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Applications of personalised signalling network models in precision oncology

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Abstract

As our ability to provide in-depth, patient-specific characterisation of the molecular alterations within tumours rapidly improves, it is becoming apparent that new approaches will be required to leverage the power of this data and derive the full benefit for each individual patient. Systems biology approaches are beginning to emerge within this field as a potential method of incorporating large volumes of network level data and distilling a coherent, clinically-relevant prediction of drug response. However, the initial promise of this developing field is yet to be realised. Here we argue that in order to develop these precise models of individual drug response, and tailor treatment accordingly, we will need to develop mathematical models capable of capturing both the dynamic nature of drug-response signalling networks, and also key patient-specific information such as mutation status or expression profiles. We also review the modelling approaches commonly utilised within this field, and outline recent examples of their use in furthering the application of systems biology for a precision medicine approach to cancer treatment.

List of Abbreviations

BNGL	BioNetGen language
CME	chemical master equation
EGFR	epidermal growth factor receptor
ER	oestrogen receptor
ERK	extracellular signal-regulated kinase
FcεRI	high-affinity immunoglobulin receptor
HER2	human epidermal growth factor receptor 2
MAPK	mitogen-activated protein kinase
MEK	mitogen-activated protein kinase kinase
ODE	ordinary differential equation
PCA	principal component analysis
PDE	partial differential equation
PLSR	partial least squares regression
SSA	stochastic simulation algorithm

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1 Introduction

Precision medicine is an emerging concept in healthcare that places an emphasis on the development of a more targeted approach to the treatment of diseases. Broadly speaking, precision medicine describes the tailoring of treatment strategies to individual patients by replacing generalised diagnostic protocols with new approaches that incorporate patient-specific information such as genetic data, lifestyle details and environmental factors (National Research Council (US) Committee on A Framework for Developing a New Taxonomy of Disease, 2011). While the idea of tailoring treatments to individual patients is well established (Ginsburg & McCarthy, 2001), it has substantially increased in popularity in recent years alongside improvements in the technology required for the successful deployment of precision medicine strategies in the clinic (Jameson & Longo, 2015; McGrath & Gherzi, 2016). This is reflected in the announcement of the Precision Medicine Initiative in the United States (Collins & Varmus, 2015) and the establishment of Genomics England in the United Kingdom (Marx, 2015), which, amongst other state-sponsored precision medicine initiatives, are explicitly designed to bring the idea of precision medicine into fruition (Minari, Brothers, & Morrison, 2018). It is important to note that while the term “personalised medicine” is often used interchangeably with “precision medicine”, its usage has fallen out of favour owing to the implication that medical professionals have previously failed to treat their patients on an individual basis, and as such the term “personalised medicine” is no longer widely used (Konig, Fuchs, Hansen, von Mutius, & Kopp, 2017; McGrath & Gherzi, 2016).

The past two decades have seen astronomical improvements in our ability to generate and process large scale ‘omics data from patient samples. One particularly prominent example of this is The Cancer Genome Atlas, an enormous repository of cancer genomic information that contains over 20,000 primary cancers and matched normal samples from 33 cancer types. This extensive collection of well annotated datasets has generated over 2.5 petabytes of genomic, epigenomic, transcriptomic, metabolomic and proteomic data since its nascence in 2006 (Blum, Wang, & Zenklusen, 2018). Another example is the Cancer Institute’s Clinical Proteomic Tumour Analysis Consortium, which aggregates proteomic and genomic information from tissue samples and clinical data from a wide range of sources, and has accumulated almost 20 terabytes of proteomic data that has been provided to nearly 20,000 individual users. (Ellis, et al., 2013). The full potential of this knowledge has not been

leveraged, however, and its introduction into general patient care is a core element of the appeal of precision medicine (Ashley, 2016).

Currently, many human diseases are treated with a procedure that closely resembles the structure of a flow chart, with a consistent frontline standard of care treatment offered to all patients that receive the same diagnosis, which will then be adjusted based on the progression of their symptoms (Day, Coombes, McGrath-Lone, Schoenborn, & Ward, 2017). Of course, in reality the same disease can manifest itself in a different manner between patients, and as such not all patients will respond to the same treatment. The potential for precision medicine strategies to fully account for these individual molecular intricacies would mark a sizeable step in the evolution of patient healthcare. Furthermore, the research and development of precision medicine approaches would also stimulate a better understanding of the underlying mechanisms by which diseases occur, promoting continued improvements for preventing, diagnosing and treating a wide range of diseases.

2 Precision medicine for the treatment of cancer

As a disease, cancer is characterised by genomic alterations that confer a selective advantage to a subpopulation of cells, leading to the development of neoplasia (Kinzler & Vogelstein, 1996). Cancer can arise in almost any part of the body and has a wide range of different anatomic and molecular types that each require their own particular approach for successful treatment (Hanahan & Weinberg, 2000). An early example of precision medicine taking root in oncology is the treatment of breast cancer. When prescribing therapy to a breast cancer patient, it is standard practice to tailor treatment based upon the tumour expression levels of oestrogen receptor (ER), progesterone receptor and overexpression of the oncogenic human epidermal growth factor receptor 2 (HER2) (Low, Zembutsu, & Nakamura, 2018). The ER-positive, or luminal subtype, cancers usually show good prognoses and often do not require chemotherapy, however in the case of advanced disease they respond most favourably to a combination of chemotherapy and endocrine therapy, such as an aromatase inhibitor (Pritchard, et al., 2013). In contrast, HER2-positive tumours tend to have a less favourable prognosis (Perou, et al., 2000). The standard of care for early-stage HER2-positive breast cancer is chemotherapy plus trastuzumab (HerceptinTM), a humanised monoclonal antibody that targets the extracellular domain of HER2 (Piccart-Gebhart, et al., 2005). While the use of this rationalised treatment approach has greatly improved the survival rates for both ER

("Tamoxifen for early breast cancer: an overview of the randomised trials. Early Breast Cancer Trialists' Collaborative Group," 1998) and HER2-positive (Cameron, et al., 2017) breast cancer patients, it is certainly not a silver bullet for this disease as a whole. Due to an array of potential compensatory and resistance mechanisms, approximately a third of all luminal breast cancer patients show no benefit from combination of endocrine and chemotherapy (Musgrove & Sutherland, 2009). Similarly, for HER2-positive breast cancer, less than half of patients respond to trastuzumab and approximately 25% will relapse within 10 years (Cameron, et al., 2017). Clearly, even within this indisputably successful precision medicine treatment paradigm there is still a need for an increased granularity in the understanding of drug response mechanisms to provide individual treatment options for the patient cohort as a whole.

Another significant advance for precision medicine in oncology has been the treatment of late-stage melanoma. Prior to 2011, melanoma was associated with a 10% 5-year survival rate, as the patient response to standard of care dacarbazine remained below 20% (Cheng, Lopez-Beltran, Massari, MacLennan, & Montironi, 2018; Eggermont & Kirkwood, 2004). However, rapid advances in genomic sequencing technologies in the early 2000s revealed that the oncogenic driver gene mutation *BRAF V600E* is present in approximately half of all patients presenting with metastatic melanoma (Davies, et al., 2002). More recently, lower frequency occurrences of *RAS* mutations (Johnson & Puzanov, 2015) and *NF1* mutations (Kiuru & Busam, 2017) have also been identified, while tumours that lack all three of these mutations are classified as Triple *wild-type* (Cancer Genome Atlas, 2015). This detailed genomic characterisation has led to the modern paradigm of melanoma treatment, where patients are screened for these oncogenic driver mutations to inform the appropriate treatment strategy (Amann, et al., 2017).

While the treatment of each of these categories has been met with mixed success, with the treatment of *RAS* mutant tumours proving to be particularly challenging (Z. Ji, Flaherty, & Tsao, 2012; Johnson & Puzanov, 2015), the development of selective BRAF inhibitors dabrafenib (Falchook, et al., 2012), vemurafenib (Bollag, et al., 2010) has significantly improved prognosis for this particular subtype (Amann, et al., 2017). However, while BRAF inhibition has been shown to be a more effective treatment option than standard chemotherapy, often eliciting an almost complete tumour regression, most patients rapidly

develop resistance to these inhibitors after only 6-8 months (Sullivan & Flaherty, 2013; Villanueva, Vultur, & Herlyn, 2011).

BRAF mutations are known to result in hyper-activation of the contiguous mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) pathway (Solit, et al., 2006). Accordingly, a number of mechanisms of resistance to *BRAF* inhibitors have emerged, including the formation of alternative *RAF* family heterodimers and the compensatory activation of parallel signalling pathways, all ultimately leading to reactivation of MEK/ERK signalling in resistant tumours (Sullivan & Flaherty, 2013; Villanueva, et al., 2011). The identification of these compensatory mechanisms led to attempts to address this resistance through the simultaneous targeting of both *BRAF* and MEK (Dossett, Kudchadkar, & Zager, 2015). Here, the inclusion of MEK inhibitors, such as trametinib, has improved response rates and lowered relapse in melanoma patients when compared to treatment with single-agent *BRAF* inhibitors (Chapman, et al., 2011; Flaherty, et al., 2010). More recently, next-generation inhibitors, such as PLX8394 have also emerged (Yao, et al., 2019). Unlike dabrafenib and vemurafenib, which only target *BRAF* monomers, PLX8394 selectively targets *BRAF* dimers, resulting in increased efficacy by preventing compensatory signalling through *RAF* family heterodimers (Yao, et al., 2019).

While these developments have led to marked improvements in the treatment of metastatic melanoma, there still exists a lack of biomarkers to tailor the optimal therapeutic combination for each individual patient (Pavlick, Fecher, Ascierto, & Sullivan, 2019). Realistically, a sophisticated, mechanistic understanding of the RAS/ERK signalling network will be required in order to establish such biomarkers that are capable of both encapsulating the extensive plasticity of this signalling network, whilst also delivering useful clinical insight. Importantly, a number of recent mathematical modelling approaches have built towards providing this type of advance by integrating both existing knowledge about the structure and dynamics of RAS/ERK signalling network with specific somatic mutations, micro-environmental influences and known drug mechanisms (L. Huang, Jiang, & Chen, 2017; Rukhlenko, et al., 2018; Sun, Bao, & Shao, 2016)

As it stands, precision medicine has presented itself as an incredibly potent tool in the treatment of cancer, and this has brought about a new era for cancer medicine where clinical trials are increasingly designed to investigate the viability of treatments targeting specific

biomarkers or genetic mutations (Fountzilas & Tsimberidou, 2018; Renfro, An, & Mandrekar, 2017). As of 2018, there were 43 FDA-approved small molecule protein kinase inhibitors for use in the treatment of tumours (Roskoski, 2019) and at least 18 monoclonal antibodies for targeted cancer therapies in experimental and clinical trials (Shabani & Hojjat-Farsangi, 2016). However, as these strategies and treatment options become available to clinicians, it is becoming increasingly apparent that the granularity of precision medicine will need to increase in order to see continued improvements in this field (Jackson & Chester, 2015). To achieve these advances, significant improvements in the ability to accurately predict an individual patient's drug response, and tailor treatment accordingly, will be required. This type of conceptual leap will only be possible with mathematical models that are capable of capturing both generally valid biological knowledge about the disease and drug-response mechanisms, and also patient-specific information such as mutation status or expression profiles (Lai, Eberhardt, Schmitz, & Vera, 2019; Saez-Rodriguez & Bluthgen, 2020; Westerhoff & Palsson, 2004; Yadav, Vidal, & Luck, 2020). Therefore, in this review we have focused upon the key systems biology concepts and methods required to achieve these aims, and the recent advances in the application of systems biology towards precision oncology.

3 Systems biology

Systems biology is an interdisciplinary field that takes elements from biology, computer sciences, bioinformatics and physics to develop computational and mathematical models that describe the behaviour of complex biological systems such as intracellular signal transduction networks (Aderem, 2005; Kholodenko, Hancock, & Kolch, 2010). This approach aims to develop a comprehensive understanding of the interactions between the components of biological systems, in contrast to the more traditional reductionist approach, where investigative efforts are focussed on individual components, such as a single protein or gene of interest (Strange, 2005) (Figure 1). It is becoming increasingly apparent that the integrated approach of systems biology is more suited to understanding the pluralism of causal relationships that exist within a biological system and identifying the emergent properties that arise as a result (Kitano, 2002; Kolch, Halasz, Granovskaya, & Kholodenko, 2015).

The potential for systems biology approaches to improve efforts to personalise treatments for cancer patients is a consistent motif throughout the literature relating to this topic (Bhinder &

Elemento, 2017; Filipp, 2017; Frantzi, Bhat, & Latosinska, 2014; Saez-Rodriguez & Bluthgen, 2020). Contrasting with the use of artificial intelligence machine-learning algorithms which can be used to personalised treatments plans based on correlations drawn from large volumes of data, with a systems biology approach we attempt to determine the molecular mechanisms that drive the disease (Fountzilias & Tsimberidou, 2018). This not only promotes an increased understanding of the disease's aetiology, but may also provide valuable insights into the development of future treatment strategies (Frantzi, et al., 2014). However, while routine methods for certain aspects of systems biology are emerging, such as feature extraction (Lin & Chien, 2009) and parameter estimation (Nim, Luo, Clement, White, & Tucker-Kellogg, 2013), the construction of an insightful mathematical model is by no means a straightforward process (Kolch, et al., 2015). Compared to other reviews on this topic, here we aim to provide a comprehensive coverage of the different modelling strategies that are currently being put into use in the field of systems biology.

4 Kinase signalling networks

A prevailing theme within many systems-based approaches to cancer medicine is a focus on protein kinases and their signalling activity, with many examples of signalling network models aimed at identifying improvements in contemporary treatment strategies (Amadoz, Hidalgo, Cubuk, Carbonell-Caballero, & Dopazo, 2018; Azuaje, 2017; Eduati, et al., 2017; Fey, et al., 2015; Gendelman, et al., 2017; Hidalgo, Amadoz, Cubuk, Carbonell-Caballero, & Dopazo, 2018; Y. Hu, Gu, Wang, Huang, & Zou, 2015; Wei, Liu, Zheng, & Li, 2019). This focus on kinase signalling in cancer is partly driven by the observation that protein kinases are the most commonly mutated protein family across all types of cancer (Futreal, et al., 2004; Greenman, et al., 2007), and that cancer 'driver' mutations are known to preferentially cluster within phospho-signalling domains (Fleuren, Zhang, Wu, & Daly, 2016).

As kinases are generally considered the master regulators of cell behaviour, it is perhaps unsurprising that dysregulation of kinase activity is often responsible for driving a cancerous phenotype and that targeting protein kinases is a common therapeutic approach in cancer (Kung, et al., 2013; Weinstein & Joe, 2008). At present, there are a number of clinically available small molecule inhibitors targeting kinases across a variety of cancer types (Roskoski, 2019), however their effectiveness is frequently limited by the development of drug resistance (Dar & Shokat, 2011; J. Zhang, Yang, & Gray, 2009). As outlined above for

the case of BRAF inhibitors, this is largely due to the complex wiring, and highly adaptive nature of kinase signalling networks, which are often able to engage redundant signalling pathways to compensate for the loss of an individual kinase (Trusolino & Bertotti, 2012; Wilson, et al., 2012).

While many oncogenic kinases have been historically characterised by considering signalling events as linear cascades, this conceptual framework ignores much of the inherent complexity of intracellular signalling networks (Kholodenko, et al., 2010). In reality, kinase signalling pathways are not independent linear pipelines with clearly defined inputs and outputs, but are in fact intertwined with each other, resulting in far more complex network structures (Vert & Chory, 2011). These structures often result in features that improve the accuracy and reliability of information flow through a cell than would otherwise be achieved through linear pathways, and allow for nuanced cell-fate decisions in response to multitude of stimuli (Murray & Miller, 2015). The most basic of these features include activation-deactivation cycles (typically involving phosphorylation and dephosphorylation), negative feedback and positive feedback, which depending on the specific context can cause different emerging behaviours, including ultrasensitivity, oscillations, bistability and hysteresis (Table 1). Different arrangements of these signalling components give rise to the characteristic complexity of intracellular signalling networks, resulting in the plasticity and adaptability that underlie their ability to overcome inhibition of an individual network node through compensatory signalling (Logue & Morrison, 2012).

Due to this inherently complexity of signalling networks, which typically contain multiple redundancies and extensive crosstalk, they are virtually impossible to understand intuitively (Ideker & Lauffenburger, 2003; Kolch & Fey, 2017; Wright & Dyson, 2015). However, computational modelling is now emerging as an approach that could rapidly combine existing knowledge of these complex dynamic processes with relevant patient-specific data, in order to generate accurate predictions of drug response within a time frame that would allow for appreciable clinical benefit. Therefore, by considering these broader network structures in an integrated manner using systems biology techniques, it may now possible to account for the inherent plasticity of signalling networks and develop clinically-relevant strategies to accurately tailor therapeutic choices to individual patients and also overcome drug resistance (Eduati, et al., 2020; Fey, et al., 2015; Kolch, et al., 2015). Although, to provide such effective precision medicine approaches, it is likely that different modelling approaches will

be required for different disease and drug contexts. Indeed, within this field, numerous different approaches have already been proposed to address different biological and clinical questions, depending upon the required level of specification, data type and predictive power. These methods, which are described in detail below, range from descriptive statistical data driven approaches to more powerful predictive mechanistic models, each with their own level of complexity, individual strengths and potential caveats (Figure 2).

5 Statistical modelling

Data-driven statistical models describe the relationship between the elements of a signalling network that most accurately reflect available experimental data. This is a relatively recent approach in the field of systems biology that has quickly risen in popularity due to its applicability in the analysis of large datasets generated by high throughput experimental techniques (Djordjevic, Rodic, & Graovac, 2019). By employing an integrated statistical analysis on high-volume, multidimensional “-omics” data, it is possible to create a comparison between the signalling events in normal, healthy cells and the aberrant signalling that occurs in diseased-state cells, thereby identifying the network structures that may promote this diseased phenotype, rather than having to focus on genes individually (Blair, Trichler, & Gaille, 2012), or infer the activity of signalling pathways based on gene expression (Schubert, et al., 2018). The types of models that are developed using this approach are essentially diagrams of signal transduction connections that are often broad in scope but lack the ability to provide detailed mechanistic insight into signal transduction dynamics, as the data used to inform these models typically lack time-related or kinetic information (Halasz, Kholodenko, Kolch, & Santra, 2016). Ultimately, while these models lack the nuance and insight into these structures that would provide the ability to predict their functional properties, they have demonstrated a clear use in their ability to visualise network topologies (Cai, Huang, & Yang, 2018).

High volume -omics data often contain a large number of individual analytes, which makes it difficult to render on a simple coordinate system. In these scenarios, variance reduction methods such as principal component analysis (PCA) and partial least squares regression (PLSR) are often used, due to their ability to display complex, multidimensional data in a more digestible format, facilitating the identification of critical connections between signalling elements within biological systems data. Both of these methods involve

redistributing the available data onto a new coordinate system, weighting the inputs in a manner that most efficiently describes the variance within the dataset (Dunn III, Scott, & Glen, 1990). The key difference between the two is that PCA transposes the input values onto a set of axes that emphasise the presence of any linear correlations in the dataset, whereas PLSR is performed on data that have been organised into discrete categories, and transposes these values onto a set of axes that best describe the variance between these categories. As a result of this, PLSR is typically preferred in cases where a strong correlation between the independent and dependent variables are known to exist (Figure 3) (Tu, Kramer, & Lee, 2012; Yi, et al., 2015). Both methods are important tools for the analysis of large datasets generated by high-throughput experimental techniques and can be used to extract associations between network nodes that would not be able to be derived from the raw data, thereby identifying elements of the dataset that require further scrutiny. This has some powerful applications, and due to their effectiveness and relative simplicity, PCA and PLSR are the *de facto* standards for dimensional reduction of large scale data in the field of molecular biology, which includes their implementations as tools of systems biology (Harada-Shoji, et al., 2019; Rajalahti & Kvalheim, 2011; Yi, et al., 2015).

An example of the utility of this type of approach is provided by Janes *et al.* in a seminal 2005 study which demonstrated that, given the appropriate input parameters, a data-driven statistical model can accurately predict cellular signalling outputs. In this study, a model of cytokine-mediated apoptosis was constructed based on a stress-apoptosis axis and a survival axis that were defined through PLSR, following extensive measurements of the apoptotic response to various cytokine stimuli (Janes, et al., 2005). Furthermore, not only did this model demonstrate the capability of predicting apoptosis as an output of these signalling pathways, but it could potentially be used to predict chemokine release, gene expression and drug sensitivity (Miller-Jensen, Janes, Brugge, & Lauffenburger, 2007).

Statistical modelling approaches have also been shown to predict phospho-protein signalling network structures within a heterogeneous population of cells. One key example of the application of this modelling strategy is provided by a 2009 study that described Ephrin-mediated cell sorting. The authors of this study employed a specialised algorithm that used statistical information to calculate the probability of an association between the amino acid motifs flanking key phosphorylation sites and either the kinases responsible for performing phosphorylation at that site, or a cognate phospho-binding domain (Linding, et al., 2007).

Using this strategy, the researchers were able to simultaneously predict the structures of the kinase networks involved in both the Ephrin receptor and ligand expressing cell populations (Jorgensen, et al., 2009).

Both studies outlined above demonstrate the ability to empirically reduce even the most complex of biological systems to a far simpler computational model that still captures the emergent properties of the system. The use of statistical models provides an opportunity to capitalise on our ability to generate large volumes of data, with which other analytical methods have not been able to keep pace. This can also be done graphically, such as through the use of a probabilistic graphical model (Blair, et al., 2012). In this approach, the network structure is determined and then separated into smaller sections that are translated into a product of conditional probabilities. This approach allows the integration of a wide variety of diverse data types and a great degree of flexibility in how this information is displayed.

Statistical modelling may also be used as a tool for predicting patient outcomes such as survival time or time to cancer relapse. The Cox proportional hazard regression is the most widely approach for this of style of survival analysis in medical research, largely due to the fact that its application relies on fewer assumptions regarding the features of the system in question (Abadi, et al., 2014). In brief, the Cox regression analysis determines the relationship between risk factors (such as age, behaviour, mutational, *etc.*) and patient outcomes based on a comparison of outputs from the hazard function for the system (George, Seals, & Aban, 2014). Overall, however, while this style of approach has proven effective at predicting cohort outcomes, it is difficult to apply to the outcomes of an individual patient (Matsuo, et al., 2019).

As computational resources improve, systems biologists have increasingly focused on the development of models that capitalise on high volume datasets of genomic, transcriptomic, metabolomic and proteomic information that have become increasingly readily available. While they are powerful in a research setting for investigating network components that have not been previously evaluated, the clinical potential of these models to predict individual patient outcomes is limited, as the data often do not contain sufficient information to reconstruct functional network structures, and can therefore be quite error prone. However, this type of data driven computational modelling has proven to be a very important tool for deepening our understanding of how cells process information, and has even produced

examples of clear clinical applications (Halsey, Yang, Walker, Hogenesch, & Thomas, 2007; Kastrup, Runyon, Shen, & Ismagilov, 2006; Kumar, Srikanth, Ahlfors, Lahesmaa, & Rao, 2007; Ma'ayan, Gardiner, & Iyengar, 2006; Oda & Kitano, 2006).

6 Logic modelling

In a logic model, the variables are represented as discrete integers that are determined by the results of logical statements that form connections between the model components (Morris, Saez-Rodriguez, Sorger, & Lauffenburger, 2010; Saez-Rodriguez, et al., 2009). Typically, time is not represented in these models and instead the system state is evaluated in discrete steps that are not necessarily of a uniform duration (Le Novere, 2015). The key advantage of these types of models lies in their versatility; variables can be used to represent virtually anything, and individual elements can be described by multiple variables for the purpose of improving model granularity (Le Novere, 2015).

By far the most frequently used variant of logic modelling is the construction of Boolean networks (Tyson, Laomettachit, & Kraikivski, 2019; Wang, Saadatpour, & Albert, 2012). In these models, variables are designated as “ON” or “OFF” and are evaluated at each time step by logical expressions using Boolean operators (OR, AND and NOT), as the name would suggest (Helikar, Kochi, Konvalina, & Rogers, 2011). These models are relatively easy to construct and are particularly effective at providing qualitative information about systems where experimental data is scarce (Wang & Albert, 2011). This approach has found applications in modelling regulatory signalling networks in a variety of biological systems, such as mammalian cortical development (Giacomantonio & Goodhill, 2010), T-cell receptor signalling (Saez-Rodriguez, et al., 2007) and epidermal growth factor receptor (EGFR) signalling (Samaga, Saez-Rodriguez, Alexopoulos, Sorger, & Klamt, 2009), and in the analyses of signalling network associated with diseases to predict pathogenesis and potential therapeutic targets (Terfve, Wilkes, Casado, Cutillas, & Saez-Rodriguez, 2015), such as in small GTPase crosstalk (Hetmanski, Zindy, Schwartz, & Caswell, 2016), hepatocyte signal transduction (Saez-Rodriguez, et al., 2011; Schlatter, et al., 2012), survival signalling in leukaemia (R. Zhang, et al., 2008) and the development of eosinophil-derived interleukin-13 mediated development of allergic airway responses (Walsh, et al., 2011).

While logic models have contributed to our understanding of the signalling events that control cell fate in cancer (Calzone, et al., 2010; Grieco, et al., 2013), their specific

characteristics generally preclude them from direct clinical applications such as predicting patient responses. Most critically, the qualitative nature of these models and the lack of a representation of time greatly increase the challenge of capturing the inherently quantitative properties of signal transduction networks.

7 Rule based modelling

In the context of systems biology, the term “rule-based model” refers to computational models that describe the biomolecular dynamics of a given system through the use of a set of locally defined rules (Hlavacek, et al., 2006; Maus, Rybacki, & Uhrmacher, 2011). These models represent a level of specification a degree higher than that of statistical models, without requiring the quantification of the system’s kinetics (Meier-Schellersheim, Fraser, & Klauschen, 2009). They are most applicable in the description of systems that can be deconstructed into modular components and are therefore well-suited to the analysis of intracellular signalling networks.

The key advantage of rule-based modelling lies in the ability to reduce the complexity of systems and describe their dynamics using simple terms. Intracellular signal transduction networks generally owe their complexity to the vast number of interacting molecular species they encompass, rather than containing large number of different process types (Mayer, Blinov, & Loew, 2009). Kinase signalling networks, for instance, are notorious for their complexity and plasticity, yet the behaviour of their components is almost entirely controlled by phosphorylation/dephosphorylation cycles and protein binding interactions. Rule-based modelling is therefore an effective means of accounting for this type of combinatorial complexity when modelling these systems (Bachman & Sorger, 2011).

Another significant advantage of rule-based models is that they tend to be comparatively simple in their implementation and do not require extensive formal training in order to be constructed (Liu & Gunawan, 2017). Rules can be described using highly specialised model-specification languages that are considerably easier to learn than general purpose programming languages (*e.g.* Python), although will still benefit from some basic knowledge of programming (Nagarajan & Upreti, 2017). The most prevalent form of model-specification language is BioNetGen language (BNGL), which has been used to accurately describe a large variety of different intracellular signal transduction networks, such as EGFR signalling (Blinov, Faeder, Goldstein, & Hlavacek, 2006) and toll-like receptor 4 (TLR4) signalling (An

& Faeder, 2009), amongst others (Chylek, Harris, Faeder, & Hlavacek, 2015; Colvin, et al., 2010). A pair of key studies by Goldstein et al. and Faeder et al. demonstrate the utility of this approach through the construction of a rule-based model for high-affinity immunoglobulin receptor (Fc ϵ RI) signalling described with BNGL. The initial model used a conserved set of rules that were able to capture the mechanisms of Fc ϵ RI signalling cascade reactions, highlighting the potential of BNGL rule-based modelling to provide simplified representations of intracellular signalling dynamics (Goldstein, et al., 2002). Upon revisiting the model and supplementing it with additional information from available literature, the model was able to predict how the bivalent properties of an Fc ϵ RI-specific ligand affect both Fc ϵ RI crosslinking and downstream signalling events (Faeder, et al., 2003). This showcases a specific advantage of using model-specification languages to describe rule-based models: existing models can be relatively easily improved upon using other models constructed with the same language. The use of BNGL in this manner has been facilitated by the development of additional software tools such as BioNetGen (Harris, et al., 2016), BioNetFit (Thomas, et al., 2016), RuleBuilder (B. Hu, Matthew Fricke, Faeder, Posner, & Hlavacek, 2009), RuleBender (Smith, Xu, Sun, Faeder, & Marai, 2012; Xu, Smith, Faeder, & Marai, 2011) and MOSBIE (Wenskovitch, Harris, Tapia, Faeder, & Marai, 2014), amongst others (Colvin, et al., 2009; Colvin, et al., 2010; Sneddon, Faeder, & Emonet, 2011; Thomas, et al., 2016).

The continued development of programming languages to foster the creation of rules-based models has increased the number of these types of models dramatically in recent years, and the flexible nature that these types of models possess does make them an attractive tool for deployment in the clinic (Tsigkinopoulou, Hawari, Uttley, & Breitling, 2018), as they can be developed to allow alterations in network output without requiring expertise in the mathematical framework that drives them.

8 Mechanistic models

By encapsulating the spatiotemporal aspects of intracellular signalling that are typically ignored elsewhere, such as reaction rates, diffusion gradients and the effects of noise on signal transduction, mechanistic models aim to provide a more comprehensive recreation of system dynamics. These models typically require extensive knowledge of a signalling network's components and structure in addition to data provided by dedicated experiments for their successful construction (Kolch, et al., 2015). As a result, mechanistic models

represent the most accurate *in silico* recreations of biological systems and have provided some key insights into intracellular signalling behaviours, such as the how emergent system properties (*e.g.* ultrasensitivity and activity pulses) allow cells to maintain robust decision-making processes in chaotic environments (Halasz, et al., 2016).

Deterministic models

There are many different formalisms that can be used to express mechanistic models (Machado, et al., 2011), but by far the most prevalent form is the approach based on ordinary differential equations (ODEs), due to their convenience, effectiveness and predictive potential (Degasperi, Fey, & Kholodenko, 2017; S.-Y. Shin & Nguyen, 2017). ODE-based models fall within the category of deterministic mechanistic models, where the dependent variables are determined as individual values neglecting potential spatial and stochastic effects. An ODE is an equation that relates a number of functions containing a single independent variable, usually time, to the derivatives of these functions (Machado, et al., 2011). In a model of a biological system these equations could express how protein expression, protein activation or the formation of protein complexes occurs over time. The standard approach in developing an ODE-based model is to create a series of time-dependent ordinary differential equations that describe reactions that occur within the system that assume mass action kinetics or an enzymatic reaction law such as Michaelis-Menten kinetics or the Hill function (S.-Y. Shin & Nguyen, 2017). This allows the dynamics of the system to be expressed, showing how the state of each network component evolves over time.

Current understanding of mitogen-activated protein kinase (MAPK) signalling dynamics is largely due to the development of ODE-based models of this pathway, highlighting the value of this tool (S.-Y. Shin & Nguyen, 2017). A seminal contribution to the field of systems biology was a 1996 study conducted by Huang and Ferrell (C. Y. Huang & Ferrell, 1996) that used ODEs and mass-action kinetics to characterise the ultrasensitive properties of the MAPK cascade. A feature of the MAPK cascade is that proteins situated further down the cascade are considerably less sensitive to small stimuli and more sensitive to large stimuli than the proteins directly upstream. By constructing kinetic rate equations to describe the reactions of MAPK cascade constituents, researchers were able to determine that this feature arose as a result of emergent properties of the MAPK cascade, rather than inherent differences in enzyme dynamics between the proteins. Subsequent ODE-driven models of

MAPK signalling have also demonstrated how fluctuations in EGF and nerve growth factor stimulations affect cell fate decision-making (Brightman & Fell, 2000; Ryu, et al., 2015; Santos, Verveer, & Bastiaens, 2007) and how the characteristic oscillations of the ERK cascade are controlled through ERK-SOS/Grb2 negative feedback and ERK-Raf kinase inhibitor protein positive feedback (S. Y. Shin, et al., 2009). Even more recent models of this network have incorporated adjacent signalling structures such as PI3K and Wnt cascades in order to provide further insight into the dynamics of this system (Borisov, et al., 2009; S. Y. Shin, et al., 2010).

While ODE-based models are capable of producing very accurate and meaningful results, they are not suitable for systems where spatial effects cannot be neglected. In these cases, a popular strategy in the development of deterministic mechanistic models is to construct the model using partial differential equations (PDE). Unlike ODEs, PDEs allow for more than one independent variable within a system. As a consequence, PDE-based models can be used to account for both the temporal and spatial distribution of network components (Materi & Wishart, 2007). This advantage comes at the cost of a significant increase in complexity and computational cost, and the effort required to develop these models often outweighs the potential benefits. Despite this, PDEs have been used to great effect in modelling certain aspects of cancer, including tumour growth (Castro, Molina-Paris, & Deisboeck, 2005; Khain & Sander, 2006; Marciniak-Czochra & Kimmel, 2007) and the interactions between solid tumours and the immune system (Matzavinos, Chaplain, & Kuznetsov, 2004).

Stochastic models

Stochastic models are another type of mechanistic model that, unlike other modelling approaches, are developed to account for the inherent randomness that exists at all levels of biological systems. Whereas deterministic models report the variables as individual values, stochastic models define a probability distribution for each variable. This is particularly important when modelling signalling networks with low population levels of molecular species, the effect of noise on the output response becomes far more significant.

Stochastic models are commonly used in the simulation of the dynamics of chemical reaction systems and are a powerful tool to describe biochemical network dynamics at the level of single cells. For example, a stochastic model of the p53-mouse double minute 2 homolog (MDM2) axis was previously used to describe how p53 levels oscillate over time in response

to radiation-induced DNA damage, indicating the presence of a negative feedback structure (Wilkinson, 2009). Discovering underlying network structures such as these will likely be an imperative for the future development of predictive models of system behaviour in the context of drug response.

One method of stochastic modelling is the use of a chemical master equation (CME), which is an application of Markov jump processes, modelling a system as a probabilistic set of states at each time point, and describing the probability of a system existing in a certain state and jumping into another state at any given time (Hahl & Kremling, 2016). Often when modelling a biological system, an assumption is made that the system is well-mixed, in thermal equilibrium and consisting of randomly distributed molecules. This in turn leads to the assertion that the probability of every molecular interaction depends only on the current state of the system, and that the progression of the system can therefore be modelled as a set of Markov processes (Bardini, Politano, Benso, & Di Carlo, 2017). By deriving and solving a CME it is possible to observe the complete distribution of all possible states the system could enter at any arbitrary time point. However, deriving such an analytical solution is usually not possible due to the nonlinear nature and inherent complexity of most biological systems. Analytical solutions restricted to simple systems with linear reaction laws have limited feasibility for clinical applications, and for this purpose less computationally expensive methods must be developed.

One such method is the stochastic simulation algorithm (SSA), otherwise known as the Gillespie method, which has proven to be the most effective and widespread approach to stochastic modelling in biology (Tyson, et al., 2019). The SSA was proposed to address problems with the CME method, namely the inability to provide analytical solutions to any but the simplest systems (Albert, 2016; Bardini, et al., 2017). By simulating the CME using random numbers, the SSA can provide a statistically complete probability distribution of systems states, provided a sufficient number of iterations have been performed. As such it has seen a number of applications in medical research, including the simulation of Hox gene regulatory mechanisms, lacZ gene expression and transcription initiation (Meng, Somani, & Dhar, 2004). Despite this success, these types of simulations will probably not be deployed in the clinic, as they remain computationally expensive, which is further compounded as the number of reactions and molecular species increase.

Both the CME and the SSA approaches are examples of discrete-state stochastic simulation. Unlike spatial stochastic methods, the discrete-state approach does not track the position and momentum of individual molecules, sacrificing some granularity of the simulation in order to greatly improve computational efficiency. These types of models are capable of simulating the activity of molecular populations over large timeframes, while still considering the variables as being comprised of discrete units, thereby allowing the simulation to provide a detailed representation of network activity that also shows the effect of signal noise on the system (Szekely & Burrage, 2014). However, it is ultimately difficult to develop a stochastic model of a dynamic signalling network with the expectation of direct clinical benefit, as despite their stated advantages, they are far too computationally resource intensive and require too large a volume of information to be practical. Regardless, they remain a powerful tool for the investigation of biological systems and will eventually become an avenue for future clinical use as computational capabilities improve over time, alongside our ability to generate large volumes of clinical data (de la Cruz, Guerrero, Calvo, & Alarcon, 2017; Waldherr, 2018).

9 Applications of systems medicine in cancer precision medicine

While systems biology is emerging as an exciting prospect in modern medicine, this approach is considered to be relatively esoteric at present (Henney & Superti-Furga, 2008), with the majority of successful attempts to bring systems biology to the clinic being conducted through drug development or clinical trial design, rather than individualised patient treatment (Stephanou, Fanchon, Innominato, & Ballesta, 2018). Perhaps the most notable early successful implementation of systems concepts in medicine have arisen from outside the field of oncology; including the computational modelling of ion channels in the heart in order to determine the method of action of the sodium channel inhibitor ranolazine, which at the time was poorly understood (Cho, Labow, Reinhardt, van Oostrum, & Peitsch, 2006). In this study, the researchers constructed an ODE-based model to simulate the electrophysiology of the Purkinje fibres, which was then used to explore the effects of ranolazine on this system (Bottino, et al., 2006). This research directly contributed to the US Food and Drug Administration's decision to approve the use of ranolazine for the treatment of chronic angina (Fredj, Sampson, Liu, & Kass, 2006). Another success story for the use of systems biology in the field of drug discovery comes from the treatment of degenerative joint disease. Through the use of Markov process based simulations, alongside other modelling strategies, it was

predicted that novel compounds such as the CD20 antibody rituximab, would be more effective at reducing bone erosion in patients than standard-of-care TNF inhibitors (Kielhorn, Porter, Diamantopoulos, & Lewis, 2008; Lindgren, Geborek, & Kobelt, 2009; Merkesdal, et al., 2010; Vera-Llonch, et al., 2008). This discovery ultimately led to the UK National Institute for Health and Care Excellence to revise their treatment guidelines for severe joint disease (Malottki, et al., 2011).

Such paradigm shifting studies have yet to occur within the field of oncology, although as outlined below, significant effort is being placed into studies that aim to either improve our understanding of tumour biology or develop more accurate methods to guide rationalised precision medicine approaches for cancer patients.

Genetic Instability

Systems-based approaches have been indeed applied to a variety of problems in the field of cancer biology, with a notable example being the investigation of the degree to which genetic instability contributes to the development of cancer. Numerous studies have employed a wide variety of strategies, including ODE-based approaches (Heng, et al., 2006; Spencer, Berryman, Garcia, & Abbott, 2004), probabilistic computer modelling (Nowak, Michor, Komarova, & Iwasa, 2004; Stringer, et al., 2005) and stochastic simulation (Spencer, Gerety, Pienta, & Forrest, 2006) to explore the impact genetic instability has on tumour progression in comparison to other classical traits of cancer such as avoidance of apoptosis, increased growth rate and increased angiogenesis. These studies came to the general consensus that genetic instability was important in the development of late stage tumours, as it fostered the acquisition of survival advantages in cancer cells (Materi & Wishart, 2007). When Hanahan and Weinberg revisited their landmark paper in 2011, genetic instability was included as an enabling characteristic of the hallmarks of cancer, potentially as a result of the findings of these studies (Hanahan & Weinberg, 2011).

Understanding Tumour Growth

Tumour growth is another prominent example of an area of cancer biology that systems-based approaches have improved our understanding. Early researchers interpreted the sigmoidal growth pattern that is commonly observed of tumours to be a consequence of the dominant role surrounding growth inhibitors (Cox, Woodbury, & Myers, 1980), however

later studies that constructed basic PDE models determined that this hypothesis could not account for the invasive branching morphology observed in tumours and showed instead that a supply of autocrine stimulatory factors more closely resembled these spatial characteristics (Castro, et al., 2005). In particular, epidermal growth factor (EGF) was identified as one of the key determinants of tumour morphology, and by incorporating it into earlier models of tumour growth a more accurate recreation of the morphological characteristics of tumours was observed (Bajzer & Vuk-Pavlovic, 2005). EGFR has long been considered to be a strong prognostic indicator of tumour malignancy, and given the implication of EGF in tumour growth, the relationship between EGFR dynamics and cancer proliferation has been thoroughly explored through various modelling strategies (Athale & Deisboeck, 2006; Mansury, Kimura, Lobo, & Deisboeck, 2002; Mayawala, Vlachos, & Edwards, 2005). Similar PDE based modelling approaches have also been used to characterise the growth and development of lung adenocarcinomas in alveolar epithelia (Marciniak-Czochra & Kimmel, 2007), and ODE simulations of the effect of vascular remodelling and angiogenesis on early stage tumour growth (Arakelyan, Vainstein, & Agur, 2002). Yet another study presented a multiscale mathematical model of how tumour progression and invasiveness changes in accordance with the tumour microenvironment. In an unexpected result, the study found that harsh tumour environmental conditions such as hypoxia or a heterogeneous extracellular matrix were strongly associated with increased tumour invasiveness and aggression, whereas a milder tumour microenvironment led to tumours with larger variety of sub-clones that formed a less invasive heterogeneous tumour (Anderson, Weaver, Cummings, & Quaranta, 2006).

From a mechanistic viewpoint, there are many examples of ODE models designed to dissect a number of growth promoting signalling pathways, including JAK1/2-STAT1 signalling (Gambin, Charzynska, Ellert-Miklaszewska, & Rybinski, 2013), EGFR/ERK signalling pathway (Orton, et al., 2009) and NF- κ B family proteins (Hoffmann, Levchenko, Scott, & Baltimore, 2002), amongst others (Aldridge, Burke, Lauffenburger, & Sorger, 2006; Di Camillo, Carlon, Eduati, & Toffolo, 2016). These many studies demonstrate the benefit of the systems biology approach. By performing an unbiased simulation of tumour biology, a well devised model is capable of informing researchers of counter-intuitive network characteristics and behaviours that may otherwise not be immediately apparent.

Optimising Treatment

Systems biology and computational modelling also possess immense potential as part of the preclinical screening process in order to determine the efficacy of available therapeutic options prior to commencing treatment (Faratian, et al., 2009; Faratian, Moodie, Harrison, & Goryanin, 2007; Jarrett, et al., 2018; Reeh, et al., 2019). In the past decade, numerous studies have emerged demonstrating the ability of unbiased systems biology analysis to take into consideration the plurality of interactions between oncogenic mutations and chemotherapy drugs, thereby obviating the need for trial and error in optimising treatments for cancer patients.

In an early example of this application, researchers constructed a statistical model that described signal transduction pathways and common oncogenic events in triple negative breast cancer (Lee, et al., 2012). Analysis of the model's behaviour indicated that a sequential application of anticancer agents would improve sensitivity and reduce the risk of acquired resistance by creating aberrations in oncogenic signalling pathways, an effect that would not be observed if the same agents were administered simultaneously. This concept was then demonstrated in practice, revealing that targeting EGFR with the kinase inhibitor erlotinib prior to treatment with doxorubicin enhanced sensitivity in 9 out of 10 TNBC cell lines. (Lee, et al., 2012). This approach has also been successful in HER2 positive breast cancer, where researchers profiled both basal signalling and pathway activation following stimulation with 22 growth factors and cytokines, then used PLSR to construct models that predicted the efficacy of therapeutics targeting these pathways. This study was able to determine the most effective ErbB-targeted therapy based on simulated GI_{50} values for individual HER2 positive breast cancer cell lines from a panel that included lapatinib, afatinib, gefitinib, AG1478 and erlotinib (Niepel, et al., 2013).

Systems biology approaches are also often an effective means of improving patient stratification and tailoring treatments to the resulting subgroups. A pertinent example of this is again in the field of HER2-positive breast cancer. In addition to the study outlined above, Kirouac *et al.* have further demonstrated this concept through the development of a computational model driven by quantitative logic (an extension of Boolean logic) that predicted molecular mechanisms of resistance to individual therapeutics. Using this model, they identified elevated ErbB3-heregulin signalling as an indicator of resistance towards

ErbB2-targeted therapies, and that a combination of trastuzumab, lapatinib and the ErbB3 inhibitor MM-111 was a more effective treatment (Kirouac, et al., 2013).

Improving patient stratification in this manner is an important clinical tool, as rarer cancer subtypes often do not respond to conventional treatment options. For instance, dedifferentiated liposarcoma is a subtype of liposarcoma that frequently resists chemotherapy. By using an ODE-based modelling approach, however, systems biologists have identified CDK4 and IGF1R as synergistic drug targets in this subtype and demonstrated that targeting these proteins in tandem results in an increase in treatment efficacy (Miller, et al., 2013). The potential for systems biology approaches to predict drug synergy in this manner is another key element of their appeal, and a further example of this is an ODE-based model of the EGFR/ERK pathway, which through *in silico* simulation of drug response dynamics and cellular outputs, was able to highlight the synergistic effects of several targeted inhibitor combinations in a variety of cancer types. Of particular interest was this model's ability to recapitulate experimental values for the synergistic effects of combination therapy targeting EGFR/BRAF, BRAF/MEK, FTI/MEK and FTI/RAF in colorectal and lung cancer cells (L. Huang, et al., 2017).

Through dynamic pathway modelling of kinase signalling networks, systems biology studies have also shown that the effectiveness of some cancer therapies can be predicted based upon protein abundance levels in the cancer cells. Specifically, it has been shown that the efficacy of targeting Akt and ERK pathway components (such as through the use of AKTVIII, U0126, BID1870 or rapamycin) were largely dependent on the abundance of signalling components within these systems (Adlung, et al., 2017). Finally, mathematical modelling of chronic myeloid leukaemia has been used to determine how a cancer cell population is affected by molecularly targeted therapies, such as imatinib, assessing their ability to eradicate leukaemic stem cells and providing quantitative insight into the dynamics of relapse following chemoresistance (Michor, et al., 2005).

Predicting Patient Response

While they have yet to break ground in the clinic, there are still many convincing examples of mechanistic, signalling network models being used to predict patient outcome on a case by case basis. Breast cancer medicine is an area that would greatly benefit from increased personalisation, as acquired resistance to targeted therapies remains a frequently observed

issue in the clinic (X. Ji, et al., 2019). A 2009 study by Faratian et al. highlighted the importance of PTEN in breast cancer aetiology by illustrating that resistance to trastuzumab can be predicted through the mathematical modelling of PI3K and MAPK signalling structures parameterised to reflect the proteomics of various different cell lines (Faratian, et al., 2009). This study further demonstrated that this model could successfully stratify the patient response to trastuzumab, providing the first successful example of systems biology concepts being used to personalise patient therapy. Another example of the implementation of a systems medicine approach is provided by Chen et al. in their 2014 study on endocrine therapy in breast cancer (Chen, et al., 2014). Chen et al. had previously utilised stochastic differential equations to simulate the transition of breast cancer cells through various states of resistance to endocrine therapy, based upon interactions between the oestrogen receptor pathway and the growth factor receptor pathways (Chen, Baumann, Clarke, & Tyson, 2013). This initial model was able to successfully predict the variance in response of different subclones within a breast cancer cell population to various endocrine therapies, based on their expression levels of HER2 and EGFR. In the subsequent study, the model was further refined to be able to simulate the effects of continuous and intermittent treatment regimens and was able to simulate how different treatment strategies could be used to reduce or reverse acquired resistance during therapy, providing an example of how systems medicine can theoretically be used to tailor treatment strategies to individual patients (Chen, et al., 2014).

Halasz et al. provide a definitive demonstration of the potential of mechanistic model approaches to predict response to colorectal cancer therapies in their 2016 study (Halasz, et al., 2016). The study described the construction of an ODE-based computational framework of molecular interactions in the EGFR and IGF1R pathways that incorporated a perturbation dataset containing common alterations observed in colorectal cancer. Using this model, they were able to predict cell-line specific network rewiring and were able to determine that colorectal cancer cell lines which displayed greater feedback inhibition of IRS1 by P70S6K were more prone to developing resistance to EGFR inhibition. Furthermore, by disrupting this feedback the sensitivity of colorectal cancer cells to treatment with the EGFR inhibitor lapatinib could be restored (Halasz, et al., 2016). Ultimately this study showed that by using cell line specific data to reconstruct the non-linear structure of a cancerous signalling network, it was possible to predict a more ideal therapeutic approach using a concept that could be readily translated to a clinical setting.

Another example of the application of a system based approach to colorectal cancer is provided by Lindner et al (Lindner, et al., 2013). In this study, an ODE-based network model of BCL2 family proteins was created in order to explore apoptotic desensitisation in colorectal cancer cells. By using this model to investigate how BCL2 family interactions process varying levels of chemotherapeutic stress, the research team were able to establish the predictive power of their systems model approach. Furthermore, by incorporating protein profiles of matched tumour and normal tissue samples derived from colorectal cancer patients, the model was able to accurately differentiate these patients based on their clinical response (Lindner, et al., 2013). More recently, the authors built upon this model to calculate apoptosis kinetics using the input proteins Apaf-1, XIAP, SMAC and Procaspase-3. Using this model, the researchers were able to develop panels of combinatorial biomarkers that were able to more effectively stratify stage III colorectal cancer patients by overall risk and response to 5-FU-based chemotherapy than single biomarkers (Salvucci, et al., 2017).

A 2015 study by Fey *et al.* also provided a very clear demonstration of the predictive capacity of mechanistic network models and demonstrated how such a model could be translated into the clinic (Fey, et al., 2015). This investigation detailed the construction of a rule-based model of the apoptotic JNK signalling network and applied it in the context of neuroblastoma. By incorporating expression data from each patient as parameters within the model, they generated patient-specific simulations of chemotherapy-induced JNK activation. Survival analysis based upon these simulations then demonstrated that an inability to activate JNK signalling *in silico* was an independent predictor of poor overall survival for these neuroblastoma patients. Clearly, as the sophistication of these models increase, the benefit of incorporating them into clinical pre-screening processes continues to grow. The challenge now remains to fully utilise these types models in order to draw clinically-actionable insights and guide alternative therapeutic options for each individual patient.

10 Conclusions

While systems medicine has only offered modest successes in the clinic at this juncture, there is a significant push for its development amongst researchers, particularly in the field of cancer medicine, where it could potentially resolve a number of pertinent contemporary issues. A prevailing trend in bringing a systems based approach to cancer medicine is the derivation of mathematical descriptions of molecular states within a drug-response signalling

network using reaction kinetic laws, and simulating them in *silico*. While these models can vary significantly in scope, they are typically constructed using ODEs or rule-based approaches. This mechanistic model-based approach is preferred in this scenario over data-driven statistical networks as they provide considerably more insight into functional properties such as temporal and spatial dynamics, the difference in effects of pulsed or continuous treatments or the combinatorial effects of targeting a single molecule with multiple different agents (Fitzgerald, Schoeberl, Nielsen, & Sorger, 2006; Halasz, et al., 2016). Data-driven models, while an effective tool for visualising network topologies or uncovering statistical correlations, typically lack this capacity and are therefore less suited to be implemented into a precision medicine application (Halasz, et al., 2016).

However, the drawback associated with these mechanistic model-based approaches is their inherent complexity and the amount of information required to correctly parameterise these models. This complexity will only increase once added layers of information, such as patient-specific mutation status or expression profiles, are included as parameters within these models. Therefore, while these approaches are considered the best candidates for generating accurate predictions of drug response for each individual patient, in order to realise the full benefit of this truly personalised, systems biology approach to cancer medicine, a balance will need to be found between model complexity, computational requirements and ease of use in clinical scenarios. The challenge associated with meeting these needs and developing a clinically-relevant, modelling-based biomarker will not be insignificant, although the benefits of predicting the patient response prior to treatment will be a hugely significant advance in the field of oncology.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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Figure Legends

Figure 1: Reductionist paradigm vs. systems biology. In the reductionist approach the focus lies on the components of a system without considering context, and as such the spatiotemporal aspects of the system are not accounted for. Systems biology addresses the shortcomings of this approach by considering these components as part of a larger network context.

Figure 2: Examples of common types of modelling strategies. Modelling strategies will differ based on several factors, including their level of specification, data requirements and predictive power. Typically, mechanistic models will be highly specified and will require dedicated experimental design but will have high predictive power. Conversely, experiments for more abstracted data-driven models will be easier to conduct but generally require high volumes of data and lack the same degree of descriptive power. Images adapted from Fey et al., 2015 (Fey, et al., 2015).

Figure 3: Comparison of supervised vs. unsupervised statistical modelling. Methods of dimensionality reduction such as PCA and PLSR use statistical processing techniques to transpose the raw data onto a new set of axes that capture a greater degree of existing variance. Supervised methods, such as PLSR, use data that has been classified into discrete categories and use the annotated label to better describe the maximum variance between the datasets. Unsupervised methods, such as PCA, are best suited for deriving linear correlations between the features of the data.

Table 1

Network Property	Example network structure	Function outputs	Description	Refs
Negative feedback			<p>A feedback system wherein some function of the output inhibits activity of the system.</p> <p>This has an attenuating effect on the system, dampening the effects of perturbations and assisting the system in reaching equilibrium, but depending on parameter can also lead to oscillations. Negative feedback loops are common in the human body as they can maintain a</p>	(Nguyen & Kholodenko, 2016)
Positive feedback			<p>A feedback system wherein some function of the output increases activity of the system.</p> <p>The resulting amplification of signal can lock the output into a high activity state. Positive feedback loops frequently act as analogue to digital converters in cellular signalling networks</p>	(Nguyen & Kholodenko, 2016)
Activation-deactivation cycle			<p>A network feature where a signalling component alternates between two states <i>e.g.</i> phosphorylated and dephosphorylated.</p> <p>The presence of an activation-deactivation cycle can cause ultrasensitivity and in combination with feedback result in various complex behaviours, including oscillations, toggle switches and excitable behaviour</p>	(Kaimachnikov & Kholodenko, 2009)

Figure 1

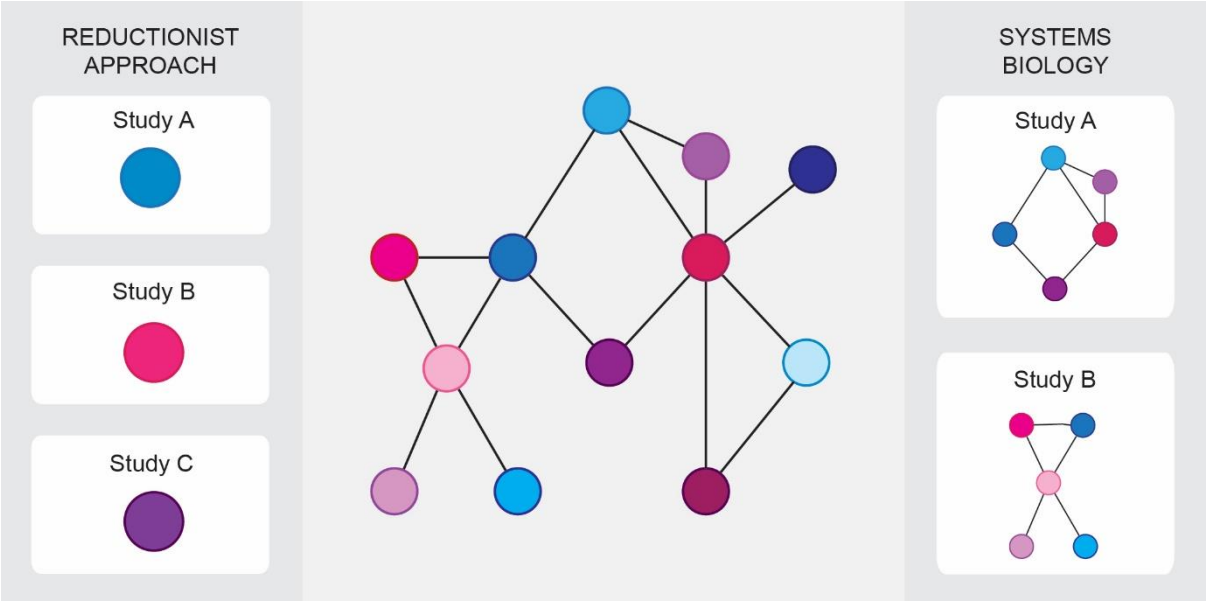


Figure 2

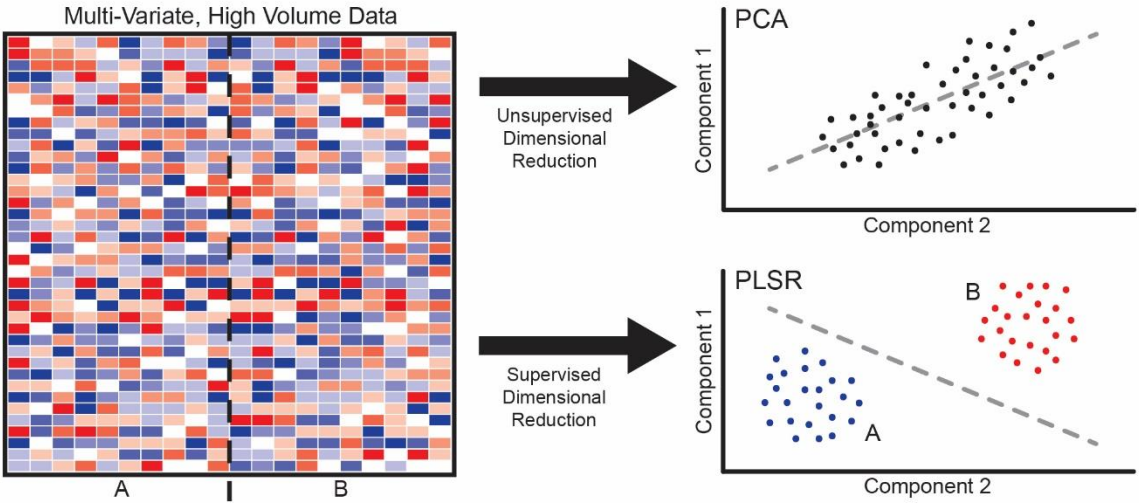


Figure 3

