Molecular biomarkers to individualise treatment: assessing the evidence

Chee K Lee, Sarah J Lord, Alan S Coates and R John Simes

ABSTRACT

- The absolute benefit of a treatment varies between individuals depending on their prognosis before treatment and whether their response to the treatment varies from the overall relative risk reduction measured in clinical trials.
- Based on these principles, biomarkers that can provide information about an individual's prognosis or predict his or her treatment response can be used to tailor treatment decisions to individual patients.
- Many novel molecular biomarkers are currently available. Although there is evidence to show that some of these can improve patient outcomes through improved biomarker-guided treatment strategies, others are yet to be adequately evaluated.
- Randomised controlled trials (RCTs) can distinguish whether a biomarker provides prognostic or predictive information and assess whether using a biomarker to guide treatment improves patient outcomes.
- Targeted RCTs can be used to demonstrate the efficacy of treatment in a restricted biomarker-defined population, and non-targeted RCTs can compare biomarker-guided versus conventional test-guided treatment strategies in broader populations.

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The development of rapid high-throughput technologies to analyse DNA, RNA and protein has led to a paradigm shift in our understanding of the molecular basis of disease. Molecules that can be used to differentiate between normal and abnormal biological processes or predict treatment responses are termed "molecular biomarkers" (Box 1).[1] Examples include DNA sequence mutations, epigenetic changes, and levels of messenger RNA or protein expression that are associated with a patient's risk of disease events, survival time or response to treatment. Use of molecular biomarkers to supplement or replace conventional clinicopathological factors has the potential to transform the practice of medicine by creating new opportunities for developing and tailoring treatments to individual patients.

The clinical value of some molecular biomarkers has been established by trials that have demonstrated that biomarker-guided treatment strategies improve patient outcomes. For example, randomised controlled trials (RCTs) have provided evidence about the value of measuring oestrogen receptor (ER) status in women with breast cancer to identify those who will benefit from targeted treatment with anti-oestrogen therapy and those who will not. However, many molecular biomarkers discovered in observational studies are yet to be adequately evaluated in clinical practice.

In this article, we describe the principles of using biomarkers to individualise treatment and discuss the role of RCTs in assessing their clinical value.

What is individualised treatment?

We rely on RCTs to identify the most effective treatment for patients with a given condition. Classically, these trials randomly assign patients to new or standard treatment and compare the risk of disease events, or time to disease events, to measure the effectiveness of the new treatment as a relative risk or hazard ratio. The absolute risk reduction for individual patients depends on their baseline risk before treatment — that is, their prognosis — and whether their response to treatment varies from the overall effect measured in RCTs — the "treatment prediction" (Box 2). If we have access to this information, we can individualise treatment decisions by weighing up the size of benefits and harms of the new treatment for individual patients.[3]

The full potential of using biomarkers to individualise treatment is not yet well understood. For example, trials have shown that anticoagulant therapy for the prevention of thromboembolic stroke in patients with non-valvular atrial fibrillation is more effective than standard care, but this does not mean that all patients will benefit. On the basis of trial results showing that anticoagulant therapy leads to a 70% relative risk reduction for major stroke, we can estimate that if 100 patients with a baseline risk of stroke of 10% were treated, seven strokes would be avoided.[3] However, some patients would not have had a stroke, regardless of whether they received the anticoagulant therapy, and others would not respond to the anticoagulant therapy and still have a stroke (90 and three patients, respectively), but both groups might still experience the side effects of treatment. Ideally, a biomarker or panel of biomarkers could be used to identify the small subgroup of patients most likely to receive a clinical benefit — those who would otherwise most certainly have a stroke (the perfect prognostic test) and also respond to treatment (the perfect predictive test) — and offer treatment only to this group. This would obviate adverse events and unnecessary costs of treatment for low-risk patients and provide the opportunity to offer an alternative therapy to high-risk patients who would not be responsive to this treatment.

Identifying biomarkers that classify prognosis or predict response

When a biomarker is discovered to be a promising classifier of patient outcome, further studies are needed to validate its reliabil-

1 Definitions

Biomarker: a "characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention".[1] Molecular biomarkers include genetic factors measured as variations or mutations of DNA sequence, epigenetic factors, and levels of messenger RNA and proteins.

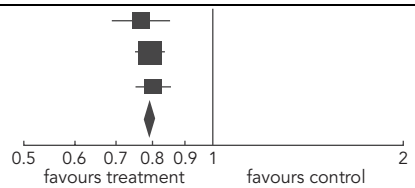
Prognostic biomarker: classifies an individual's baseline risk of having a clinical event.

Predictive biomarker: classifies the magnitude of an individual's response to treatment. ◆

3 Meta-analyses demonstrating prognostic and predictive value of biomarkers

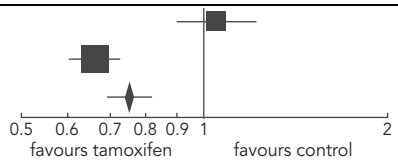
Risk of major vascular events following cholesterol-lowering therapy versus no cholesterol-lowering therapy, by total serum cholesterol level[9]

Total cholesterol (mmol/L)	Treatment events	Control events	Relative risk (95% CI)
⩽ 5.2	1465 (13.5%)	1808 (16.6%)	0.76 (0.69–0.85)
> 5.2 to 6.5	3312 (13.9%)	4159 (17.4%)	0.79 (0.75–0.83)
> 6.5	1457 (15.2%)	1992 (19.7%)	0.80 (0.76–0.86)
All*	6354 (14.1%)	7994 (17.8%)	0.79 (0.77–0.81)



Breast cancer mortality in women with early breast cancer after tamoxifen treatment versus control, by ER status[12]

ER status	Tamoxifen events	Control events	Tamoxifen/control death rate ratio (95% CI)
Negative	407 (17.8%)	402 (17.2%)	1.04 (0.90–1.21)
Positive	812 (19.3%)	1111 (27.1%)	0.66 (0.60–0.72)
All†	6492 (19.0%)	6450 (23.3%)	0.76 (0.70–0.82)



ER = oestrogen-receptor. * All patients were included in meta-analysis. † Includes cases where ER status was unknown. ◆

marker test and the subsequent treatment intervention. However, it is an inefficient design as a large number of patients are needed but only patients with a biomarker test result that disagrees with the conventional test result contribute to the expected outcome from the treatment intervention.[15] All patients testing positive by both investigations will receive the same treatment intervention, hence the biomarker does not discriminate outcomes for these patients.

Targeted trials

An alternative approach is a targeted RCT,[16,17] also referred to as an enrichment design, which involves upfront testing of all patients for the biomarker of interest and selecting only patients with biomarker results that will lead to a change in outcome with the proposed treatment. This is more efficient than a classical design as those without the biomarker of interest are excluded from the study, hence fewer patients are enrolled (Box 4). A positive trial result provides evidence about the effectiveness of using the biomarker to guide treatment. The design is generally used when there is robust a priori biological evidence that treatment is effective or more effective in the biomarker-defined population.

As targeted trials recruit patients with the same biomarker status, they can be used to assess the efficacy of a new treatment in a biomarker-defined population, or the efficacy of an existing treatment in a subgroup of patients newly defined by a biomarker. Recent oncology trials provide examples of these scenarios.

Testing a new treatment in a biomarker-defined population. The pivotal trial for trastuzumab, a monoclonal antibody against the human epidermal growth factor receptor 2 (HER2) protein, was undertaken in a targeted population of women with metastatic breast cancer who were positive for HER2 (Box 5). This trial showed the efficacy of the combined strategy of testing for HER2 status and treating women with HER2-positive tumours with trastuzumab.[18] Subsequent research has focused on improving assay methods and the cut-off level of HER2 amplification or expression to optimise the predictive performance of HER2, to identify women who will benefit from treatment with trastuzumab.[23]

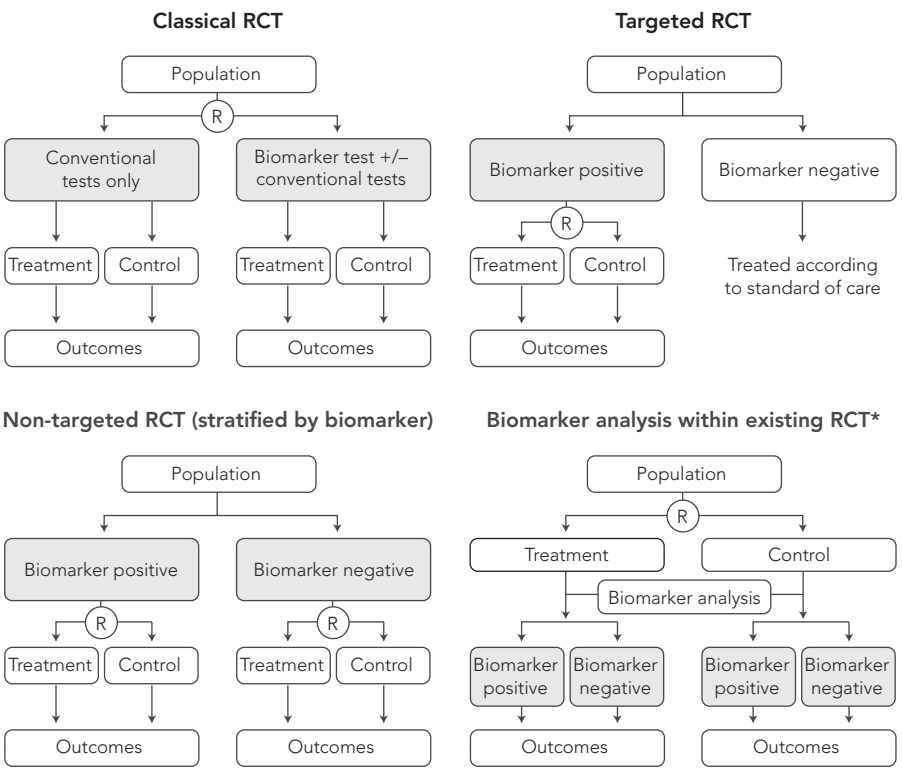
Testing an existing treatment in a subgroup of patients newly defined by a biomarker. Onco*type* DX is a 21-gene prognostic assay developed to classify women with node-negative, ER-positive breast cancer into three categories according to their risk of developing recurrent disease (low, intermediate and high risk). It has been proposed to guide treatment decisions by sparing women who are at low risk unnecessary chemotherapy, and identifying those who are at high risk and need treatment. Onco*type* DX is currently being prospectively assessed in the TAILORx trial (Trial Assigning Individualized Options for Treatment [Rx]).[19] The primary objective of the trial is to investigate the efficacy of chemotherapy as an addition to hormone therapy in women who are at intermediate risk (recurrence score, 11–25) (Box 5). The working premise is that patients in the intermediate-risk group will do no worse with hormone therapy alone than they would with hormone therapy plus chemotherapy. This study assumes that chemotherapy does not improve outcomes in patients at low risk (recurrence score, < 11) but will be beneficial in patients at high risk (recurrence score, > 25).

Targeted RCTs offer an efficient method to show proof of concept for the efficacy of treatment in biomarker-selected patient groups. They can be used where there is a biological assumption that only a biomarker-defined patient group will benefit from the new treatment; hence restricting the trial to this group (eg, biomarker-positive patients) is more efficient and potentially more ethical. However, molecular pathways of tumour pathogenesis are complex and often not clearly understood, so questions about the efficacy of treatment in biomarker-negative patients may also warrant consideration. For example, trastuzumab was recently reported to have efficacy in HER2-negative patients in a retrospective analysis of a broader patient population that included both HER2-positive and HER2-negative patients.[24]

Non-targeted trials

In contrast to targeted trials, non-targeted (or unselected design) trials do not restrict recruitment to a single biomarker-defined subgroup of patients. This design is needed to assess the predictive value of a biomarker to distinguish between patients who will

4 Randomised controlled trial (RCT) designs for biomarker evaluation



R = randomisation. * Research hypotheses may be pre-specified in the trial protocol (prior to study unblinding) or defined retrospectively. ◆

respond differently to the same treatment (Box 4), or compare the efficacy of a new biomarker-guided treatment strategy with current best practice using conventional testing.

The International Breast Cancer Study Group Trial IX, which randomly assigned postmenopausal node-negative early-stage breast cancer patients, stratified according to ER status, to chemotherapy followed by tamoxifen versus tamoxifen alone, is an example of the first situation. An example of the second situation is the ongoing MINDACT (Microarray In Node-negative Disease may Avoid ChemoTherapy) trial, which is comparing the prognostic value of a 70-gene signature for breast cancer with established clinicopathological criteria to identify women with node-negative early-stage breast cancer who can safely avoid adjuvant chemotherapy (Box 5). Unlike the TAILORx trial, the MINDACT trial does not restrict randomisation to one biomarker-defined patient subgroup. All women are assessed by using both the new biomarker gene signature and conventional criteria to classify their risk of disease recurrence. Only women with discordant results (biomarker-negative and conventional criteria-positive, or vice versa) are randomly assigned to receive chemotherapy or no chemotherapy; women with concordant results are not randomised but treated according to the standard of care (women at low risk with observation, and women at high risk with chemotherapy). This trial will provide data that compare patient outcomes when chemotherapy is selected according to the biomarker versus conventional criteria.

Non-targeted trials generally require a large sample size, particularly if the incremental effects of offering treatment to patients reclassified using a biomarker are small. Hence, their main disadvantage is that they may not be feasible if a small proportion of patients tested with the target condition are eligible — for example, if biomarker prevalence is low. Design variations to optimise the efficiency of non-targeted trial designs are discussed elsewhere and are beyond the scope of this article.[17]

Biomarker analysis within an existing RCT

A pre-specified analysis of prospective data collected from an existing RCT of treatment can sometimes be used to test a hypothesis about the prognostic or predictive value of a biomarker (Box 4). This study design was used to assess K-*ras* status as a predictor of response to panitumumab, an epidermal growth factor receptor inhibitor, in patients with metastatic, chemotherapy-refractory colorectal cancer. Investigators used archival tissue from an earlier RCT, which demonstrated the efficacy of panitumumab compared with best supportive care in a non-targeted population, to test the hypothesis that K-*ras* status predicts treatment response (Box 5).[22,25]

This study design has the advantage of time and cost efficiency. Its major disadvantage is the potential for selection bias if archival tissue or serum data are not available for all patients. Other disadvantages include measurement bias if measurements of the biomarker or statistical analyses are not blinded, and chance false-positive findings due to multiple comparisons, particularly with trial data used to explore multiple candidate biomarkers. These problems reduce the validity of study results, so analyses should only be considered if the biomarker can be measured on all or a large representative sample of all patients. Furthermore, the design needs a biologically plausible hypothesis, a prospectively defined protocol for the biomarker assays, and statistical analysis plans.

Implications for future trials

The current availability of genomic, transcriptomic, proteomic, metabolic and other similar technologies provides unprecedented opportunities for individualised treatment through the discovery of molecular biomarkers and the development of molecularly targeted therapies. The conventional rules of evidence for evaluating these new technologies have not changed, but the need for more efficient RCTs has led to innovations in the design of targeted and non-targeted trials. If an existing treatment trial has archived specimens from all, or most, participants, then an efficient and reliable evaluation of a new biomarker may be achievable by further analysis of these specimens. Future trials should be designed to anticipate these data needs.

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5 Purposes of randomised controlled trials (RCTs) used to evaluate biomarkers: recent examples in oncology

Trial	Target condition	Inclusion criteria	Treatment comparison	Purpose
Targeted trials				
HER2 trastuzumab trial[18]	Progressive metastatic breast cancer	HER2-positive tumour	Trastuzumab + chemotherapy versus chemotherapy alone	Test for effect of new treatment in a subgroup of patients defined by an existing biomarker
TAILORx[19]	Operable node-negative, ER-positive, HER2-negative early-stage breast cancer	Oncotype DX score 11–25 (intermediate risk of recurrence)	Chemotherapy + tamoxifen versus tamoxifen alone	Test for effect of existing treatment in a subgroup of patients defined by a new biomarker
Non-targeted trials				
IBCSG Trial IX[20]	Operable node-negative, postmenopausal early-stage breast cancer	ER-positive, ER-negative and ER-unknown	Chemotherapy + tamoxifen versus tamoxifen alone	Test for differences in treatment effect according to biomarker status
MINDACT[21]	Operable node-negative, ER-positive early-stage breast cancer	Genomic test result discordant with clinical criteria for classification of risk of recurrence: genomic low risk, clinical high risk; or genomic high risk, clinical low risk	Chemotherapy versus no chemotherapy	Test for differences in treatment effect between biomarker-guided and conventional test-guided treatment strategies
Biomarker analysis within an existing RCT				
K-*ras* panitumumab trial[22]	Progressive, metastatic, chemotherapy-refractory colorectal cancer; ⩾ 1% EGFR-positive	Subset of trial participants with measurable K-*ras* mutation status of tumour (mutant versus wild-type)	Panitumumab + best supportive care versus best supportive care alone	Test for differences in treatment effect according to biomarker status

EGFR = epidermal growth factor receptor. ER = oestrogen receptor. HER2 = human epidermal growth factor receptor 2. IBSCG = International Breast Cancer Study Group. MINDACT = Microarray In Node-negative Disease may Avoid ChemoTherapy. TAILORx = Trial Assigning Individualized Options for Treatment (Rx). ◆

Competing interests

None identified.

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