) through the repair of base excisions, which are normally repaired by error-free homologous recombination [49]. In cells deficient in this repair mechanism, inhibition of PARP enzymes results in the generation of irreparable DNA SSBs that cause the accumulation of DNA double-strand breaks (DSBs), which trigger apoptosis [50]. There are at least five PARP inhibitors that are in clinical trials, and two of these agents, BSI-201 (BiPar Pharmaceuticals) and olaparib (Astra Zeneca), have been evaluated in Phase II trials in women with *BRCA1* mutations who have metastatic breast cancer [51]. Furthermore, niraparib (MK-4827; Merck) has been shown to radiosensitise a variety of human tumour xenografts with differing p53 status, including the triple-negative MDA-MB-231 human breast carcinoma [52]. Thus, combining these agents with RT appears

promising, but clinical trials to test the efficacy and toxicity of this combination are warranted.

POTENTIAL CAVEATS FOR CONSIDERATION

Studies aimed at determining patient response to RT and identifying factors influencing response suffer from the complexity of establishing the appropriate cohorts of breast tumours for analysis. This is owing to the heterogeneous nature of the disease and the many different treatment modalities available for its effective management. Single modality adjuvant RT in the absence of systemic therapy for the treatment of breast cancer is uncommon and is only administered to low-risk patients where the risk of LR is low. Large patient numbers are therefore needed in prospective clinical trials. This becomes even more of an issue when separating these patients further into individual subtypes. As such, many of the published studies include those patients receiving additional treatments alongside RT. For example, in the study conducted by Nguyen et al [29], 90% of the patient cohort also received adjuvant systemic therapy as well as RT. Under these circumstances, it becomes difficult to dissect whether LRs are the direct result of radioresistance or resistance to the other treatment modalities given. Furthermore, LR after BCS and RT is low in all subtypes and was found to be <10% in the study conducted by Nguyen et al [29], again highlighting the need for large patient numbers for accurate data interpretation.

The question also remains as to whether certain subtypes are just more biologically “aggressive”. HER2 and triple-negative phenotypes are not only associated with a higher risk of LR following RT but are also associated with higher grade, larger size, nodal positivity, younger age at presentation and a higher rate of distant metastases—all of which influence prognosis. Likewise, it remains unknown whether LRs are simply the result of a lack of alternative therapies available for some subtypes, e.g. triple-negative breast cancers. It is also possible that ER/PR negativity defines radioresistance rather than any intrinsic characteristics associated with the HER2 and triple-negative breast cancers. If this is true, the discovery of new radiosensitising strategies will be problematic, as it is more difficult to target a lack of expression than it is to target specific biomarkers known to influence response. For these reasons, there will continue to be limited treatment options for the

ER/PR-negative breast cancer patients for the foreseeable future.

Specific subtypes are also subject to more difficult classification based on the expression of only ER, PR and HER2. As described previously, the triple-negative phenotype is often used as a proxy for the basal-like breast cancers owing to the absence of a basal-like specific biomarker. Although the triple negative phenotype greatly enriches for basal-like breast cancers, a recent study has shown that, using this proxy, some basal-like breast tumours are missed and some non-basal-like cancers are included compared with classifications based on gene expression profiles [53]. Thus, some patients may not be offered the most optimal treatments using the triple-negative phenotype to predict response to a particular agent. At present, it is also difficult to say that some radiosensitising agents will only be efficient for the treatment of specific subtypes of breast cancers. For example, an overexpression of EGFR is not only associated with the basal-like phenotype but is also inversely related to the expression of ER [54]. Therefore, some of the EGFR-specific therapies (e.g. cetuximab) may also be effective for the radiosensitisation of those breast cancers lacking ER expression. This not only includes the basal-like breast cancers but also those breast cancers of the HER2 subtype. Likewise, HER2+ luminal B breast cancers may also benefit from the radiosensitising effects of trastuzumab and/or EGFR inhibitors. Since breast cancer is not a single disease, and with the recent discovery of further subtypes [55], treating tumours individually may ultimately prove more beneficial, owing to their unique genotypes, but is not yet feasible and presents profound biostatistical challenges.

Furthermore, classification based on receptor expression can only approximate the underlying genotype. This may have profound ramifications when using these

classifications to predict whether or not breast tumours may benefit from certain radiosensitising agents. Agents targeting HER2 and/or EGFR would not be effective for the treatment of those breast tumours exhibiting mutations in key downstream players of these pathways. For example, mutations in the *K-Ras* gene have been implicated with resistance to EGFR-TKIs in non-small cell lung cancer [56]. Unfortunately, this information cannot be inferred by the immunohistochemical pattern of these receptors.

CONCLUSION

RT remains an important treatment for the local control of breast cancers, yet there are currently no valid predictive factors that reliably identify patients who would derive greater than average benefit from this treatment and treatment decisions are still made on the basis of stage and standard histopathological criteria. We have entered into an era in which molecular markers, gene expression profiling and other molecular prognostic indicators are being investigated as a means of individualising adjuvant systemic therapies, and this same approach should be applied to RT. The challenge now will be to apply identified biomarkers to the different breast cancer subtypes to predict RT outcome using techniques already utilised within the clinic. Indeed, the evidence suggests that subtypes do exhibit differential responses to RT, with HER2 and triple-negative breast cancers responding less well compared with luminal cancers. There may also be certain characteristics associated with specific subgroups that may be targeted by radiosensitising agents to increase the efficacy of RT, which can only benefit patients in the future.

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REFERENCES

1. Early Breast Cancer Trialists' Collaborative Group. Effects of radiotherapy and surgery in early breast cancer. An overview of the randomized trials. *N Engl J Med* 1995;333:1444–55.
2. Eifel P, Axelson JA, Costa J, Crowley J, Curran W Jr, Deshler A, et al. National Institutes of Health Consensus Development Conference Statement: adjuvant therapy for breast cancer.

- J Natl Cancer Inst 2001;93: 979–89.
3. Hoskin P, ed. Radiotherapy in practice: external beam therapy. Oxford, UK: Oxford University Press; 2006.
 4. Clarke M, Collins R, Darby S, Davies C, Elphinstone P, Evans E, et al. Effects of radiotherapy and of differences in the extent of surgery for early breast cancer on local recurrence and 15-year survival: an overview of the randomised trials. *Lancet* 2005;366:2087–106.
 5. Overgaard M, Nielsen H, Overgaard J. Is the benefit of post-mastectomy irradiation limited to patients with four or more positive nodes, as recommended in international consensus reports? A subgroup analysis of the DBCG 82 b&c randomized trials. *Radiother Oncol* 2007;82:247–53.
 6. Ly BH, Nguyen NP, Vinh-Hung V, Rapiti E, Vlastos G. Loco-regional treatment in metastatic breast cancer patients: is there a survival benefit? *Breast Cancer Res Treat* 2010;119:537–45.
 7. Whelan T, Levine M, Julian J, Kirkbride P, Skingley P. The effects of radiation therapy on quality of life of women with breast carcinoma: results of a randomized trial. *Cancer* 2000;88:2260–6.
 8. Holli K, Saaristo R, Isola J, Joensuu H, Hakama M. Lumpectomy with or without postoperative radiotherapy for breast cancer with favourable prognostic features: results of a randomised study. *Br J Cancer* 2001;84:164–9.
 9. Lilla C, Ambrosone C, Kropp S, Helmbold I, Schmezer P, von Fournier D, et al. Predictive factors for late normal tissue complications following radiotherapy for breast cancer. *Breast Cancer Res Treat* 2007;106:143–50.
 10. Davis SD, Yankelevitz DF, Henschke CI. Radiation effects on the lung: clinical features, pathology, and imaging findings. *Am J Roentgenol* 1992;159:1157–64.
 11. Jaén J, Vázquez G, Alonso E, León A, Guerrero R, Almansa J. Changes in pulmonary function after incidental lung irradiation for breast cancer: a prospective study. *Int J Radiat Oncol Biol Phys* 2006;65:1381–8.
 12. Krengli M, Sacco M, Loi G, Masini L, Ferrante D, Gambaro G, et al. Pulmonary changes after radiotherapy for conservative treatment of breast cancer: a prospective study. *Int J Radiat Oncol Biol Phys* 2008;70:1460–7.
 13. Schultz-Hector S, Trott KR. Radiation-induced cardiovascular diseases: is the epidemiologic evidence compatible with the radiobiologic data? *Int J Radiat Oncol Biol Phys* 2007;67:10–18.
 14. Bird BR, Swain SM. Cardiac toxicity in breast cancer survivors: review of potential cardiac problems. *Clin Cancer Res* 2008;14:14–24.
 15. Clark RM, McCulloch PB, Levine MN, Lipa M, Wilkinson RH, Mahoney LJ, et al. Randomised clinical trial to assess the effectiveness of breast irradiation following lumpectomy and axillary dissection for node-negative breast cancer. *J Natl Cancer Inst* 1992;84:683–9.
 16. Stadler W. Fuzzy thinking on biomarkers. *Urol Oncol* 2007;25: 97–100.
 17. Hu Z, Huang G, Sadanandam A, Gu S, Lenburg M, Pai M, et al. The expression level of HJURP has an independent prognostic impact and predicts the sensitivity to radiotherapy in breast cancer. *Breast Cancer Res* 2010;12:R18.
 18. Woolston CM, Storr SJ, Ellis IO, Morgan DA, Martin SG. Expression of thioredoxin system and related peroxiredoxin proteins is associated with clinical outcome in radiotherapy treated early stage breast cancer. *Radiother Oncol* 2011;100: 308–13.
 19. Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. *Nature* 2000;406: 747–52.
 20. Brenton JD, Carey LA, Ahmed AA, Caldas C. Molecular classification and molecular forecasting of breast cancer: ready for clinical application? *J Clin Oncol* 2005;23:7350–60.
 21. Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci U S A* 2001;98: 10869–74.
 22. Sørlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. *Proc Natl Acad Sci U S A* 2003;100:8418–23.
 23. Sotiriou C, Neo SY, McShane LM, Korn EL, Long PM, Jazaeri A, et al. Breast cancer classification and prognosis based on gene expression profiles from a population-based study. *Proc Natl Acad Sci U S A* 2003;100:10393–8.
 24. Rakha EA, Tan DS, Foulkes WD, Ellis IO, Tutt A, Nielsen TO, et al. Are triple-negative tumours and basal-like breast cancer synonymous? *Breast Cancer Res* 2007;9:404.
 25. Schneider BP, Winer EP, Foulkes WD, Garber J, Perou CM, Richardson A, et al. Triple-negative breast cancer: risk factors to potential targets. *Clin Cancer Res* 2008;14:8010–18.
 26. Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, Conway K, et al. Race, breast cancer subtypes,

- and survival in the Carolina breast cancer study. *JAMA* 2006;295: 2492–502.
27. Wang Y, Yin Q, Yu Q, Zhang J, Liu Z, Wang S, et al. A retrospective study of breast cancer subtypes: the risk of relapse and the relations with treatments. *Breast Cancer Res Treat* 2011;130:489–98.
 28. Smith L, Qutob O, Watson MB, Beavis AW, Potts D, Welham K, et al. Proteomic identification of putative biomarkers of radiotherapy resistance: a possible role for the 26S proteasome. *Neoplasia* 2009;11:1194–207.
 29. Nguyen PL, Taghian AG, Katz MS, Niemierko A, Abi Raad RF, Boon WL, et al. Breast cancer subtype approximated by estrogen receptor, progesterone receptor, and HER-2 is associated with local and distant recurrence after breast-conserving therapy. *J Clin Oncol* 2008;26: 2373–8.
 30. Kyndi M, Sørensen FB, Knudsen H, Overgaard M, Nielsen H, Overgaard J, et al. Estrogen receptor, progesterone receptor, HER-2, and response to postmastectomy radiotherapy in high-risk breast cancer: the Danish Breast Cancer Cooperative Group. *J Clin Oncol* 2008;26:1419–26.
 31. Stål O, Sullivan S, Wingren S, Skoog L, Rutqvist L, Carstensen J, et al. c-erbB-2 expression and benefit from adjuvant chemotherapy and radiotherapy of breast cancer. *Eur J Cancer* 1995;31A: 2185–90.
 32. Pirolo KF, Tong YA, Villegas Z, Chen Y, Chang EH. Oncogene-transformed NIH 3T3 cells display radiation resistance levels indicative of a signal transduction pathway leading to the radiation-resistant phenotype. *Radiat Res* 1993;135: 234–3.
 33. Pietras RJ, Poen JC, Gallardo D, Wongvipat PN, Lee HJ, Slamon DJ. Monoclonal antibody to HER-2/*neu* receptor modulates repair of radiation-induced DNA damage and enhances radiosensitivity of human breast cancer cells over-expressing this oncogene. *Cancer Res* 1999;59:1347–55.
 34. Broniscer A, Baker SJ, Stewart CF, Merchant TE, Laningham FH, Schaiquevich P, et al. Phase I and pharmacokinetic studies of erlotinib administered concurrently with radiotherapy for children, adolescents, and young adults with high-grade glioma. *Clin Cancer Res* 2009;15:701–7.
 35. Joensuu G, Joensuu T, Nokisalmi P, Reddy C, Isola J, Ruutu M, et al. A Phase I/II trial of gefitinib given concurrently with radiotherapy in patients with nonmetastatic prostate cancer. *Int J Radiat Oncol Biol Phys* 2010;78:42–9.
 36. Harrington KJ, El-Hariry IA, Holford CS, Lusinchi A, Nutting CM, Rosine D, et al. Phase I study of lapatinib in combination with chemoradiation in patients with locally advanced squamous cell carcinoma of the head and neck. *J Clin Oncol* 2009;27:1100–7.
 37. Normanno N, Campiglio M, De LA, Somenzi G, Maiello M, Ciardiello F, et al. Cooperative inhibitory effect of ZD1839 (Iressa) in combination with trastuzumab (Herceptin) on human breast cancer cell growth. *Ann Oncol* 2002;13:65–72.
 38. Brunner TB, Gupta AK, Shi Y, Hahn SM, Muschel RJ, McKenna WG, et al. Farnesyltransferase inhibitors as radiation sensitizers. *Int J Radiat Biol* 2003;79:569–76.
 39. Hsieh CH, Jeng KS, Lin CC, Chen CK, Liu CP, Lin CP, et al. Combination of sorafenib and intensity modulated radiotherapy for unresectable hepatocellular carcinoma. *Clin Drug Investig* 2009;29: 65–71.
 40. Shannon AM, Telfer BA, Smith PD, Babur M, Logie A, Wilkinson RW, et al. The mitogen-activated protein/extracellular signal-regulated kinase kinase 1/2 inhibitor AZD6244 (ARRY-142886) enhances the radiation responsiveness of lung and colorectal tumor xenografts. *Clin Cancer Res* 2009; 15:6619–29.
 41. Anders CK, Carey LA. Biology, metastatic patterns, and treatment of patients with triple negative breast cancer. *Clin Breast Cancer* 2009;9:S73–81.
 42. Gonzalez-Angulo AM, Timms KM, Liu S, Chen H, Litton JK, Potter J, et al. Incidence and outcome of BRCA mutations in unselected patients with triple receptor-negative breast cancer. *Clin Cancer Res* 2011;17:1082–9.
 43. Nielsen TO, Hsu FD, Jensen K, Cheang M, Karaca G, Hu Z, et al. Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. *Clin Cancer Res* 2004;10: 5367–74.
 44. Baselga J. Why the epidermal growth factor receptor? The rationale for cancer therapy. *Oncologist* 2002;7:2–8.
 45. Huang SM, Harari PM. Modulation of radiation response after epidermal growth factor receptor blockade in squamous cell carcinomas: inhibition of damage repair, cell cycle kinetics, and tumor angiogenesis. *Clin Cancer Res* 2000;6:2166–74.
 46. Ciardiello F, Caputo R, Troiani T, Borriello G, Kandimalla ER, Agrawal S, et al. Antisense oligonucleotides targeting the epidermal growth factor receptor inhibit

- proliferation, induce apoptosis, and cooperate with cytotoxic drugs in human cancer cell lines. *Int J Cancer* 2001;93:172–8.
47. Baumann M, Krause M, Dikomey E, Dittmann K, Dörr W, Kasten-Pisula U, et al. EGFR targeted anti-cancer drugs in radiotherapy: pre-clinical evaluation of mechanisms. *Radiother Oncol* 2007;83:238–48.
 48. Bonner JA, Harari PM, Giralt J, Cohen RB, Jones CU, Sur R, et al. Radiotherapy plus cetuximab for locoregionally advanced head and neck cancer: 5-year survival data from a phase 3 randomised trial, and relation between cetuximab-induced rash and survival. *Lancet Oncol* 2010;11:21–8.
 49. Dantzer F, de La Rubia G, Ménissier-De Murcia J, Hostomsky Z, de Murcia G, Schreiber V. Base excision repair is impaired in mammalian cells lacking Poly(ADP-ribose) polymerase-1. *Biochemistry* 2000;39:7559–69.
 50. Ashworth A. A synthetic lethal therapeutic approach: poly(ADP) ribose polymerase inhibitors for the treatment of cancers deficient in DNA double-strand break repair. *J Clin Oncol* 2008;26:3785–90.
 51. Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M, et al. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. *N Engl J Med* 2009;361:123–34.
 52. Wang L, Mason KA, Ang KK, Buchholz T, Valdecanas D, Mathur A, et al. MK-4827, a PARP-1/-2 inhibitor, strongly enhances response of human lung and breast cancer xenografts to radiation. *Invest New Drugs* 2012; 30:2113–20.
 53. Bertucci F, Finetti P, Cervera N, Esterni B, Hermitte F, Viens P, et al. How basal are triple-negative breast cancers? *Int J Cancer* 2008;123: 236–40.
 54. Bossuyt V, Fadare O, Martel M, Ocal IT, Burtneß B, Moïnfar F, et al. Remarkably high frequency of EGFR expression in breast carcinomas with squamous differentiation. *Int J Surg Pathol* 2005;13:319–27.
 55. Curtis C, Shah SP, Chin SF, Turashvili G, Rueda O, Dunning MJ, et al. The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. *Nature* 2012;486:346–52.
 56. Massarelli E, Varella-Garcia M, Tang X, Xavier A, Ozburn N, Liu D, et al. KRAS mutation is an important predictor of resistance to therapy with epidermal growth factor receptor tyrosine kinase inhibitors in non-small-cell lung cancer. *Clin Cancer Res* 2007;13: 2890–6.