Cancer Treatment Reviews 67 (2018) 88–96

Contents lists available at ScienceDirect

Cancer Treatment Reviews

journal homepage: www.elsevier.com/locate/ctrv

Laboratory-Clinic Interface

Metabolomics in breast cancer: A decade in review

Amelia McCartney[a,1], Alessia Vignoli[b,1], Laura Biganzoli[a], Richard Love[c], Leonardo Tenori[b,d], Claudio Luchinat[b,e], Angelo Di Leo[a,*]

[a] "Sandro Pitigliani" Medical Oncology Department, Hospital of Prato, Istituto Toscano Tumori, Prato, Italy
[b] Centre for Magnetic Resonance (CERM), University of Florence, Via Sacconi 6, Sesto Fiorentino 50019, Italy
[c] Department of Mathematics, Statistics and Computer Science, Marquette University, Milawaukee, WI, USA
[d] Department of Clinical and Experimental Medicine, University of Florence, Largo Brambilla 3, Florence 50100, Italy
[e] Department of Chemistry, University of Florence, Via della Lastruccia 3, Sesto Fiorentino 50019, Italy

ARTICLE INFO

Keywords:
Breast cancer
Biomarker
Metabolomic
Prognosis
Screening
Chemotherapy

ABSTRACT

Breast cancer (BC) is a heterogeneous disease which has been characterised and stratified by many platforms such as clinicopathological risk factors, genomic assays, computer generated models, and various "-omic" technologies. Genomic, proteomic and transcriptomic analysis in breast cancer research is well established, and metabolomics, which can be considered a downstream manifestation of the former disciplines, is of growing interest. The past decade has seen significant progress made within the field of clinical metabolomic BC research, with several groups demonstrating results with significant promise in the setting of BC screening and biological characterisation, as well as future potential for prognostic metabolomic biomarkers.

Introduction

Metabolomics is the study of the multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modifications [1]. The metabolome, a quantitative ensemble of metabolites, is established via analysis of various biological samples (for example, blood, urine, saliva, tissue). The metabolome is influenced by both exogenous and endogenous factors, such as age, gender, race, diet, presence of disease, and drug exposure. As such, a metabolomic fingerprint – the conglomerate pattern of many different, individually expressed metabolites – reflects the idiosyncratic biological milieu of the individual from whom a sample is drawn [2]. As cells cycle – for example, during neoplastic transformation and subsequent proliferation, or through an inflammatory or immunological response to malignancy – the metabolites that form as byproducts of cellular activity differ from those found in normal, non-malignant cellular turnover [3,4]. Furthermore, the metabolome expresses dynamic change over time, concordant with evolving disease trajectory [5]. Metabolomics, which can be thought of as a downstream manifestation of proteomics, transcriptomics and genomics, presents potential for a relatively non-invasive liquid biopsy method that may be utilised in the future to diagnose and characterise cancer, assess treatment response and toxicity, and predict outcome from the outset of diagnosis [6,7].

The main analytic metabolomic platform utilised in metabolomic research is proton nuclear magnetic resonance (HNMR) spectroscopy, which produces a metabolic spectrum with a number of peaks. The idiosyncrasies of these peaks allow the carbon-hydrogen framework of an organic molecule to be characterised. Nuclear magnetic resonance (NMR) spectroscopy combines this technique with statistical data analysis methods, in order to assess and describe metabolic status. Other metabolomic platforms, such as mass spectroscopy (which often includes a separation stage via gas chromatography or liquid chromatography) are also utilised by researchers. No single technology is considered superior; indeed, each has unique strengths and limitations [8].

Since a seminal review of metabolomics was published by our group in 2007 [8], the past decade has seen further progress made with regard to its potential utility within the field of BC. This article intends to serve as a clinical update and companion to that original review. The original review also provides a targeted introduction to various technical approaches to metabolomic identification and analyses.

Utility in screening

The sensitivity of screening mammography is approximately 84%, with variability according to factors such as age and breast density [9]. A metabolomic fingerprinting approach via nuclear magnetic resonance and mass spectroscopy has demonstrated 100% accuracy of subsequent

* Corresponding author at: Hospital of Prato, Via Suor Niccolina 20, Prato 59100, Italy.
E-mail address: angelo.dileo@uslcentro.toscana.it (A. Di Leo).
[1] Contributed equally to this work.

https://doi.org/10.1016/j.ctrv.2018.04.012
Received 29 March 2018; Received in revised form 9 April 2018; Accepted 10 April 2018

Random Forest (RF) analyses to identify between plasma samples from healthy controls and women with BC [10]. This suggests a potential role in metabolomic screening for BC in the general population, perhaps in time replacing or enriching the current gold-standard imaging-centred approach. Sampling and analysis of ductal fluid aspirated from patients with unilateral early BC (eBC) (with aspirates from the contralateral breast serving as a control) has demonstrated some feasibility in BC detection [11]. However, as the microenvironment sampled from the control breast may not have been representative of a truly disease-free state (as the microenvironment and resultant metabolome could potentially be altered by the presence of cancer on the contralateral side), this confounding element could have led to an overestimation of screening accuracy. The reasonably invasive procedure required by this sampling technique also raises doubt regarding the desirability and practicality of its employment in population-based screening. A less invasive approach with a relatively simple analysis method has been described, wherein a targeted analysis of 23 amino acids and 26 acylcarnitines was conducted on dried blood spot samples [12]. A resultant model, based on multivariate and regression analysis, demonstrated sensitivity of 92.2% and specificity of 84.4% in distinguishing between patients with newly diagnosed BC and normal controls. In an attempt to identify biomarkers to assist in diagnosis of BC, another group combined bioinformatic pattern recognition techniques with NMR metabolomic analysis of blood and urine samples collected from BC patients and healthy controls. Nine different metabolites arising in serum, and two in urine differed significantly in concentration between the groups, although this metabolomic profile was not confirmed in alternate cohorts or applied prospectively [13]. Finally, a recent prospective study comparing serum from patients with invasive BC with healthy, gender and ethnicity-matched controls showed gas chromatography–mass spectroscopy (GC–MS) metabolomic profiling differentiated between the groups with a sensitivity of 96% and specificity of 100% [14].

Metabolomics role in tumour biology characterisation

Differences in metabolomic profiles according to race

An untargeted metabolomic analysis of malignant breast tumours derived from African American patients via comprehensive GC–MS and liquid chromatography methods identified 418 separate metabolites, of which 31.8% occurred at statistically different rates between oestrogen receptor positive (ER+) and triple negative patients [15]. Increases in activity of biochemical pathways involved in energy metabolism (several metabolites notably involving the glycolytic pathway) and transmethylation were noted in the triple negative BC cohort. The same group previously showed via PCR analysis that methylation of multiple genes occurs at higher frequency in African American women with hormone receptor negative disease, compared to Caucasian women [16]. This confirms similar, previously reported findings which compared a native Korean cohort to a Caucasian population from the United States [17], wherein the former cohort demonstrated promoter hypermethylation. Distinguishing differences in global metabolic profiles were observed by another group when comparing plasma samples from African American and Caucasian American women [18]. Recently, comparative high resolution magic angle spinning (HR-MAS) NMR analyses of triple negative and luminal A breast tissue derived from African-American and Caucasian women revealed different metabolic profiles according to subtype and race alike [19]. Triple negative tumours in African-American women exhibited higher levels of glutathione, choline and glutamine, as well as metabolic changes consistent with decreased mitochondrial respiration and increased glycolysis in parallel with decreased levels of ATP. Conversely, triple negative tumours in Caucasians showed indication of increased pyrimidine synthesis. These collective findings prompted the authors to surmise that such unique metabolic alterations may in the future guide possible novel treatment targets for triple negative disease.

Differences between molecular subtypes according to metabolomic analysis

Significant differences in metabolomic spectra have been observed when comparing between triple negative and triple positive disease (77.7% accuracy), ER+ and oestrogen receptor negative (ER−) status (72.2% accuracy) and human epidermal growth factor receptor 2 (HER2) positive and negative status (69.1% accuracy) [20]. This group observed that triple negative disease was characterised by lower levels of glutamine and higher levels of glutamate, consistent with increased glutaminolysis-based metabolism. Increased transcriptional activity of c-MYC with consequent alterations in glutaminergic metabolism has been shown by others to be a hallmark of triple negative disease [21,22]. Liquid chromatography of serum collected from BC patients and healthy controls tested the levels of 15 amino acids, eight of which were elevated in pre (but not post-) operative BC samples [23]. These amino acids were elevated most markedly in those with basal-like BC, compared to the more indolent luminal A subtype.

Another group has demonstrated metabolomic divergence associated with different BC molecular subtypes, with metabolomic phenotypes exhibiting different patterns of metabolite concentrations observed between HER2+ and HER2− disease, and ER+ and ER− status [24]. Receiver operating characteristic (ROC) analysis of a panel of eight metabolites tested their performance in classifying BC subtypes, yielding a potential diagnostic value of 0.89. The metabolomic phenotype of HER2+ disease was characterised by increased glycolysis, as well as increased fatty acid biosynthesis and gluconeogenesis. The Warburg effect describes the phenomenon in which cancer cells rely upon glycolysis rather than mitochondrial oxidative phosphorylation to generate ATP, regardless of available oxygen levels [25,26], though the reasons behind this predilection for a less efficient form of metabolism are not clear. Many metabolomic studies have referenced the presence of glycolytic markers as an indication of the Warburg effect driven by malignancy, and it remains a metabolic pathway of ongoing interest in cancer research [27,28].

Potential to refine existing molecular subtypes

A study combining transcriptomic and metabolomic analysis of malignant breast tumours via HR-MAS magnetic resonance spectroscopy (MRS) showed potential for metabolomic analysis to add refinement to existing molecular classification [29]. Metabolomic and gene expression data were merged by multivariate analysis as a means of identifying different intrinsic groups, with the majority of the samples analysed falling under luminal A classification. HR-MAS MRS identified three distinct metabolomic clusters within the luminal A group, which suggests established molecular groups may have potential for further sub-classification down metabolomic lines. Intriguingly, one of these sub-clusters showed significantly lower glucose and higher alanine levels than the other luminal A clusters, metabolites that may be regarded as surrogate markers of glycolic activity. A luminal A subcluster exhibiting a higher Warburg effect than others might be hypothesised to represent a comparatively more aggressive clinical subphenotype, though unfortunately the group had no clinical data to correlate with this finding. The potential to subcategorise within heterogeneous molecular subgroups is compelling, in that this may facilitate personalised refinement of treatment options. Metabolomic, proteomic and transcriptomic integrative clustering has also demonstrated a clinically significant split into further parts within the luminal A subtype [30], further establishing the potential for finer subdivisions. Haukaas et al. merged transcriptomic, metabolomic and protein expression data (including PAM50 subtyping) derived from a larger, untreated BC cohort to establish clusters based on metabolic expression [31]. Three distinct clusters were identified, with one cluster sharing similarities with that previously described by Borgan et al. [29], wherein surrogate markers of Warburg metabolism were noted. All three clusters identified by Haukaas et al. [31] included non-luminal A,

basal and HER2 enriched samples, suggesting that metabolomic clustering functions not just as a subdivider of existing heterogeneity within predefined molecular groups.

Pre-clinical implications for BRCA mutated and/or triple negative disease

NMR-based metabolomic characterisation of the metabolic responses of three different BC cell lines in response to the poly (ADP-ribose) polymerase (PARP) inhibitor, veliparib, revealed several cell line independent metabolic changes [32]. PARP inhibition was associated with enriched nitrogen metabolism, glycine, serine and threonine metabolism, aminoacyl-tRNA biosynthesis and taurine and hypotaurine metabolism in all three cell lines. PARP inhibition and radiation appeared to induce a similar metabolic response in BRCA-mutant HCC1937 cells, but not in MCF7 or MDAMB231 lines. The same group also conducted NMR analyses of the metabolic responses observed in cell lines in response to different classes of DNA-damaging agents, which demonstrated the ability of metabolomics to classify the type of DNA damage seen [33]. Another group successfully utilised liquid chromatography-mass spectrometry (LC-MS) based metabolomic profiling in delineating between BC cell lines according to their BRCA1 genotype [34]. Based on those in vitro findings, the group performed metabolomic analysis on plasma of patients (n = 35) with triple negative hereditary breast cancer syndrome, who were carriers and non-carriers of BRCA1 mutations. In the carrier group, plasma levels of adenine, N6-methyladenosine and 1-methyladenosine were significantly lower than in non-carriers, and could distinguish between the two groups, leading the authors to postulate these metabolites may be potential BC biomarkers connected to BRCA1.

Predicting treatment response

A pilot study conducted by our group ran metabolomic analysis via NMR spectroscopy on serum collected from patients enrolled in the EGF 30,001 trial, which saw patients with advanced BC randomised to paclitaxel plus lapatinib or placebo [35]. Metabolomic profiles were compared to time to progression, overall survival and treatment toxicity. A notable aspect of EGF 30,001 was that the majority of subjects enrolled had HER2- disease, and as such, this trial was not an ideal source for a retrospective study that set out, in part, to test a correlation related to response to anti-HER2 treatment. Overall, no significant correlation was found with outcome or toxicity in the unselected population. However, the small subset of HER2+ subjects ($N = 49$) who received lapatanib showed the metabolomic profile had significant predictive accuracy with regards to time to progression and overall survival, though the small number of subjects in this subset obviously limits the conclusions that may be drawn from these findings.

These findings were contrasted by analysis of preoperative serum samples collected from eBC patients prior to uniformly mandated neoadjuvant chemotherapy (epirubicin plus cyclophosphamide, then three weekly docetaxel ± trastuzumab), to assess whether metabolomics could predict response to chemotherapy [36]. Following neoadjuvant chemotherapy, patients ($N = 28$) were divided according to radiological and histological response into complete (pCR), partial, or no response/stable disease groups, with pre-operative serum samples being analysed via a combination of NMR and LC-MS. The concentrations of four metabolites – linolenic acid, glutamine, threonine and isoleucine – were identified as statistically different when comparing response to chemotherapy, and a predictive model based on ROC analysis accurately identified 80% of those patients who did not achieve pCR. However, explaining the mechanisms underlying the correlation between altered metabolite levels and responsive disease was beyond the scope of this pilot study.

A later study reduced the potential bias of histological heterogeneity by enrolling only HER2+ eBC patients ahead of receiving neoadjuvant paclitaxel-trastuzumab treatment, subsequently correlating targeted serum-based metabolomic data to histological response. In serum collected prior to neoadjuvant treatment, higher levels of spermidine and lower levels of tryptophan were observed in patients who achieved pCR [37]. Not unlike its predecessors, this study was limited by a small sample size ($N = 34$), and such results are also difficult to interpret in that it is not clear whether the observed result comments upon the response to a treatment combination, or more specifically to a single agent.

Within a neoadjuvant setting, another group attempted to correlate HR MAS MRS derived metabolomic profiles expressed in pre-treatment tumour core biopsies with pathological response [38]. No single metabolite was identified via univariate analysis that showed statistical significance with regard to pathological response, though there was a trend towards lower levels of choline-containing metabolite concentrations and phosphocholine/creatine ratios in the pCR group. Discrimination between treatment outcome groups was attained via multivariate analyses in OPLS-DA models built from the metabolomic profiles. These findings provide contrast to results published a year earlier which described significant differences in tumour metabolomic changes in response to neoadjuvant chemotherapy, that predicted BC survival, but not clinical response [39]. Pre- and post- treatment biopsies were analysed by MR MAS MRS and correlated to patient outcome. Subjects who died within five years of receiving treatment were found to have post-treatment increases in lactate (perhaps reflective of increased glycolysis), whereas patients who were alive beyond five years had an increase in glucose and decrease in glycine and choline-containing compounds. Clinical response to neoadjuvant therapy was not related to metabolic response, in contrast to the clear correlation between metabolic response and clinical outcome. Similar metabolic responses were observed in patients who received neoadjuvant paclitaxel, compared to those who received epirubicin.

Anticipating diagnosis of recurrence

Perhaps of most interest to clinicians is the potential metabolomics may have in generating prognostic biomarkers. In 2010, the first evidence supporting metabolomics as a potential biomarker of recurrent disease was published [40]. A retrospective analysis was performed on 56 patients with eBC, all of whom had serial serum samples collected over six years. Twenty subjects were diagnosed with recurrent disease during that period. Via multivariate analysis, eleven metabolite markers that differentiated between those with recurrent disease and those without were identified; this model performing with a sensitivity of 86% and a specificity of 84%. Metabolomic signatures predicted recurrence an average of 13 months before CA27.29 counts rose in 55% of patients with recurrence, though it must also be acknowledged that tumour markers are not routinely used or recommended in the surveillance of otherwise asymptomatic patients under surveillance following curative treatment [41,42]. Early detection of ipsilateral recurrence or a second contralateral primary is known to improve survival [43,44], but early detection of asymptomatic metastatic disease is of less clinical utility. Nevertheless, such strategies could arguably benefit those patients with new, symptomatically undetectable metastatic BC, who would be considered eligible for targeted palliative therapies in the setting of low volume disease. By making a diagnosis before the burden of metastatic disease progresses to crisis levels, aggressive upfront cytotoxic salvage intervention may also be avoided, and less onerous targeted therapies instituted in the first line instead.

Estimating prognosis in eBC patients

In the era of personalised medicine, development of tailored oncological treatment for breast cancer is lagging. Biomarkers such as hormone receptors and HER2 over-expression has allowed some precision in prognostication and treatment decisions, but the heterogeneous nature of BC has meant that discriminating within biological and

molecular classifications still requires refinement. The ability to discern between patients with eBC at high risk of recurrent disease, and those cured by locoregional therapy alone would be invaluable – allowing clinicians to selectively offer more aggressive adjuvant therapies to the former group, whilst sparing those at lower risk exposure to potentially toxic treatments which offer little, if any commensurate benefit. Historically, the risk of recurrent or eventual metastatic disease in eBC has been determined by assessing clinicopathological features, with certain factors (for example, high tumour grade, nodal involvement or HER2 over-expression) known to be associated with a poorer prognosis. Further refinement in prognostic risk estimation has been brought by validated online prognostic tools such as Adjuvant!Online and PREDICT – models built from data derived from cancer registries, trials group estimates and population-based mortality and morbidity databases – which provide an estimate of the potential benefit of systemic therapy relative to manifested clinicopathological features. However, these tools of prediction are blunt, and the level of authority they command is limited by shortcomings such as inability to generalise to certain patient subgroups [45,46], and restrictions in applying these models across ethnic demographics [47]. Evolving knowledge of BC molecular subtypes has enhanced clinical decision making. However, formally ascertaining the molecular subtype of all eBCs via mRNA expression profiling as routine practice is prohibitively time consuming and expensive. Whilst surrogate clinical definitions of subtypes have been proposed [48], they are yet to reach international consensus [49–51]. Furthermore, there is evidence to suggest a great deal of heterogeneity exists within defined intrinsic subtypes, suggesting that further delineation within and between groups is still required [52–54].

Genomic assays such as Oncotype DX and Mammaprint can provide some reassurance in decision making when residual uncertainty exists after potential risk is initially evaluated. However, these assays are not without shortcomings, and perhaps most significantly, genomic assays can over-estimate the risk of recurrence. For example, in the NSABP B20 trial, which studied a node negative, ER+ eBC cohort, of those with a high Oncotype recurrence score who received hormonal therapy alone, 60.5% remained free of distant disease at ten years [55]. Similarly, retrospective analysis of ER+, node positive eBC subjects enrolled in the SWOG S8814 trial demonstrated 43% of those with a high Oncotype score who received adjuvant tamoxifen alone remained event-free at ten years [56]. The first data emerging from the MINDACT trial demonstrates that of those enrolled subjects who were at both high genomic and clinicopathological risk (and therefore recommended to receive chemotherapy), 90.6% remained free of distant disease at five years – a significant proportion of this cohort will have been cured with surgery alone [57]. This echoes outcome data derived from seminal BC trials, wherein 40% of patients with ER−, node negative eBC treated with surgery alone remained recurrence free at twenty years. Of those with node positive disease treated with surgery alone, 22% were recurrence free at thirty years [58]. These examples underline the dilemma of treating luminal disease: in this subgroup, which is characterised by an overall relatively favourable prognosis, there remains an unmet need for refinement in prognostic techniques, as current assays still over-estimate the risk of recurrence in some cases, thus overstating the relative potential benefit of adjuvant chemotherapy.

Whilst existing prognostic markers assess the potential, proportional benefit of adjuvant therapies by examining the primary tumour, metabolomic analysis, derived from the periphery, detects the presence of residual disease, which represents the absolute risk of eventual disease relapse (see Fig. 1). One criticism of existing prognostic markers is that they are based on analysis of centrally-derived tumour tissue. Most cases of eBC are treated with upfront surgery, which by definition physically removes the factor upon which risk of recurrence is calculated. Once a malignancy has been macroscopically cleared, the estimation of *residual* risk should therefore be made according to what potentially remains: occult micrometastatic disease, rather than solely inferring risk and prospective benefit from adjuvant therapies according to characteristics of a tumour which has been excised in the interim.

Our group has established a reproducible method of quantifying individual metabolomic fingerprints, and we have shown that metabolomics has an ability to accurately discern between advanced and early disease [59]. Serum samples of patients with eBC were analysed by NMR spectroscopy, with metastatic samples serving as a control reference. Characteristic clustering of metabolites – the metabolomic "fingerprint" – consistently differed between the two groups, allowing reliable separation between the early and metastatic cohorts. Some metabolomic fingerprints expressed in subjects with early disease more closely resembled a metastatic fingerprint, with a concordant metabolomic high risk of recurrence (reflective of the hypothesis that a misclassification of "metastatic" in an eBC sample indicated the presence of micrometastatic disease): the closer to the metastatic cluster barycentre, the greater the estimated metabolomic risk. Accurate discrimination between early and metastatic groups was made with 75% sensitivity, 69% specificity and a predictive accuracy of 72%. Metabolomic fingerprinting was employed by another group who confirmed the ability of metabolomics to discriminate between early and metastatic BC [60]. Their profiling model performed with sensitivity and specificity of 89.8% and 79.3% respectively; slightly higher than the results observed by our group. While the concentration patterns of individual metabolites found in metastatic samples were not identical between the two studies, alternations in glucose and lipids were noted by both groups. Oakman et al. [59] compared metabolomic risk for each sample to the corresponding estimated 10 year mortality prediction by Adjuvant!Online, wherein poor concordance was found between the two. This remains the main limitation of this study: metabolomic risk was compared to an estimate of mortality according to the computer model of Adjuvant!Online, rather than actual follow up data.

Building on our findings published in 2011 [59], our group retrospectively tested a metabolomic prognostic model for disease relapse in biobank-derived samples of patients with operable, predominantly ER− eBC, treated at a single institution [61]. Metabolomic fingerprints were derived from post operative serum samples, and compared against a control group (comprised of samples from subjects with metastatic BC) via an RF classifier [62] algorithm, which was utilised to build discriminative models between the two groups. Similar to our previous study, the metabolomic risk of each eBC subject was based on the degree of similarities observed when compared to control metastatic BC profiles, with the methodology underpinning the design of the RF algorithm described in detail elsewhere [61,63]. A low RF score derived from an eBC sample correlated with a low probability of recurrence due to a presumed absence of occult micrometastatic disease, whereas a high score reflected a greater estimated risk of recurrence (see Fig. 2). Next, subsequent ROC analysis compared the RF scores to actual clinical outcome in both a training and validation set – in contrast to the group's previous study which relied up on Adjuvant! Online estimates as a surrogate for follow up data. Relapse was predicted with high sensitivity and specificity in the training set (90% sensitivity, 67% specificity, and 73% predictive accuracy), and subsequently reproduced within the independent validation eBC set (82% sensitivity, 72% specificity, and 75% predictive accuracy). Comparatively worse disease-free survival rates were seen in those with high estimated risk of recurrence (see Fig. 3).

We subsequently reproduced similar findings, using the same methodology, within an ER+ eBC cohort, analysing serum collected in several clinical centres in South-East Asia as a part of an unrelated Phase III adjuvant trial, with serum collected as a part of a metastatic BC trial serving as a model calibrator [63]. The RF model demonstrated an accuracy of 84.9% in correctly discriminating between eBC and metastatic disease. Fig. 4 illustrates the correlation between nodal status and estimated metabolomic risk. In correlating the estimated metabolomic risk to actual recurrence rates, the model performed with 71.3% specificity and 70.8% specificity. Again, there were clear

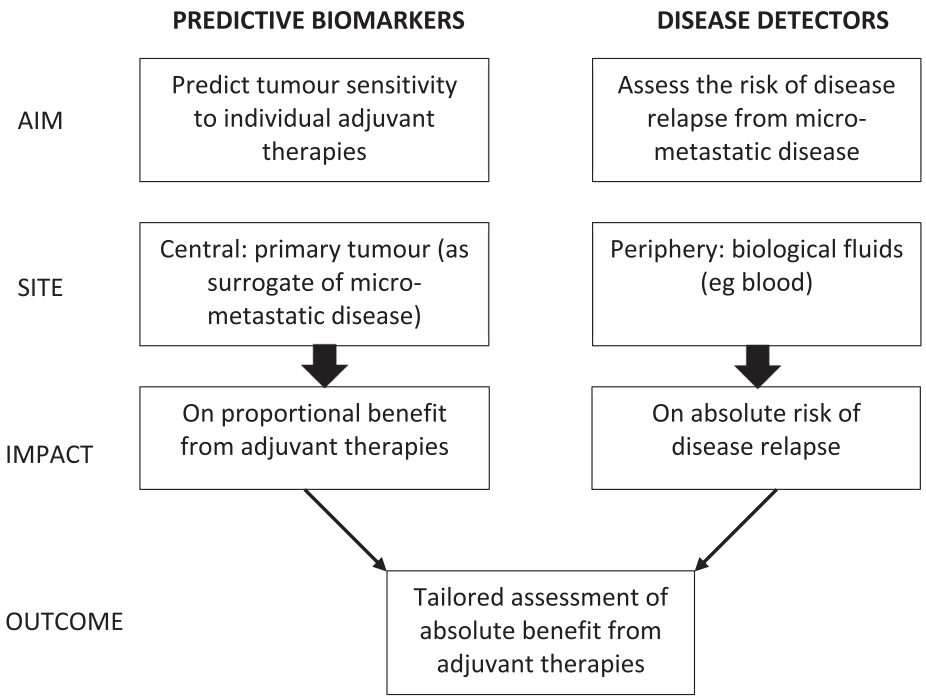

Fig. 1. Comparison between the concept of predictive biomarkers, and alternative "disease detector" measures that are capable of detecting residual, micro-metastatic disease.

differences in outcome between high and low risk groups as estimated by metabolomic profiling (see Figs. 5 and 6). The majority of studied samples came from women with relatively advanced disease. The majority had node positive disease, with 27% having 1–3 involved lymph nodes, and a further 31% with ≥3 positive nodes. Two thirds of the cohort had primary tumours measuring 2–5 cm (67.1%), and 27% had T3 disease (tumour dimension > 5 cm). Whilst this preponderance of advanced disease is reflected by an overall high rate of disease recurrence, which is indeed not representative of the generally favourable outlook normally seen in eBC (see Fig. 5), subset analysis of a subgroup with pathologically lower risk disease (node negative, primary tumour > 2 cm) illustrates fewer recurrence events, yet still offers a strong indication of the prognostic power of metabolomic analyses nonetheless (see Fig. 6). This study also differed from its biobank-based predecessor, in that samples were derived from multiple centres in several countries, in the preoperative setting – further demonstrating the ability of metabolomic analysis to meaningfully estimate risk of eventual relapse, even prior to the primary malignancy being surgically

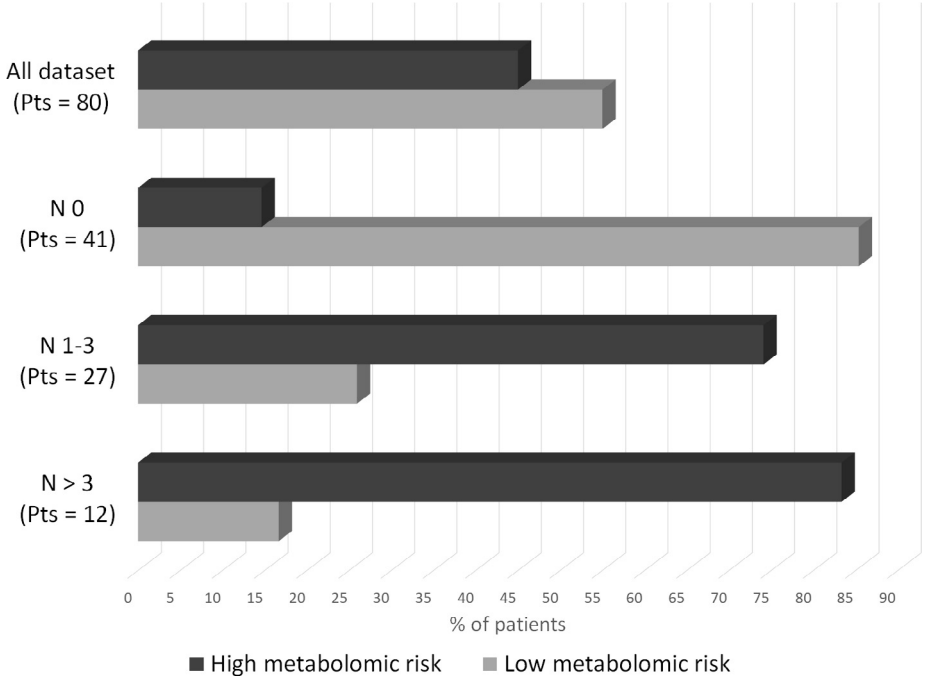

Fig. 2. Entire ER negative dataset described in Tenori et al. [61], illustrating estimated risk of recurrence as calculated by metabolomic profile, subdivided by nodal status.

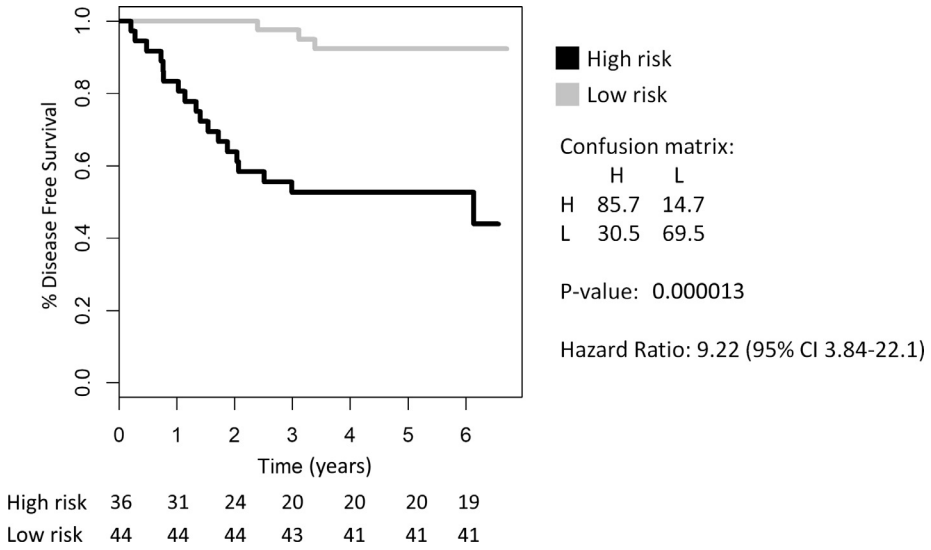

Fig. 3. Overall ER negative dataset, plotting actual disease-free survival over time (measured in years) according to estimated metabolomic risk score [61].

excised. Additionally, as serum samples were sourced from archived trial material, this guaranteed each patient received uniform treatment (bilateral oophorectomy and tamoxifen) as mandated by trial protocol, thus reducing confounding potential in subsequent metabolomic analyses.

These two studies are in partial accordance with previously published data [65]. Metabolomic predictions of five-year survival within an ER+ subgroup made via HR MAS MRS analysis of tumour tissue was found to outperform predictions made according to clinical parameters, with five-year BC survival correctly predicted by metabolomic analysis with a sensitivity of 71.6%, and specificity of 70.4%. Higher levels of glycine and lactate correlated with poorer survival. In contrast to positive results demonstrated by Tenori et al. [61], discriminating metabolic differences were not observed in a corresponding ER negative subgroup, though this cohort was comparatively small ($N = 24$) and may have lacked sufficient numbers to demonstrate efficacy. It is also unclear as to whether either group contained HER2+ subjects, which may also have had some bearing on prediction and outcome alike. Nevertheless, these studies collectively demonstrate capacity for metabolomics in identifying patients with eBC who are at increased risk of eventual relapse. This may in turn facilitate greater sophistication in clinical decision making with regards to adjuvant therapies, by adding further clarity to existing prognostic markers such as clinicopathological risk factors and genomic assays.

Future strategies

To expand upon work already completed by our group, our next

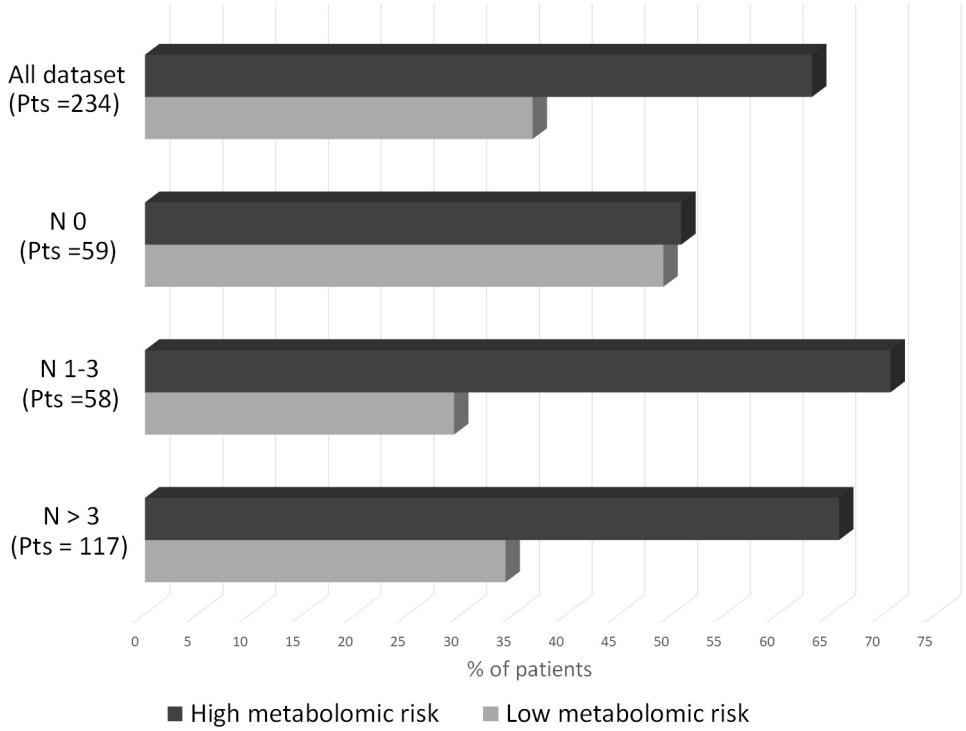

Fig. 4. Entire ER positive dataset described by Hart et al. [63], illustrating estimated risk of recurrence as calculated by metabolomic profile, subdivided by nodal status.

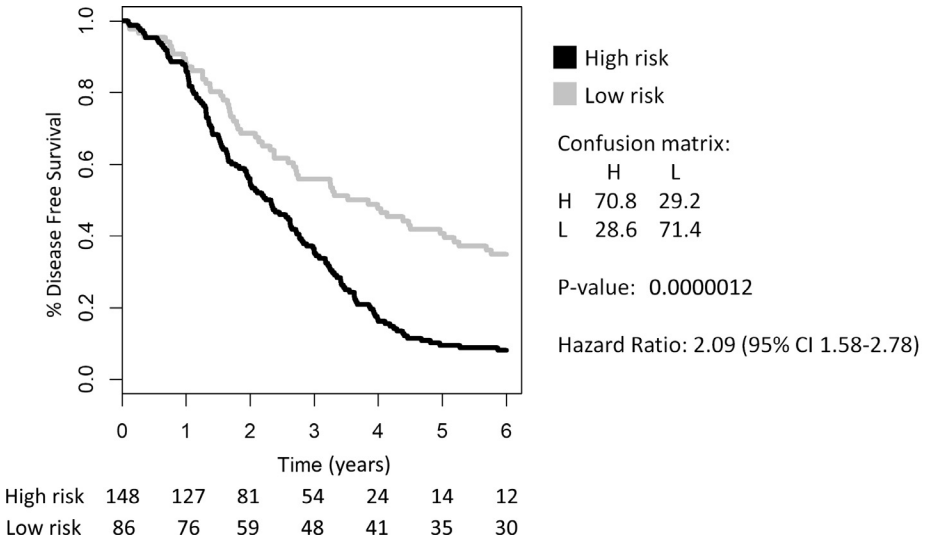

Fig. 5. Overall ER positive dataset, plotting actual disease-free survival over time (measured in years) according to estimated metabolomic risk score [63].

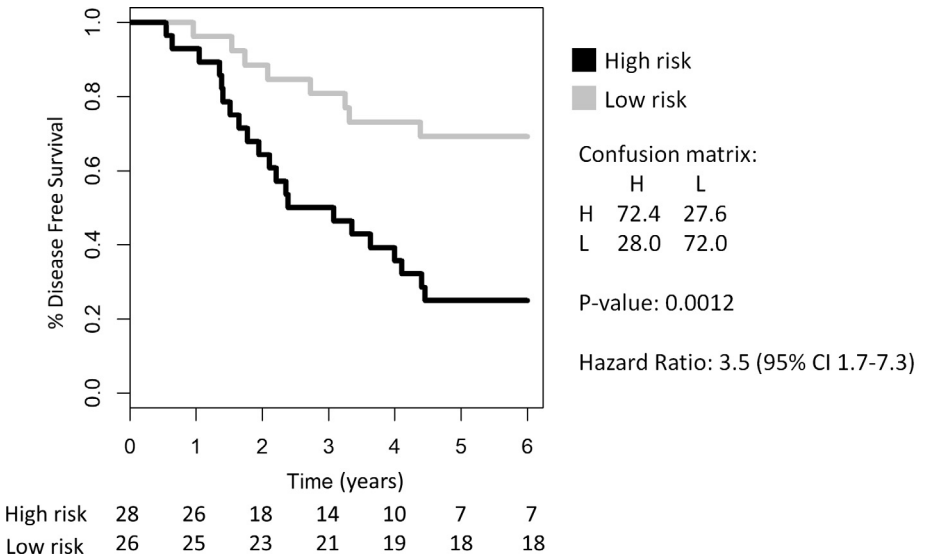

Fig. 6. ER positive tumour size > 2 cm, node negative subset, plotting actual disease-free survival over time (measured in years) according to estimated metabolomic risk score [63,64], figure reprinted with permission.

objective is to provide prospective validation to previous findings with regards to the potential role for metabolomics as a prognostic biomarker. The risk of recurrence as calculated by Oncotype DX will be combined with metabolomic serum analysis in patients with ER+, HER2- eBC in an attempt to achieve greater precision in recurrence risk prediction. We anticipate that the low, intermediate and high Oncotype recurrence risk categories will be subdivided again by metabolomic profiling, and that within single Oncotype subsets, those with a predicted low risk of recurrence according to metabolomic analysis will demonstrate greater disease-free survival than those with predicted high metabolomic risk. This prospective cohort study will commence enrolment in two cancer centres in Italy in 2018.

The Breast Cancer to Bone (B2B) Metastases Research Program is a large Canadian multi-project initiative that has proposed a core plan designed for further discovery into the role metabolomic profiles may play in the prediction and early diagnosis of bone metastases [66]. Recruitment is underway, having begun in 2010, and results are awaited with interest.

Conclusion

In the absence of a conclusive and refined understanding of BC subtypes and the heterogeneity that exists within this large disease entity, a truly personalised management approach cannot be achieved at the individual level. Metabolomics, particularly when harnessed with other validated tools of prognostication, may prove a key figure in ultimately achieving this aim in the setting of prediction of recurrence and estimating prognosis via liquid biopsy. Similarly, metabolomics may potentially offer a relatively non-invasive alternative or enhancement to BC screening, biological tumour characterisation and prediction of treatment response. In the domain of –omic sciences, metabolomics may once have been considered an esoteric field, but should now be regarded in the mainstream.

Acknowledgements

The authors wish to acknowledge the Sandro Pitigliani Foundation, Prato, Italy for their ongoing support. This work was partially funded by a grant from the Breast Cancer Research Foundation, New York USA

(grant number BCRF 16-055).

Disclosure of potential conflicts of interest

No potential conflicts of interest were declared by the authors.

References

[1] Nicolson JK, Lindon JC, Holmes E. "Metabonomics": under standing the metabolic responses of living systems to pathological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. Xenobiotica 1999;29:1181–9.
[2] Beger RD, Dunn W, Schmidt MA, et al. Metabolomics enables precision medicine: "A White Paper, Community Perspective". Metabolomics 2016;12:149. http://dx.doi.org/10.1007/s11306-016-1094-6.
[3] Griffin JL, Shockcor JP. Metabolic profiles of cancer cells. Nat Rev Cancer 2004;4:551–61.
[4] Teicher BA, Linehan WM, Helman LJ. Targeting cancer metabolism. Clin Cancer Res 2012;18:5537–45. http://dx.doi.org/10.1158/1078-0432.CCR-12-2587.
[5] Chen R, Mias GI, Li-Pook-Than J, et al. Personal omics profiling reveals dynamic molecular and medical phenotypes. Cell 2012;148:1293–307. http://dx.doi.org/10.1016/j.cell.2012.02.009.
[6] Spratlin JL, Serkova NJ, Eckhardt SG. Clinical applications of metabolomics in oncology: a review. Clin Cancer Res 2009;15:431–40. http://dx.doi.org/10.1158/1078-0432.CCR-08-1059.
[7] Hart CD, Tenori L, Luchinat C, Di Leo A. Metabolomics in breast cancer: current status and perspectives. Adv Exp Med Biol 2016;882:217–34. http://dx.doi.org/10.1007/978-3-319-22909-6_9.
[8] Claudino WM, Quattrone A, Biganzoli L, et al. Metabolomics: available results, current research projects in breast cancer, and future applications. J Clin Oncol 2007;25:2840–6.
[9] Breast Cancer Surveillance Consortium (BCSC). Performance measures for 1,838, 372 screening mammography examinations from 2004 to 2008 by age – based on BCSC data through 2009. National Cancer Institute; 2013. < http://breastscreening.cancer.gov/data/performance/screening/2009/perf_age.html > .
[10] Jové M, Collado R, Quiles JL, et al. A plasma metabolomic signature discloses human breast cancer. Oncotarget 2017;8:19522–33. http://dx.doi.org/10.18632/oncotarget.14521.
[11] Matos Do Canto L, Marian C, Varghese RS, et al. Metabolomic profiling of breast tumors using ductal fluid. Int J Oncol 2016;49:2245–54. http://dx.doi.org/10.3892/ijo.2016.3732.
[12] Wang Q, Sun T, Yunfeng C, et al. A dried blood spot mass spectrometry metabolomic approach for rapid breast cancer detection. Onco Targets Ther 2016;9:1389–98. http://dx.doi.org/10.2147/OTT.S95862.
[13] Zhou J, Wang Y, Zhang X. Metabolomics studies on serum and urine of patients with breast cancer using 1H-NMR spectroscopy. Oncotarget; 2017 Mar 15. http://dx.doi.org/10.18632/oncotarget.16210. [Epub ahead of print].
[14] Hadi NI, Jamal Q, Iqbal A, et al. Serum metabolomic profiles for breast cancer diagnosis, grading and staging by gas chromatography-mass spectrometry. Sci Rep 2017;7:1715. http://dx.doi.org/10.1038/s41598-017-01924-9.
[15] Kanaan YM, Sampey BP, Beyene D, et al. Metabolic profile of triple-negative breast cancer in African-American women reveals potential biomarkers of aggressive disease. Cancer Genom Proteom 2014;11:279–94.
[16] Mehrotra J, Ganpat MM, Kanaan Y, et al. Estrogen receptor/progesterone receptor-negative breast cancers of young African-American women have a higher frequency of methylation of multiple genes than those of Caucasian women. Clin Cancer Res 2004;10:2052–7.
[17] Lee JS, Lo PK, Fackler MJ, et al. A comparative study of Korean with Caucasian breast cancer reveals frequency of methylation in multiple genes correlates with breast cancer in young ER, PR-negative breast cancer in Korean women. Cancer Biol Ther 2007;6:1114–20.
[18] Shen J, Yan L, Lui S, et al. Plasma metabolomic profiles in breast cancer patients and healthy controls: by race and tumour receptor subtypes. Transl Oncol 2013;6:757–65.
[19] Tayyari F, Nagana Gowda GA, Olopade OF, et al. Metabolic profiles of triple-negative and luminal A breast cancer subtypes in African-American identify key metabolic differences. Oncotarget 2018;9:11677–90. http://dx.doi.org/10.18632/oncotarget.24433.
[20] Cao MD, Lamichhane S, Lundgren S, et al. Metabolic characterization of triple negative breast cancer. BMC Cancer 2014;14:941. http://dx.doi.org/10.1186/1471-2407-14-941.
[21] Alles MC, Gardiner-Garden M, Nott DJ, et al. Meta-analysis and gene set enrichment relative to ER status reveal elevated activity of MYC and E2F in the "basal" breast cancer subgroup. PLoS ONE 2009;4:e4710. http://dx.doi.org/10.1371/journal.pone.0004710.
[22] Koranath P, Teo WW, Sadik H, et al. Targeting glutamine metabolism in breast cancer with aminooxyacetate. Clin Cancer Res 2015;21:3263–73. http://dx.doi.org/10.1158/1078-0432.CCR-14-1200.
[23] Poschke I, Yumeng M, Kiessling R, de Boniface J. Tumor-dependent increase of serum amino acid levels in breast cancer patients has diagnostic potential and correlates with molecular tumor subtypes. J Transl Med 2013;11:290. http://dx.doi.org/10.1186/1479-5876-11-290.
[24] Fan Y, Zhou X, Xia TS, et al. Human plasma metabolomics for identifying differential metabolites and predicting molecular subtypes of breast cancer. Oncotarget 2016;7:9925–38. http://dx.doi.org/10.18632/oncotarget.7155.
[25] Warburg O, Wind F, Negelein E. The metabolism of tumors in the body. J Gen Physiol 1927;8:519–30.
[26] Warburg O. On the origin of cancer cells. Science 1956;123:309–14.
[27] Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 2009;324:1029–33. http://dx.doi.org/10.1126/science.1160809.
[28] Kim J-W, Dang CV. Cancer's molecular sweet tooth and the Warburg effect. Cancer Res 2006;66. http://dx.doi.org/10.1158/0008-5472.CAN-06-1501.
[29] Borgan E, Sitter B, Lingjærde OC, et al. Merging transcriptomics and metabolomics – advances in breast cancer profiling. BMC Cancer 2010;10:628. http://dx.doi.org/10.1186/1471-2407-10-628.
[30] Aure MR, Vitelli V, Jernström S, et al. Integrative clustering reveals a novel split in the luminal A subtype of breast cancer with impact on outcome. Breast Cancer Res 2017;19:44. http://dx.doi.org/10.1186/s13058-017-0812-y.
[31] Haukaas TH, Euceda LR, Giskeødegård GF, et al. Metabolic clusters in breast cancer in relation to gene- and protein expression subtypes. Cancer Metab 2016;4:12. http://dx.doi.org/10.1186/s40170-016-0152-x.
[32] Bhute VJ, Ma Y, Bao X, Palecek SP. The poly (ADP-ribose) polymerase inhibitor veliparib and radiation cause significant cell line dependent metabolic changes in breast cancer cells. Sci Rep 2016;6:36061. http://dx.doi.org/10.1038/srep36061.
[33] Bhute VJ, Palecek SP. Metabolic responses induced by DNA damage and poly (ADP-ribose) polymerase (PARP) inhibition in MCF-7 cells. Metabolomics 2015;11:1779–91. http://dx.doi.org/10.1007/s11306-015-0831-6.
[34] Roig B, Rodríguez-Balada M, Samino S, et al. Metabolomics reveals novel blood plasma biomarkers associated to the BRCA1-mutated phenotype of human breast cancer. Sci Rep 2017;7:17831. http://dx.doi.org/10.1038/s41598-107-17897-8.
[35] Tenori L, Oakman C, Claudino WM, et al. Exploration of serum metabolomic profiles and outcomes in women with metastatic breast cancer: a pilot study. Mol Oncol 2012;6:437–44. http://dx.doi.org/10.1016/j.molonc.2012.05.003.
[36] Wei S, Lingyan L, Zhang J, et al. Metabolomics approach for predicting response to neoadjuvant chemotherapy for breast cancer. Mol Oncol 2013;7:297–307. http://dx.doi.org/10.1016/j.molonc.2012.10.003.
[37] Miolo G, Muraro E, Caruso D, et al. Pharmacometabolomics study identifies circulating spermidine and tryptophan as potential biomarkers associated with the complete pathological response to trastuzumab-paclitaxel neoadjuvant therapy in HER-2 positive breast cancer. Oncotarget 2016;7:39809–22. http://dx.doi.org/10.18632/oncotarget.9489.
[38] Choi JS, Baek H-M, Kim S, et al. Magnetic resonance metabolic profiling of breast cancer tissue obtained with core needle biopsy for predicting pathologic response to neoadjuvant chemotherapy. PLoS ONE 2013;8:e83866. http://dx.doi.org/10.1371/journal.pone.0083866.
[39] Cao MD, Giskeødegård GF, Bathen TF, et al. Prognostic value of metabolic response in breast cancer patients receiving neoadjuvant chemotherapy. BMC Cancer 2012;12:39. http://dx.doi.org/10.1186/1471-2407-12-39.
[40] Asiago VM, Alvarado LZ, Shanaiah N, et al. Early detection of recurrent breast cancer using metabolite profiling. Cancer Res 2010;70:8309–18. http://dx.doi.org/10.1158/0008-5472.CAN-10-1319.
[41] Khatcheressian JL, Hurley P, Bantug E, et al. Breast cancer follow-up and management after treatment: American Society of Clinical Oncology practice guideline update. J Clin Oncol 2013;31:961–5. http://dx.doi.org/10.1200/JCO.2012.45.9859.
[42] Senkus E, Kyriakides S, Ohno S, et al. Primary breast cancer: ESMO clinical practice guidelines. Ann Oncol 2015;26(suppl 5):v8–30. http://dx.doi.org/10.1093/annonc/mdv298.
[43] Lu WL, Jansen L, Post WJ, et al. Impact on survival of early detection of isolated breast recurrences after the primary treatment for breast cancer: a meta-analysis. Breast Cancer Res Treat 2009;114:403–12. http://dx.doi.org/10.1007/s10549-008-0023-4.
[44] Houssami N, Ciatto S, Marinelli F, et al. Early detection of second breast cancers improves prognosis in breast cancer survivors. Ann Oncol 2009;20:1505–10. http://dx.doi.org/10.1093/annonc/mdp037.
[45] Plakhins G, Irmejs A, Gardovskis A, et al. Underestimated survival predictions of the prognostic tools Adjuvant! Online and PREDICT in BRCA1-associated breast cancer patients. Fam Cancer 2013;12:683–9. http://dx.doi.org/10.1007/s10689-013-9646-7.
[46] De Glas NA, van de Water W, Engelhardt EG et al. Validity of Adjuvant! Online program in older patients with breast cancer: a population-based study. Lancet Oncol 2014;15:722–29. http://dx.doi.org/10.1016/S1470-2045(14)70200-1.
[47] Bhoo-Pathy N, Yip CH, Hartman M, et al. Adjuvant! Online is overoptimistic in predicting survival in Asian breast cancer patients. Eur J Cancer 2012;48:982–9. http://dx.doi.org/10.1016/j.ejca.2012.01.034.
[48] Goldhirsch A, Wood WC, Coates AS, et al. Strategies for subtypes – dealing with the diversity of breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. Annals Oncol 2011;22:1736–47. http://dx.doi.org/10.1093/annonc/mdr304.
[49] Bastien RRL, Rodríguez-Lescure A, Ebbert MTW, et al. PAM50 breast cancer subtyping by RT qPCR and concordance with standard clinical molecular markers. BMC Med Genom 2012;5:44. http://dx.doi.org/10.1186/1755-8794-5-44.
[50] Maisonneuve P, Disalvatore D, Rotmensz N, et al. Proposed new clinicopathological surrogate definitions of luminal A and luminal B (HER2-negative) intrinsic breast cancer subtypes. Breast Cancer Res 2014;16:R65. http://dx.doi.org/10.1186/bcr3679.
[51] Ades F, Zarddavas D, Bozovic-Spasojevic I, et al. Luminal B breast cancer: molecular characterization, clinical management and future perspectives. J Clin Oncol 2014;32:1803–2794. http://dx.doi.org/10.1200/JCO.2013.54.1870.

[52] Prat A, Perou CM. Deconstructing the molecular portraits of breast cancer. Mol Oncol 2011;5:5–23. http://dx.doi.org/10.1016/j.molonc.2010.11.003.
[53] Metzger-Filho O, Tutt A, de Azambuja E, et al. Dissecting the heterogeneity of triple-negative breast cancer. J Clin Oncol 2012;30:1879–87. http://dx.doi.org/10.1200/JCO.2011.38.2010.
[54] Russnes HG, Lingjærde OC, Børresen-Dale AL, Caldas C. Breast cancer molecular stratification: from intrinsic subtypes to integrative clusters. Am J Pathol 2017; pii: S0002–9440(17)30134–7. http://dx.doi.org/10.1016/j.ajpath.2017.04.022. [Epub ahead of print].
[55] Paik S, Tang G, Shak S, et al. Gene expression and benefit of chemotherapy in women with node-negative, estrogen-positive breast cancer. J Clin Oncol 2006;24:3726–34. http://dx.doi.org/10.1200/JCO.2005.04.7985. Epub 2006 May 23.
[56] Albain KS, Barlow WE, Shak S, et al. Prognostic and predictive value of the 21-gene recurrence score assay in a randomized trial of chemotherapy for postmenopausal, node-positive, estrogen receptor-positive breast cancer. Lancet Oncol 2010;11:55–65. http://dx.doi.org/10.1016/S1470-2045(09)70314-6. Epub 2009 Dec 10.
[57] Cardoso F, van't Veer LJ, Bogaerts J, et al. 70-gene signature as an aid to treatment decisions in early-stage breast cancer. N Engl J Med 2016;375:717–29. http://dx.doi.org/10.1056/NEJMoa1602253.
[58] Bonadonna G, Moltierni A, Zambetti M, et al. 30 years' follow up of randomised studies of adjuvant CMF in operable breast cancer: cohort study. BMJ 2005;330:217.
[59] Oakman C, Tenori L, Claudino WM, et al. Identification of a serum-detectable metabolomic fingerprint potentially correlated with the presence of micrometastatic disease in early breast cancer patients at varying risks of disease relapse by traditional prognostic methods. Annals Oncol 2011;22:1295–301. http://dx.doi.org/10.1093/annonc/mdq606.
[60] Jobard E, Pontoizeau C, Blaise BJ, et al. A serum nuclear magnetic resonance-based metabolomic signature of advanced metastatic human breast cancer. Cancer Lett 2014;343:33–41. http://dx.doi.org/10.1016/j.canlet.2013.09.011.
[61] Tenori L, Oakman C, Morris PG, et al. Serum metabolomic profiles evaluated after surgery may identify patients with oestrogen receptor negative breast cancer at increased risk of recurrence. Results from a retrospective study. Mol Oncol 2015;9:128–39.
[62] Breiman L. Random forests. Mach Learn 2001;45:5–32.
[63] Hart CD, Vignoli A, Tenori L, et al. Serum metabolomic profiles identify ER-positive early breast cancer patients at increased risk of disease recurrence in a multi center population. Clin Cancer Res 2017;23:1422–31. http://dx.doi.org/10.1158/1078-0432.CCR-16-1153.
[64] McCartney A, Vignoli A, Hart C, et al. De-escalating and escalating treatment beyond endocrine therapy in patients with luminal breast cancer. Breast 2017;34(Suppl 1):S13–8. http://dx.doi.org/10.1016/j.breast.2017.06.021.
[65] Giskeødegård GF, Lundgren S, Sitter B, et al. Lactate and glycine – potential MR biomarkers of prognosis in estrogen receptor-positive breast cancers. NMR Biomed 2012;25:1271–9. http://dx.doi.org/10.1002/nbm.2798.
[66] Brockton NT, Gill SJ, Laborge SL, et al. The Breast Cancer to Bone (B2B) Metastases Research Program: a multi-disciplinary investigation of bone metastases from breast cancer. BMC Cancer 2015;15:512. http://dx.doi.org/10.1186/s12885-015-1528-y.