Institution: University of Chester		
Unit of Assessment: 10 Mathematical Sciences		
Title of case study: Mathematical modelling leads to advances in immunology and drug development		
Period when the underpinning research was undertaken:		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	**Role(s) (e.g. job title):**	**Period(s) employed by submitting HEI:**
Gennady Bocharov	Leverhulme Professor	2002-2004
Neville J Ford	Professor	1986-ongoing
Christopher T H Baker	Professor, Reader	2006-2016
Jason A Roberts	Dr, Senior Lecturer	2008-2020
Sarah Andrew	Professor, VL	1998-2017
Period when the claimed impact occurred: 2014 – 2020		
Is this case study continued from a case study submitted in 2014? N		

1. Summary of the impact (indicative maximum 100 words)

Research involving mathematical modelling is helping to unravel the complexities of key areas of biomedicine. Our study of the mammalian immune system focuses on two areas: (1) genetic evolution of HIV within the host during infection, and (2) dendritic-cell-based immunotherapy. The research has influenced understanding by biomedical practitioners of control parameters, the immune response and viral resistance to drugs. Our consequent involvement in developing the COMPIT tool has enabled mathematical modelling to play a key part in 3Rs (Replacement, Reduction and Refinement) of animal experimentation in the development of new drugs as well as reducing the cost and the time taken to bring a new drug to market.

2. Underpinning research (indicative maximum 500 words)

The Leverhulme Trust invested in an initiative over a period of two years (2002-4) with the specific objective of establishing research activity in the areas indicated in the Chester Mathematical Sciences Research Group through the employment of Bocharov as a Leverhulme international professor. As a consequence, a team was established (Ford, Baker, Andrew and Roberts), augmented by others (Lumb, Norton, Mc Auley and Kavallaris) working in this area. The team had two targeted areas of research.

Area 1 - HIV-1. The Type 1 HIV virus is characterised by an extreme variability resulting from two major processes acting in parallel:

- changes in the viral genome (point mutations), and
- the multi-infection of target cells in conjunction with the recombination of viral genomes.

These processes enable the virus to escape the immune response and acquire resistance to drugs by tuning its genomic sequence. The complexity of the virus dynamics in vivo compounds the difficulty of understanding intra-patient HIV evolution. While HIV-1 infection poses a great challenge to the development of strategies for an effective cure, there has been major progress in controlling the infection with highly active antiretroviral therapy (HAART).

Many groups have analysed viral kinetics following HAART and have made inferences about HIV dynamics. The major focus in previous studies of drug-resistance was on the effect of point mutations. By contrast, we are one of only a few to have attempted to simulate HIV sequence evolution and to examine recombination effects. In seeking to appreciate the impact on viral evolution of a high frequency of multi-infected cells, with attendant recombination, one of the contributions of the Team has been to develop an *in-silico* stochastic model [1] to explore the effects of major microscopic parameters (e.g., the point-mutation and recombination rates, and the proviral copy number per cell), on the dynamics of macroscopic characteristics. This model

showed that the time to build up n-point mutants is enhanced by multi-infection. Previous studies were of infection with a single provirus per cell rather than of multi-infection.

In 'neutral' (random) evolution, where no selection occurs, mutants can be temporarily fixed over numerous rounds of replication before becoming extinct. This suggests that the majority of mutations observed in cross-sectional analyses do not arise from strong selection in reaction to drug therapy. Whenever a strong selection pressure is applied via the administration of drugs to a few sites, as under antiviral treatment, there is a rapid emergence of variants encoding the selected traits, showing partial or full resistance. Thus, under the conditions of an initial homogeneous infection, the selection of n-point mutants, which are fundamental to the development of multi-drug resistance, is generally accelerated by multi-infection and recombination, even though there is great variation in the kinetics of fixation.

Area 2 - Cancer vaccines. Growing knowledge of the molecular identity of tumour-specific antigens has opened new avenues for effective cancer vaccines.

Dendritic-cell-based (DC) immunotherapeutic approaches appear particularly promising, as indicated by a series of preclinical experimental studies in mice which demonstrated that anti-tumour immunity can be induced using DC. Significantly, in 2010 the US Food and Drug Administration approved the first DC vaccine for prostate cancer.

The research reported by Ludewig et al. [2] developed a mathematical model to determine the major parameters controlling DC-Cytotoxic T-lymphocyte (CTL) interaction. It found that T-cell receptor avidity greatly affects the pattern of CTL dynamics in response to single or multiple immunisations with DC. For induction of high avidity CTL, the number of adoptively transferred dendritic cells was of minor importance once a minimal threshold of cells-per-spleen had been reached. The Unit's study indicated that as long as significant numbers of activated CTL persist and ensure rapid elimination of antigen-expressing DC, any further application of DC has only a limited 'enhancement' effect. Nevertheless, such repeated DC application is apparently necessary to maintain high levels of activated CTL. These findings impinge in particular on the use of DC in anti-tumour therapy, where the availability of high-avidity CTL against the chosen immune-therapeutical target antigen should be carefully examined. The research suggests that the translation of successful preclinical studies is likely to be hampered by complexities associated with the clinical situations encountered by medical practitioners.

Members of the Chester Mathematical Sciences Research Group have continued to work on this theme [3,4]. In addition to ongoing interactions with previous collaborators and users (as above) work has included modelling of DNA methylation [5] and its implications for health and ageing [6], work on mouse models to understand the transition to autoimmunity in uncontrolled type-I diabetes (a collaboration with Immunosys at Alderley Park, Cheshire), and our research which provided the mathematical modelling insights that influence the decision-making by the COMPIT tool developed by Immundnz Ltd, also at Alderley Park, Cheshire.

The COMPIT vision is to help with decision-making about which new candidate drugs should proceed to clinical trials. The aim is to provide answers that are faster, cheaper and have less dependence on animal testing than conventional methods. The fundamental approach is to use *in-vitro* methods combined with mathematical principles and methodologies to automate parts of the decision-making within the life cycle of drug development and testing.

The key question is to determine whether or not a drug induces cell death, DC maturation or T cell activation when it has been introduced *in-vitro* to a cell culture under laboratory conditions. The tests are both expensive and conventionally involve the use of animals. The objective, which was achieved, was to identify an appropriate sequence of *in-vitro* tests to be completed, so that decisions to reject a drug candidate can be taken after as few tests as possible, speeding up decision-making and saving costs. Further mathematical work enabled us to identify tests that are redundant in the process, and to further speed up the time taken and reduce the cost of identifying which drugs are most appropriate to proceed to expensive clinical trials.

3. References to the research (indicative maximum of six references)

[1] Bocharov, G., Ford, NJ., Edwards, JT., Breinig, T., Wain-Hobson, S., Meyerhans, A., A genetic-algorithm approach to simulating human immunodeficiency virus evolution reveals the strong impact of multiply infected cells and recombination, JOURNAL OF GENERAL VIROLOGY, 86, pp. 3109-3118, 10.1099/vir.0.81138-0, 2005.

[2] Ludewig, B., Krebs, P., Junt, T., Metters, H., Ford, NJ., Anderson, RM., Bocharov, G., (2004) Determining control parameters for dendritic cell-cytotoxic T lymphocyte interaction EUROPEAN JOURNAL OF IMMUNOLOGY, 2004, 34(9), pp. 2407-2418

[3] Baker, CTH., Bocharov, G., Ford, JM., Lumb, PM., Norton, SJ., Paul, CAH., Junt, T., Krebs, P., Ludewig, B., Computational approaches to parameter estimation and model selection in immunology JOURNAL OF COMPUTATIONAL AND APPLIED MATHEMATICS, 184(1), pp. 50-76, 10.1016/j.cam.2005.02.003, 2005.

[4] Andrew, SM., Baker, CTH., Bocharov, G., Rival approaches to mathematical modelling in immunology, JOURNAL OF COMPUTATIONAL AND APPLIED MATHEMATICS, 205(2), pp. 669-686, 10.1016/j.cam.2006.03.035, 2007.

[5] Larson, K., Zagkos, L., Mc Auley, M., Roberts, J., Kavallaris, N. I., Matzavinos, A., Data-driven selection and parameter estimation for DNA methylation mathematical models. JOURNAL OF THEORETICAL BIOLOGY 467, pp. 87-99, 10.1016/j.jtbi.2019.01.012, 2019.

[6] Zagkos, L., Mc Auley, M., Roberts, J., Kavallaris, N., Mathematical models of DNA methylation dynamics: Implications for health and ageing. JOURNAL OF THEORETICAL BIOLOGY 462, pp. 184-193, 10.1016/j.jtbi.2018.11.006, 2019.

4. Details of the impact (indicative maximum 750 words)

Overall, the research team's work has promoted awareness of the benefits of applying novel, 'high-end' techniques in computational modelling to help unravel the complexities and causalities in the cited areas of biomedicine. The Group's research described here has had a far-reaching effect, not just on the academic community, and not just in the field of mathematics, changing the way different specialisms can work together. Recognising that research papers do not necessarily attract the attention of medical practitioners, and the actions of medical practitioners are circumscribed by protocols overseen by regulators, our contacts with colleagues have been used to facilitate access to and interaction with clinicians. In affecting the perceptions and approaches of our collaborators we have had an impact on practitioners, not least by identifying potentially relevant programmes of testing. Prof Dr Burkhard Ludewig (one of our co-authors and collaborators) is Head of the Institute of Immunology, which is part of the medical research centre and is an independent research unit of the Kantonal Hospital St. Gallen, Switzerland.

Our initial research activities were focussed on immunopathological processes in the cardiovascular system, the development of new immunotherapeutical approaches against cancer, and the biology of coronaviruses. The Institute also supports basic research projects of physicians from the clinical departments.

Prof Dr Andreas Meyerhans (another collaborator and co-author with Bocharov) is group leader of the Infection Biology Group at UPF, Barcelona with research interests in virus evolution; lymphocyte responses in persistent human infections (HIV, HCV, CMV and Mycobacterium tuberculosis). His group's work is in two main areas:

1. describing fundamental features in virus evolution i.e. the characteristics of HIV quasispecies, HIV and HBV hypermutation, and HIV multi-infection of single cells *in vivo*.
2. quantifying human T cell responses in viral (HIV, CMV, poliovirus), bacterial (Mycobacterium tuberculosis) and yeast (Candida albicans, etc.) infections directly from the blood of patients. Part of the work is funded by two international grants from the Bill and Melinda Gates foundation and the EU euco.net programme.

Burkhard Ludewig wrote of the ongoing joint work in 2013: *Changed perceptions arising from the work of Bocharov and his co-workers [...] relate to issues of cause-and-effect in the complex pathological states encountered in real life illness. Ultimately, though (because of the natural conservatism in applied medicine) not in the short term, this should lead to increased understanding of the effects of clinical treatment procedures and the discovery of new or changing forms of medication in specific therapeutic interventions*.

There have been further developments since 2014 based on the joint work. There has been an added understanding that mathematical modelling approaches (from our 2004 paper [2], for example) have a real impact on drug development [7]. There has also been a growing acceptance that these mathematical and computational models (developed from our work) can be used to enhance the insights from experimental work. For example, *in vivo* imaging technologies have been used to quantitatively characterise target cell scanning and migration of lymphocytes within lymph nodes. The translation of these quantitative insights into a predictive understanding of immune system functioning in response to various perturbations critically depends on computational tools linking the individual immune cell properties with the emergent behaviour of the immune system [8].

Likewise, Andreas Meyerhans expresses the view that the Chester Group's work has contributed to changed perceptions. He remarks [9] that: *our joint paper from 2004 on HIV recombination was the first to use a genetic algorithm approach to address the impact of HIV recombination in virus evolution. It inspired subsequent work on HCV recombination and possible affects on evolution and drug resistance development. Subsequently, we also generated a quantitative mathematical description of the complex HIV life cycle in infected cells that adequately mirrors the experimentally observed kinetic appearance of HIV components within infected cells.* [10]

Largely as a consequence of this successful work on the mathematics of immunology members of the Chester Group were invited to provide the mathematical modelling for **COMPIT (**Comprehensive In-vitro human Immune-response Testing) [11], a tool developed by Immundnz that studies the comprehensive immunogenicity of drug compounds. This platform has been designed to enable **preclinical** *in-vitro* testing of drugs for an in-depth immunogenicity analysis, including prediction of the risk of immune mediated pathology. It has been shaped by mathematical modelling approaches that have helped guide a pathway through the in vitro tests that optimises both the speed and the efficiency of decisions to progress to further drug trials.

*COMPIT can be used during preclinical development to assess drug-induced tissue damage and immunogenicity and to predict the **human** immune response as a result of a drug and drug-induced tissue damage. COMPIT brings numerous benefits:*

- *Prediction of immune-mediated adverse drug reaction and avoiding risk in the clinical phase*
- *Better translation from preclinical studies to humans*
- *Reduction of the number of animal tests*
- *A more robust and comprehensive immunologic profile: improved safety and risk prediction thus reducing human health hazard risk*
- *Drug development can be terminated before costly advanced stages of drug development: reduced investments in non-viable drugs and reduced attrition rate*
- *Potential to improve drugs, generate blocking agents against immune effectors and make non-viable drugs accessible to patients*
- *Meeting changes to expected regulatory requirements on safety.* [12]

The NC3Rs website [11] draws attention to the following features of COMPIT that lead to 3Rs (Replacement, Reduction and Refinement) of animal experimentation in the development of new drugs:

COMPIT: an in vitro platform that enables the comprehensive analysis and prediction of the human immune response to drug molecules. Uniquely, the platform can be used to assess the immune response evoked by cells that have been damaged by drugs. This is neglected in previous models

for assessing and analysing human immune response. Using human cells, COMPIT has the potential to deliver better translation from preclinical studies to humans and provides users with currently unattainable information on the safety of drugs.

Preclinical studies are performed in animals. Classically, non-transgenic mice, rats and non-human primates are used for immunogenicity studies. A typical study consists of 60 experimental animals per compound to be studied, including tests such as lymph node assays, histopathology, and determination of antigen-specific- and auto-antibodies. Such animal models are in many cases not relevant for translation to humans, especially in the case of studying immune response. There is a pressing market and regulatory need for more physiologically relevant in vitro approaches to reduce reliance on these in vivo models. COMPIT can robustly predict the human immune response, offering a viable alternative approach to replace some animal studies in immunogenicity testing. Additionally, by enabling the comprehensive analysis and prediction of the human immune response to drug molecules in vitro, COMPIT will reduce the number of compounds entering regulatory animal studies and therefore the number of animals used overall.

5. Sources to corroborate the impact (indicative maximum of 10 references)

[7] dePillis, L.G., Eladdadi, A. & Radunskaya, A.E. Modeling cancer-immune responses to therapy, JOURNAL OF PHARMACOKINETICS AND PHARMACODYNAMICS 41, pp. 461–478, 10.1007/s10928-014-9386-9, 2014.

[8] Grebennikov, D., Bouchnita, A., Volpert, V., Bessonov, N., Meyerhans, A., Bocharov, G., Spatial Lymphocyte Dynamics in Lymph Nodes Predicts the Cytotoxic T Cell Frequency Needed for HIV Infection Control. FRONTIERS IN IMMUNOLOGY 2019 10:1213, 10.3389/fimmu.2019.01213, 2019.

[9] Letter from Principal Investigator, Department of Experimental and Health Sciences, Universitat Pompeu Fabra, 17 November 2020.

[10] Shcherbatova O., Grebennikov D., Sazonov I., Meyerhans A., Bocharov G., Modeling of the HIV-1 Life Cycle in Productively Infected Cells to Predict Novel Therapeutic Targets, PATHOGENS 9(4):255, 10.3390/pathogens9040255, 2020.

[11] In vitro human immune response testing - COMPIT, NC3Rs Innovation Platform, NC3Rs, 2018. https://nc3rs.org.uk/crackit/vitro-human-immune-response-testing-compit

[12] Visit to Immundnz in February 2018, DISCOVERY ANALYTICAL CONSULTING, 2018. https://www.discoveryanalyticalconsulting.com/blog/2018/3/5/visit-to-immundnz-in-february-2018

[13] Report to Immunosys Ltd., Confidential report, February 2019.