

CLINICAL BIOMARKERS IN DRUG DISCOVERY AND DEVELOPMENT

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Biomarkers enable the characterization of patient populations and quantitation of the extent to which new drugs reach intended targets, alter proposed pathophysiological mechanisms and achieve clinical outcomes. In genomics, the biomarker challenge is to identify unique molecular signatures in complex biological mixtures that can be unambiguously correlated to biological events in order to validate novel drug targets and predict drug response. Biomarkers can stratify patient populations or quantify drug benefit in primary prevention or disease-modification studies in poorly served areas such as neurodegeneration and cancer. Clinically useful biomarkers are required to inform regulatory and therapeutic decision making regarding candidate drugs and their indications in order to help bring new medicines to the right patients faster than they are today.

BIOLOGICAL MARKER (BIOMARKER)

A characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.

During the past decade, the greatest risk reduction in drug development decision making has resulted from the earlier involvement of drug metabolism in the optimization stage of drug design rather than the late stage characterization of lead molecules¹. The benefit can be seen by comparing rates of attrition in 1991 with those in 2000 (FIG. 1). *In vitro* screens for absorption² and metabolism³ have been validated by subsequent correlation with clinical measurements, which are then used for their predictive power to select compounds and design dosing regimens with a high likelihood of meeting the pharmacokinetic criteria for a new drug. One goal of BIOMARKER development is to enable this same reduction in risk for drug safety and efficacy, thereby reducing attrition of drugs during the clinical phases of development and, hence, the overall cost of drug development.

To that end, biomarkers are proposed to measure the delivery of drugs to their intended targets, and to understand and predict pathophysiology, and how it is altered by therapy, through monitoring variables known to have clinical relevance (FIG. 2). Biomarkers are especially valuable, as they can help to prioritise drug discovery resources by enabling early proof-of-concept studies for novel therapeutic targets. This is especially important for therapeutic indications in which assumptions regarding the relevance of animal models to clinical

disease are tested only in large late-phase, long-term clinical trials that can require extensive dose ranging.

Biomarkers can often be developed using animals *in vivo* before transferring the methodology to the clinic, although some technologies, such as functional brain imaging, can be compromised by constraints of experimental protocols. Biomarkers are especially valuable for providing early tests of key programme hypotheses, particularly if changes can be measured in normal volunteer subjects during initial clinical trials. The choice of biomarkers should always factor in the feasibility and ease of clinical use in the specific setting in which the biomarker will be deployed. It is important to anticipate that use by planning, in advance, rigorous validation in appropriate preclinical and clinical study designs. The time required to achieve this can be substantial, and therefore biomarker development should begin simultaneously, and proceed in parallel with, the search for new therapeutics.

Biomarkers that monitor specific physiological or pharmacological mechanisms can be used to select between multiple therapeutic targets for a drug by identifying those that are most sensitive to the intervention. Biomarkers can also reveal drug targets as well as optimize selection of molecules that interact with these targets for further development. For drugs with a large therapeutic index, these biomarkers can allow fast

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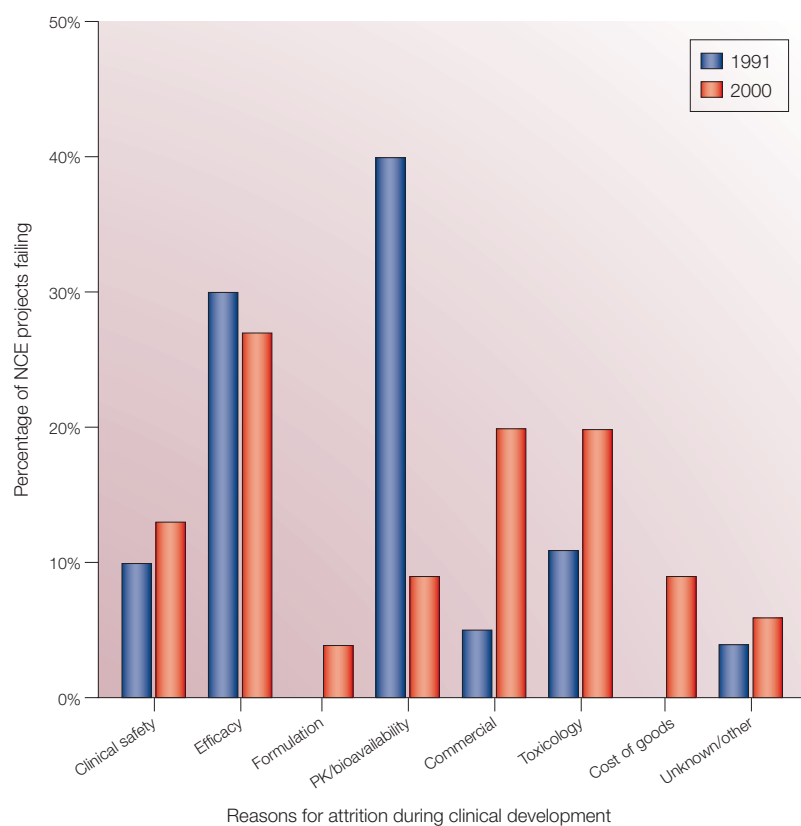


Figure 1 | Reasons for attrition. A survey of pharmaceutical companies comparing reasons for attrition between 1991 and 2000, expressed as a percentage of all drug projects stopped during clinical development, reveals a large reduction in losses to pharmacokinetic (PK) failures. NCE, new chemical entity.

progression from Phase I to Phase II clinical studies on the basis of quantification of target modulation (exposure–effect relationships), rather than the achievement of maximum tolerated dose.

Pharmacogenetics and pharmacogenomics offer the potential to predict disease occurrence, decipher variability in drug response and tailor individualized drug therapies in which the window between efficacy and toxicity is optimized for every patient. However, it is recognized that these fields of research have still to deliver on their promises. It is now clear that understanding the multivariate nature of disease and drug responses will usually depend on an integrated approach encompassing the use of genetic, messenger RNA expression, clinical, epidemiological, proteomic and related molecular phenotype data collectively termed ‘molecular profiling’⁴. The use of genomic approaches as biomarkers could, however, have an earlier impact on the efficiency and probability of success in the preclinical drug discovery process by identifying profiles characteristic of unwanted toxicity in early drug candidate screening. Phenotypic or genotypic biomarkers could assist prognosis⁵ and underpin population-enrichment strategies⁶ that increase the signal in early proof-of-concept clinical trials.

Clinical relevance is particularly important for clinical biomarkers of potential toxicities, as spurious findings could unnecessarily restrict dosing regimens if they

become a limiting factor in the calculation of the maximum recommended starting dose⁷ or the rate of dose escalation. Ultimately, however, it is unlikely that secure safety decisions could be made on the basis of biomarker data alone.

Rolan *et al.*⁸, de Gruttola, *et al.*⁹, and Meyer and Shapiro¹⁰ have recently published useful synopses of the biomarker experience of the global pharmaceutical industry that are complementary to this review.

Terminology

Under the auspices of the Office of the Director, National Institutes of Health, the ‘Biomarkers and Surrogate Endpoint Working Group’ agreed a classification system for biomarkers¹¹.

This system recognizes important differences among biomarkers. Type 0 biomarkers are markers of the natural history of a disease and correlate longitudinally with known clinical indices, such as symptoms over the full range of disease states. Type I markers capture the effects of an intervention in accordance with the mechanism of action of the drug, even though the mechanism might not be known to be associated with clinical outcome. Type II markers are considered SURROGATE ENDPOINTS because a change in that marker predicts clinical benefit. Unambiguous definitions were proposed to distinguish biomarkers from CLINICAL ENDPOINTS, enabling debate on the validation and application of surrogate endpoints (BOX 1).

The definition of ‘surrogate endpoint’ has evolved significantly during the past decade, driven by the urgency to find effective treatments for unmet medical needs in cancer and HIV infection. Early statistical definitions¹² were unrealistic, but seductive in the precision of language and potential for quantitative testing, which persists even today¹³. However, experience in testing the null hypothesis that “...requires the surrogate variable to ‘capture’ any [all] relationship between the treatment and the true endpoint...”¹² has taught us that random error in measuring biomarkers and ‘true’ endpoints makes it impossible to know with certainty that the net benefit of a drug has been captured fully by a change in a single biomarker; neither is it possible to estimate precisely the proportion of treatment effect^{14–16}. Surrogacy, as defined by Prentice¹², might require studies of virtually the whole population, or be applicable only to drugs with massive effects¹⁷, and therefore render useless the concept of biomarkers as an efficient means of assessing drug benefit.

Another relevant definition is provided by the US FDA: “A surrogate endpoint, or ‘marker’, is a laboratory measurement or physical sign that is used in therapeutic trials as a substitute for a clinically meaningful endpoint that is a direct measure of how a patient feels, functions, or survives and is expected to predict the effect of the therapy”¹⁸. Using this definition, Temple¹⁹ has listed the benefits and drawbacks of surrogates and gives examples of some markers, and the clinical endpoints for which they have been used as surrogates.

There is often confusion over terminology used to describe biomarkers in the scientific literature, but one

SURROGATE ENDPOINT

A biomarker intended to substitute for a clinical endpoint. A clinical investigator uses epidemiologic, therapeutic, pathophysiologic, or other scientific evidence to select a surrogate endpoint that is expected to predict clinical benefit, harm, or lack of benefit or harm.

CLINICAL ENDPOINT

A characteristic or variable that reflects how a patient feels or functions, or how long a patient survives.

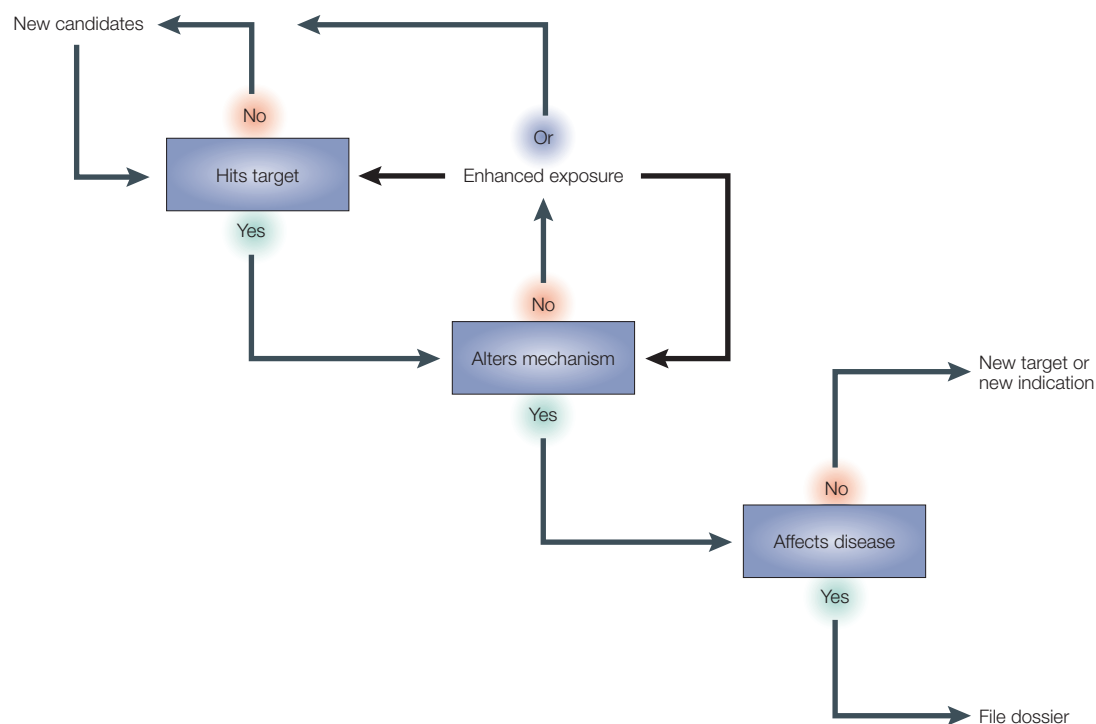


Figure 2 | **Biomarker categories: target, mechanism and clinical.** Biomarkers can be categorized into three distinct categories on the basis of their contribution to the logic of a clinical plan. Although they seem to parallel the three phases of drug development, the objective is to deploy them as early as possible, first to confirm hitting the target and then to test two concepts, namely, that hitting this target alters the pathophysiological mechanism and altering this mechanism affects clinical status.

useful way of conceptualizing a ‘biomarker hierarchy’ is using the body of evidence needed to support classification. These levels are plausibility, correlation/association, predictive power (that is, prognostic value) and cause.

Validation

The standard concepts of test–re-test reliability and validity apply with equal force to clinical biomarkers as they do in any assay system^{20,21}. Quality standards are well established for biological assays used in clinical pathology laboratories²². The Standards for Reporting of Diagnostic Accuracy initiative has resulted in a set of criteria²³ by which the potential for

bias, or the generalizability and applicability of the results, can be assessed in published studies of diagnostics (see Further information). The work required to establish the reliability and validity of a new biomarker should not be underestimated in general, and in particular needs planning for each combination of clinical indication and mechanism of action.

Type 0 markers can be characterized in phase 0 clinical studies, in which a reliable assay is used in a well-defined patient population for a specified period of time. Ideally, a linear (positive or negative) relationship is established with the ‘gold standard’ clinical assessor. Such studies are presently underway in **osteoarthritis** (see Further information, Osteoarthritis imaging initiative) and are planned for **Alzheimer’s disease** (see Further information, Alzheimer’s disease neuroimaging initiative). Often overlooked are test–re-test reliability, manufacturer differences in hardware and software, and cross-site differences in acquisition or interpretation of data. For instance, quantification of volumetric changes in brain images can be standardized by automated, software-based evaluations of digital data²⁴, which can be subjected to rigorous quality assurance (see Further information, Principles of software validation).

A priori validation of Type I biomarkers is impossible for truly novel targets without an effective positive control treatment. By definition, the more innovative the target, the less validated will be the associated biomarkers²⁵. Therefore, for novel targets the biomarker will be validated in parallel with the drug candidate, and the risk in

Box 1 | **Biomarkers Definitions Working Group**¹¹

Biological marker (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.

Clinical endpoint

A characteristic or variable that reflects how a patient feels or functions, or how long a patient survives.

Surrogate endpoint

A biomarker intended to substitute for a clinical endpoint. A clinical investigator uses epidemiological, therapeutic, pathophysiological, or other scientific evidence to select a surrogate endpoint that is expected to predict clinical benefit, harm, or lack of benefit or harm.

decisions made on the basis of that biomarker will improve over time. Alternatively, validating a marker can sometimes be achieved using a closely related treatment. By way of example, a battery of cognition markers validated with the anticholinergic scopolamine²⁶ were used to confirm the pharmacological effect of a novel agonist directed against the $\alpha 7$ nicotinic acetylcholine receptor²⁷, for which there is at present no candidate drug sufficiently well characterized in humans that it could serve as a positive control. Good type I biomarkers will capture the effects of both agonists and antagonists²⁸.

Type II biomarkers (or surrogate end-points) must be relevant both to the mechanism of action of the drug and to the pathophysiology of the disease. Changes in the biomarker should reflect treatment benefit and therefore effective therapy is necessary for this validation. Temple¹⁹ and Lonn²⁹ have made the case that to become surrogates biomarkers need to be correlated with outcome in clinical trials of more than one drug with the same mechanism of action targeted at the same indication. Validation can therefore best be gained from trials of common design, whether or not they contribute to a meta-analysis^{30–32} and can require long-term follow up to ensure predictability. Extrapolation of a validated Type II marker to drugs with different mechanisms for the same clinical indication, or to drugs with the same mechanism for different indications, needs careful consideration of those differences. It is likely that some biomarkers will be restricted to a particular class of drug defined according to mechanism in a specific indication.

The most commonly used surrogate endpoint for regulatory purposes is plasma concentration of drug in 'bioequivalence' studies³³, which proves the limiting case that a biomarker well-correlated with both the mechanism of action and the clinical effect needs only a single set of clinical trials for validation; that is to say, the Phase III studies supporting claims of effectiveness for the innovator drug are necessary and sufficient. The quality of evidence supporting the validity of a new biomarker has been described as similar to that used to support the validity of new therapies³⁴, in terms of the number and size of clinical validation trials and the concordance among those trials. The risk of using biomarkers for decision making is directly related to the level of validation for the purpose to which it is applied. The ideal trial to validate a surrogate endpoint is not necessarily the ideal trial to gain drug approval using the surrogate endpoint. Indeed, it can be argued that to establish a surrogate is to lower the hurdle for competitors coming behind in the same therapeutic area, but has the advantage that the validator is the primary holder of the data that establishes the value of the surrogate with respect to the clinical endpoint.

The reasons biomarkers can lead to erroneous conclusions have been sorted into four categories³⁵, which apply equally to business decisions as to regulatory decisions (FIG. 3). However, there is also a fifth, and more subtle, reason that a biomarker might 'fail', and this is by providing potentially misleading information. Erroneous conclusions can be drawn regarding the usefulness of a biomarker when the biomarker is actually a better assessor of

disease than the gold-standard clinical assessor³⁶. For example, poor correlations can be found between early biomarkers and gold-standard neuropsychiatric assessments during the early stages of neurodegenerative diseases such as Alzheimer's disease, in which the disease can progress asymptotically for many years^{37–39}. Indeed, in Alzheimer's disease symptomatic assessments will not discern the unique benefits of disease-modifying therapy from those of simple symptomatic treatment even during clinical stages of the disease. The development of biomarkers for diagnostic and prognostic use in diseases with asymptomatic phases is particularly challenging and can take a long time, as their validation is by necessity often linked to long-term clinical outcomes. Nonetheless, such biomarkers could have a big impact on healthcare by facilitating the assessment of new medicines that prevent costly chronic progressive disease.

Biomarker examples

Cardiology. Non-invasive imaging and biochemical biomarkers are being developed for the identification of 'vulnerable plaque', that is, lipid core vascular plaque vulnerable to rupture, in the carotid and coronary vasculature for studies of primary prevention. Asymptomatic carotid stenosis is associated with an 11% chance of ipsilateral stroke within five years, despite medical management, which justifies endarterectomy or placement of a stent. Classically, ultrasonography has been used to assess intima-media thickness⁴⁰ at baseline and following treatment. Most recently, magnetic resonance imaging (MRI) has been used to study heart structure and function, and to assess plaque composition^{41,42} and its regression in the coronary vasculature⁴³. Thermography⁴⁴ and fluorodeoxyglucose positron emission tomography (¹⁸FDG-PET)⁴⁵ have been used to image the inflammatory component of vulnerable plaque. The best-validated imaging technique for monitoring coronary atherosclerotic disease is now intravascular ultrasonography (IVUS). IVUS, although invasive, probably surpasses conventional coronary angiography (QCA) in its ability to identify atheromatous lesions in the vessel wall and then measure their distribution and size accurately^{46,47}. IVUS is, at present, being used as an end-point in several trials that study coronary artery disease regression. Multislice spiral computed tomography (CT) is quickly establishing itself as a relatively simple and clinically reliable technology for non-invasive quantitative coronary angiography. Multislice CT has great potential to characterize early coronary artery disease, assess atherosclerotic plaque burden and study the effectiveness of new-vessel-wall-modifying therapies⁴⁸.

Few biomarkers have attained the status of surrogate endpoints for drug approval, but examples of these can be found in the cardiovascular field¹⁹, in which blood pressure and cholesterol reduction are clearly linked to mortality as a result of heart attack and stroke. It is, however, noteworthy that extensive clinical experience, such as first detailed in the Scandinavian Simvastatin Survival Study (4S)⁴⁹, and most recently in the Heart Protection Study⁵⁰, have been required to achieve validation.

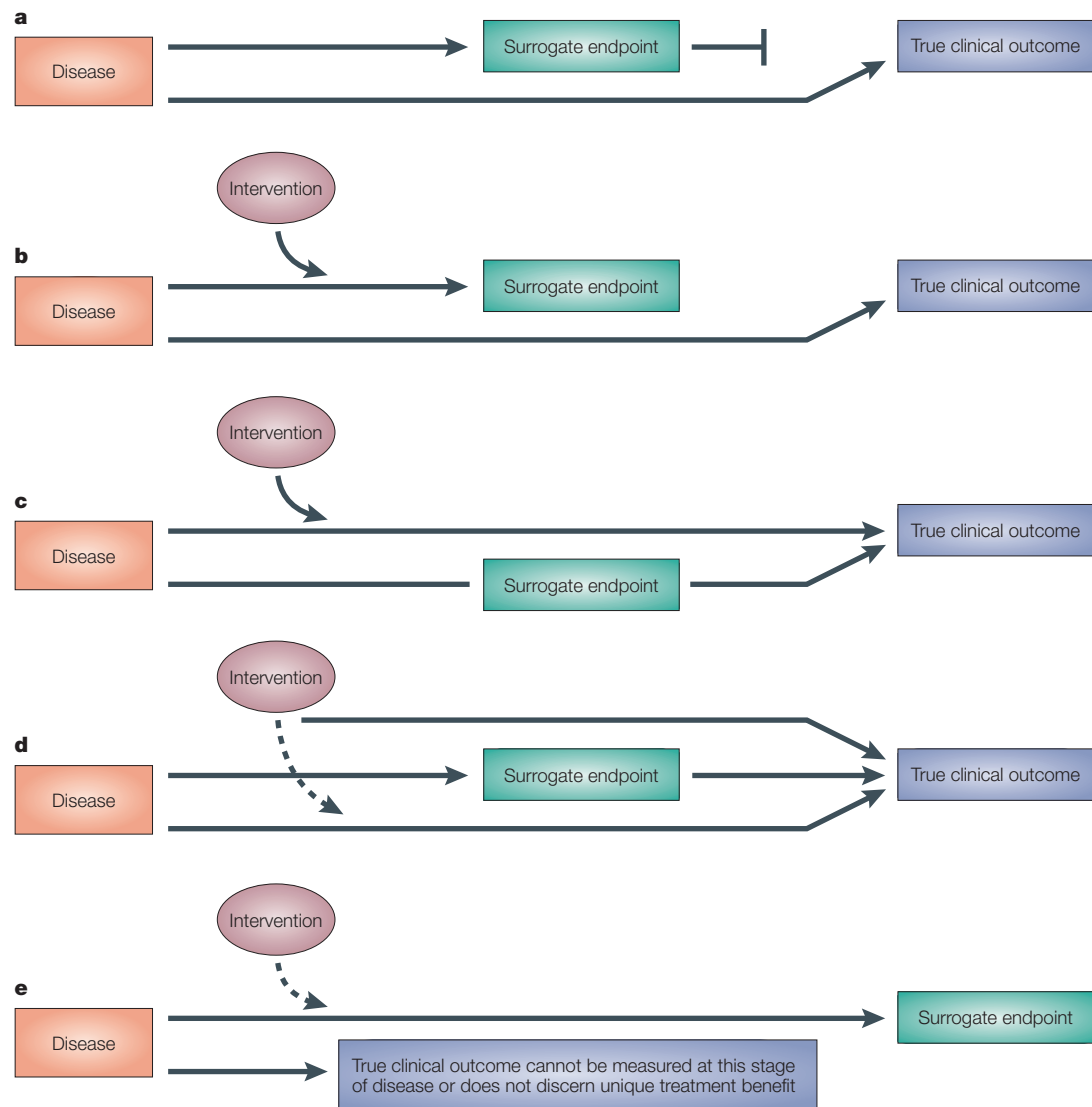


Figure 3 | **Reasons surrogate endpoints 'fail'.** **a** | Changes in the biomarker reflect the effect of treatment but are irrelevant to the pathophysiology of the indicated disease (false positive). **b** | changes in the biomarker reflect an effect of treatment on an element of the pathophysiology, but this element is clinically unimportant (false positive). **c** | changes in the biomarker would reflect clinically relevant changes in pathophysiology but do not capture the mechanistic effect of the treatment (false negative). **d** | changes in the biomarker reflect one effect of the treatment but there are other, more significant effects on outcome that are not captured (false negative or positive) and might even be unintended (for example, an adverse event resulting from toxicity or unwanted pharmacology). **e** | the biomarker might not correlate well with classical clinical assessors because the biomarker is more sensitive or the classical assessor is irrelevant to a subset of the patient population, a novel mechanism, or a new indication.

C-reactive peptide (CRP), an acute-phase reactant, has recently been recommended as a predictive biochemical biomarker for risk of coronary disease to inform primary prevention strategies including lifestyle changes and pharmacotherapy³⁴. That CRP should be a useful biomarker might seem surprising because it is a nonspecific biomarker of inflammation and correlates very poorly with extent of atherosclerosis by angiography, a classical biomarker of risk. However, the recommendation is based on the cumulative evidence of many prospective clinical trials of risk (Class1A in accordance with the classification system of the American College of Cardiology and American Heart Association)^{51,52}. CRP contributes to risk assessment

independently of serum cholesterol measures; it is further consistent with histopathological findings of active inflammatory cells in plaque⁵³. In clinical trials of the statins, a proportion of the treatment effect is thought to be captured by reductions in CRP⁵⁴. However, when examined on a patient-by-patient basis, reductions in CRP are quite heterogeneous and the correlation to outcome is unclear, perhaps as a result of genetic polymorphism⁵⁵. So, until better understood, the value of monitoring CRP in clinical trials of atherosclerosis therapies remains questionable. Biomarkers such as QT prolongation⁵⁶ and troponin T⁵⁷ have proven value to evaluate potential for cardiac electrophysiological toxicities and prognosis after heart attack.

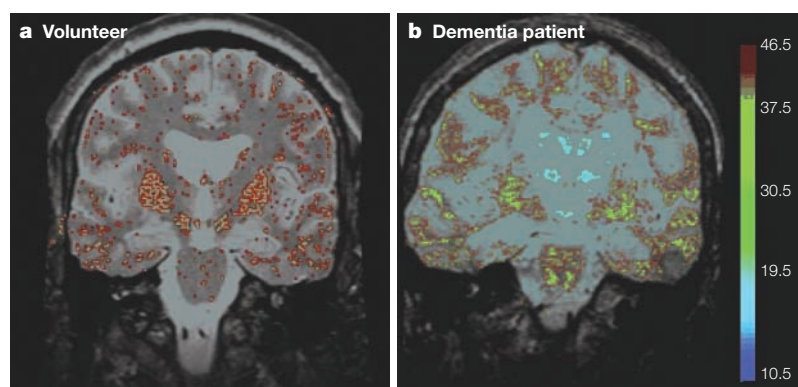


Figure 4 | Correlation of brain iron staining and T2 mapping. Iron in basal ganglia, white matter, and the boundary of the cortical ribbon can be visualized by Perl's staining of post-mortem tissue and correlate with low signals on T2-weighted magnetic resonance imaging. Comparison of iron distribution in a healthy volunteer (**a**) with that of an Alzheimer's patient (**b**) shows that it is clearly abnormal in the disease state. This could indicate a regional variation in the chemical form of the iron or the state of aggregation of iron-rich particles, which can be applied to the study of the role of iron storage abnormalities in Alzheimer's, Parkinson's and other neurodegenerative diseases. FIGS 4, 5 and 6 courtesy of GE Medical Systems.

Neurology. There is a pressing need to develop new therapies both for acute and chronic neurodegeneration. In the acute setting of stroke and head injury, it has been difficult to conduct proof-of-concept clinical trials because of the heterogeneous nature of the patient population. Attempts have been made to use diffusion and perfusion MRI⁵⁸ and perfusion CT⁵⁹ measurements as entry criteria and as biomarkers that can detect, assess and predict final infarct size, thereby providing potential endpoints for intervention studies. In contrast to acute neurodegenerative processes, diseases such as **multiple sclerosis**, **Parkinson's disease** and Alzheimer's disease generally remain asymptomatic for many years after the neurodegenerative process has begun^{60–62}. This attests to the remarkable capacity of the human brain to accommodate insult, but also reveals an opportunity for intervention. As distinguished from presently available symptomatic treatments, the advent of experimental disease-modifying therapies makes it crucial to identify pre-symptomatic neurodegenerative changes, because they might be stabilized before symptoms present. Early drug treatment could be the most efficacious therapeutic approach. To date, the most successful use of a neuroimaging biomarker in chronic neurodegenerative disease has been MRI for the diagnosis, prognosis and treatment of multiple sclerosis. Indeed, MRI studies have been used to support the registration and labelling of interferon-1- β and have shown the benefits of early intervention⁶³.

A wide range of imaging-based biomarkers are presently being studied for Alzheimer's disease. These include volumetric MRI of whole brain or brain regions^{64–67}, magnetic resonance spectroscopy, FDG-PET^{38,68} single-photon-emission computed tomography (SPECT) and PET for amyloid plaque⁶⁹ or microglial tracers⁷⁰. High-resolution 3 tesla (3T) MRI has also recently revealed abnormalities in iron distribution that

might be a characteristic of chronic neurodegenerative disease (FIG. 4). Multiple biochemical analytes⁷¹ in blood, urine or cerebrospinal fluid have been proposed, the most obvious of which are β -amyloid and Tau proteins, as they seem to be intimately involved in the pathology of Alzheimer's disease⁷². However, none of the candidate biomarkers seem to be linearly related to stage of disease throughout the full course of disease⁷³, and there are many different therapeutic approaches⁷⁴ predicated on different pathophysiological hypotheses that might require different mechanistic markers. It is therefore likely that a multi-modal biomarker approach stratified for ease of use, sensitivity and specificity will be needed in Alzheimer's disease.

New biomarkers of pre-symptomatic disease will be important for population-enrichment strategies and confirmation of efficacy during the assessment of novel Alzheimer-disease-modifying therapies.

Many individuals with mild cognitive impairment (MCI) do not progress to become Alzheimer's disease patients⁷⁵. Indeed, the conversion rate from MCI to Alzheimer's disease is only about 12% per annum⁷⁶. So, clinical trials that use treatment response rate (conversion for MCI to mild Alzheimer's disease) as an endpoint will require prohibitively large numbers of subjects, even if studies are long in duration. Enrichment of trials with patients of similar prognosis according to the presence of a particular biomarker or combination of biomarkers could speed up proof-of-concept and dose-ranging studies.

An alternative trial design might be to study disease-modifying treatments in mild Alzheimer's disease, but the standard of care in this population includes presently available symptomatic treatment, which could confound interpretation of the results. Traditional primary clinical measures of cognition and function⁷⁷ cannot distinguish between palliative agents that simply improve cognitive function from those that modify disease progression. Sophisticated study designs, such as staggered start or withdrawal, do not allow for direct comparisons of treatment effect and are impractical because of lack of cooperation by patients, who are understandably reluctant to delay initiation of treatment or to withdraw from a therapy which had shown benefit. Clearly, for Alzheimer's disease a good biomarker strategy could significantly reduce drug development timelines and optimize resources, thereby facilitating the evaluation of multiple molecules and therapeutic approaches.

Oncology. Biochemical biomarkers have long contributed to the assessment of risk and benefits in cancer, and routine clinical assays are available for such markers as prostate-specific antigen and carcinoembryonic antigen^{78,79}. More recently, imaging of tumour size has gained acceptance⁸⁰. Multi-dimensional imaging adds precision, whereas multi-modal imaging such as PET-CT adds quantification of metabolic activity or receptor status. Primary prevention cannot rely on assessments of tumours, but will require the validation of biochemical or genomic biomarkers.

As compared with biopsies or biochemical biomarkers, imaging methods offer the benefit of staging or quantifying therapeutic response, both for single lesions and the global tumour burden, which can be used as a good broad ‘clinical’ biomarker (FIG. 2). CT is presently used at all stages of cancer management, including early disease detection, differential diagnosis of suspected lesions and the assessment of therapeutic response⁸¹.

High-resolution CT combined with automated computational analysis⁸² can, for example, determine whether lesions in the lung exhibit growth consistent with lung cancer⁸³. FIGURE 5 shows a lung cancer lesion that was scanned with high-resolution CT after initial discovery (left) and during a follow-up scan one month later (right). In this case, the doubling time was estimated to be 103 days, indicating a malignant process. A biopsy subsequently confirmed the lesion to be large-cell carcinoma. It is noteworthy that recent communications at the Radiological Society of North America have shown that higher-resolution CT scanning and better correction for partial volume effects can improve the accuracy with which lesion volume can be measured.

FDG-PET offers the unique advantage of assessing tumour metabolism⁸⁴, and has been used for prognosis in gastrointestinal stromal tumours, documenting after only a week of therapy the likelihood of tumour shrinkage assessed by classical means⁸⁵. FDG is particularly

useful in localizing occult primaries or for staging when metastases might be missed⁸⁶. The interpretation of FDG signals can be confounded by tumour flare or inflammation following the treatment of certain tumour types⁸⁷, whereas cell proliferation tracers such as fluorine-labelled thymidine (FLT)⁸⁸ are more sensitive than FDG to the effects of cytostatic therapy. Neuroendocrine neoplasms are best identified by tracers such as 5-hydroxytryptophan that target their unique metabolic activity⁸⁹. Determining receptor status by PET⁹⁰ allows non-invasive, *in vivo* correlation with treatment benefit, albeit initially validated by correlation with *ex vivo* histology, and might be more reliable than biopsy because of heterogeneity within and across tumour masses⁸⁷. The multi-modal combination of PET and CT provides metabolic assessment with optimal spatial resolution⁹¹. PET-CT hardware has been developed for both animal and human studies^{92,93} (FIG. 6).

Expression profiling has been achieved by immunohistochemistry for at least a decade⁹⁴ and will find much wider application in diagnosis and prognosis with targeted cancer therapies, informing selection of the ‘cocktail’ of drugs to be used as treatment. For primary prevention strategies, expression profiles can also serve both to identify candidates for treatment and also assess the success of therapy⁵. Databases are being built to facilitate such studies^{95,96}. Predictive expression profiles have been determined for diffuse large B-cell lymphoma, **breast cancer**, and **melanoma**^{5,97–99} that might be superior to the gold-standard International Prognostic Index¹⁰⁰, but do not ensure therapeutic success. For example, enrichment of a breast cancer population by fluorescence *in situ* hybridization (FISH) for **ERBB2** (also known as HER2/neu) positivity resulted in less than 50% success following treatment with trastuzumab, an antibody directed against ERBB2 (REF. 101).

Genetic profiling in association with chemotherapeutic outcome also contributes to the understanding of oncogenesis or tumour growth, thereby indicating new targets for treatment¹⁰². However, attention must be paid to protocol design and analysis if confounders are to be managed and these huge volumes of data are to be distilled into useful bioinformatics^{103,104}.

Psychiatry. Functional models in psychiatry can use biomarkers, such as serum cortisol and adrenocorticotropic hormone, and clonidine-stimulated growth hormone release as probes for interactions with central serotonin and noradrenaline pathways¹⁰⁵. Other functional clinical experimental models, such as fear-potentiated startle reflex^{106,107}, simulated public speaking¹⁰⁸, and drug-, lactate- or CO₂-induced panic^{109–111}, some of which include endocrine and autonomic nervous system reflex responses, have also been used to discern the activity of potential anxiolytic agents.

The need for validation of biomarkers in psychopharmacology is generated by the poor sensitivity and precision of presently available clinical assessment tools^{21,112}. Non-invasive imaging could be particularly

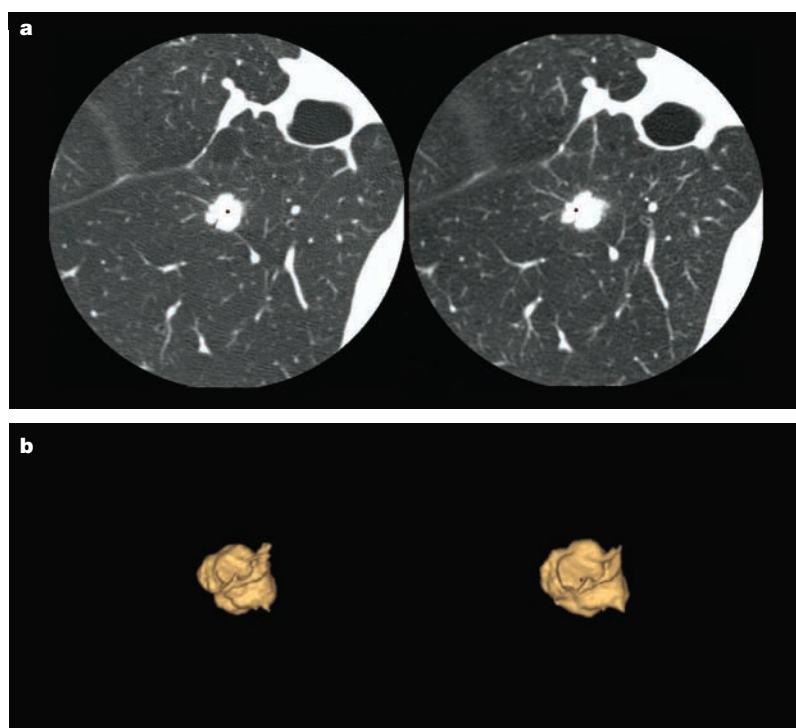


Figure 5 | **Computed tomography lung nodule.** Advanced lung image analysis can now be used to estimate the growth in volume of a lung cancer lesion scanned at two time points spaced one month apart. This image shows 250 mA, 120 kVp 1.25 mm thin computed tomography sections of the lung nodule (a), and a volume rendering of the lesion after it has been automatically separated from surrounding vasculature (b). Analysis of the lesion revealed an increase in volume of 24% and an estimated doubling time of 103 days. Biopsy confirmed the lesion to be large cell carcinoma.



Figure 6 | **Whole-body PET-CT.** Multi-modality imaging provides the best of both worlds; in this case, the anatomic specificity of computed tomography (CT) with the metabolic quantification by positron emission tomography (PET) of fluorodeoxyglucose uptake in a lung tumour.

useful because of the inaccessibility of the human brain. Conceptually, neuroimaging in drug discovery and development can be divided into four interrelated categories. First, neuroreceptor mapping (for example, with PET or SPECT tracers) to examine the involvement of specific neurotransmitter systems in central nervous system (CNS) diseases, drug occupancy characteristics and perhaps examine mechanisms of action. Second, structural imaging to examine morphological changes and their consequences. Third, metabolic mapping (for example, ^{18}F FDG and magnetic resonance spectroscopy) to provide evidence of central activity and the neuroanatomy of drug effects. Finally, functional mapping (for example, FDG-PET or functional MRI (fMRI)) to examine disease–drug interactions. Metabolic and

functional mapping are sometimes characterized as ‘fingerprinting’¹¹³ for their potential use as clinical screens for drugs in development.

An important objective in the clinical development of any novel CNS therapy is to establish a clear correlation between the dose or plasma level of the drug and the receptor occupancy achieved. The recent advent of small animal PET cameras enables these occupancy data, together with plasma drug levels in patients and *in vivo* animal models, to be used to model the dose–receptor occupancy relationship achieved in successful clinical studies and predict effective dosing regimens for subsequent trials with the lead or back-up compounds¹¹⁴. This is especially valuable in studies in CNS indications for which traditional clinical endpoints are plagued by high placebo responses.

A good example is the recent development of the PET ligand [^{18}F] SPA-RQ, a radioactive, brain-penetrant, non-peptide tracer that binds to the neurokinin-1 (NK_1) receptor with high affinity and selectivity to study the distribution of NK_1 receptor in the normal human brain and the occupancy of those receptors by exogenous administration of the drug aprepitant¹¹⁵.

In patients treated with cisplatin chemotherapy, an initial daily dose of 40 mg of aprepitant followed by 25 mg daily doses showed some efficacy, but a higher dosing regimen (initial dose 125 mg, followed by subsequent daily doses of 80 mg) was considerably more effective in the prevention of nausea and vomiting. No further efficacy improvements of the 125 mg over the 80 mg aprepitant regimen were observed with a higher initial dose of aprepitant of 375 mg, followed by 125 mg daily dose¹¹⁶, thereby establishing the dose of the oral NK_1 antagonist aprepitant for chemotherapy-induced nausea and vomiting.

A series of PET studies examined the relationship between dose (FIG. 7a), plasma concentration, and NK_1 receptor occupancy for the substance P NK_1 receptor antagonist aprepitant. The distribution of NK_1 receptors in the CNS of healthy male volunteers was evaluated using [^{18}F] SPA-RQ before and after treatment with placebo or increasing doses of aprepitant. A clear dose and plasma concentration versus receptor occupancy relationship was observed. The data led to the prediction that high levels of central NK_1 receptor occupancy (>90%) were required to achieve optimal anti-emetic effects. This finding also guided dose selection for trials of drugs with this mechanism in other CNS indications (antidepressant) (FIG. 7b) (see Further information, Gastrointestinal Drugs Advisory Committee).

Depression and pain: fMRI. Picture stimuli (for example, fearful or emotional faces) are a common way to induce emotion for experimental purposes in human subjects, and can be used in conjunction with imaging techniques including fMRI and functional PET (fPET)^{117,118}. Recently, hyperarousal of the amygdala in response to facial images expressing fear has been demonstrated in depressed patients. This hyperarousal was normalized by treatment with the serotonin-reuptake inhibitor and antidepressant sertraline in an uncontrolled pilot study

with fMRI¹¹⁹. Compared with still photographs, short clips from commercial films produce greater intensity and longer-duration experiences of particular emotions, including sadness^{120,121}, but the potential response to antidepressants has not been tested. These fMRI methods might, however, be confounded by cognitive and attentional differences between subjects or test sessions. Consequently, differences might require further examination using challenge tasks that can dissociate brain systems involved in attention and emotion¹²². In pain research, fMRI-based psychophysical studies can assess brain activation by pain, its anticipation and modulation by attention¹²³ providing

simple fingerprints for the rapid assessment of the potential anxiolytic and analgesic activity of novel compounds, as demonstrated with remifentanyl¹²⁴ and the benzodiazepine midazolam (I. Tracey, personal communication). As pain is not a unitary sensation, functional neuroanatomical studies with fMRI could allow the detection and evaluation of novel drug molecules that affect cognitive and affective, as well as sensory, responses¹²⁵.

Osteoarthritis. There is growing interest in the development of disease-modifying agents whose primary action is focused on the inhibition of the processes that lead to the breakdown of articular cartilage in osteoarthritis, rather than relieving pain and improving joint function (see Further information, Osteoarthritis Imaging Initiative). Although conventional radiography continues to be the method of choice for the evaluation of osteoarthritis, it is limited in its ability to image the cartilage directly. In contrast, MRI, especially at the higher field strength of 3T, with its excellent soft-tissue contrast and full tomographic capability, is able to provide a full three-dimensional view of the joint and cartilage, allowing detailed analysis for potential biomarker indices. Recent studies indicate that MR measurements of bone marrow oedema might be a good predictor of progression. Furthermore, the use of a contrast-enhanced MRI sequence (dGEMERIC) using chelated gadolinium (Gd-DTPA, gadolinium diethylene-triamine-penta-acetic acid) could be a promising technique to probe the biology of the cartilage by mapping glycosaminoglycans, which are present in excess at damaged sites¹²⁶.

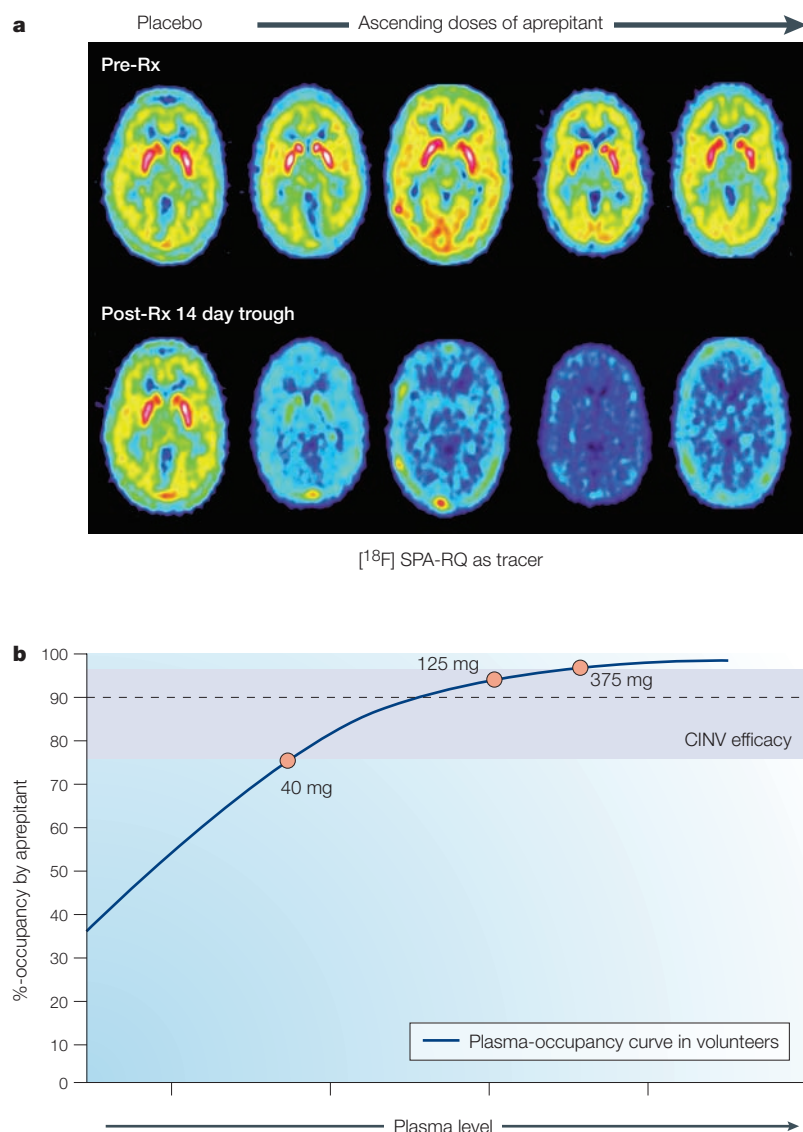


Figure 7 | NK₁ receptor occupancy required for efficacy. The relationship of dose and plasma concentration of aprepitant to central nervous system (CNS) receptor occupancy was defined in healthy volunteers in order to predict the occupancy of central NK₁ receptors that was likely to have been achieved by the doses of aprepitant used in chemotherapy-induced nausea and vomiting (CINV) trials. The analysis clearly indicated that the levels of CNS NK₁ receptor occupancy were linked to the efficacy of aprepitant against CINV. Importantly it explained why the 375 mg dose of aprepitant had no advantage over the 125 mg dose in the treatment of CINV as the NK₁ receptor occupancies achieved by these two dose strengths were essentially the same.

Microimaging

There are many developments in the field of imaging that could be incorporated into drug discovery and development programmes¹²⁷. The advent of translational research technologies, in the form of small animal microCT, micro-ultrasound and microPET detectors¹¹⁴ is now beginning to allow identical protocol design and *in vivo* assessment across species, enabling truly comparative pharmacology, directing proof-of-concept studies and reducing risk in decision making (FIG. 8).

The next generation

The next generation of biomarkers will include many emerging technologies, and will be tailored both to disease and to mechanism of action of potential treatments.

Pharmacogenetic/pharmacogenomic biomarkers. Clinically significant polymorphisms are found in the genetic coding for proteins (exons), promoter regions or the cofactors¹²⁸ that drive transcription of that protein, or in post-translational modifications of that protein. These can be used not only to identify patients at risk of disease, but also those most likely to benefit from a particular therapy, or most at risk of adverse effects. In drug-treatment studies, linking outcomes with genotypes can be biased if the populations are not well characterized or the study is underpowered. Other potential confounding

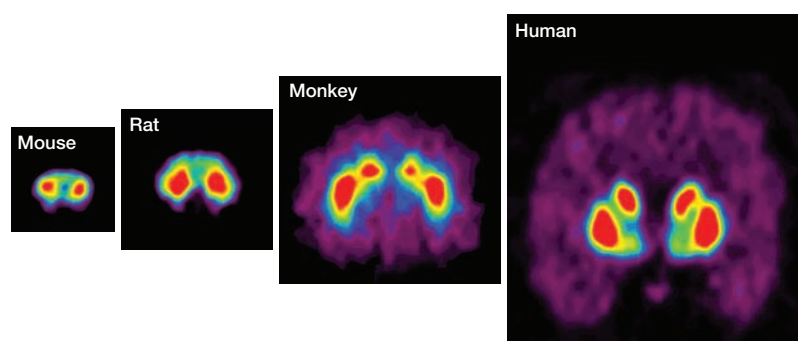


Figure 8 | **Positron emission tomography across species.** In four different species [^{11}C]WIN35428 (^{11}C CFT) positron emission tomography can be used to quantify the dopamine transporter in the striatum even when this structure is small in rodents. Courtesy of PETNet Pharmaceuticals.

factors were considered by Weiss *et al.*¹²⁹. These concerns also apply to potential genomic markers of toxicities, but do not deter us from studying them (see Further information, Technical Committee of Application of Genomics to Mechanism-based Risk Assessment).

Most pathophysiological processes result from a complex interaction between genetic predisposition and environmental factors¹³⁰. Therefore analyses of variants of individual genes will generally be insufficient to characterize disease states or define risk as a basis for prevention strategies. For even those diseases dominated by genetic factors, the estimation of risk and the identification of targets for new drugs through sophisticated statistical analyses can be complicated by the fact that few diseases result from single gene mutations, and their phenotypic manifestation is confounded by incomplete penetrance. Furthermore, low prevalence often limits their contribution to the overall incidence of disease, and, hence, the potential contribution of narrowly targeted therapies.

Genotypes, SINGLE-NUCLEOTIDE POLYMORPHISMS (SNPs) and haplotypes can serve as biomarkers of clinical phenotypes. Phenotypic variation can result from the effect of a single gene, such as **Tangier disease** or drug metabolism by the cytochrome P450 enzyme **CYP2D6**, or can be multigenic and multifactorial, as in diabetes or responsiveness to statin therapy. Genotypes represent sequence variations of base pairs in a gene encoding a specific variant of a protein. Variants might not result in phenotypic differences, because the substitution of a single base pair can be neutral (that is, does not change the amino acid encoded by the sequence of base pairs) or the substituted amino acid might not result in a protein with distinguishable differences in function. Post-translational changes (which themselves can be under genetic control) might also render a variant insignificant, or could result in a phenotypically important difference despite the primary protein sequence being normal.

Genotypic biomarkers of drug metabolism and transport. The cytochrome P450 (CYP) system of drug-metabolising enzymes represents the best-studied set of

pharmaceutically important genotypic markers⁶. Despite that, subjects are still phenotyped for stratification or as an entry criterion for drug metabolism and interaction studies, and allometry is used to predict human exposure on the basis of *in vivo* animal studies¹³¹. Pitfalls in relying on genotypic markers to predict drug concentrations in the central compartment (plasma) are exemplified by efforts to predict drug metabolic phenotype. Differences across drugs in enzyme inhibition and induction, and the multiplicity of enzymes catalysing the same metabolic step¹³², render unpredictable the impact of variation in a single gene. Furthermore, age-related phenotypic deficiencies of flavin-containing monooxygenase-3 (**FMO3**) or **CYP1A2** in genotypically normal neonates and infants^{133–135} make the apparent genotype clinically irrelevant, even misleading, in those patient populations.

In tissue compartments, where drugs usually have their effects, concentrations are also affected by transport processes¹³⁶ that can vary widely between patients¹³⁷ and across organs¹³⁸, and at present are too poorly understood to be incorporated into regulatory guidances¹³¹. Perhaps best characterized are the MULTI-DRUG RESISTANCE (MDR) system in tumours^{139,140} and the corresponding P-glycoprotein transport systems in the intestinal mucosa¹⁴¹, placenta¹⁴² or blood–brain barrier^{143,144}. Predicting exposure concentrations from genotype would be even more difficult for tissues than for plasma.

Haplotype and SNP biomarkers. Because haplotypes include the linkage of multiple SNPs, they will generally occur at lower prevalence than individual gene variants, and the more SNPs involved in a haplotype, the narrower and more precisely defined will be the patient subgroups. In clinical studies with sufficient numbers of well-characterized patients, important associations between large variations in drug response and particular haplotypes can be discerned. The gene encoding β_2 -adrenoceptors ($\beta_2\text{AR}$, a G-protein-coupled receptor that mediates the actions of catecholamines) has multiple SNPs, facilitating a haplotype-association study. In one example (FIG. 9), a clear correlation was found in asthmatics¹⁴⁵ between outcome from treatment with albuterol, a widely used β_2 -adrenergic asthma medication, and specific haplotypes of the gene encoding $\beta_2\text{AR}$.

RNA expression profiling. Profiling studies are designed to correlate RNA expression signatures with key biological events related to efficacy or safety outcomes. The combination of genetic analyses (explaining variation in physical traits) and gene expression studies (an index of active genes) to identify expression quantitative trait loci (QTLs) has been discussed as the key to delivering the promise of genomics in healthcare⁴.

Molecular signatures can be useful to guide basic research when obtained experimentally from animal tissues that are inaccessible in the clinic. Signatures can sometimes be obtained from patients by biopsy of target organs or tumours, but these will not be available from normal subjects in early-stage clinical trials. Indeed,

SINGLE-NUCLEOTIDE POLYMORPHISM (SNP). A variant in a single base pair which can occur in any region of the gene including promoters, introns, exons, splice junctions or even untranslated regions.

MULTI-DRUG RESISTANCE (MDR). A set of proteins which oppose tumour uptake of chemotherapeutic drugs by transporting them back into the bloodstream.

signatures from blood are most accessible and would have the widest clinical usefulness, assuming that the cascade of events precipitated by a drug will alter RNA expression in white blood cells. It is important to initiate expression profiling early in any discovery programme in order to maximize its usefulness. Success in molecular profiling will be dependent on the continued development of sophisticated bioinformatics tools to interpret huge, complex sets of data and to create pattern-recognition routines that identify, classify and quantify unique molecular signatures. Future challenges involve gaining acceptance of the use of patterns as biomarkers, rather than single measurable elements, and the development of clear guidelines on what to do if minor changes in expression patterns are observed in suspected dangerous genes¹⁴⁶.

Metabonomics and proteomics

Metabonomics and proteomics can be thought of as differential phenotyping of patient samples¹⁴⁷ by a range of new methods, such as laser scanning cytometry¹⁴⁸, or liquid chromatography–mass spectrometry (LC-MS) with electrospray ionization for the quantitative measurement of enzymatically digested peptide fragments in serum and other fluids and tissues¹⁴⁹, or specific immunoassays for quantification of trace proteins in serum. However, although plasma samples provide an easy source of material for proteomic studies, the complexity of serum

makes protein profiling extremely challenging. Proteomics and metabonomics have been used to provide insight into important diseases of unmet medical need and to make risk assessments. These have been exemplified recently by studies of DRUSEN proteome in age-related macular degeneration¹⁵⁰ and non-invasive imaging, through proton nuclear magnetic resonance (NMR), of the chemical composition of lipoproteins in patients with elevated serum lipid levels, potentially replacing angiography¹⁵¹. Proteomics has also been used to identify patients with ovarian cancer¹⁵².

Metabonomics and proteomics also have great potential for the subclinical detection¹⁵³ of toxic effects of new therapies, but will require similar validation as for markers of efficacy¹⁵⁴ if they are to become factors in determining dosing regimens for clinical studies¹⁵⁵.

As an example, advanced rheumatoid arthritis patients ($n = 20$, American College of Rheumatology score 3 or 4) have different molecular profiles than age- and gender-matched controls ($n = 20$) (FIG. 10)¹⁵⁶. Differences include expected differences in cell populations including more granulocytes and fewer CD8 T cells, as well as expected increased α_1 -antitrypsin, α_1 -acid-glycoprotein and ceruloplasmin, and reduced transferrin concentrations. Other differences represent novel, yet to be characterized, peptide fragments of other circulating proteins and cell surface markers, which might yield novel targets or biomarkers for rheumatoid arthritis.

Once identified as characteristic of a patient population, neo-epitopes in synovial fluid, plasma or urine can be used for prognosis and therapeutic monitoring of immunomodulation in rheumatoid arthritis¹⁵⁷, or inhibition of enzymes like collagenase¹⁵⁸, matrix metalloproteinase inhibitors¹⁵⁹ or aggrecanase¹⁶⁰ for rheumatoid or osteoarthritis, with the potential to assess treatment benefits in animals or humans. Ultimately, multi-modal assessment of biofluids might be necessary, but still has the potential to give simple, rapid and sensitive readouts that could complement or replace classical radiography measures of disease progression and speed up clinical trials (see Further information, Guidance for Industry).

Environmental factors

Beyond genomics, the impact of environment in some of the most important targets for prevention, such as cardiovascular disease and cancer, was demonstrated long ago by studies showing that when people migrate from one place to another, their risk changes to that of the new environment¹⁶¹. For systemic lupus erythematosus, the genetic predisposition is best described as a susceptibility in the immune system which likely is triggered by environmental factors such as infectious agents, which accounts for the relatively low apparent penetrance. The underlying aetiology can include both overactive immune responses and deficiencies in complement, impairing clearance of infectious agents¹⁶². By contrast, obesity and diabetes are approaching epidemic proportions in the United States. Spontaneous genetic rodent models of obesity and diabetes have been available

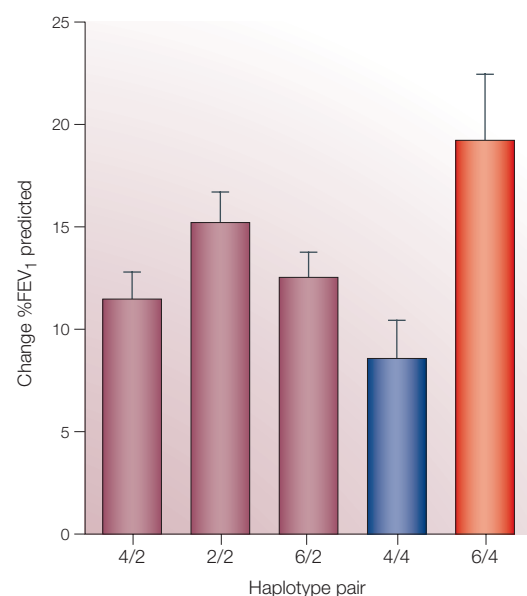


Figure 9 | **In vivo response depends on haplotype pair.**

In a population of 121 asthmatics, the gene encoding the β_2 -adrenoceptor was found to have thirteen single-nucleotide polymorphisms (SNPs) organized into 12 haplotypes out of the theoretically possible 8,192 combinations. By assessing the bronchodilator response to β -agonist¹⁴⁵, it was observed that response to albuterol, measured as change in percent predicted forced expiratory volume in 1 second (FEV₁), was significantly related to haplotype pair ($p = 0.007$ by ANCOVA) but not to individual SNPs. In fact, mean responses by haplotype pair varied by more than twofold. Adapted with permission from REF. 145 © (2000) National Academy of Sciences. Courtesy of Genaissance Pharmaceuticals, Inc.

DRUSEN

Small yellowish protein–lipid deposits in the retina that develop in the early stages of dry (atrophic) macular degeneration.

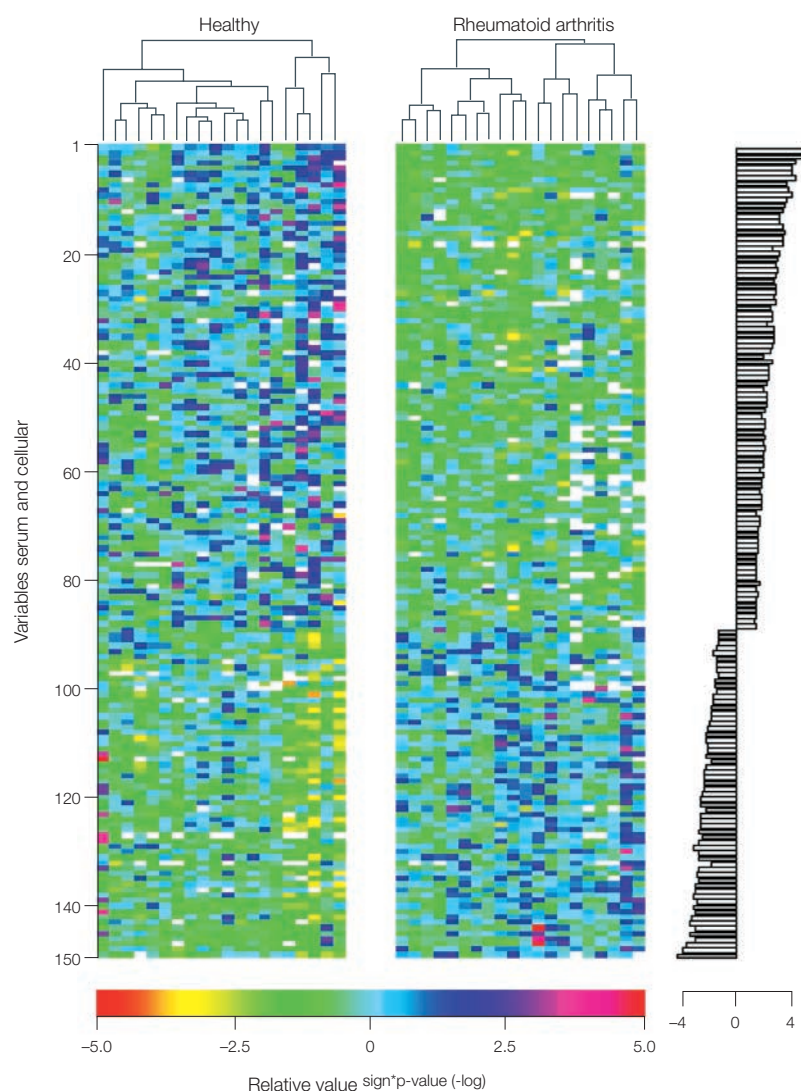


Figure 10 | Metabonomics array. In this example, advanced rheumatoid arthritis patients ($n = 20$, American College of Rheumatology score 3 or 4) have different molecular profiles than age- and gender-matched controls ($n = 20$). Data are shown for 36 variables from cellular assays and 114 variables from mass spectrometry analysis of the serum proteome sorted by effect size. Each pixel is a normalized expression of the variable (z-score). A rainbow colour scale is shown on the bottom. Missing values are grey. All displayed variables have a univariate $p < 0.05$. The actual p-values (negative log) are shown by the bars on the right. Larger bars indicate greater significance. The direction (sign) of the bar indicates whether the change in mean was positive or negative. For proteins with multiple peptides, the peptides are clustered and a representative peptide from each cluster group showing Spearman correlation coefficient of < 0.75 is included in the plot. Courtesy of Surromed.

since the early 1980s, with more refined mouse models aimed at specific molecular targets being developed throughout the 1990s. Novel treatments for obesity are now due if this area of research is to keep pace with usual drug development timelines¹⁶³. In obesity and

diabetes, the environmental impact of diet and exercise is clear, but lifestyle decisions continue to drive the need for new pharmacotherapy. The closely related dysmetabolic syndrome X has been assigned a single ICD-9-CM code (see Further information, American Association of Clinical Endocrinologists) but is probably a collection of different pathophysiologies contributing to the risk of coronary artery disease, which will be teased apart only when effective therapies can target specific aspects of this syndrome. These benefits must ultimately be measurable by a reduction in risk of coronary artery disease, but will be much more efficiently and accurately measured by biomarkers specific to the action of that therapy.

Conclusions

The development of new biomarkers is an imperative that will best be achieved by collaboration between pharmaceutical and diagnostics companies and, for imaging biomarkers in particular, hardware manufacturers¹⁶⁴. If, as in the case of serum cholesterol measurements, the diagnostic tool can both identify patients requiring therapy and confirm the treatment effect, then primary prevention will be served not only by the identification of persons at risk, but also by encouraging compliance with a regimen that might not yield immediate feedback in symptomatic improvement. Conversely, without effective therapy (for example, a disease modifier for Alzheimer's disease) there is little use for a diagnostic.

The collaborative development of new diagnostics in the same clinical studies as new therapeutics will contribute to the validation of each, particularly if the diagnostic captures treatment benefit in Phase III studies of pre-symptomatic patients. The early involvement of regulatory agencies¹⁶⁵ will help speed up the availability of new medicines to under-served patient populations by allowing biomarker studies to be evaluated off the crucial review path of a therapeutic drug dossier. By definition, the application of emerging technologies as biomarkers will always be dependent on the development of trained scientists and a collaborative effort between industry and academia, with a sensitivity to the unique needs of each^{166–168}. The drug development plan which makes effective use of a biomarker strategy is inherently lower risk and should be regarded as a higher priority than those which are otherwise equal but lack biomarkers. Biomarkers allow the prudent management of opportunity costs, facilitate business and regulatory decision making and enable expansion of effective therapy to broader patient populations. The likelihood of success is greatest, and the patient has the most to gain, from primary prevention and pre-symptomatic treatment, which depend crucially on the development of appropriately validated biomarkers.

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