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Relevance of systems pharmacology in drug discovery

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The pharmaceutical industry is in the process of re-inventing its pipeline in an attempt to overcome its increasing phase II and III attrition rates. Here, we describe how systems pharmacology can be used as a risk assessment tool to alleviate this problem before bringing in larger investments. We propose that this translational research tool could provide a valuable, complementary addition to other emerging innovative approaches for target identification and validation in discovery and, ultimately, for aiding clinical trial optimization.

The major cause of attrition in the pharmaceutical industry is the lack of efficacy in phase II and III clinical trials [1,2]. Failures in phases I and II trials resulting from negative readouts towards proof of concept (POC) represent the largest proportion of the cost of drug development [3]. Achieving strong confidence in rationale (CIR), mechanism of action (CIM) and drug safety (CIS) of a therapy under development can be powerful milestones in overcoming these problems. However, the process of collecting evidence to feed this confidence needs to be initiated at the point of inception of the target idea [4–6], and not just as projects move from discovery to development [7,8]. This means that research and development (R&D) discovery efforts should shift their heavy weight from compound design and synthesis to a truly shared load with target and pathway validation [5,9]. This cultural shift has begun with the adoption of 'target engagement' [10,11] or '3 pillars of survival' [7] strategies, and the identification and use of different categories of biomarkers [4,9,12]. In this context, systems pharmacology could play a central role as a translational research [4,5] modeling and simulation tool [3].

Systems pharmacology is a recently coined term that refers to the area of systems biology dealing with the representation of disease mechanisms of action (i.e. with the pharmacology of drug targets). Perhaps the clearest attempt to define it formally has been described in a release of a white paper by the US National Institutes of Health (NIH) [13], where Sorger *et al.* define systems pharmacology as 'an approach to translational medicine that combines computational and experimental methods to elucidate, validate and apply new pharmacological concepts to the development and use of small molecule and biologic drugs'. This brief definition is expanded in depth in the paper with a proposed working definition that we recommend as further reading.

Although the definition of systems pharmacology is currently affected by the fluctuations typical of a newly emerging discipline, it is apparent that this approach could enable the necessary transition to a new cultural paradigm. An important reason for this is the early applicability of systems pharmacology in the discovery pipeline, and a sufficient flexibility that enables its development in parallel with the project program, thereby constantly representing an integrated body of knowledge for the project [11]. In this article, we use an example in the Pfizer pipeline to showcase how systems pharmacology could guide an inflammatory project from an early stage towards its optimal strategy to achieve efficacy, by using a mathematical formulation and analysis of the existing knowledge of the relevant pathway.

In particular, we discuss a direct application of systems pharmacology to influence and guide an exploratory RNA interference (RNAi)/antisense research project on chronic obstructive pulmonary disease (COPD). The drug target of the project was I-κB kinase 2 (IKK-2), an important player in inflammatory responses. Systems biology methods were used at the time to inform three key decisions: (i) is the target an appropriate point of intervention? (ii) by how much does the target need to be downregulated at the protein

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level to suppress the inflammatory signal? and (iii) can a systems pharmacology model be used for setting lead compound selection criteria? These key questions were addressed by combining a model of the IKK-2 pathway kinetics with a range of mathematical tools, such as simulations, sensitivity analysis and parameter scans. The use of these *in silico* approaches increased confidence in the decisions made by the project team, which eventually spared further investments in compounds with low success potential. In an era where the pharmaceutical industry is more than ever in need of innovative approaches to redesign its current R&D model, and of tools to prioritize its investments [14], this example illustrates how systems pharmacology modeling is emerging as a real solution.

Description of the research project

COPD is a devastating disease of the lungs, which manifests as a severe, chronic shortness of breath ultimately requiring continuous oxygenation of the patient. The progression of the disease is characterized by the loss of structure and surface area in the deep lung (alveolae) as a result of chronic, commonly cigarette smoke-induced, inflammation, which in turn reduces the efficiency of gaseous exchange and oxygen uptake. Moreover, during exacerbations, a host of different inflammatory stimuli, such as bacteria and viruses, contribute to the disease by causing constriction of the bronchi and enhancement of the underlying inflammatory responses. Therefore, means by which the inflammatory component of the disease can be curbed have been intensely sought after [15,16].

The nuclear factor (NF)-κB pathway has a well-described, central role in the signaling cascades initiated by a host of different inflammatory stimuli, which differ slightly between cell types and tissues, leading to differential expression of up to 500 genes [17]. Thus, inhibition or ablation of key components of this cellular machinery presents a unique opportunity for controlling aberrant inflammatory responses [18,19].

Description of the systems biology model

The IKK–NF-κB signaling module is a crucial pathway in drug research on inflammation, and has been the object of study of systems biology for several years, starting with Hoffmann's modeling of the temporal control of NF-κB activation by the coordinated degradation and synthesis of inhibitors of κB (IκB) proteins [20]. Several refinements have followed [21], including the incorporation of additional IκB isoforms, [22,23] or linking IKK to the specific upstream signaling stimulus [22,24,25].

The case study presented here is based on the mathematical analysis of Ihekwaba's version of the NF-κB signaling model [26]. This model describes the inflammatory pathway from the point of IKK stimulation to the translocation of NF-κB into the nucleus, where it initiates the transcription of cytokines, such as interleukin 8 (IL-8) and NF-κB inhibitors (Fig. 1). In the absence of an inflammatory stimulus, NF-κB forms a complex with the IκB inhibitor in the cytoplasm. When IKK is stimulated, this kinase

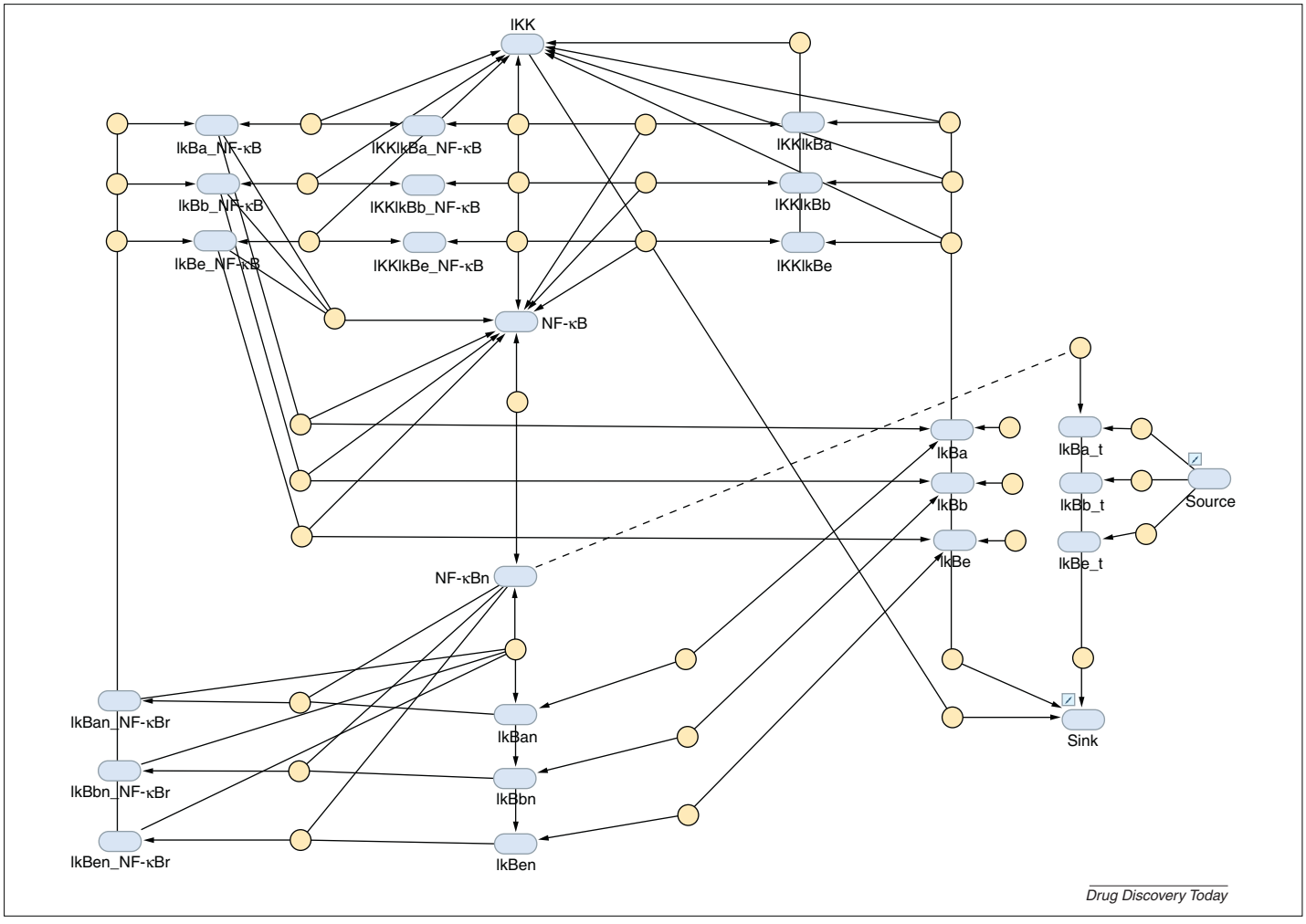

FIGURE 1

Schematic diagram of the nuclear factor (NF)-κB mechanism triggered by I-κB kinase (IKK) stimulation.

phosphorylates the IκB moiety of the complex, inducing its ubiquitination and proteosome-mediated degradation. This leaves NF-κB free to translocate from the cytoplasm to the nucleus, where it modulates the genetic transcription of the newly synthesized IκB inhibitor as well as several inflammatory cytokines. The nuclear IκB inhibitor closes a negative feedback loop by binding again to NF-κB and translocating to the cytoplasm.

The model summarizes all the chemical interactions that are involved in the pathway as time-dependent ordinary differential equations (ODEs), which contain a total of 64 parameters (rate constants) and 26 variables (biochemical species). This allows for the simulation of time profiles for all the species, such as, for example, the concentration levels of NF-κB in the nucleus after an IKK-2 insult.

Is the target an appropriate point of intervention?

Of the three components of the NF-κB complex, IKK-1, IKK-2 and IKK-3 (aka NEMO), IKK-3 is a scaffold protein that is believed to be essential for complex stabilization, and IKK-2 carries the enzymatic activity of the NF-κB complex. Thus, at first glance, IKK-2 and IKK-3 constitute primary points of intervention in this signaling cascade. To assess whether the mechanism chosen contained a better point of intervention than IKK-2, a sensitivity analysis was performed on the *in silico* model described. Given that the nuclear concentration of NF-κB is the inflammatory response biomarker, the focus of this analysis was to observe how sensitive it was to the changes in concentration of other species in the system.

The sensitivity analysis used here was based on the calculation of the time-dependent derivatives of the NF-κB nuclear concentration with respect to the concentration of each of the other species in the system [27,28]. In short, the closer to zero the values of those derivatives were, the less sensitive the biomarker would be to the specific species.

The result of this analysis is summarized in the sensitivity plot in Fig. 2. It shows the degree of sensitivity (*Y* axis) of the NF-κB nuclear species to all the other species present in the system (*X* axis). It can be seen that the transcription factor exhibited the maximum sensitivity to IKK-2. This result confirmed to the project team that IKK-2 is the most appropriate point of intervention in the mechanism, because its concentration needs to be altered only slightly to achieve a big change in nuclear NF-κB levels. This would be the optimal situation in a research project, because the level of inhibition of the target would be the lowest required to achieve an effect via this pathway.

By how much does the target need to be downregulated?

Once IKK-2 was confirmed as the optimal target in the pathway, the next logical question was to ask how much IKK-2 would have to be inhibited to reduce the inflammatory signal. The analysis that was used at this point is known as 'scan analysis' [28]. The idea in this approach is to run a baseline simulation and then compare it to additional simulations where the concentration values of one or multiple species have been altered. Figure 3 shows the time profile of NF-κB in the nucleus after a typical IKK-mediated insult. The oscillatory nature of the signal is a consequence of the negative-feedback loop present in the pathway, and is a measure of the intensity of the inflammatory response following stimulus. This is typically measured through biomarkers, such as cytokine release (e.g. of IL-8).

To find out which target levels would yield a significant dampening effect on this NF-κB time profile, a series of simulations were run at

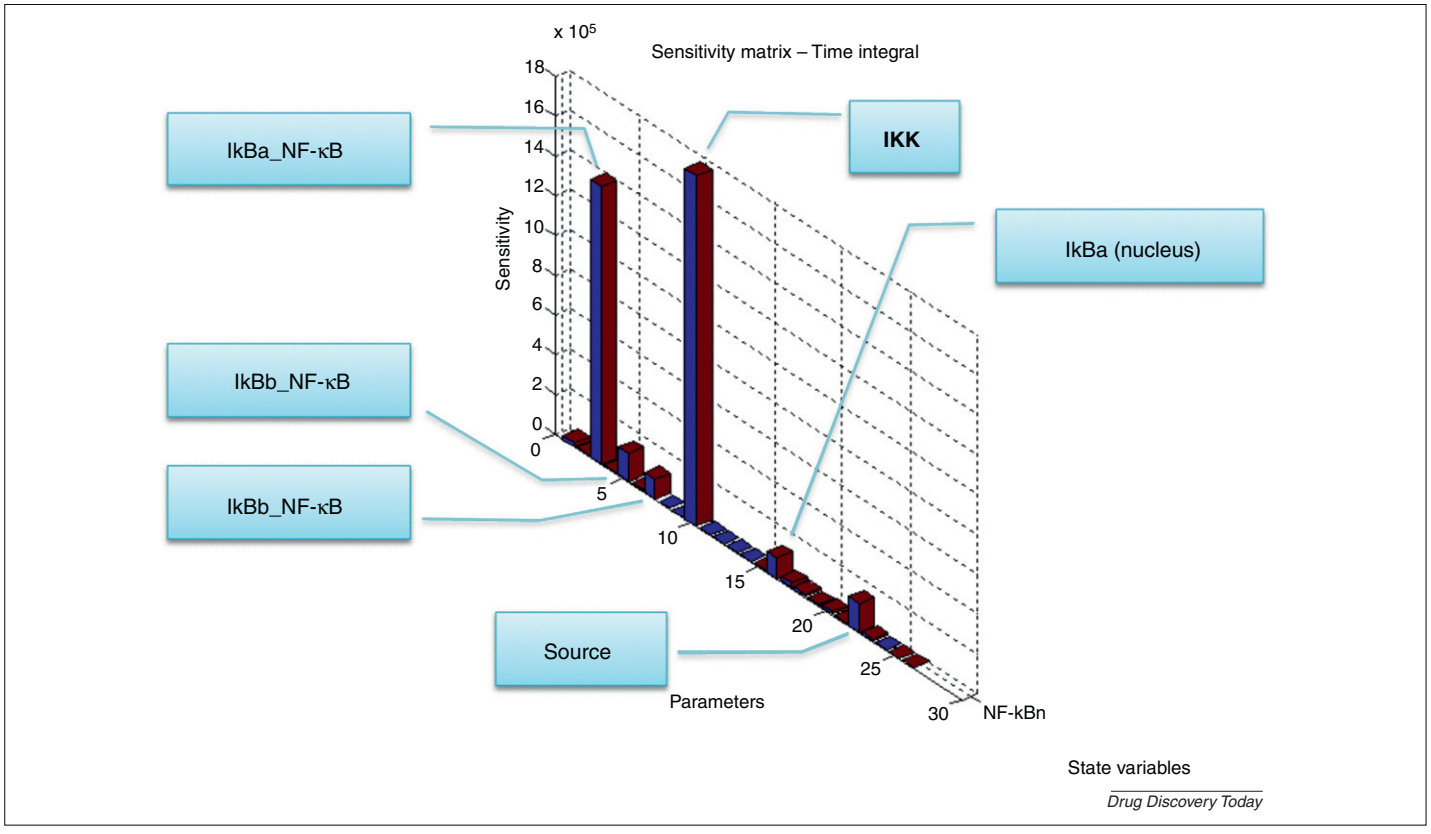

FIGURE 2

Sensitivity plot for nuclear factor (NF)-κB with respect to concentrations of other species present in the pathway.

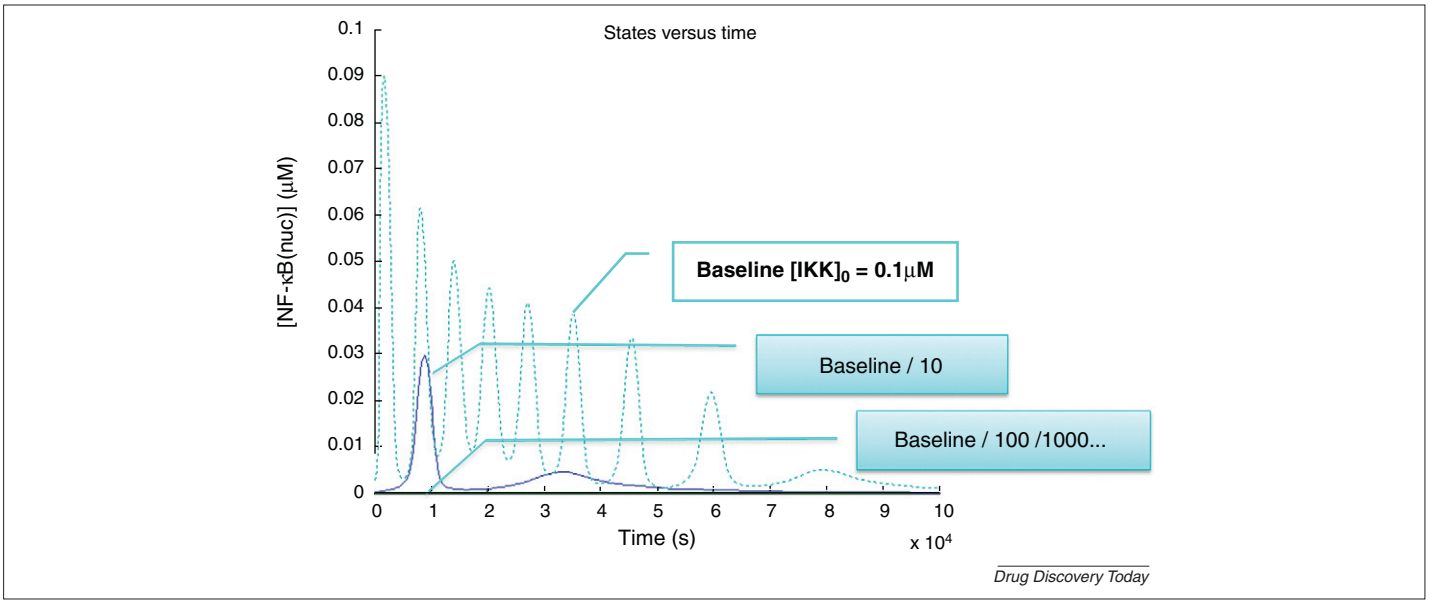

FIGURE 3

Effect of different knockdown levels of I-κB kinase (IKK) on the time profile of nuclear factor (NF)-κB.

gradually lower concentrations of IKK-2, expressed as fractions of the baseline level. As can be seen in Fig. 3, both the typical amplitude and frequency of the oscillatory signal of nuclear translocation of NF-κB tended to diminish as the concentration of IKK-2 protein was reduced by 10 times, 100 times, 1000 times, and so on.

It could be concluded from this analysis that to suppress the signal completely it would be necessary to decrease the concentration of IKK-2 protein in the system by 20 times or more.

Can the model set lead compound selection criteria?

To evaluate the power of RNAi in achieving this modulation target, a series of seven commercially available small interfering (si)RNA against IKK-2 were tested *in vitro* in the A549 type II alveolar epithelium cell line. The most efficient siRNA achieved a 4-times reduction of IKK-2 mRNA across a 7 day time course, with a 2-times and 5-times reduction of IKK-2 protein concentration achieved within 4 and 7 days of transfection, respectively. The question that followed, therefore, was whether this degree of IKK-2 protein concentration reduction, although still not as extensive as desired (i.e. 20-times), could reduce the inflammatory signal to a significant extent.

The answer was provided with a simulation interrogating the impact of a 5-times reduction of IKK-2 protein concentration on the area under the curve of the corresponding NF-κB nuclear translocation time profile [29]. Both are shown in Fig. 4, where they are also compared to the baseline and ideal (i.e. 20-times reduced) profiles of IKK-2 protein levels. It can be seen that 5-times reduction of IKK-2 dampens the oscillatory signal to a limited extent, reducing its intensity (area under the curve) by 70%. This contrasts substantially with the oscillatory signal intensity reduction of 91.7% achieved when IKK-2 protein concentration is reduced by 20 times.

To determine the model utility in predicting inflammatory stimulation outcomes following controlled NF-κB ablation, a well-characterized, IL-1β-induced IL-8 release *in vitro* inflammation model was used [30]. In this model, within 24 h of IL-1β stimulation of the type II alveolar epithelial cell line A549, a 24.66 (±1.12)-fold increase of [IL-8] in culture supernatant is typically induced through NF-κB signaling. By contrast, use of dexamethasone, a classic glucocorticoid receptor inhibitor that arrests NF-κB-mediated transcriptional events, such as IL-8 production, ablates the induction of this biomarker by 80.11% (±0.27). Thus, the best-performing commercial IKK-2 siRNA was transfected into A549 cells, which were then stimulated with IL-1β 7 days after transfection. This would instigate IL-8 release at the point

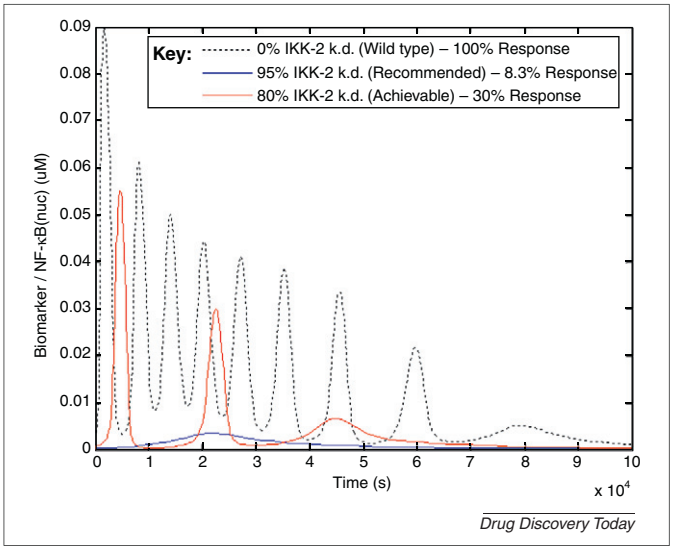

FIGURE 4

Comparison of the nuclear translocation time profiles of nuclear factor (NF)-κB under baseline, 5-times and 20-times reduced levels of I-κB kinase 2 (IKK-2) protein concentration.

where a 5-times reduction of IKK2 protein concentration would have been achieved by the siRNA. On day 8, supernatants were collected and [IL-8] was measured by sandwich ELISA. Thus, a 5-times reduction in IKK-2 protein concentration by means of RNAi resulted in only an approximately 30% reduction of IL-1β-induced IL-8 release, which, although statistically significant, was of limited extent and in agreement with the *in silico* simulation.

Concluding remarks

The exercise presented in this case study demonstrates the power of systems pharmacology to predict the required and achievable extent of target knock-down and the ensuing pathway interruption and biomarker modulation efficiency. This helped to set clear goals for target and biomarker modulation that could be considered as efficacious, or indicators of success. An innovative approach in a research project was taken to: (i) confirm that the target was suitable to reduce the inflammatory response via the NF-κB pathway; (ii) define that, for a compound to suppress the inflammatory response successfully, it would have to knock-down IKK-2 protein levels by 95%; and (iii) contribute to the assessment of efficacious versus statistically significant extent of biomarker modulation needed to achieve, thus setting out clear lead compound selection criteria.

This case shows how, ultimately, the timely and appropriate use of systems pharmacology can impact significantly on project progression by providing the criteria and risk-assessment tools that enable project teams to discriminate between successful and unsuccessful targets, mechanisms and compounds. The starting point for this strategy can be a model published in the literature, a model shared by the systems biology markup language (SBML) community, a model built from scratch with all the mechanistic information available from literature or internal sources, or a combination of the three. The main aim is to describe the knowledge of the target mechanism at a molecular level, including the kinetics of the relevant processes. Thus, regardless of the source, the model should incorporate all the existing evidence that links the target to the mechanism believed to regulate the disease, bearing in mind that it might need to be expanded and/or refined as new experimental evidence appears.

The impact of systems pharmacology has been illustrated here at the preclinical stages of the pipeline. However, in our opinion, it can be easily extended to clinical studies, as pharmacokinetic and pharmacometric data start to accumulate within projects. Notably, this information can be complementary to human genetic and epidemiological data, which are now known to have demonstrable clinical utility on patient stratification [e.g. gefitinib and epidermal growth factor receptor (EGFR) mutation status [31] or Vectibix™ and KRas mutation status [32]], but whose contribution to a systems pharmacology model can be accurately integrated only following experimental assessment.

Implementation of systems pharmacology models at the exploratory and preclinical stage, particularly in translational models integrating multiple data sources often across species and with partial data coverage, empowers project teams to map out the explicit information that is used in study design and to review the principles supporting CIR. As with any other *in silico*, *in vitro* or *in vivo* modeling approach, the uncertainties of quantification of effects across animal species, and the inherent noise in any data measured experimentally, will have to be taken into consideration. However, the advantage of systems pharmacology modeling is that those potential sources of variability can be simulated in high detail, and their impact on the therapy can be evaluated before running any studies. In addition, metabolic control analysis of the model can indicate which sources of variability would have the highest impact and therefore need to be measured as extensively, accurately and precisely as possible on experimental models as close as possible to humans.

Utilized as a powerful communication tool within and outside the team, transparent summaries of target mechanisms can be prepared, thus highlighting key underlying assumptions and eliciting challenging questions. As exemplified in this case study, the semiquantitative nature of these models harbors the capacity to supply decision-making answers or at least achieve caveat awareness. Importantly, continuous assimilation of emerging data is pivotal in maintaining model efficiency and reliability. Updated model utility can additionally manifest through periodic pathway interrogation to monitor the impact of underlying assumptions, or towards identifying alternative targets of equal or higher importance. Concomitantly, rational design of appropriate assays and clear outcome expectations is ensured. Collectively, these models represent a valuable instrument for target and biomarker selection at the early preclinical stage.

The integration of emerging pharmacokinetic data and general physiology principles in these models also facilitates their expansion from mechanism-centered, systems pharmacology to pharmacokinetic/pharmacodynamic (PK/PD) modeling. At this stage, the quantitative nature of such models efficiently focuses efforts into achieving a clear mechanistic understanding of dose prediction, its links to effect and relevant biomarker modulation. Therefore, the impact of different dosage regimens, therapeutic modalities or routes of administration can be compared before heavy investment. In fact, van der Graaf and Benson already define systems pharmacology as the interface between PK/PD and systems biology [33], and some achievements in this direction have been put in the public domain, such as, for example, the understanding the susceptibility of patients with hepatitis C to interferon-a therapy [34].

Further expansion to incorporate safety-relevant findings achieves a net result of a combination of data and up-to-date modeling parameters. Together, these facilitate strategy optimization towards clear compound selection criteria, underpinning CIM and CIS studies in the clinic with coherent, quantifiable, high confidence outcome expectations. Having facilitated early acknowledgement of key assumptions at a molecular level, and their monitoring across project progression, clinical studies can then be enriched with powerful secondary readouts. Hand in hand with epidemiological pharmacogenomic data, a better understanding of the unavoidable, intrinsic causes of differences in response within the patient population can be achieved, giving rise to what has been called 'systems clinical pharmacology' [35].

In conclusion, the integration of systems pharmacology approaches from the early stages of drug discovery through to clinical evaluation provides an instrumental tool for establishing clear decision making criteria, mapping out project development and furnishing the foundations towards achieving high confidence POC studies in humans, all with the long-term potential to develop personalized therapies.

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