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Review

Anti-cancer drug resistance: Understanding the mechanisms through the use of integrative genomics and functional RNA interference

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ABSTRACT

Primary or acquired drug resistance remains a fundamental cause of therapeutic failure in cancer therapy. Post-hoc analyses of clinical trials have revealed the importance of selecting patients with the appropriate molecular phenotype for maximal therapeutic benefit, as well as the requirement to avoid exposure and potential harm for those who have drug resistant disease, particularly with respect to targeted agents. Unravelling drug resistance mechanisms not only facilitates rational treatment strategies to overcome existing limitations in therapeutic efficacy, but will enhance biomarker discovery and the development of companion diagnostics. Advances in genomics coupled with state-of-the-art biomarker platforms such as multi-parametric functional imaging and molecular characterisation of circulating tumour cells are expanding the scope of clinical trials – providing unprecedented opportunities for translational objectives that inform on both treatment response and disease biology. In this review, we propose a shift towards innovative trial designs, which are prospectively set up to answer key biological hypotheses in parallel with the RNA interference elucidation of drug resistance pathways in monotherapy pre-operative or 'window of opportunity' early phase trials. Systematic collection of paired clinical samples before and after treatment amenable to genomics analysis in such studies is mandated. With concurrent functional RNA interference analysis of drug response pathways, the identification of robust predictive biomarkers of response and clinically relevant resistance mechanisms may become feasible. This represents a rational approach to accelerate biomarker discovery, maximising the potential for therapeutic benefit and minimising the health economic cost of ineffective therapy.

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1. Introduction

Despite the expanding repertoire of new anti-cancer drugs, treatment failure due to primary or acquired resistance remains an almost inevitable outcome in most advanced solid tumours.[1,2] The sequential or simultaneous exposure of tumours to cytotoxics with non-overlapping mechanisms of action has dominated medical oncology practice – traditionally implemented to increase cell kill and to avoid the selection of tumour cell clones harbouring resistance mutations to a single chemotherapy agent.[3–6] Progress in molecular biology has led to a shift towards mechanism-based drug discovery[7] that target specific molecular pathways, such as epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKI), e.g. gefitinib as well as those targeting the microenvironment, e.g. multi-targeted Vascular Endothelial Growth Factor receptor TKI such as sunitinib. Yet, despite these therapeutic advances, drug resistance remains a pervasive problem. In this review we highlight the potential for the combined analysis of gene function related to drug sensitivity, using whole genome RNA interference approaches, with tumour genomics analysis of clinical material from defined clinical trial cohorts, to illuminate clinically relevant mechanisms of drug response and resistance. Such strategies may accelerate predictive biomarker discovery and identify novel therapeutic targets in cancer medicine (Fig. 1).[8]

2. Overview of drug resistance mechanisms

2.1. Multi-drug resistance

A multitude of drug resistance mechanisms have been reported.[2,9–11] These are often specific to a mechanism of drug action, such as increased excision repair cross-complementing 1 protein expression resulting in enhanced removal of DNA adducts caused by from platinum chemotherapy[12] or modification of drug metabolism, such as through upregulation of thymidylate synthase resulting in resistance to 5-fluorouracil.[13] Mechanisms of multi-drug resistance include expression of resistance efflux proteins, which are members of the ABC transporter superfamily involved in the transport of both hydrophobic and hydrophilic compounds, e.g. MDR1 protein.[14,15] Interestingly, an RNA interference (RNAi) screen has revealed molecular pathways that influence response to non-cross-resistant cytotoxic agents appear to be significantly more common than drug-specific resistance mechanisms,[16] suggesting that common signalling pathways can be targeted to circumvent the acquisition of multi-drug resistance.

2.2. Cancer stem cells

Cancer stem cells (CSCs) are typified by a capacity for self-renewal, relative quiescence and the ability to differentiate[14,17]

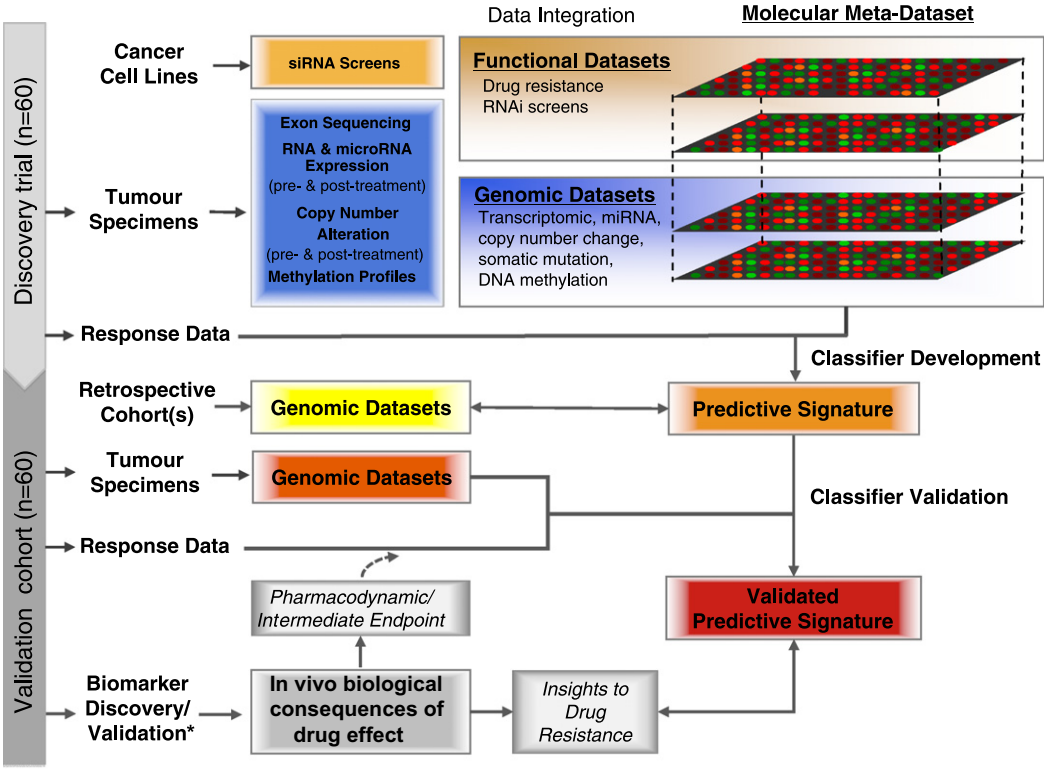

Fig. 1 – Integrating prospective clinical trials with functional genomics and validation datasets. (*) Biomarker discovery and validation should occur in tandem with the clinical trial, and can serve as pharmacodynamic markers, e.g. change in blood flow on DCE-MRI or proliferation with [18F] FLT-PET, intermediate endpoints e.g. circulating tumour cell counts, as well as provide insights to drug resistance.

– potentially subverting the effects of cytotoxic chemotherapy that typically target rapidly proliferating cells. The identification and characterisation of CSCs in primary tumours has proved challenging due to inconsistent classical stem cell properties that vary in frequency and phenotype between individual tumours. Nevertheless, it is becoming increasingly apparent that CSCs may share some common functional characteristics,[18] such as the ability to undergo epithelial-mesenchymal transition or activation of key pathways, e.g. Akt and nuclear factor-κB (NF-κB) signalling. Such pathways have been implicated in the drug resistance phenotype, thereby revealing common traits to focus novel therapeutic strategies.

2.3. *Tumour somatic alterations*

Genetic alterations in the drug target or downstream regulators of target signalling can have an impact on drug response and resistance. Trastuzumab, a monoclonal antibody targeting the HER-2 receptor illustrates this concept.[19,20] Trastuzumab is routinely administered to patients with breast cancers strongly positive for the HER-2 protein by immunohistochemistry and to those in whom HER-2 amplification is detected by fluorescence *in situ* hybridisation.[21] Resistance mechanisms can involve upregulation of HER-2 downstream signalling pathways, which can result from *PTEN* loss, *PI3K* mutations, *AKT1* mutations or PDK1 overexpression.[22] RNAi screening technology further established *PTEN* silencing as a key mediator of trastuzumab resistance and highlighted the importance of this approach to identify drug resistance mechanisms with relevance *in vivo*; *PTEN* loss and *PIK3CA* mutation status were associated with shorter progression free survival in breast cancer patients following trastuzumab-based treatment.[23] In lung adenocarcinoma, activating point mutations in EGFR (e.g. *L858R*) in the kinase domain predict sensitivity to EGFR TKI *in vitro* and *in vivo*, as well as clinical benefit.[24,25] However, presence of a *T790M* mutation that increases ATP-binding affinity results in re-constitution of downstream signalling and acquired resistance to these agents, with recent data further suggesting that this mutation can also account for *de novo* resistance.[26,27]

The observations from genomics analyses from phase III trials of EGFR inhibitors in tumours with and without tyrosine kinase domain mutations are noteworthy. In the Iressa Pan-Asia Study (iPASS), whilst upfront gefitinib resulted in superior progression-free survival compared to paclitaxel-carboplatin in those patients harbouring *EGFR* mutations, those with wild-type EGFR did worse compared to those treated with chemotherapy.[28] Similarly, in patients with mutated *KRAS* in the Cetuximab Combined With Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer (Crystal) trial, the addition of cetuximab resulted in trend towards reduced response rates.[29] Moreover, while the identification of activating *KRAS* mutations is becoming routine clinical practice in the decision to utilise cetuximab therapy in colorectal cancer, this has not been the case in advanced non-small cell lung cancer,[30] contrary to early evidence in the context of EGFR TKI therapy.[31] While the precise interaction between *KRAS* and EGFR signalling has yet to be fully elucidated, recent experimental data have shed light on an associated oncogene and emerging therapeutic target, *BRAF*. In melanoma cell lines, BRAF inhibitors and kinase-dead BRAF were found to activate downstream CRAF and MEK-ERK signalling, in the presence of oncogenic *RAS*, causing paradoxical enhancement of tumourigenesis.[32]

These findings emphasise the critical importance of predictive biomarker development in order to direct treatment to patients most likely to respond and limit potentially harmful exposure to therapy in drug resistant disease.

2.4. *Tumour microenvironment*

The tumour microenvironment is also emerging as a key mediator of drug resistance, with experimental data revealing a tremendous degree of stromal adaptation, potentially mediated by cancer cell signalling activity, to circumvent drug effects.[33,34] Prime examples are highlighted by the mechanisms of resistance to anti-angiogenic therapies, which include upregulation of alternative pro-angiogenic signals,[35,36] recruitment of bone marrow progenitors,[37] increased pericyte coverage[38] and a pro-invasive adaptive response.[39] Recently, through an analysis of sunitinib-treated clear cell renal cell carcinoma xenograft models and validation in patient RCC samples, we discovered that sunitinib resistance can be mediated by elevation of interleukin-8 (IL-8) in both tumour and plasma. Importantly, reversal of acquired sunitinib resistance could be achieved specifically in the context of concurrent administration of IL-8 antibody treatment, with no effect of IL-8 antibody in treatment naïve tumours or when given as a single agent.[36]

It is also increasingly recognised that resistance to drug therapies may arise from the non-tumour cellular compartment. Our preclinical data have recently shown that sunitinib primarily acts on endothelial cells rather than on tumour cells.[40] Tumour-associated endothelial cells derived from patient hepatocellular tumours have been reported to be more resistant to doxorubicin and sorafenib[41] and fibroblasts are emerging as important players in promoting tumour progression and drug resistance.[42,43] Finally, in a transgenic model of pancreatic ductal adenocarcinoma, gemcitabine resistance was attributed to inefficient drug delivery poorly vascularised tumours with impaired perfusion, and was reversed through depletion of stromal tissue through hedgehog targeting.[44]

2.5. *Challenges in drug resistance research*

Despite extensive efforts to comprehend individual drug resistance mechanisms, only a handful of strategies have successfully been implemented to overcome drug resistance or predict drug sensitivity; and there remains a paucity of robust predictive biomarkers used in the clinic.[45] It is possible that inter-patient somatic and germline genomic heterogeneity mitigates against the formal discovery of predictive biomarkers to distinct therapeutic agents suitable for implementation across a large cohort of patients.

However, another possible explanation is that traditional biomarker discovery and validation processes have often been undertaken in retrospective trial cohorts with limited parallel assessment of the underlying molecular mechanisms of drug resistance to support the functional relevance of

Table 1 – Examples of genetic alterations that occur in malignancies and their relation to therapeutic response.

Level	Genetic Alteration	Example	Implication on Drug Response	Validation	References
DNA	Somatic Mutations	*EGFR L858R mutation, exon 19 deletion*	Response to gefitinib EGFR smTKI in lung cancer	Clinical: Prospective (phase III)	[25]
		EGFR T790M mutation	Resistance to gefitinib in lung cancer	Clinical: Prospective (phase II)	[26]
		KRAS codon 12 and 13 mutation (exon 2)	Resistance to cetuximab EGFR mAb in colon cancer	Clinical: Post-hoc (phase III)	[29]
	Copy Number Alterations	*HER2 amplification > 2.2 (by FISH)*	Response to herceptin HER2 mAb in breast cancer	Clinical: Prospective (phase III)	[21]
		PTEN loss	Resistance to herceptin in breast cancer	Clinical: Retrospective	[23], [142]
	Chromosomal Translocation	*BCR-ABL*	Response to imatinib (c-kit smTKI) in CML	Clinical: Prospective phase III	[141]
		TMPRSS2-ERG	Response to abiraterone (17-alpha hydroxylase C17, 20-lyase inhibitor) in prostate cancer	Clinical: Post-hoc (phase I/II)	[143]
		EML4-ALK	Response to PF-02341066 (ALK and MET smTKI) in lung cancer	Clinical: Prospective (phase I/II)	[131]
mRNA	Expression profiles	Stroma signature	Resistance to neoadjuvant FEC chemotherapy in breast cancer	Clinical: Post hoc (phase III)	[144]
		70-gene signature "Poor Prognosis"	Response to neoadjuvant chemotherapy in breast cancer	Clinical: Prospective (phase II)	[145]
		CIN signature "High-CIN"	Relative resistance to paclitaxel in ovarian cancer and sensitivity to carboplatin in ovarian cancer	Clinical: Retrospective	[100]
	Alternative Splicing	Splicing factor SPF45 overexpression	Resistance to doxorubicin and cyclophosphamide (multiple cancer types, *in vitro* only)	Preclinical	[146]
		BCR-ABL (35INS) btw exon 8 & 9	Resistance to imatinib in CML	Clinical: Retrospective	[147]
microRNA	Expression profiles	19-microRNA signature (18 downregulated, 1 upregulated)	Resistance to imatinib in CML	Clinical: Retrospective	[148]
		miR-214 (via *PTEN* downregulation)	Resistance to cisplatin in ovarian cancer	Clinical: Retrospective	[149]
		miR-26	Response to adjuvant interferon in HCC	Clinical: Retrospective	[150]
Epigenetic	Methylation	MGMT silencing via promoter hypermethylation	Response to temozolomide in GBM	Clinical: Post hoc (phase III)	[151]
	Histone modification Methylation/ acetylation	Increase in H3 acetylation and induction of H3K4 at MDR1 gene locus	Resistance to daunorubicin and etoposide (*in vitro* only)	Preclinical	[152]
Tumour	Clonal diversity	Variation in genetic alterations within each sector of a solid tumour	N.A.	N.A.	[101]

EGFR: epidermal growth factor receptor; mAb: monoclonal antibody; smTKI: Small molecule tyrosine kinase inhibitor; FISH: fluorescent in situ hybridization; FEC: 5-Fluorouracil, Epirubicin, Cyclophosphamide; GBM: glioblastoma multiforme; HCC: hepatocellular carcinoma; CML chronic myeloid leukaemia Examples in italics require compulsory testing prior to use of drug as recommended by European Medicines Agency (EMEA).

genes associated with drug resistance in patients, with many gene expression analyses reliant on associative predictive learning strategies. Multiple testing approaches in genome-wide mRNA expression and CGH copy number datasets also have an inherent tendency to produce false positive results exemplified by early associative gene expression studies that have provided minimal information regarding the contribution of individual genes to drug sensitivity, resistance and clinical response.[46–48] Moreover, inadequately controlled sample collection methodology,[49] specimen handling (e.g. time to processing),[50] archival storage conditions,[51] extent of microdissection,[52] stromal contamination[53] and slide processing (e.g. fixation)[54] may all contribute to limit effective biomarker discovery.

Higher resolution strategies based on next generation sequencing and genomic and transcriptomic analyses of tumour samples delivered by trials designed to provide high quality material for these analyses combined with the high-throughput analyses of gene function discussed in this review, promise to improve both data integrity and opportunities to explore the complexity and heterogeneity of drug resistance. Such parallel 'integrative genomics' strategies may accelerate predictive biomarker discovery and identify novel drug resistance pathways suitable for therapeutic intervention.

3. Molecular classification of solid tumours

The ability to characterise tumours in great detail by readouts of RNA expression, alternative splicing, genome-wide somatic mutations, and of epigenetic alterations can efficiently annotate key genetic events in tumours.[55,56] Such approaches represent a first step in the development of a personalised therapeutic approach in cancer medicine (Table 1).

Expression microarray studies, through the analysis of thousands of genes, have resulted in the identification of molecular subclasses within individual cancer types,[57,58] and revealed transcriptomic signatures reflecting key oncogenic pathways associated with tumour progression. Such molecular classifications can be particularly useful in delineating subtypes that can be challenging to distinguish with routine histopathology,[59–62] yield novel diagnostic biomarkers,[63–65] as well as new therapeutic targets.[66–69] The discovery of upregulated hepsin and PIM-1 serine/threonine kinase from early microarray studies in prostate cancer[70] has led to the potential clinical application of hepsin-targeted imaging for early prostate cancer detection[71] and a PIM kinase small molecule inhibitor, SGI-1776,[72] currently undergoing evaluation in a phase I clinical trial (http://clinicaltrials.gov, NCT00848601).

In breast cancer, gene expression profiles from historical datasets have yielded several distinct subtypes such as basal-like, luminal-A, -B and *Erb-B2+*.[73] In addition, prognostic gene expression panels have been developed that may contribute to risk stratification and the adjuvant chemotherapy decision process. Mammaprint is a 70-gene biomarker that has been found to be predictive of survival in early stage breast cancer,[74–76] while the Oncotype Dx (21 genes) classifies patients into three categories of risk based on a recurrence score.[77] Their utility in the risk stratification of women with node negative early breast cancer for adjuvant chemotherapy is presently being evaluated prospectively in clinical trials. More recently, the integration of gene expression microarray data from cell lines and eight independent clinical cohorts led to the classification of hepatocellular carcinoma into three subclasses – characterised by WNT pathway/TGFβ activation, *MYC/AKT* activation and a molecular profile of differentiated hepatocyte function.[78]

Next generation sequencing technology provides extraordinary speed and resolution for whole genome analysis at a less prohibitive cost compared to the traditional sequencing technologies (approximately 12,000 euros for whole genome sequencing and 6000 euros for exon sequencing).[79,80] The breadth and depth achieved through these approaches are beginning to elucidate the genomic landscape of cancers,[81] identifying key genetic alterations associated with specific tumour types.[82–84] Importantly, these studies have revealed the marked variation in mutation profile between individual tumours (i.e. inter-tumour heterogeneity).[85] For example, while on average only approximately 80 different genetic alterations which affect the amino acid sequence or the respective protein products were found in the analysis of 11 breast cancers and 11 colorectal cancers, the number of mutations per tumour ranged from 38 to 193 and 49 to 111 respectively. It is noteworthy that less than 15 of them were thought to be 'driver' aberrations involved in initiation, progression, or maintenance of the tumour.[86] In pancreatic cancer, the average number of mutations affecting protein sequences was 63, and these could be functionally classified into 12 core signalling pathways.[87] Finally, using a similar approach, genetic events that occur at greater frequency were elucidated in glioblastoma multiforme, e.g. *IDH1*, providing important insights into disease pathogenesis as well as new prognostic markers.[88] These sequencing studies have revealed the marked DNA somatic mutational heterogeneity even in cancers of the same histopathological subtype, indicating the need to consider diverse drug resistance mechanisms responsible for therapeutic failure in patient cohorts.[86,89]

4. Tumour heterogeneity

Intra- and inter-tumoural heterogeneity can arise through processes such as structural and numerical chromosomal instability, microsatellite instability or DNA methylation (Table 1). It has further been suggested that environmental or iatrogenic pressures due to either physiologic changes such as hypoxia, or chemotherapy exposure, can induce heterogeneity and clonal evolution.[90,91]

Elegant *in vitro* models have demonstrated that acquisition of multi-drug resistance can occur at a higher rate in aneuploid cell lines compared to diploid cells. This process may be 'catalysed' through chromosome re-assortments selected following drug exposure. The 'selectable phenotype', may in turn be associated with multiple 'unselected phenotypes', such as resistance to diverse non-cross-resistant cytotoxics, by virtue of fluxes in gene dosage and gain or loss of genes encoded on the mis-segregated chromosome.[92–95] In support of the hypothesis that genome instability enhances tumour

biological fitness, the accumulation of clonal diversity has been found to independently predict for the progression of oesophageal carcinoma[96] and chromosomal instability has been associated with poor prognosis across several solid tumours,[97,98] as well as implicated in chemotherapy resistance.[99,100]

Recent data further underscore the heterogeneity that exists within tumours.[97,98] Navin and colleagues employed sector-ploidy-profiling (SPP), where CGH profiling was performed on distinct tumour subpopulations separated by virtue of geographic sector and fluorescent activated cell sorting separation based on ploidy status, revealing distinct clonal subpopulations within the same tumour with differing genetic alterations.[101] Thus within a single tumour, or even within a specific sector, there can be several populations of diploid and aneuploid cells, with varying DNA copy number alterations, exemplified in this case by amplification of the *KRAS* locus. Hence the capacity of tumours to "acquire" drug resistance may be influenced by the presence of drug resistant clones within the tumour prior to treatment exposure. Importantly, intra tumoural heterogeneity is often under represented due to limitations in tumour sampling, and therefore can affect the sensitivity and specificity of genomic predictive biomarkers of response.

Systematic *in vitro* studies of gene function by RNA interference techniques have provided information about the complexity of drug resistance and the myriad of potential genes that are capable of altering drug response.[16] Such high dimensional data, particularly when integrated with genomic and proteomic datasets, present a formidable challenge for extracting meaningful information, resulting in the frequent adoption of relatively simple models that merely facilitate visualisation of data structure like hierarchical clustering.[102] Furthermore, with the race to sequence the complete human genome at USD$1000 still ongoing,[85,103] it is anticipated that the increase in the resolution, speed and detail of analysis – perhaps even to a single-cell level[104] – will require novel approaches to integrate such vast genetic information. Given the multidimensional nature of drug resistance, a systems biology approach that integrates established factors, e.g. histopathology and TNM staging, with protein–protein interaction networks, gene and kinase networks and pathway simulation may prove informative.[105,106]

A practical consideration is that it is unlikely to be clinically feasible to target all individual mechanisms of every resistant clone selected from a background of tumour heterogeneity driven by genomic instability. Conceivably, strategies aimed at targeting survival pathways associated with specific mechanisms of genome instability may reduce the acquisition of genetic alterations that may create the diversity necessary for survival and 'natural selection' of a neoplastic clone following drug exposure.

5. Integration of clinical datasets with tumour genomics to elucidate drug resistance mechanisms

Early studies on imatinib resistance illustrate the capacity of genomics technologies to inform clinically relevant drug resistance mechanims in small patient cohorts. From sequencing clinical material in 9 patients, point mutations in the threonine residue of the ABL kinase domain were found to interfere with drug binding causing reactivation of BCR-ABL signal transduction.[107] This clinical insight led to the preclinical development of a novel highly competitive inhibitor at the ATP-binding site of BCR-ABL, BMS354825,[108] or dasatinib. A subsequent phase I trial of dasatinib, revealed tumour responses in imatinib-resistant patients in all phases of CML.[109] The elegant application of genomics in a small subset of patients is an important proof of concept of this approach and demonstrates that with knowledge of tumour biology, drug target and therapeutic response in well-characterised cancer types, small patient sample sizes are sufficient to reveal dominant tumour drug resistance mechanisms.

Next generation sequencing has been employed to provide insights into the evolutionary process that occurs during the natural history of the tumour. In a landmark study, Shah and colleagues profiled the mutational evolution of an oestrogen receptor alpha positive metastatic lobular carcinoma in depth, demonstrating 19 new somatic mutation events through a comparative analysis of the metastasis and primary tumour.[110] In addition, of several hundred RNA editing events discovered, the integration of genome and RNA-seq data yielded two novel RNA editing events that recode *COG3* and *SRP* genes, as well as high expression of *ADAR* gene which encodes a key RNA editing enzyme. This illustrates the value of complementing molecular profiling techniques – such as the study of protein binding sites e.g. through Chip-seq, DNA methylation profiles and whole exome sequencing – where it is now possible to depict the key genetic events in parallel that likely contribute to tumour phenotype, facilitating personalised treatment approaches.[89,111]

Indeed the integration of complex genomic datasets, derived from identification of common structural DNA aberrations in cancer and parallel laboratory studies exploring the roles of genes encoded within these regions on drug response mechanisms, is illustrated by recent examples. Chromosome 8q22 copy number gains were identified in poor prognosis breast tumours. These discrete 8q22 DNA copy number gains were associated with increased expression of metadherin (MTDH) encoded within this region. Laboratory experiments subsequently established the role of MTDH in metastatic seeding and enhanced chemoresistance.[112] Recently, other genes within this region have been identified that are also overexpressed and associated with early recurrence after adjuvant anthracycline therapy. Two genes encoded within this region, *YWHAZ* and *LAPTM4B* reproduced anthracycline resistance in laboratory studies when overexpressed and enforced expression of *LAPTM4B* led to sequestration of doxorubicin in the lysosome.[113] Similar approaches have been employed in the discovery of mechanisms of resistance to poly ADP ribose polymerase (PARP) inhibitors and carboplatin through an in-frame deletion in *BRCA2*,[114] development of MSI and a methylator phenotype in glioblastoma multiforme post-temozolamide relapse,[115] and *T790M* mutations in acquired gefitinib resistance in lung adenocarcinoma.[26]

6. Integration of RNA interference approaches with clinical genomics datasets

A powerful method for interrogating gene function in a high-throughput manner is the use of RNAi screens, which entail the use of small interfering RNAs (siRNA) and short hairpin RNAs (shRNA).[116] siRNA screens systematically knock down a series of genes individually in cell lines that can now be employed in a genome-wide manner. The precise influence of individual genes on cell survival (or other relevant measurable phenotypes) can be studied in the presence or absence of defined cytotoxics or targeted agents to explore their roles in drug response. Such an approach has proven successful in the discovery of specific mediators of resistance to cytotoxic, targeted and hormonal agents,[23,116–121] as well as the identification of synthetic lethal genes that influence cell fate for gain-of-function (e.g. activated *RAS*) and loss-of-function (e.g. *VHL*) genetic alterations.[122–124] Successful application of this technology is further illustrated in the identification of novel tumour suppressor genes in human hepatocellular carcinoma (HCC) through the integration of DNA copy number changes in HCC with *in vivo* mouse shRNA screens.[125] Finally, RNAi screens on patient derived primary cancer cells have been shown to be feasible, yielding novel putative therapeutic targets in leukemia.[126]

Coupling functional genomics with gene expression datasets has also provided corroborative data in defining clinically relevant drug sensitivity pathways.[100,127] Using RNAi screening approaches, we have recently determined that genes permissive for polyploidy or chromosomal instability are determinants of taxane sensitivity *in vitro* and in parallel demonstrated that chromosomal instability is associated with relative paclitaxel resistance and carboplatin sensitivity *in vivo*.[100]

In a proof of concept analysis, we have recently shown that genes which consistently influence paclitaxel sensitivity across multiple cell lines in an RNAi screen, appear to confer paclitaxel-specific predictive power in patients treated with paclitaxel-combination chemotherapy in primary breast cancer.[8] These data suggest that RNAi drug resistance screening datasets are enriched with genes that predict for drug response *in vivo* and that these approaches, when combined with genomics analyses of clinical trial tumour tissue from patients, may be used to identify predictive biomarkers of drug response.[128] Such integrative functional genomics techniques may lead to more rapid predictive biomarker discovery methods in cancer medicine.

7. Integrative genomics opportunities and drug development

As integrative genomics studies and enhanced molecular understanding of disease biology begin to yield new targets and biomarkers that can potentially predict for drug response, such markers require prospective validation in independent trial cohorts.[129] Such predictive biomarkers may comprise of a single or multi-gene expression signature, somatic mutation or DNA copy number analysis, or a combination of protein/RNA/DNA analytical processes. Indeed, enrichment for sensitive patient cohorts can translate to clinical benefit to patients even early in the drug development process. For example, in the phase I trial of PLX4032, a BRAF inhibitor, 9 of 15 melanoma patients harbouring ^{V600E}*BRAF* mutations, demonstrated partial response. All 5 patients without ^{V600E}*BRAF* mutations progressed within 3 months of experimental therapy.[130] In another phase I trial of PF02341066, an oral MET and ALK inhibitor, an enriched population of lung cancer patients harbouring the ALK fusion

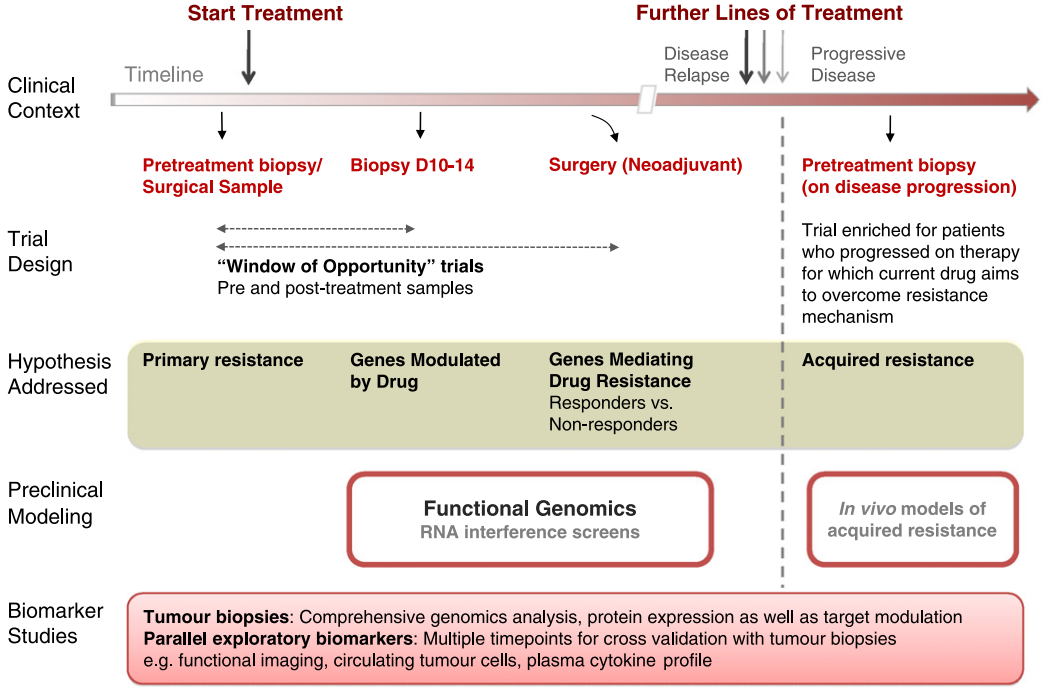

Fig. 2 – Hypothesis-testing clinical trial designs with parallel integration of genomic datasets and biomarker discovery.

protein resulted in an overall response rate of 10 of 19 (53%) and a disease control rate at 8 weeks of 79%.[131] Of note, a phase II trial of PF02341066 in non-small cell lung cancer specifically harbouring a translocation or inversion event of the *ALK* gene is currently ongoing (http://clinicaltrials.gov, NCT00932451), within 3 years of the discovery of EML4-ALK fusion protein[132] – representing an impressive timeline for preclinical target discovery to clinical testing. Other examples where such enrichment strategies have been employed include the inclusion of patients with germline *BRCA* mutations in a phase I trial of a PARP inhibitor,[133] as well as enrichment for basal cell carcinomas – that commonly have mutations in *PTCH1* and *SMO* – in a phase I trial of a hedgehog inhibitor.[134]

Identification of predictive biomarkers for early trial enrichment with pre-defined tumour subtypes is likely to reduce late stage attrition from the drug development pipeline, lowering the financial cost of drug development, but crucially, limiting unnecessary exposure of patients to ineffective drugs.[135,136] The ability to select patients that respond to therapy also has significant health economic implications, and would in theory improve the cost–benefit ratio of modern therapeutics.[137] The integrative genomics approaches discussed in this review provide a rational basis to accelerate biomarker discovery, when conducted in parallel with clinical trials with pre-specified planned analysis of biological material with defined tissue collection protocols for appropriate handling, collection and storage (Fig. 1). More sensitive imaging techniques for monitoring tumour response when assessed in parallel with integrative genomics approaches have the potential to provide further mechanistic insights through measurement of biological consequences, such as tumour blood flow using dynamic contrast enhanced MRI or tumour metabolic activity with PET imaging.[138,139] Developments in the molecular characterisation of single circulating tumour cells may permit a dynamic assessment of tumour behaviour and evolution whilst on treatment in parallel with novel functional imaging approaches, without the need for invasive biopsies in order to annotate the molecular evolution of genetic alterations as drug resistance develops (Fig. 2).[136,140] Stringent consideration for pre-analytical processes, as well as scientific and technical validation, will also enhance the confidence in molecular marker studies, and increase the chance for development of companion predictive biomarker assays for a specific drug.[136]

8. Conclusion

Advances in high-throughput RNAi screening and next generation sequencing strategies may be harnessed to enhance clinical trial design and improve the efficiency of drug and predictive biomarker development. These approaches should optimise patient selection for therapy and reduce patient exposure to ineffective and toxic treatments that may harm patients relative to standard of care, with the goal of improving patient survival and quality of life outcomes. Unravelling the mechanisms of treatment failure and the identification of mediators of drug response and resistance, using such integrative genomics approaches, should promote the discovery of predictive biomarkers to enrich sensitive patient cohorts. As a consequence, new opportunities for therapeutic intervention in drug resistant disease will be identified to support the development of rational drug combinations to limit the acquisition of drug resistance. Ultimately, improved patient selection will reduce the health economic costs associated with ineffective therapy, thereby reducing quality adjusted life year (QALY) costs and accelerating the delivery of effective therapies to more patients with drug sensitive disease.

Conflict of interest statement

None declared.

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