

Applications of Mechanistic Modelling to Clinical and Experimental Immunology: An emerging technology to accelerate immunotherapeutic discovery and development

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Summary

The application of *in silico* modelling is starting to emerge as a key methodology to advance our understanding of mechanisms of disease pathophysiology and related drug action, and in the design of experimental medicine and clinical studies. In this perspective, we will present a non-technical discussion of a small number of recent and historical applications of mathematical, statistical and computational modelling to clinical and experimental immunology. We focus specifically on mechanistic questions relating to human viral infection, tumour growth and metastasis, and T-cell activation. These exemplar applications highlight the potential of this approach to impact on human immunology informed by ever expanding experimental, clinical and ‘omics’ data. Despite the capacity of mechanistic modelling to accelerate therapeutic discovery and development and to de-risk clinical trial design, it is not widely utilised across the field. We outline ongoing challenges facing the integration of mechanistic modelling with experimental and clinical immunology, and suggest how these may be overcome. Advances in key technologies including multi-scale modelling, machine learning and the wealth of ‘omics’ datasets, coupled with advancements in computational capacity are providing the basis for mechanistic modelling to impact on immunotherapeutic discovery and development over the next decade.

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Introduction

What is a model in the context of experimental human immunology?

The Medical Research Council (UK) defines experimental medicine as: “Investigation undertaken in humans, relating where appropriate to model systems, to identify mechanisms of pathophysiology or disease, or to demonstrate proof-of-concept evidence of the validity and importance of new discoveries or treatments”. In this context, a model system is viewed as any non-human organism or other self-contained biological system used to experimentally assess a particular biologic activity, disease process or therapeutic intervention. To immunologists, the word ‘model’ frequently refers to a mouse or a cell culture system rather than a set of mathematical equations. In the physical sciences, models are based on equations, including both mathematical and stochastic multi-scale computational approaches, and are at the core of methods used to understand complex chemical and physical systems. These mechanistic models (also known as mechanism-based models) are complemented and supported by data-driven or statistical models of large datasets.

In contrast to the physical sciences, in biology and clinical medicine, systems-based approaches have almost entirely focused on utilising data driven approaches based on ever expanding ‘omics’ datasets (*e.g.* transcriptomics, proteomics, metabolomics). This has had a profound impact on clinical immunology, by providing quantitative insights into biological systems and identifying novel disease biomarkers. However, data-driven approaches are generally neither determined nor constrained by prior biological knowledge. Approaches range from simple curve-fitting to machine learning and artificial intelligence methods, but all provide only limited insight into the biological mechanisms leading to the data and have limited predictive value under extrapolation [1, 2]. Specifically, the hypotheses and predictions generated by data-driven models are generally abstract, relating to patterns or correlations, instead of biological mechanisms or understanding.

In contrast, a mechanistic model is a quantitative representation of a biological system whose definition (*e.g.* equation set) is determined and constrained by relevant knowledge of the system. In particular, its inputs, outputs and represented biological processes are analogues of

real-world counterparts. The validity of the model is then determined by how well it predicts one or more known real behaviours of the system and its capacity to predict previously unobserved behaviours. Mechanistic models can be improved by iterative comparison to real behaviours leading to a cycle of model refinement. Analogous to conducting experiments on a biological system, *in silico* experiments can be performed on mechanistic models. These *in silico* studies can drive mechanistic insights and lead to novel hypotheses that would have been difficult to formulate otherwise, and that may be further evaluated by human experimental medicine or animal based experiments on the biological system. Although this approach has so far had relatively little impact in immunology, in other areas of biology it is widely used. In cardiovascular biology, multi-scale computational models based on Hodgkin and Huxley’s mechanistic model have a predictive value for human toxicology reported to surpass experimental rabbit models, and which are now accepted by the FDA as an appropriate methodology to understand therapy induced cardio-toxicities [3].

Why apply mechanistic models to clinical and experimental immunology?

Mathematical and computational models may be used to simulate real-world patients and experiments and test hypotheses, whose corresponding outputs can be compared to their real-world counterparts. Thus, applied mechanistic modelling is well aligned with the aims of experimental and clinical immunology to (i) gain deeper mechanistic understanding into protective immune responses to pathogens, triggers and drivers for inflammatory and autoimmune disease pathophysiology and treatment responses; (ii) provide a mechanistic context for the analysis and interpretation of clinical datasets; (iii) improve the design of future experimental medicine and clinical trial studies, potentially leading to lower rates of therapeutic failure. In 2016, over 80 billion USD were spent by pharmaceutical and biotechnology companies in R&D, with a significant percentage focused on tumour immunotherapy and treatments for inflammatory and autoimmune disease. Despite this expenditure, over 90% of potential therapies fail due to lack of efficacy or off-target toxicology, thus the potential for *in silico* models to impact on human experimental immunology is massive.

Over the last 30 years there have been many different mechanistic models of immunological

processes, such as TLR signalling and APC–T-cell dynamics based on 4D imaging of mouse immune responses (see sections below). In clinical pharmacology, an increasing number of *in silico* models have been developed and applied to model disease processes and treatment efficacy and toxicology. The Food and Drug Administration has recommended the application of mechanistic models to the field [4] and even recently used one to aid evaluation of a potential dosing regimen [5]. However, despite their potential and the increasing number of models, the deep integration of mechanistic modelling with experimental or clinical research (Figure 1), particularly in immunology, is relatively uncommon. This perspective will present a small number of recent examples of models that have had an impact on human medicine, including viral–innate immune dynamics, anti-tumour immune responses and T-cell activation dynamics, before providing thoughts on how to best integrate and utilise mechanistic modelling to support future experimental and clinical human immunology studies.

Applications of mechanistic models to human experimental medicine and clinical immunology

Application of models to optimise interferon–anti-viral small molecule combination therapy of chronic HCV infection

Mechanistic models of viral dynamics have long supported and impacted experimental medicine and clinical studies, originally developed for HIV [6]. Their utility and capacity to support antiviral clinical development was dramatically increased by the acceptance and use of viral load as a surrogate for longer-term, clinically meaningful outcomes. Applications include gaining deeper mechanistic insights into antiviral mechanisms of action and/or viral disease pathophysiology, as well as enabling “virtual clinical trials” to inform clinical trial designs (*e.g.* [7]). An early mathematical model of Hepatitis-C Virus (HCV) [8, 9] described the infection of healthy cells and the action of type I IFNs (Figure 2a). At the time, the effect that IFN α had on HCV was not clear, so parameters to reduce the infection and viral production rates were both included in the model. Fitting the model to patient viral loads [9] indicated that the drug acts by reducing viral production rate. The model was also able to explain

the biphasic decline of viral load: a fast initial decline followed by a more gradual one. The fast decline was predicted to be due to rapid clearance of free virus, and the other due to the slower death rate of infected cells producing the virus. The “shoulder” of constant viral load sometimes seen between the two phases was predicted to occur when the rate of infected cell death is equal to the combined rates of healthy cell infection and infected cell proliferation [10]. The balance is predicted to eventually be lost if healthy cells proliferate faster than and out-compete infected cells. These insights and associated predictions would have been difficult to identify from innumerable possibilities in an experimental study unguided by these insights, but they were simple to verify. Similarly, a model of HIV has been used to reconcile the observations that $CD8^+$ T-cells do not appear to contribute to the death rate of infected cells, yet viral load increases significantly after they are depleted, by assuming that CTL kill-rate varies with the life cycle of the infected cell [11]. Models such as these showcase the ability for mechanistic modelling to reveal phenomena and complexities that are ‘obvious’ in hindsight but would not have been easily discovered without a model to act as a guide.

Subsequently, multiple mechanistic models have been developed to explain or predict clinical trial results where type I interferons have been utilised to stimulate innate anti-viral defences. A model of evolution of HCV in response to a protease inhibitor (Telaprevir) [12] could reproduce observed rebound in viral load during treatment due to the expansion of resistant strains (Figure 2b). The model was parameterised using Phase I and II trials that included telaprevir, and subsequently used to accurately predict sustained virologic response rates in Phase II and III trials [13]. Another was used to simulate a population of *in silico* patients, leading to the prediction that a potent combination therapy would avoid resistance and lead to sustained virologic response in most patients [14]. This has since been confirmed in the clinic [8, 15, 16]. Mechanistic modelling that can predict key clinical responses may be used to conduct virtual clinical trials across a range of proposed trial designs to inform the actual design to carry through into future trials [8, 17, 18]. We believe that this approach is particularly important when clinical or experimental data are sparse or difficult to generate.

Application of models to predict tumour progression and response to treatment, a potential paradigm for human immune modelling

Both the experimental medicine and clinical study of cancer have benefited from application of mechanistic modelling, which has deepened understanding of cancer disease biology and responses to treatment (*e.g.* radiotherapy), and informed clinical trial designs (timing, dose, and the window between efficacy and toxicology). Models have enhanced mechanistic insight into tumour progression and metastasis, providing unexpected insights into the role of pH in the microenvironment to facilitate invasion of surrounding tissue (Figure 3a). The mathematical equations of one such model [20] predicted the existence of a pH gradient from the tumour periphery into healthy tissue, which was hypothesised to induce an acellular gap around the tumour and the remodelling of tissue to facilitate tumour invasion. These hypotheses have been subsequently supported by experimental studies [20, 21]. A more recent model [22] proposed a mechanism for the Warburg effect to arise even in oxygenated tumours, despite the apparent loss of fitness (Figure 3b). The model showed that it may arise as a ‘bet-hedging’ strategy in response to variable oxygen concentration. These examples illustrate how mechanistic hypotheses can be used to develop, explore and refine new concepts *in silico*, to inform subsequent definitive experimental studies.

Models have been used to optimise human tumour therapy and determine how best to treat tumours. Models have been used to develop new concepts, one of which is that increasing treatment strength does not necessarily improve patient survival, but rather counter-intuitively could potentially decrease patient survival. Models indicated that maintaining enough radiosensitive cells alive is important to out-compete slower dividing but therapy resistant cells, thus improving patient survival [23]. This hypothesis was subsequently confirmed in mice (Figure 3b). A similar conclusion was determined by modelling a heterogeneous tumour with dynamic radiation resistance [24] (Figure 3c). The predicted improved schedule derived from this model, designed to avoid heavily irradiating a resistant population, yielded better survival compared to the standard radiotherapeutic schedule for both *in silico* and mouse models. This therapeutic strategy is currently being assessed in a clinical trial [25].

One class of models that have increasingly supported clinical trial designs are tumour

kinetic (TUK) models. These models describe early (*e.g.* 8-week) patient-level changes in tumour burden over time and in response to treatment. The resulting models are used to generate patient-level metrics such as estimated on-treatment tumour growth rates. Multivariate survival models may then be developed that link baseline patient characteristics and model derived metrics, such as tumour growth rates, to longer-term overall survival (OS) [2, 26]. TUK models have been used to successfully predict the overall survival of patients receiving a drug in a phase III clinical trial, by developing a survival model from phase III trial data for a different drug and a TUK model from phase II data for both drugs [2] (Figure 3d). TUK models have also been used to inform phase III clinical trial designs, or to inform clinical dosing regimens, as originally considered by Goldie and Coldman [27, 28]. For example, an *in silico* model [29] was used to demonstrate that tumours that become small in response to therapy may become resistant, so would require dense, intensive therapy to prevent remission. This observation was “contrary to popular belief” at the time, but was later confirmed by clinical studies to improve survival. TUK models have also been recently applied to immunotherapy. Standard early oncology endpoints (objective response rate, progression free survival) are poor predictors of OS for immunotherapies. TUK metrics were recently explored as a potential surrogate endpoint for OS. Claret *et al* (2017) [26] explored the predictive performance of TUK metrics using early data from clinical trials of the checkpoint inhibitor atezolizumab (anti-PDL1) in patients with non-small cell lung cancer with promising early results, suggesting that such approaches may be used to inform the design of immunotherapy clinical studies [26]. Mechanistic models in human cancer offer massive potential to impact on clinical development; modellers have started to create a plethora of models focused on non-immune processes [2, 30] which has recently been extended to model anti-tumour immune responses [26].

Application of models to quantify or predict T-cell activation and dynamics

Enhanced or reduced T-cell activation has an essential role in the pathophysiology of autoimmune diseases and immune responses to cancers. Mechanistic models of T-cell activation within lymph nodes have complemented experimental medicine studies to drive a deeper

mechanistic understanding than was possible by experimentation alone. One such model was used to explore whether chemokines could attract T-cells to antigen expressing dendritic cells [31] (Figure 4a). These authors found that an optimum search strategy cannot include such an effect, as although it increases the number of T-cell–dendritic cell contacts per unit time, it sharply reduces the number of *unique* contacts due to crowding around dendritic cells. Another model was used to determine whether the ‘run-and-tumble’ motion exhibited by T-cells (as opposed to diffusion) is due to an intrinsic, stochastic process, or whether it can be explained by the lymph node environment [32], and was developed in parallel with 4D multi-photon imaging experiments to validate model predictions. It was found that the pattern of motion can be explained entirely by collisions with other cells (Figure 4b). A combined modelling and imaging study has shown that T-cell receptor (TCR) affinity affects the duration of T-cell–dendritic cell contacts and T-cell proliferation [33] (Figure 4c). Matching to the imaging data resulted in the prediction that, though the contact duration depends on T-cell receptor affinity, T-cells can ‘integrate’ subsequent short-duration contacts with a dendritic cell over a duration of hours before activation. There are also many models for the activation and proliferation of T-cells that are consistent with observations of lymph node output [34, 35], even under acute or prolonged infection [35]. Another approach that combined a model with *in vivo* imaging was developed to quantify the minimum number of antigen-bearing dendritic cells required to activate CD4⁺ T-cells in the lymph node [36] (Figure 4d). We ourselves have extended this model to quantify the ‘trade-off’ between the number of dendritic cells and the amount of antigen that they are carrying [37]. Through the development of mechanistic models, virtual clinical trials akin to that described for tumour modelling [2] could be developed and applied to immunological modelling. The resulting capacity to better design protective and therapeutic vaccines has the potential to optimise and potentially individualise vaccines for cancer, chronic infection and regulate autoimmune disease [38].

Issues, limitations and prospects for modelling to support experimental and clinical immunology

In this perspective, we have discussed applications of mechanistic modelling to experimental medicine and clinical immunology through examples of *in silico* models that have made useful contributions, with a focus on pathogen infections, cancer biology, T-cell dynamics and vaccine design. Through providing more detailed descriptions of a small number of models, we have focused on how their conclusions are directly impacting on both understanding of biological mechanisms and on therapeutic strategy; both the technical process and more in-depth applications to non-clinical immunology and cancer biology have been previously reviewed in detail [39–42]. Despite the emerging successes of mechanism driven systems immunology, most models receive little attention and very few clinicians would use models that make clinical predictions in decision making. Why do so few models get developed and used, or conversely, why are so few models useful? Mechanistic models are often produced by researchers who are not, or do not have strong collaborative links with, experimentalists. Often, such models are developed in isolation from the clinic and do not address specific questions, in which case they cannot be verified by experimental study. Conversely, data reported by experimental studies are often not easily applicable to models, due to a lack of quantitative information or data spanning short and long timescales, and multiple patients. Similarly, many human parameters required by models are not reported in experimental literature, so either parameter values that are estimated (educated guess) or based on values from mouse experiments (which are also models) are used, leading to predictions with unknown relevance to human disease.

To remedy these problems, mechanistic modelling must be more deeply integrated into experimental workflows. Models should be question-driven, taking the form of a verifiable *in silico* experiment, and their inputs and outputs must align with clinically measurable assessments and endpoints, so that they generate clinically meaningful predictions. In turn, human parameters need to be measured and experimentalists should endeavour to report quantitative information that aids mechanistic analysis by a model. Experimentalists and

modellers should endeavour to design complementary experimental and modelling studies, where data from each study can support the other and produce something greater than the sum of its parts.

In the clinic, models are also hindered by the lack of clinical endpoints used for drug regulatory approval (*e.g.* clinical measures of depression or patient reported pain assessments) that are definable as variables in mechanistic models, restricting the use and impact of models to support clinical trials or conduct virtual trials. Though mechanistic models can predict “process driven” pathological outcomes (*e.g.* number of bone erosions), they will never easily be able to predict complex clinical measurements that are compounded by social factors. Analysis of data-driven models have hit similar road blocks in finding correlations to patient reported outcomes, thus requiring a step change in clinical trial design. Clinical efficacy studies of HIV antivirals, for example, were dramatically accelerated by the regulatory agency approval of plasma HIV RNA (viral load) as a surrogate endpoint for risk of AIDS progression and death. This subsequently increased the utility of mechanistic modelling to support clinical development programs. Mechanistic HIV viral dynamic models that explicitly included the predicted time course of viral load were developed and used to conduct virtual clinical trials that contributed to the design and interpretation of real world clinical trials (*e.g.* [6, 7]).

A further weakness preventing the take up of modelling frameworks is the general disconnect between mechanistic modelling across multiple scales and with data-driven approaches. There are numerous pragmatic difficulties with the coupling of large scale physiological modelling with smaller scale mechanistic models such as intracellular signalling networks or molecular biology simulations. These difficulties include the determination of human model parameters and the challenges associated with multiple biological timescales. Many immunological events (such as binding, interaction or migration of individual cells) have timescales much less than a second, but correspond to processes with a timescale of hours or days (*e.g.* immune activation, maturation or migration of a population). The acquisition of data at high enough frequencies and for long enough timescales is experimentally and computationally challenging. However, modelling can help identify key data points and reduce the amount of experimental data required to parameterise a model system. There have been numerous investigations that

illustrate the feasibility of this methodology, for example to link ion channel dynamics to action potentials in cardiac electrophysiology [43]. Such multiscale studies, driven by focussed questions and with careful parameterisation, have enormous potential to inform our scientific reasoning on how perturbations at the molecular or cellular level impact on pathophysiology, with subsequent opportunities for exploitation in experimental medicine. Similarly, the coupling of disparate scales or integration of mechanistic modelling with data driven approaches has great potential in exploiting rapidly developing and rich datasets. For example, constraints gleaned from mechanistic models may be incorporated into machine learning analyses to guide outputs, or machine learning analyses may be used to parameterise mechanistic models using ‘omics’ data. One such study used fluxomic, proteomic and metabolomic data to improve estimates of kinetic parameters for cellular reactions and, in turn, the predictions of a model of *E. coli* metabolism [44]. Rapid advances in computational modelling methodologies coupled with generation of parameter values from ‘omics’ data sets provides the basis for deeper integration of *in silico* modelling with experimental human immunology.

Experimental Immunology 2020: Key role for mechanistic modelling

The development of immune-therapeutics and vaccines has a key role in developing the next generation of treatments for cancer, cardiovascular disease, neurological conditions and endocrine diseases. Utilising the rapidly expanding wealth of clinical data, whole genome sequencing, characterisation of individual microbiomes and ‘omics’ data sets will provide fertile opportunities to apply mechanistic and data-driven models together to accelerate and de-risk therapeutic discovery and development. Key challenges where models can generate impact are in the determination or identification of: (i) mechanisms of pathology, (ii) next generation correlates of efficacy *i.e.* ‘tissue biomarkers’, (iii) how to combine different constituents (small molecules, vaccines and biologics) and modulate the microbiome to drive maximal efficacy, (iv) what doses should be provided and how, and how dosing effects toxicity, (v) how outcomes vary between patients, (vi) the personalisation of treatment and novel clinical trial design.

We started this perspective by highlighting the parallels between the workflows of mechanistic modelling and experimental medicine, and how the former can thus support the latter. We have shown how hypothesis-driven mechanistic models have been used to produce testable predictions, that have been experimentally confirmed. This hypothesis-driven, prediction-generating approach is exemplary of the potential synergy between modelling and experimental immunology. We believe that this approach is critical for driving forward our understanding of mechanisms in humans, that we believe will significantly impact on clinical and experimental immunology in the coming decades.

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Competing interests

The authors declare no competing interests.

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Figure captions

Figure 1: Overview of experimental/clinical study workflow, with blue labels indicating how the application of modelling can support each step. Constrained by biological knowledge, mechanistic modelling has the potential to support all steps in this workflow, including the generation of testable hypotheses or predictions *in silico*, or the prediction of experimental or clinical study outcomes across a range of possible study designs.

Figure 2: A summary of key mechanistic models of viral kinetics. **a)** A model that predicted that the action of IFN α on HCV is to inhibit viral production rather than infection, that the biphasic decline of viral load is due to early viral clearance followed by infected cell death, and that the ‘shoulder’ of constant viral load sometimes observed is due to the temporary balance of infected cell death with division and infection [8, 9, 19]; **b)** A model that provides a mechanistic basis for HCV rebound due to random mutations, and could predict sustained virologic response in phase II and III clinical trials [12, 13].

Figure 3: A summary of key mechanistic tumour models. Each panel shows a model(s) that, **a)** predicted various features of tumour invasion mediated by acid-producing cells [20]; **b)** provided an evolutionary basis for the Warburg effect, as neoplastic cells that outcompete other cells in low oxygen concentrations (green shaded region) have a fitness advantage in an environment with variable oxygen concentrations (shown by the black line) and take over the tumour population (background of plot) [22]; **c)** showed how moderate therapy improves patient survival over intensive therapy, that may select for resistant cells [23]; **d)** showed that a less intense, more frequent therapy schedule improves survival in a dynamic model of resistance [24]; **e)** predicted patient survival in a phase III clinical trial of a drug by parameterising tumour and survival models [2].

Figure 4: A summary of key mechanistic models of T-cell activation and dynamics. Each panel shows a model that, **a)** found that chemokines cannot attract T-cells to antigen-bearing dendritic cells in an optimum search strategy [31]; **b)** showed that the dynamics of T-cell movement can be explained entirely by interactions with their environment (as opposed to

e.g. chemokines) [32]; **c)** investigated how T-cells can integrate many low-affinity interactions with dendritic cells to activate [33]; **d)** considered the minimum number of dendritic cells required for T-cell response [36].