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Combining imaging and pathway profiling: an alternative approach to cancer drug discovery

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Conventional drug discovery strategies are typically 'target centric' based on the selection of lead compounds with optimised 'on-target' potency and selectivity profiles. However, high-attrition rates are often the result of compensatory or redundant cancer mechanisms and the fact that tumours do not find it difficult to escape inhibition of a single pathway. In this article, we highlight two emerging and complimentary technologies; namely phenotypic imaging and post-translational pathway profiling, which when combined with relevant disease models can provide pharmacodiagnostic and drug combination strategies that predict and counteract inherent and adaptive drug resistance. The implementation of such approaches at early stages of the drug discovery process enables more informed decisions on candidate drug selection and how to maximise and predict efficacy before clinical development.

Despite significant scientific and technical advances over the past two decades, including the identification of an unprecedented number of potential new drug targets, increased research and development (R&D) investments have not provided the anticipated return of more effective new drugs. Indeed, the number of novel medicines approved by regulatory agencies such as the US Food and Drug Administration (FDA) has been in steady decline, with 50% fewer new molecular entities (NME) approved during the past decade [1]. Data accumulated from the top 11 pharmaceutical companies indicate that success rates in clinical drug development (in oncology) are approximately 5% when defined as 'first-time-in-man' to drug registration [2]. Increasing R&D costs, impending patent expirations, increased competition from generic drug manufacturers and financial liability associated with high attrition in late-stage drug development, are all evidence of the reality that conventional drug discovery strategies are unsustainable to most R&D enterprises [3,4].

Although advances in combinatorial chemistry, therapeutic antibody development and high throughput screening have undoubtedly improved the quality of novel agonist and antagonists on the basis of potency and selectivity, the high attrition of candidate drugs during the later stages of preclinical development, or in clinical trials, continues. Attrition resulting from poor efficacy is particularly acute in solid cancers, where underlying disease mechanisms are heterogeneous within and between individual patient groups, or where cancer mechanisms can readily adapt to therapeutic treatment. Tumour heterogeneity and multiple target mechanisms between patient subgroups mean that many patients are ineligible, or unresponsive, to specific targeted drug classes. Moreover, compensatory and redundancy mechanisms that drive inherent or adaptive resistance severely limit drug efficacy and response duration [5,6]. Thus, combinations of targeted agents will be more effective in treating solid tumours than will single agents, particularly if it is possible to identify the biochemical networks and driver mechanisms that enable cancer cells to subvert single-agent monotherapies. For example, despite strong disease linkage data correlating Src kinase activity with poor prognosis across several tumour types, only modest single-agent activity has been observed with small molecule Src inhibitors. Indeed, evidence suggests that Src inhibitors will have greater clinical utility when used in rational combination with other agents [7–10]. Networks and pathway switching enable rapid tumour evolution and therapeutic evasion. Thus, new approaches are required to understand cancer cell signalling 'driver' networks and 'driver' pathways in a broad sense, so as to guide optimal drug combinations that collapse

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the robustness of networks across tumour types, and reduce the likelihood of therapeutic evasion and recurrence.

Innovations in drug discovery

Recent advances in next-generation sequencing, quantitative proteomics, small interfering (siRNA) screening technology, quantitative *in vitro* and *in vivo* imaging and systems biology approaches all embrace the biological complexity of disease and offer alternative strategies for target selection, target validation, candidate drug testing and patient stratification [3,4]. In this article, we focus on recent advances in two emerging technologies: optical *in vitro* and/or *in vivo* imaging and reverse phase protein arrays (RPPA), which together could offer a highly sensitive, unbiased and quantitative approach to profiling the drug mechanism of action and disease heterogeneity at pathway, cellular and pathophysiological levels. We address how advanced imaging approaches enable the direct visualisation of cancer-associated behaviours in more relevant and informative *in vitro* and *in vivo* model systems, including 3-dimensional (3D), co-culture, primary tumour and genetically engineered mouse (GEM) models. We discuss how the latest advances in imaging and pathway modelling tools provide the necessary biological context for the rational design, validation and prioritisation of novel drug combinations, using biology to guide such combinations and develop companion biomarker strategies. Finally, we also describe how the latest advances in imaging- and RPPA-based functional proteomics can help maximise the value of targeted therapies and thus complement the conventional target-directed approach by anticipating, predicting and interrogating drug resistance mechanisms at earlier stages in the discovery process.

Image-based high-content phenotypic screening

Microscopic imaging of cell behaviour *in vitro* and tissue pathology *in vivo* represents a more holistic approach to the evaluation of drug efficacy, providing an unbiased assessment of a drug response in complex biological systems, where crosstalk between multiple target pathways, and the inherent system complexity, remain intact. Recent advances in automated fluorescent microscope systems, together with associated image analysis algorithms that provide quantitation of cellular phenotypes and/or intracellular pathway activity, has raised the potential value of phenotypic screening. High-content analysis describes the quantification of multiparametric features extracted from fluorescent or bright-field images of cells, usually in an automated fashion. High-content imaging microscopes and associated analytical tools have improved hugely over the past 10 years, with the design of more efficient and user-friendly platforms. These have facilitated expansion of both endpoint- and live cell-based studies into multiwell plate formats suitable for drug or siRNA/small hairpin (sh)RNA and micro- (mi)RNA screening applications [11].

The increasing adoption of high-content analysis by the pharmaceutical and biotechnology industry indicates a willingness to incorporate more complex biological endpoints into early phase drug discovery. Phenotypic screening has typically been viewed as a secondary screening strategy to confirm the quality of hits identified from high-throughput enzyme-based primary screening. Integration of automated high-content microscopy with optimised image-informatics and/or data-handling protocols increases throughput and speed; thus, phenotypic assays are emerging as a more common primary screening strategy [12,13]. Indeed, such approaches have been proposed as a strategy to reduce attrition at later stages of drug development, essentially by 'front loading' the efficacy and safety evaluation of novel target classes, chemical libraries and putative biological therapeutics [13]. However, the case for this would be stronger if physiologically relevant cell systems were used; that is, they not only relied on the rather 'tired' established cancer cell lines that do not reflect human disease, but also made use of fresh patient-derived material that retains features and heterogeneity more akin to the original tumour. Recent publications describe advanced applications of multiparametric high-content imaging for profiling mode-of-action, providing new biological insight into mechanisms of drug responses, and the necessary context for guiding structure–activity relationships based on phenotypic outcomes [14–16]. Fig. 1a details how a multiparametric high-content imaging approach can be applied to provide further information on the efficacy of DNA-damaging agents. Through parallel analysis of apoptosis, cell-cycle and a DNA repair response marker (gamma-H2AX phosphorylation; pH2AX) following cisplatin treatment of the patient-derived ovarian cancer cell line PEO23, it is possible to determine whether induction of DNA repair impairs drug-induced apoptosis, potentially representing a mechanism of relapse. In the example provided (Fig. 1a), cisplatin-induced PH2AX in PEO23 cells is associated with increased apoptosis, suggesting that cisplatin-induced DNA damage-repair response is largely ineffective in this cell line. Such advances in multiparametric high-content profiling have been used to classify small molecules by mechanism-of-action, and have the potential to provide greater logic for selecting mechanistically distinct drug candidates for drug and/or drug combination studies; in turn these could enhance efficacy [14–16].

Imaging drug response in more relevant biological assays

A key advantage of image-based analysis is quantification of functional endpoints in more complex assay formats, including those that extend beyond routine 2-dimensional (2D) culture of cell lines (or panels of cell lines), typically performed on tissue culture plastic. Newer assay formats could include appropriately matched cell-type co-cultures, inclusion of 3D-extracellular matrix, organotypic models and fresh patient-derived material, grown in ever more sophisticated conditions that better mimic the probable *in vivo* environment [17]. These can be designed such that they reflect the complex pathophysiology of cancer, and so represent distinct disease segments. For example, cancer 'stem' cell subpopulations, cancer–host stroma or inflammatory interactions and metastatic tumour microenvironments have rarely been incorporated into drug-screening cascades thus far [18,19]. The example model system provided in Fig. 1b represents an *ex vivo* culture of a GFP-labelled pancreatic cancer cell line (PANC-1) on human peritoneal omentum tissue, a major site of pancreatic cancer metastasis. The detailed mechanistic information and flexibility provided by high-content analysis enables drug mechanism and efficacy to be explored in detail across a suite of distinct assay formats that together begin to recapitulate the heterogeneity and complexity of malignant disease. In turn, this will enable more evidence-based and rational decisions around which preclinical

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animal modelling, and potential clinical development strategies, should be adopted.

Advances in phenotypic imaging: probes, platforms and image analysis

Advances in the design of live cell-fluorescent reporter molecules, including optical biosensors and fluorescent proteins, in parallel with automated kinetic imaging microscopes, provides the opportunity to include live cell and molecular dynamic imaging applications in drug discovery (Table 1). In turn, quantification of dynamic processes, for example, transient or oscillating signalling events, dynamic turnover of cancer-associated adhesion or growth factor receptors or oncoproteins, and detailed mechanistic analysis of tumour cell migration and invasion that are not provided by single endpoint assays, could be of enormous benefit [20,21]. Kinetic imaging also enables more sophisticated understanding of the pharmacokinetics of drug responses and can inform on optimal time points for the co-mapping of mechanism of action studies by genomic, transcriptomic or proteomic pathway modelling. Pharmacokinetic monitoring of phenotypic responses will also help to guide optimal drug combination schedules by evaluating more sophisticated data sets before *in vivo* testing.

Advances in image analysis and informatics further support the implementation of microscopic screening into routine drug discovery. A constraint of conventional preprocessed image analysis algorithms that accompany commercial high-content analysis platforms is their limitation to cell lines, endpoints and 2D culture systems amenable to basic image-based object definition and analysis. Consequently, large-scale high-content screens are often restricted to immortalised cell lines, such as HeLa or U2OS cells, the criteria being that they exhibit homogenous cell morphology when cultured on 2D substrates. Hence, the relative simplicity (and so quality) of high-content analysis to date has limited its ability to predict clinical efficacy and so to impact on drug discovery *per se*. Clearly, the biological models need to be improved, and there is much work to be done. Recent advances in generating increasingly sophisticated context-aware image analysis software solutions and image-based machine-learning approaches are beginning to influence the design of bespoke image analysis algorithms that are tailored to more complex and relevant biological models and tissue samples [14,22,23]. Adaptive image-analysis approaches can be used to leverage quantitative information from complex 2D or 3D biological models, incorporating heterogeneous cell populations, fresh patient-derived material or co-cultures derived from cell lines or primary cell isolates to get closer to mimicking the tumour environment.

Image-based phenotypic screening: future prospects

Development of bespoke high-content assays that enable parallel efficacy and toxicity screening across both disease (e.g. cancer cells) and host cell populations enable the optimisation and guided search of chemical and target space away from toxic liability towards enhanced efficacy [24,25]. Image informatics solutions are available from both academic and commercial providers that streamline the application of image analysis algorithms across large image data sets, and integrate secondary multivariate statistical data analysis and address bottlenecks associated with downstream analysis of image-based screening data [14,26–28]. In principal, such

TABLE 1

Live cell-imaging reagents

Optical reporter	**Phenotypic application**	**Supplier**
Lysotracker & LysoSensor	Lysosomes	Invitrogen
TMRE	Mitochondria function	Invitrogen
pHrodo™ Indicators	Phagocytosis and endocytosis	Invitrogen
NucView	Apoptosis (caspase activity)	Biotium
MitoView633	Mitochondrial function	Biotium
DiO/DPA FRET pair	Membrane potential	Biotium
Premo™ FUCCI Cell cycle sensor	Cell cycle	Invitrogen
Premo™Autophagy Sensor (LC3B-RFP or GFP)	Autophagy	Invitrogen
Premo™Calcium Sensor	Ca^{2+} signalling	Invitrogen
CellLight™Histon2BGFP	Nuclear morphology	Invitrogen
CellLight™Actin/Tubulin GFP/RFP	Cytoskeletal dynamics	Invitrogen
CellLight™Talin GFP/RFP	Adhesion dynamics	Invitrogen
Human EGFR live cell fluorescent biosensor assay	EGFR signalling dynamics	Sigma
IntegriSense	Angiogenesis and/or tumour cell metastasis	Perkin Elmer
ReninSense	Renin activity	Perkin Elmer
Neutrophil Elastase	Neutrophil elastase activity	Perkin Elmer
CatK	Cathepsin K proteinase activity	Perkin Elmer
CatB	Cathepsin B protease activity	Perkin Elmer
Qtracker quantum dots	Cell and/or vascular labelling	Invitrogen
FM 4-64	Membrane label	Invitrogen
Acrivlavin	Cell label and/or mask	Sigma

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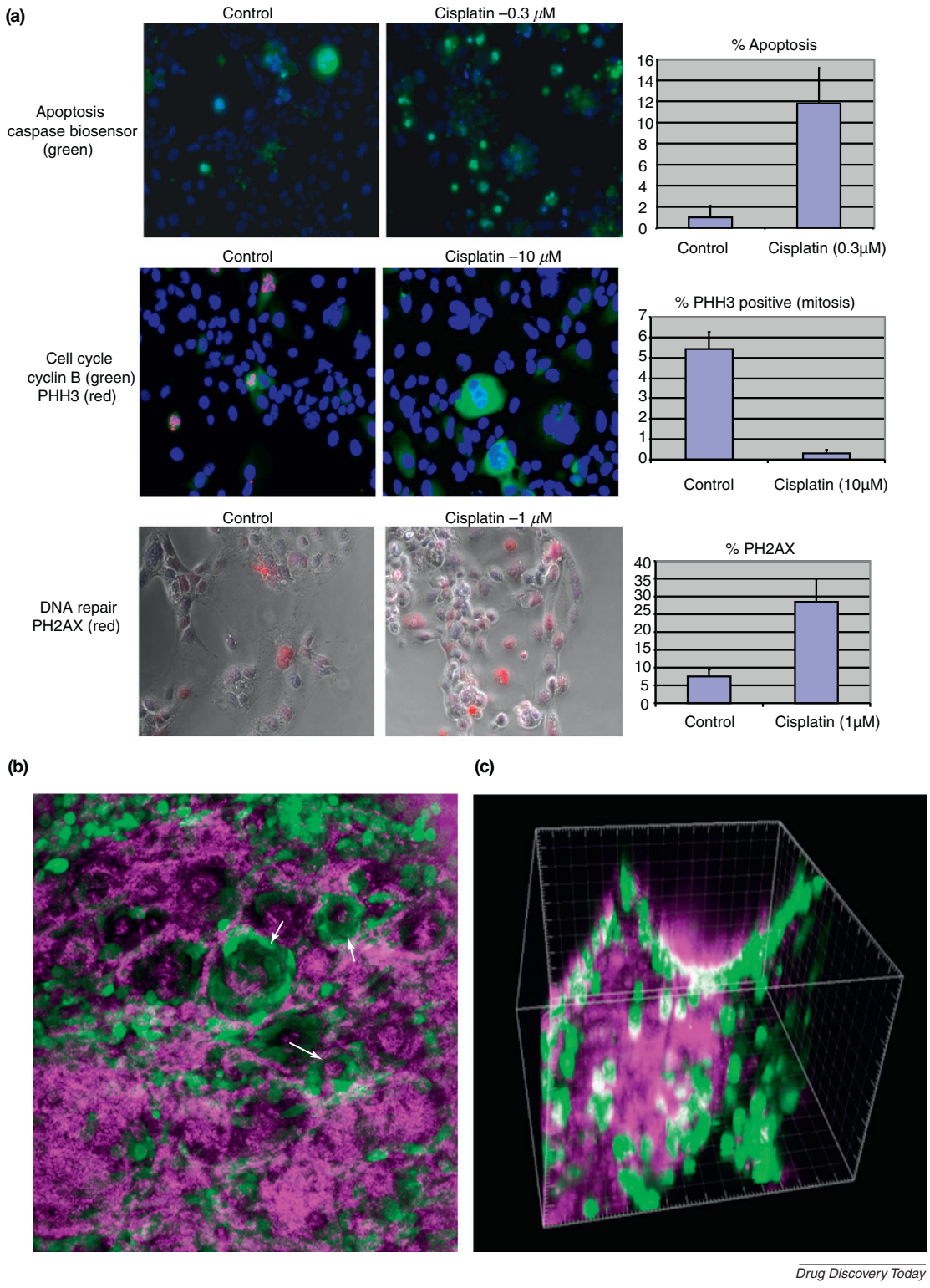

FIGURE 1

Quantitative high-content cancer phenotypic assays. **(a)** High-content microplate phenotypic cancer assays monitoring: apoptosis (cell-permeable caspase 3 biosensor: NucView™); cell-cycle M phase: anti-phospho histone H3 (PHH3-red); cell-cycle G2 phase: anti-cyclin B (cytoplasmic green) and DNA-damage repair response (anti-phospho histone H2AX – red) following cisplatin treatment of the PEO23 ovarian cancer cell line. Images acquired on the Olympus ScanR high content assay platform (×20 objective magnification). Data represent mean values and standard deviation across triplicate wells. **(b)** Advanced high content imaging of three-dimensional (3D) metastatic tumour microenvironment. Images represent 10-day culture of GFP-labelled pancreatic cancer cell line (PANC-1; green) cultured on human peritoneal omentum tissue scaffold. A two-dimensional (2D) projection of 100 μm 3D image series. Arrows point to circular 'structures' of PANC1 cells surrounding adipocytes within the omentum tissue scaffold. **(c)** 3D reconstruction of PANC1/omentum organotypic assay using Imaris software (Bitplane AG) enables accurate quantification of cell number and phenotypic response of pancreatic cells within the peritoneal metastatic microenvironment following drug exposure. Confocal reflectance of extracellular tissue (purple). Images acquired with an Olympus FV1000 confocal microscope.

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BOX 1

Advantages offered by high-content phenotypic screens

- No bias towards any specific target hypothesis.
- Categorise compounds according to mechanism of action.
- Identify compounds that have a novel mechanism of action.
- Large 'target space' can be tested in each assay, so no need to develop unique assays for every project.
- Identify compounds and combinations that target multiple pathways, facilitating, rational polypharmacology and drug combination screening.
- Identify new targets and off-target activity by cluster analysis across reference compound sets followed by target deconvolution strategies.
- Frontload cytotoxicity assessment (hepatotoxicity, genotoxicity and cardiotoxicity assays).
- Validate drug and/or target hypothesis across range of complex co-culture and 3D assay formats.
- Provide precise biological context and functional readouts for pharmacogenomic and pharmacoproteomic studies.

developments can provide a single cost-effective solution for efficacy testing across multiple target classes. A significant advantage of placing high-content image analysis at the earliest phase of drug discovery is that it will provide more informed validation of targets, hit series and chemical sublibraries. Some of the advantages as an alternative to traditional high-throughput biochemical assays are listed in Box 1. These could help reduce the high attrition rate of compounds at late stages in preclinical and clinical development, which is expensive. Integrated cellular systems have a higher chance of retaining relevant pathway networks, and the compensatory and redundant mechanism that prevent efficacy of targeted therapies in the clinic. Closer iteration between high-content analysis and medicinal chemistry could yield drug candidates with significantly greater efficacy response in complex biological systems.

Quantitative intravital imaging: *in vivo* drug response monitoring

Although high-content imaging often refers to the analysis of cell-based assays in primitive culture systems, there have been technological advances in confocal and/or two-photon microscopes and optical reporter probes facilitating functional *in vivo* imaging. Combined with 'in-tumour' resolution that is possible with newer intravital imaging techniques, the potential to image cancer cell behaviour and dynamic molecular processes by direct visualisation of live *in vivo* environments, is becoming realised [29–32]. High-resolution intravital *in vivo* imaging provides a unique opportunity to expedite the quantitative analysis of tumour and/or host responses *in vivo* following drug exposure. Incorporation of fluorescent proteins into the design of animal experiments, complemented with the application of spectroscopic techniques, such as fluorescence resonance energy transfer (FRET), fluorescence lifetime imaging (FLIM), photoactivation and photobleaching, enable high-definition and quantitative biological exploration *in vivo*. Therefore, a wide variety of cancer processes and primary endpoints associated with drug responses can be quantified at tissue, cellular and subcellular levels *in vivo* [33,34] (Fig. 2). Implantation of optic-enhancing tissue-window devices, such as clear glass coverslips in the skin, or over the mammary fat pad, enable long-term repeated high-resolution *in vivo* imaging, particularly when combined with optimised recoverable anaesthesia. These offer several advantages over alternative surgical exposure methods, including the so-called 'skin flap' method. These advantages include sample stability, a more consistent and faithful tumour microenvironment and the potential for repeated long-term imaging. This can provide important kinetic information in live animals, including the tracking of tumour cell invasive migration, proliferation, adhesion dynamics, autophagy, cell death, angiogenesis, vascular disruption and/or ingression and potentially metabolic and signalling events and/or enzyme kinetics [31–34]. Multiparameter monitoring of temporospatial biological responses in high definition, will inform on dynamic drug responses in a way that cannot be achieved with current industry-standard preclinical studies (Fig. 2).

Intravital imaging: added value and prospects in drug discovery

Dynamic intravital imaging provides a rapid readout of drug responses in the complex biological environment, thereby accelerating detailed evaluation of drug responses *in vivo*. In turn, this could facilitate lead optimisation cycle times and iterative medicinal chemistry might be guided by informative functional *in vivo* response data. *In vivo* imaging can also reduce the need for extensive histopathological examination at autopsy, reducing animal numbers and expense for *in vivo* drug-profiling studies. We believe that development of intravital imaging approaches that monitor cellular phenotypes deep inside tumour tissue will represent wise drug discovery investments. It will also provide much greater mechanistic definition in a way that could change the parameters for making key go–no go decisions.

The clinical predictivity of high-content screens and intravital imaging approaches remain to be fully determined; this will probably be a function of the physiological relevance of the biological models and phenotypic endpoints examined, and how best this information can be integrated with advancing genomic and proteomic technologies, and informatics, so as to build robust target, drug and companion diagnostic hypotheses. Key advantages in high-content *in vitro* and intravital imaging approaches are that they can improve the value of preclinical models by taking account of tumour environments that are 'closer' to real cancers, which are known to affect therapeutic responses. Through multiparameter phenotypic analyses, the relevant biological context can be provided for associated sequencing, genomic and proteomic network studies. Kinetic live cell-imaging applications will further inform on the optimal time points for modelling of dynamic pathway events. Thus, high-resolution *in vitro* and *in vivo* imaging should facilitate biomarker discovery and understanding of drug mechanisms, and will probably improve the prediction of clinical efficacy by providing precise and relevant biological context to genomic and proteomic pathway analysis studies.

Integrated pathway profiling tools: optimising combinations and biomarkers

Pharmacogenomics

Prognostic and predictive biomarker identification and diagnostic validation strategies underpin the ambitions of personalised medicine [35] and, in theory, should have important roles in guiding

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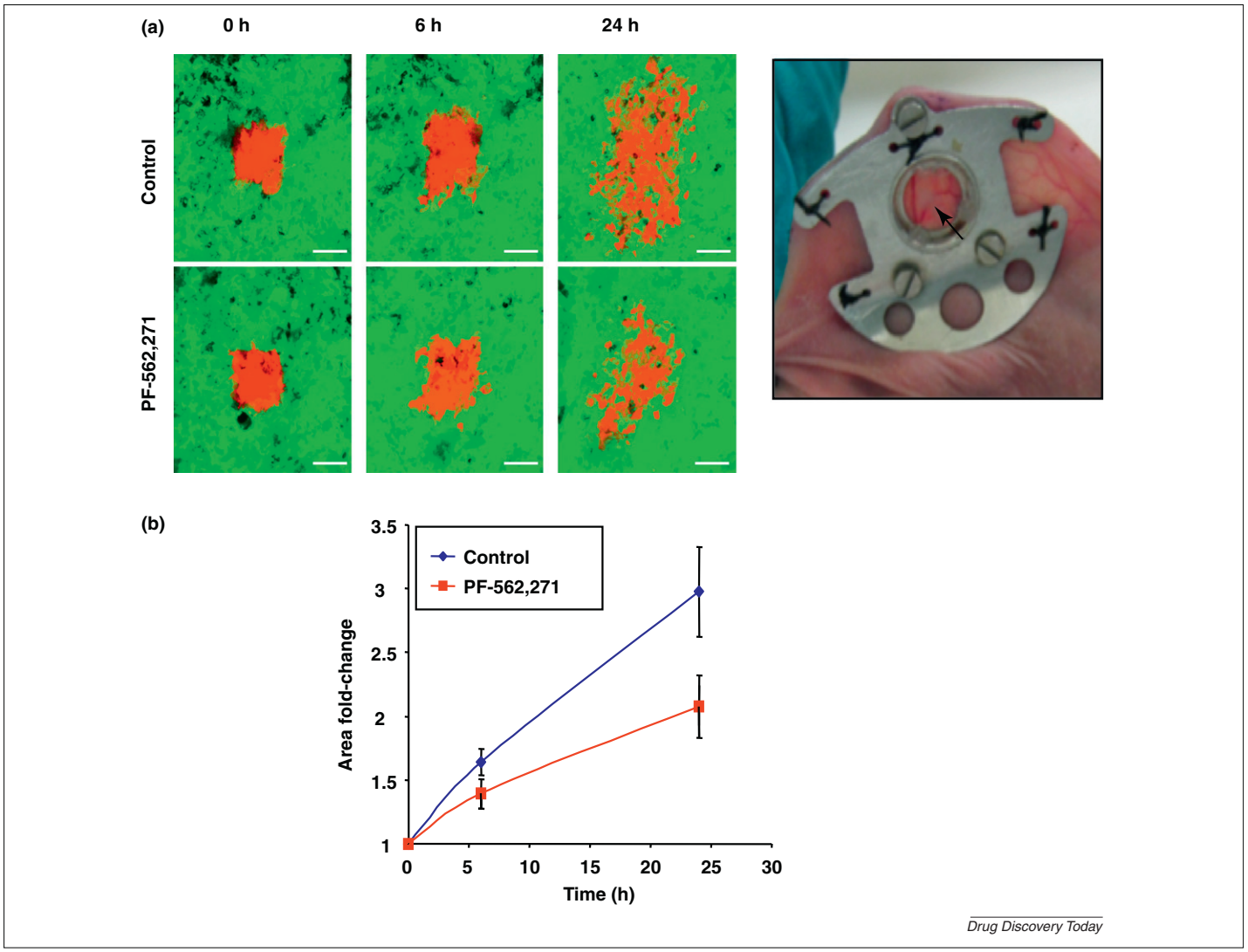

FIGURE 2

High-resolution quantitative *in vivo* imaging. Quantitative intravital imaging of tumour invasion *in vivo* using confocal microscopy and a photoswitchable probe. **(a)** Images represent the application of intravital optical imaging of functional tumour response phenotypes (e.g. invasion). In the example data shown, the A431 cell line derived from a human squamous cell carcinoma (LGC Promochem) was transfected with a photoswitchable pDendra2 reporter construct (Evrogen), using the Amaxa nucleofector transfection system (Amaxa GmbH). pDendra2-expressing A431 cells were subsequently grown as a xenograft under an optical window device implanted on the dorsal skin of a CD-1 nude mouse. Images represent A431 cells expressing the photoswitchable Dendra2 probe in tumours of untreated mice or mice treated with the FAK kinase inhibitor PF-562,271 (33 mg/kg in 0.5% methylcellulose, p.o. by gavage bid). Tumours were imaged at 0, 6 and 24 h post-switching providing a quantitative readout of *in vivo* tumour invasion (green, unswitched; red, switched). All images were captured using an Olympus FV1000 confocal microscope equipped with a UMPLFLN 20× 0.5 N.A. water immersion objective. Scale bar = 100 μm. **(b)** Quantification of the area covered by red fluorescence representing a photolabelled tumour cell at shown time-points following photoswitching. Values are the mean from at least five independent experiments performed in the CD-1 nude mouse background. Error bars: standard error of means.
Reproduced with permission from Alan Serrels and Marta Canel, Edinburgh Cancer Research Centre UK. Data represent a modified version of studies previous published by Canel *et al.* [34], where further experimental detail is given.

optimal drug combination strategies that improve clinical efficacy across patient subgroups. Pharmacogenomic studies have informed most biomarker discovery programs and pharmacodiagnostic strategies to date [36]. Although there have been some limited successes in using pharmacodiagnostics, for example in areas of drug metabolism and targeted kinase inhibitors of the epidermal growth factor receptor (EGFR)/ErbB family, broader impact of predictive genomic biomarkers on routine clinical practice and drug development has generally been disappointing. It is more than a decade since the human genome was sequenced, yet genome-wide association studies (GWAS) and mutational analysis have had modest impact on routine clinical practice and patient stratification thus far. Despite listings of 11 166 biomarkers in the GVK BIO Online Biomarker (GOBIOM) database at the beginning of 2011 (http://www.gobiomdb.com), only 32 validated genomic biomarkers have been incorporated into FDA-approved drug labels across all disease areas. In complex polygenetic disease settings, including solid cancers, it is now clear that multiple redundant, compensatory and cooperative mechanisms influence drug efficacy. Hence, it is probable that multiple genetic and post-translational markers of disease resistance, as opposed to single biomarkers, will be necessary to guide optimal drug combinations

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and to provide the desperately needed predictive power that will guide therapeutic regimens. The identification and validation of multiparametric genetic or protein biomarkers to guide treatment options, and adaptive combinations, as well as clinical drug development trials, represents a formidable challenge. Intratumour heterogeneity, nonlinear coding regions and overwhelming bioinformatic demands limit the immediate impact of advances in 'next-generation sequencing' on drug discovery and clinical practice. Although several multiparametric genetic signatures have demonstrated prognostic value (e.g. Mammaprint and Oncotype DX), there are currently no multiplex *in vitro* diagnostic tests that have been incorporated into FDA-approved drug labels.

Functional proteomics

Although many of the underlying causes of cancer occur at genetic and epigenetic levels, tumour cell phenotypes and drug responses are governed at the protein level. Recent advances in proteomic technologies have stimulated the field of functional proteomics that promises to provide new insights into the biochemical pathways driving cancer cell survival, proliferation and invasion [37]. Functional proteomics can elucidate protein modifications, and 'activities', providing details of the dynamic state of biochemical pathway networks perturbed in cancer, including following drug treatment. Our view is that these might be more predictive of crucial events than are genomic and/or transcriptomic data in many instances. It is by studying the 'cancer driver proteome' that it might be possible to obtain a clear understanding of adaptive responses that overcome drug mechanisms, and to establish the relationship between pathway states and therapeutic responses. Crucially, this will also promote rational choices of combination therapies to target multiple pathway nodes, with a view to collapsing the robustness of cancer cell biochemical networks.

Traditionally, functional proteomic methodology has relied on quantitative mass-spectrometry techniques, such as isobaric tags for relative and absolute quantitation (ITRAQ) and stable isotope labelling with amino acids in cell culture (SILAC), which remain the standard approaches for *de novo* identification of post-translation markers [37]. However, limitations relating to speed, cost, sensitivity and reproducibility of quantitative mass-spectrometry approaches have restricted their routine application across multiple samples. The evolution of antibody-based RPPA, combined with more sophisticated sample handling, optical detection and better quality (validated monospecific) antibody reagents, provide an alternative approach enabling exquisite sensitivity and appropriate throughput of functional proteomics across sets of cancer driver pathways [38,39]. RPPA provide precise quantitative analysis of pathway states and responses at the post-translational level across multiple biological samples, including preclinical and clinical drug development samples [39–41]. Recent applications include drug and disease mechanistic studies that have been directly or indirectly linked to biomarker research, and to the production of data for systems biology-based pathway network analysis, to guide effective drug combinations [40,42]. Tangible benefits of using an RPPA approach over alternative genomic or mass spectrometric proteomic methods include: (i) optimal throughput: sample numbers are limited by neither reagent costs nor instrument throughput, thereby enabling proteomic analysis across multiple clinical samples and/or dynamic dose and time-series following chemical screening or drug treatment; (ii) precise and sensitive quantification of multiple pathway responses at a post-translational level, including ratiometric analysis of low abundant 'druggable' pathways that can be mapped directly to drug–target hypotheses (e.g. rational combinations); (iii) unlimited multiplexing of appropriate antibody based reagents; (iv) high sensitivity in protein detection and high-throughput capability enable multiple sampling of single tumours, including microdissected samples to record intratumour heterogeneity; and (v) application of antibody-based detection reagents that can be readily adapted to single or small multiplex diagnostic-based assays.

Advances in RPPA technology

Advances in RPPA platform technologies and validated antibody reagents are exemplified by activity of the core group at MD Anderson, and commercial enterprises, such as Baypoint Biosystems, Theranostics Health and Zeptosens [38,41,43,44]. A typical dedicated RPPA platform uses the following core processes; total protein extracts are prepared from cell culture, or mouse or clinical tissue using quality-assured procedures, and samples are spotted onto nitrocellulose or a hydrophobic chip surface. Immobilised protein microarrays are then incubated with monospecific antibodies to detect individual proteins, or their post-translationally modified forms. Most RPPA platforms require nanolitres of protein lysate and picogram-to-femtogram quantities of protein, so enabling analysis of small preclinical and clinical samples. The ZeptoMARK platform developed by Zeptosens uses proprietary planar waveguide technology encompassing nanostructured glass protein array chips, further enhancing sensitivity (Fig. 3) [38]. Excitation laser light is directed into the wave-guiding layer by means of a nanostructured diffractive grating on the chip surface. The evanescent measurement of labelled antibodies by the ZeptoREADER is confined to the sample surface, minimising background interference from unbound antibodies or excitation light. This provides exquisite sensitivity and reproducibility by maximising the signal:noise ratio, regardless of the low levels of individual proteins [38]. The enhanced sensitivity provided by the advances in optical detection and protein microarray design enables further miniaturisation of sample (down to 400 pl) and reagent volumes (Fig. 3).

The new generation of RPPA platforms provides a cost-effective solution for high-throughput post-translational pathway analysis, supporting a variety of clinical and preclinical applications (Table 2). An expanding set of validated monospecific antibodies ensures that RPPA methods can be used to profile broad pathway responses simultaneously. Pathways typically covered in oncology RPPA studies include well-recognised cancer driver pathways; Akt/PI3K (Fig. 3), RAS–MAPK, receptor tyrosine kinases, Src/FAK, Rb/cell-cycle, p53, NF-κB, JAK/STAT, Wnt, mTOR and TGF-β effectors, multiple DNA repair, cell-cycle, apoptosis-regulating proteins, transcription factors, epigenetic histone modifications and many more. The technical advances in RPPA methodologies are complemented by huge improvements in sample handling and sample spotting, tailored to the needs of complex mixtures of cell- or tissue-derived protein extracts. Environmentally controlled liquid-handling instruments that create highly uniform arrays of complex protein and/or antibody mixtures are provided by manufacturers, such as Aushon Biosystems and GeSiM, and these can aid throughput and

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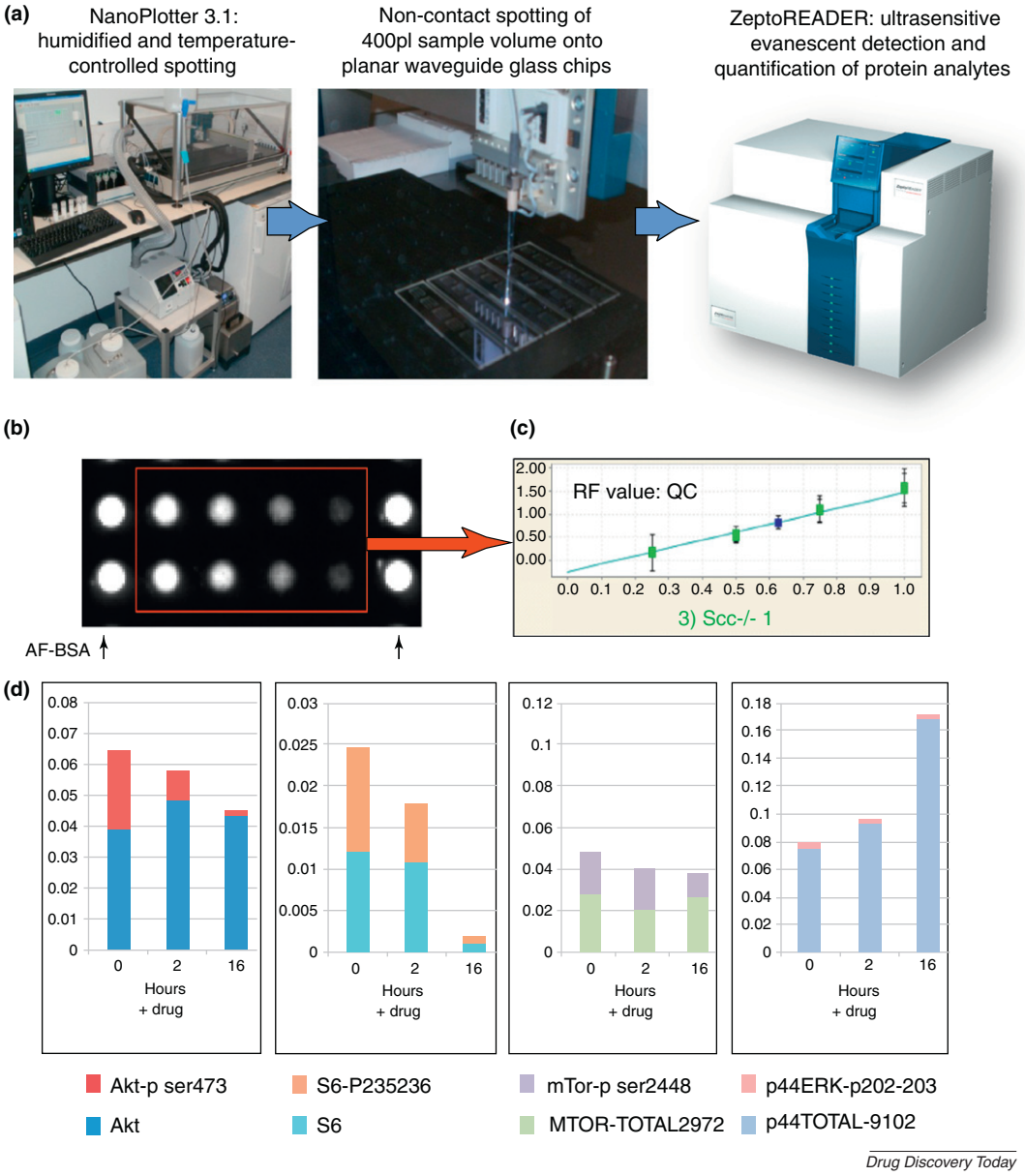

FIGURE 3

Zeptosens reverse-phase protein array (RPPA) analysis. **(a)** The Zeptosens RPPA platform incorporating environmentally controlled non-contact spotting and ultrasensitive optical nanotechnology-developed protein microarrays. **(b)** Data represent a typical Zeptosens RPPA study, each sample is spotted onto the microarray chip in 2 × 4 dilutions between Alexa-Fluor-conjugated BSA standards. Fluorescence intensity signals of each sample are calculated by optimised image analysis algorithms and normalised to intensity values of BSA standards through a local two-dimensional (2D) quadratic function. **(c)** A single relative fluorescence intensity (RFI) value (blue square) is obtained by a weighted linear fit through sample dilutions. Quality-control parameters for each sample are obtained by Shapiro–Wilk statistical test of intensity distributions across each dilution range. **(d)** An excerpt from a broad pathway analysis demonstrating suppression of the Akt/S6/mTOR signalling pathway and compensatory upregulation of p42/p44 ERK/MAPK following temporal drug exposure study. Specific phosphoepitope residues detected by Zeptosens RPPA are indicated.

reproducibility of protein and/or antibody array-based proteomics (Fig. 3).

RPPA-based pathway modelling: prospects

The advances described above are poised to complement alternative genomic and mass spectrometry technologies by reducing the knowledge gap between drug and disease mechanisms at the level of post-translational protein modification. Thus, compensatory and redundant post-translational mechanisms identified in preclinical and clinical material can be mapped to publicly available, or proprietary, drug–target databases, and these could be used to generate new drug–target and drug and/or drug combination hypotheses underpinned by strong mechanistic evidence. Potential biomarker strategies might also emerge to support drug development. Routine application of high-throughput RPPA methods during early drug discovery phases (e.g. target validation, evaluation of hit series through to lead-identification, lead-optimisation and evaluation of drug candidates) will provide an unbiased and

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TABLE 2

Applications of RPPA in drug discovery

Application	Outcomes
Drug candidate profiling *in vitro*	Establish broad pathway-activity mechanisms
Compound screening	Define EC_{50} values across multiple pathways mediators to determine on- and off-target activity
Identify compensatory or cooperative drug–target mechanisms	Identification and validation of drug combination strategies
Predictive *in vivo* pharmacodynamics	Monitor organ-specific pathway response correlating with functional drug response
Biomarker discovery	Detection of post-translational markers of therapeutic outcome from clinical biopsy or surrogate body fluids
Confirming mechanism of functional genomic screens and pathway crosstalk	Characterise impact of siRNA knockdown on key pathway nodes

comprehensive pharmacodynamic assessment of drug–target mechanisms in biological samples. This information will enable an information-based view of drug portfolios, and potentially guide more optimal clinical development strategies.

In our opinion, it is now crucial to take the bold steps to link sensitive analysis of cancer driver pathways and networks to quantifiable monitoring of phenotypic responses following drug treatment, as judged by imaging of multiple cancer-associated processes. Subsequent testing of key combination hypotheses, informed by multiple integrated biochemical and imaging technologies, can be carried out in complex genetic models of cancer. Although these models have not yet proven to be better in terms of predicting clinical efficacy, they are certainly closer in pathophysiology to human cancer than are conventional xenograft models in immunocompromised animals. When adequately informed by imaging and pathway analysis, their use could improve preclinical drug and drug combination testing for both response monitoring and biomarker development in a significant way.

Computational biology and systems network analysis

Successful implementation of phenotypic- and pathway-level data into early-phase drug discovery requires robust computational biology. Investment in resources that collate and annotate biological networks provides useful tools to study broad pathway crosstalk and subnetworks that drive resistance or predict drug response [45–48]. However, such pathways are often derived from text mining of published literature encompassing data collated from diverse (nondesigner) studies, hence reflecting composites of multiple experimental, biological and clinical scenarios. In addition, several published mathematical models have considered compensatory mechanisms of drug resistance, but these have generally been restricted to a few discrete pathways, and limited data points, reducing their value in predicting novel targets, or novel drug combinations, [49]. Thus, a major limitation of many systems and network biology studies to date is 'information quality' and 'quality control'; hence, incorporation of pure systems biology approaches into the drug-discovery process has yet to be realised [50].

Therefore, the advances in high-throughput phenotypic profiling, intravital imaging and functional proteomics platforms are creating a foundation for more integrated and informative systems-level analysis of dynamic pathway responses 'mapped on to' cancer biology. These should be based on empirical data generated from valued preclinical and clinical sources. Further integration of high-resolution drug and/or pathway response data with target selectivity databases and structure–activity relationships will further support systems-level analysis and advance the emerging field of network pharmacology, incorporating rational design and testing of polypharmacology (multitargeting drugs) and rational combinations of distinct drugs. The further development of computational methodologies and dedicated databases that integrate orthogonal, image-based phenotypic, genomic and dynamic proteomic drug-profiling data are essential to ensure more refined biomarker and/or drug combination studies and robust clinical predictivity.

Concluding remarks

Embracing grand challenges through new technology platforms

New advances in functional proteomic array platforms and sophisticated monitoring of drug response phenotypes by imaging, provide new opportunities in early-stage drug discovery. They provide the necessary throughput and resolution to pair efficiently drug mechanism-of-action biological data with pathway network analysis. Why will this provide substantial advantages? Application to valued *in vitro* and *in vivo* models can help decipher drug mechanism-of-action, and the response elicited by complex biological systems to drug exposure, so guiding accurate and robust determination of drug-response markers to inform rational combinations. More informative drug profiling in complex models represents a return to traditional physiology studies of drug exposure that existed before high-throughput target-directed enzyme-based screens becoming the standard. There is now great incentive to return to biologically led approaches, fuelled by improvements in imaging and functional proteomic technologies that enable more in-depth analysis of the perturbation of complex biological systems.

Key to successful implementation of imaging and post-translational pathway modelling approaches into routine cancer drug discovery is close and early integration and iteration with target-directed drug discovery programs. Confirming efficacy and safety profiles of hit compound series in phenotypic models, before expensive medicinal chemistry, offers a potential solution to unsustainable attrition rates and cost. Crucial to enhancing clinical predictivity and efficacy of drug discovery is objective and unbiased prioritisation, and timely termination of project compounds based on compelling biomarker and drug combination

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data from robust phenotypic and pathway analysis. This will be optimal if there is productive collaboration and partnerships across pharmaceutical company portfolios and translational cancer medicine centres, maximising the value of the most promising drug candidates by: (i) imaging the full range of cancer-associated phenotypes *in vivo* in the best-available preclinical models; (ii) understanding detailed cancer driver network responses and acquired compensatory mechanisms; and (iii) developing and validating optimal drug combination and pharmacodiagnostic strategies to support more rational and adaptive phase II/III clinical study designs.

An alternative to conventional drug discovery

We propose an alternative drug project operating model (DPOM) where combined imaging and pathway modelling data guide key investment decisions before large-scale medicinal chemistry and drug metabolism and pharmacokinetics (DMPK) activities (Fig. 4). Precedence for guiding lead-optimisation activities by phenotypic imaging and pathway modelling are provided by recent innovations in identifying structure–activity relationships based on multiparametric phenotypic and pathway endpoints [16,51], supporting a return to lead identification and optimisation based upon complex physiological outcomes. However, a step change in delivery of higher quality drug candidates will be provided by close integration of advances in phenotypic pathway profiling and target-directed approaches. Further tangible outcomes of the proposed model include the provision of robust pharmacodynamic markers to confirm proof-of-mechanism *in vivo* and guide optimal dosing schedules. Parallel development of new drugs, pharmacodiagnostic biomarkers and drug combination hypotheses support adaptive

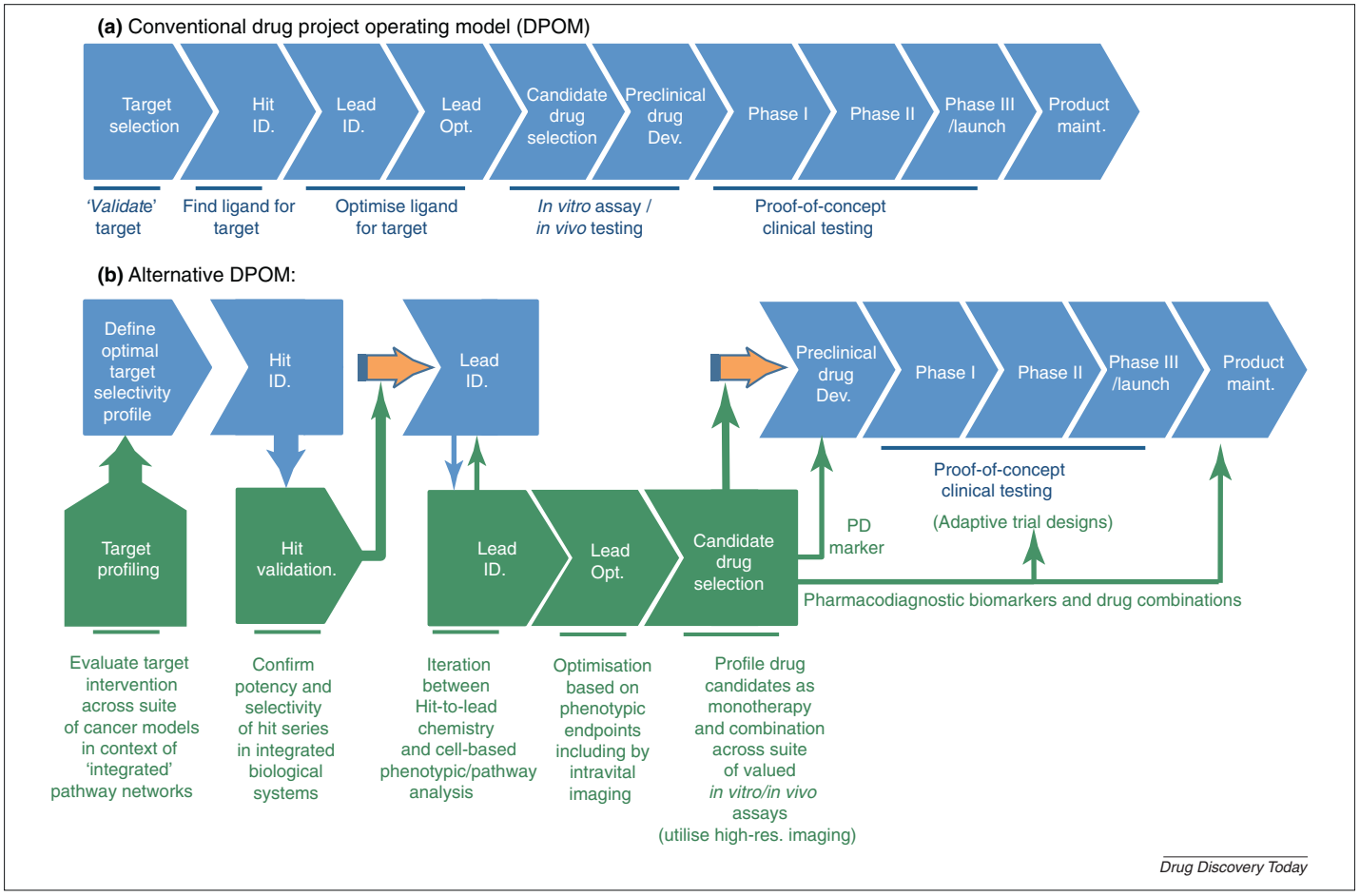

FIGURE 4

An alternative drug discovery model. **(a)** The conventional drug project operating model (DPOM) is represented as the standard linear process of well-defined activities (blue chevrons) that is commonly used across industry and academia to define drug discovery project milestones and investment decisions. **(b)** We propose an alternative DPOM that frontloads the evaluation of target and putative drug mechanism in complex biological systems through combined imaging and pathway profiling tools (green block arrows). Image and pathway profiling provides the necessary insight into cellular phenotypes, pathophysiology and drug mechanism to enable informed decisions on further investment and clinical positioning of drug–target mechanism hypothesis before expensive medicinal chemistry and absorption, distribution, metabolism, and excretion/drug metabolism and pharmacokinetics (ADME/DMPK). Selection and optimisation of hit series and lead compounds based on desired phenotypic and mechanistic characteristics feeds into objective investment decision points (orange arrows) that considers both competitor portfolios and current standards-of-care and so are appropriately tailored to the clinical indication. The alternative mechanistic profiling model further reveals the most robust pharmacodynamic (PD) markers facilitating *in vivo* dosing and scheduling studies. In addition, combined imaging and pathway profiling supports early and rational prioritisation of pharmacodiagnostic biomarker and drug combination strategies that predict and mitigate inherent or adaptive drug resistance in patient populations, further increasing the value and confidence in candidate drugs. Abbreviations: Dev, development; ID, identification; maint, maintenance; and res, resolution.

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trial designs that might also enhance efficacy and reduce attrition rates during clinical development. Drug combination and pharmacodiagnostic strategies might also protect registered drug franchise from generic competition, further increasing return on investment for pharmaceutical R&D (Fig. 4). The implementation of streamlined 'phenotypic and pathway profiling' provides an opportunity to expand the search of biological target space to uncover novel drug–target hypotheses in greater depth and sophistication (rational polypharmacology and drug combinations). Combined with earlier attrition of ineffective therapeutic strategies and more agile adaptive trial designs the multiparameter mechanistic model proposed would support innovative drug discovery at reduced R&D costs, and make better use of advances in the basic understanding of cancer driver mechanisms and cancer biology.

Investment in discovery of innovative medicines through partnerships

The overarching aim of our proposed model (Fig. 4) is to provide a cost-effective solution to enable discovery and development of innovative medicines that both transform phase II/III clinical trial success rates and provide a significant impact on patient survival. To ensure that most patients with cancer, healthcare providers and payers benefit from novel treatments, it is necessary that costs of drug discovery, clinical development and drug pricing in the clinic are constrained. More agile and cost-effective clinical development routes to drug registration are also needed to exploit the full value of novel targeted therapies, companion diagnostics and rational combinations. High attrition rates and poor financial return currently associated with discovery and development of novel medicines in oncology favour a swing towards perceived lower risk development of 'me-too' and generic drug programs that are in fact higher risk, because they fail to have substantial clinical impact.

Strong partnerships between academic research groups, pharmaceutical companies and regulators are required to implement innovative solutions that both reduce pharmaceutical R&D costs and provide more informative and predictive drug discovery and development. There are surely renewed incentives for investment in the development of novel and more effective drug development routes. We do not underestimate the challenges of bringing academic, pharmaceutical and regulatory authorities together to work towards the common goal of 'beating cancer'. However, innovative partnerships that embrace the grand challenges of drug discovery and deliver on the promise provided by new technology platforms are well placed to reap the rewards of transforming poor performing and expensive drug discovery programs.

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References

1 Paul, S.M. *et al.* (2010) How to improve R&D productivity: the pharmaceutical industry's grand challenge. *Nat. Rev. Drug Discov.* 9, 203–214
2 Kola, I. and Landis, J. (2004) Can the pharmaceutical industry reduce attrition rates? *Nat. Rev. Drug Discov.* 3, 711–715
3 Butcher, E.C. (2005) Can cell systems biology rescue drug discovery? *Nat. Rev. Drug Discov.* 4, 461–467
4 Sams-Dodd, F. (2005) Target-based drug discovery: is something wrong? *Drug Discov. Today* 10, 139–147
5 Dancey, J.E. and Chen, H.X. (2006) Strategies for optimizing combinations of molecularly targeted anticancer agents. *Nat. Rev. Drug Discov.* 5, 649–659
6 Stommel, J.M. *et al.* (2007) Coactivation of receptor tyrosine kinases affects the response of tumor cells to targeted therapies. *Science* 318, 287–290
7 Chen, Y. *et al.* (2009) Combined Src and aromatase inhibition impairs human breast cancer growth *in vivo* and bypass pathways are activated in AZD0530-resistant tumors. *Clin. Cancer Res.* 15, 3396–3405
8 Aleshin, A. and Finn, R.S. (2010) SRC: a century of science brought to the clinic. *Neoplasia* 12, 599–607
9 Mayer, E.L. and Krop, I.E. (2010) Advances in targeting SRC in the treatment of breast cancer and other solid malignancies. *Clin. Cancer Res.* 16, 3526–3532
10 Zhang, S. *et al.* (2011) Combating trastuzumab resistance by targeting SRC, a common node downstream of multiple resistance pathways. *Nat. Med.* 17, 461–469
11 Bickle, M. (2010) The beautiful cell: high-content screening in drug discovery. *Anal. Bioanal. Chem.* 398, 219–226
12 Alcock, P. *et al.* (2010) High content cell based primary screening for oncology targets—a perspective. *Eur. Pharm. Rev.* 3
13 Bickle, M. (2008) High-content screening: a new primary screening tool? *IDrugs* 11, 822–826
14 Caie, P.D. *et al.* (2010) High-content phenotypic profiling of drug response signatures across distinct cancer cells. *Mol. Cancer Ther.* 9, 1913–1926
15 Perlman, Z.E. *et al.* (2004) Multidimensional drug profiling by automated microscopy. *Science* 306, 1194–1198
16 Young, D.W. *et al.* (2008) Integrating high-content screening and ligand-target prediction to identify mechanism of action. *Nat. Chem. Biol.* 4, 59–68
17 Truong, H.H. *et al.* (2012) Automated microinjection of cell-polymer suspensions in 3D ECM scaffolds for high-throughput quantitative cancer invasion screens. *Biomaterials* 33, 181–188
18 Carragher, N.O. (2009) Profiling distinct mechanisms of tumour invasion for drug discovery: imaging adhesion, signalling and matrix turnover. *Clin. Exp. Metastasis* 26, 381–397
19 Isherwood, B. *et al.* (2011) Live cell *in vitro* and *in vivo* imaging applications: accelerating drug discovery. *Pharmaceutics* 3, 141–170
20 Nelson, D.E. *et al.* (2004) Oscillations in NF-kappaB signaling control the dynamics of gene expression. *Science* 306, 704–708
21 Friedl, P. (2009) Dynamic imaging of cancer invasion and metastasis: principles and preclinical applications. *Clin. Exp. Metastasis* 26, 269–271
22 Jones, T.R. *et al.* (2009) Scoring diverse cellular morphologies in image-based screens with iterative feedback and machine learning. *Proc. Natl. Acad. Sci. U. S. A.* 106, 1826–1831
23 Beck, A.H. *et al.* (2011) Systematic analysis of breast cancer morphology uncovers stromal features associated with survival. *Sci. Transl. Med.* 3, 108ra113
24 Pilling, J. *et al.* (2010) Development of a quantitative 96-well method to image glycogen storage in primary rat hepatocytes. *Mol. Cell. Biochem.* 341, 73–78
25 Castoreno, A.B. *et al.* (2010) Small molecules discovered in a pathway screen target the Rho pathway in cytokinesis. *Nat. Chem. Biol.* 6, 457–463
26 Durr, O. *et al.* (2007) Robust hit identification by quality assurance and multivariate data analysis of a high-content, cell-based assay. *J. Biomol. Screen.* 12, 1042–1049
27 Goldberg, I.G. *et al.* (2005) The Open Microscopy Environment (OME) Data Model and XML file: open tools for informatics and quantitative analysis in biological imaging. *Genome Biol.* 6, R47
28 Kozak, K. *et al.* (2010) Workflow-based software environment for large-scale biological experiments. *J. Biomol. Screen.* 15, 892–899
29 Brown, E. *et al.* (2010) *In vivo* imaging of tumors. *Cold Spring Harb. Protoc.* prot5452
30 Bullen, A. (2008) Microscopic imaging techniques for drug discovery. *Nat. Rev. Drug Discov.* 7, 54–67
31 Condeelis, J. and Segall, J.E. (2003) Intravital imaging of cell movement in tumours. *Nat. Rev. Cancer* 3, 921–930
32 Beerling, E. *et al.* (2011) Intravital microscopy: new insights into metastasis of tumors. *J. Cell Sci.* 124 (Pt 3), 299–310
33 Canel, M. *et al.* (2010) Use of photoactivation and photobleaching to monitor the dynamic regulation of E-cadherin at the plasma membrane. *Cell. Adh. Migr.* 4, 491–501

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34 Canel, M. *et al.* (2010) Quantitative *in vivo* imaging of the effects of inhibiting integrin signaling via Src and FAK on cancer cell movement: effects on E-cadherin dynamics. *Cancer Res.* 70, 9413–9422
35 Frank, R. and Hargreaves, R. (2003) Clinical biomarkers in drug discovery and development. *Nat. Rev. Drug Discov.* 2, 566–580
36 Stoughton, R.B. and Friend, S.H. (2005) How molecular profiling could revolutionize drug discovery. *Nat. Rev. Drug Discov.* 4, 345–350
37 Kolch, W. and Pitt, A. (2010) Functional proteomics to dissect tyrosine kinase signalling pathways in cancer. *Nat. Rev. Cancer* 10, 618–629
38 Voshol, H. *et al.* (2009) Antibody-based proteomics: analysis of signaling networks using reverse protein arrays. *FEBS J.* 276, 6871–6879
39 Weissenstein, U. *et al.* (2006) Protein chip based miniaturized assay for the simultaneous quantitative monitoring of cancer biomarkers in tissue extracts. *Proteomics* 6, 1427–1436
40 Carey, M.S. *et al.* (2010) Functional proteomic analysis of advanced serous ovarian cancer using reverse phase protein array: TGF-beta pathway signaling indicates response to primary chemotherapy. *Clin. Cancer Res.* 16, 2852–2860
41 Tibes, R. *et al.* (2006) Reverse phase protein array: validation of a novel proteomic technology and utility for analysis of primary leukemia specimens and hematopoietic stem cells. *Mol. Cancer Ther.* 5, 2512–2521
42 Iadevaia, S. *et al.* (2010) Identification of optimal drug combinations targeting cellular networks: integrating phospho-proteomics and computational network analysis. *Cancer Res.* 70, 6704–6714
43 Grote, T. *et al.* (2008) Validation of reverse phase protein array for practical screening of potential biomarkers in serum and plasma: accurate detection of CA19-9 levels in pancreatic cancer. *Proteomics* 8, 3051–3060
44 Wilson, B. *et al.* (2010) Monitoring proteins and protein networks using reverse phase protein arrays. *Dis. Markers* 28, 225–232
45 Ekins, S. *et al.* (2007) Pathway mapping tools for analysis of high content data. *Methods Mol. Biol.* 356, 319–350
46 Kuchaiev, O. *et al.* (2011) GraphCrunch 2: software tool for network modeling, alignment and clustering. *BMC Bioinform.* 12, 24
47 Lamb, J. *et al.* (2006) The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease. *Science* 313, 1929–1935
48 Shannon, P. *et al.* (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* 13, 2498–2504
49 Hendriks, B.S. *et al.* (2006) Computational modelling of ErbB family phosphorylation dynamics in response to transforming growth factor alpha and heregulin indicates spatial compartmentation of phosphatase activity. *Syst. Biol.* 153, 22–33
50 Ho, R.L. and Lieu, C.A. (2008) Systems biology: an evolving approach in drug discovery and development. *Drugs R. D.* 9, 203–216
51 Kunkel, E.J. *et al.* (2004) Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. *Assay Drug Dev. Technol.* 2, 431–441

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