Modelling Resistance Evolution

Theoretical Methodology Symposium

Book of Abstracts

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About

Drug resistance has become a global threat to human health. The rise of drug resistance has triggered a large number of mathematical studies aimed at obtaining a better understanding of its evolution and how to prevent or delay its emergence. These studies addressing different resistance problems, such as antimicrobial or cancer drug resistance, often use similar theoretical approaches. These approaches involve methods from branching processes, Markov chains, population dynamics, and stochastic numerical simulations. For the modelling of patient treatments, the models need to take into account several factors related to the dynamics of drugs and the host, including, for example, drug interactions, availability of resources in the host, or pharmacodynamics of the drugs.

Given the large number of factors that come into play, a large variety of models on resistance evolution exist. There is no "one-size-fits-all" model to study resistance in bacteria or cancer. While constructing or already working with a model, components of it may change since its advantages and disadvantages become apparent only then. This process is often not shared, although it could benefit other researchers. Generally, there is often no room for detailed discussions of the methods applied to model resistance evolution in scientific meetings.

With this symposium, we want to bring together scientists working on resistance problems (both in bacteria and cancer) to give place to discussions on the theoretical methods employed in the study of resistance evolution. For this purpose, this meeting will include "method sessions" aimed at discussing specific topics relevant to the modelling of antimicrobial resistance (e.g., drug-drug interactions, pharmacodynamics, and population growth models).

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Timetable

: Keynote Talk, : Contributed Talk, : Method Session

Wednesday 26th

8:45–9:00	**Welcome**	
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10:30–11:00	**Giorgio Boccarella** KU Leuven	The importance of persistence for the evolvability of antibiotic resistance
11:00–11:30	**Pierre Lafont** University of Edinburgh	Mathematical models of collective antibiotic tolerance
11:30–12:00	**Ian Dewan** MPI for Evolutionary Biology	Evolution of multidrug resistance from plasmid-mediated heterozygosity
12:00–12:30	**Muhittin Mungan** University of Cologne	Memory and Hysteresis in the adaptive evolution of bacterial resistance in environments of varying antibiotic concentration
12:30–12:35	**Group picture**	
12:35–13:30	**Lunch break**	
13:30–14:30	**Jasmine Foo** University of Minnesota	Computational methods for inferring tumor evolution and heterogeneity
14:30–15:00	**Coffee break**	
15:00–15:30	**Jona Kayser** MPZ für Physik und Medizin & MPI for the Science of Light	Multi-step Resistance Evolution in Compact Populations
15:30–16:00	**Michael Raatz** MPI for Evolutionary Biology	What to target in evolving populations - population size, growth or survival?
16:00–16:30	**Arne Traulsen** MPI for Evolutionary Biology	Ecological vs. game theoretical models for interaction (opening to method sessions)
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Thursday 27th

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10:30–11:00	**Linda Aulin** Leiden University & Freie Universität Berlin	Model-based design of innovative treatment strategies to suppress antimicrobial resistance using collateral sensitivity
11:00–11:30	**Eshan King** Case Western Reserve University School of Medicine	Fitness seascapes reveal heterogeneous mutant selection windows in clinically-relevant pharmacokinetic models
11:30–12:00	**Anuraag Bukkuri** Moffitt Cancer Center & Lund University	Modeling Stress-Induced Responses in Bacterial and Cancer Therapeutic Resistance
12:00–12:30	**Irina Kareva** Northeastern University	Mechanisms of non-genetic resistance to cancer therapy
12:30–13:30	**Lunch break**	
13:30–14:30	**Jacob Scott** Cleveland Clinic	TBA
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Friday 28th

9:00–10:00	**Barbora Trubenová** ETH Zurich	Modeling drug resistance in bacterial biofilms and parasitic worms
10:00–10:30	**Coffee break**	
10:30–11:00	**Joachim Krug** University of Cologne	Competing effects of mutation bias and selection on resistance evolution
11:00–11:30	**Jessica Renz** University of Bergen	Learning and predicting the pathways of AMR evolution with hypercubic inference
11:30–12:00	**Malgorzata Weh** H. Lee Moffitt Cancer Center & University of South Florida	Modeling selection for evolvability in the evolution of cancer therapy resistance
12:00–12:30	**Suman G. Das** University of Cologne	Repeatability of antibiotic resistance evolution for heavy-tailed distributions of fitness effects
12:30–13:30	**Lunch break**	
13:30–14:30	**Tobias Bollenbach** University of Cologne	Quantitative descriptions of bacterial responses to antibiotics
14:30–15:00	**Coffee break**	
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Abstracts – Keynote talks

Wednesday 26th

Stochastic models of resistance evolution

Hildegard Uecker

Max Planck Institute for Evolutionary Biology, Germany

Stochasticity affects several steps in the evolution of resistance. The appearance of resistant types by mutation or transfer of resistance genes from a source population is a stochastic process. Once resistant types have appeared, they might suffer stochastic loss while rare. During the infection, bottleneck events can lead to further randomness in the dynamics of resistant pathogens. Finally, to persist at the level of the host population, resistant pathogens need to spread to new hosts, which again involves stochasticity – host encounter (or whatever transmission route is relevant) is not guaranteed, and even if hosts meet, pathogens need to go through a potentially narrow bottleneck and establish a new infection. In this talk, I will present a general and flexible framework based on branching process theory to study aspects of stochasticity at various points on the path to resistance. I will apply it to several scenarios such as the transfer of resistance plasmids from commensals to pathogens or the spread of resistance in a host population.

Computational methods for inferring tumor evolution and heterogeneity

Jasmine Foo

University of Minnesota, USA

Tumors are typically comprised of heterogeneous cell populations exhibiting diverse phenotypes. This heterogeneity, which is correlated with tumor aggressiveness and treatment-failure, confounds current drug screening efforts to identify effective candidate therapies for individual tumors. In the first part of the talk I will present a modeling-driven statistical framework that enables the deconvolution of tumor samples into individual subcomponents exhibiting differential drug-response, using standard bulk drug-screen measurements. In the second part of the talk I will present some efforts towards obtaining insights about tumor evolution from standard genomic data. In particular, we analyze the site frequency spectrum (SFS), a population summary statistic of genomic data, for exponentially growing tumor populations, and we demonstrate how these results can in principle be used to gain insights into tumor evolutionary parameters.

Thursday 27th

Stochastic emergence of drug resistance in variable environments

Helen Alexander

University of Edinburgh

In initially drug-sensitive populations of pathogens or cancerous cells, resistance emerges during drug treatment in some, but not all, populations. This observation of variable outcomes motivates the use of stochastic mathematical models to describe and predict de novo evolution of resistance. Both rate of appearance and fate of resistant mutants depend on the environment, which varies over the course of drug treatment: firstly due to drug dosing and pharmacokinetics, and secondly because the focal population feeds back on its own environment (by consuming and producing substrates). In this talk, I will first outline basic mathematical equations for the probability of emergence of resistance, and how these equations could incorporate environmental variation over time. I will then describe a theoretical case study modelling the emergence of drug resistance in chronic viral infections. This case study illustrates both a general analytical approach, and an example of how environmental feedbacks influence stochastic emergence of resistance. Next, I will present experimental results with Pseudomonas aeruginosa (an opportunistic bacterial pathogen) that give insights into how the environment shapes emergence of resistance. In particular, initial density of the sensitive population plays a surprisingly complex role. This empirical evidence motivates ongoing extensions to our mathematical models.

Perturbing the ecological forces underlying drug resistance in cancer: towards evolutionary therapy

Jacob Scott

Cleveland Clinic, USA

Tumors are not just collections of mutated cells, they are complex ecosystems of interacting clones and host elements. This type of system is well known to theoretical ecologists, who have been using mathematical models to understand, and even bias, naturally occurring systems like fisheries and game reserves. In this spirit, we have been working to develop mathematical models to describe tumors in this way, and further, to connect these models directly to experimental measurements. Specifically, we have developed an in vitro assay to directly parameterize an evolutionary game theory model, and have begun characterizing cell-cell interactions in heterogeneous model tumors. Using this assay, we have documented evidence of frequency dependent fitness, a necessary condition for adaptive therapy and significant ecological effects on cell fitness which strongly affects the emergence of drug resistance. I will describe our findings in EGFR+ and ALK+ non-small cell lung cancer, and propose both clinical and biological next steps to making personalized adaptive (evolutionary) therapy a reality.

Friday 28th

Modeling drug resistance in bacterial biofilms and parasitic worms

Barbora Trubenová

ETH Zurich

Both bacterial and helminth infections are commonly treatable by suitable drugs. However, these pathogens are constantly evolving ways to escape drug treatment.

Bacteria often protect themselves by forming biofilms - high-density colonies attached to a surface or each other. Such a sedentary lifestyle of biofilm cells comes associated with costs and benefits. While the growth rate of biofilm populations is often significantly lower than that of their free-living counterparts, this cost is repaid once the colony is subjected to antibiotics: biofilms can survive in antibiotic concentrations up to a thousand times higher than those killing their free-living counterparts. Studies have shown that such phenotypic protection influences the evolution of drug resistance in a non-intuitive way.

Helminths, particularly nematodes, are a diverse group of macroscopic parasites causing a plethora of human and animal diseases. Nematodes are diploid organisms with complex sexual reproduction and life cycles often involving multiple hosts, which makes it extremely difficult to study them experimentally. Therefore, many extrinsic and intrinsic factors affecting drug resistance evolution in these creatures are yet poorly understood.

Despite the significant biological differences between these two taxa, fundamental evolutionary principles governing resistance evolution are the same. Here, we take advantage of this similarity and develop a framework combining pharmacodynamics and pharmacokinetics with population genetics, which we apply to investigate drug resistance evolution in bacterial biofilms and parasitic worms. We explore the effects of various phenotypic mechanisms present in bacterial biofilms on the population dynamics of bacterial populations and investigate their consequences for the evolution of antibiotic resistance. In helminths, we show the effect of population size on the rate of resistance evolution.

Quantitative descriptions of bacterial responses to antibiotics

Tobias Bollenbach

University of Cologne

When multiple antibiotics are combined, they can interact in diverse and difficult-to-predict ways. Two antibiotics may synergize or antagonize, inhibiting bacterial growth more or less than expected. Such drug interactions can strongly influence the dynamics of resistance evolution and, in extreme cases, lead to selection against drug resistance. I will present how drug interactions are quantitatively characterized and show that a theoretical description based on bacterial growth laws, combined with drug uptake and binding kinetics, allows direct prediction of a large fraction of observed drug interactions between antibiotics targeting translation. Additional interactions are explained by "translation bottlenecks": points in the translation cycle where antibiotics block ribosomal progression. In particular, extremely strong antagonistic interactions, where the addition of one antibiotic facilitates bacterial growth in the presence of another, are faithfully captured by a theoretical description based on the totally asymmetric simple exclusion process (TASEP). Finally, I will show how similar theoretical descriptions of bacterial growth can capture quantitative features of the bacterial response to antibiotics with other cellular targets, and discuss the relevance of these phenomena for the dynamics of resistance evolution.

Abstracts – Contributed talks

Wednesday 26th

Talk Session 1

The importance of persistence for the evolvability of antibiotic resistance

Giorgio Boccarella, Piet van den Berg, and Jan Michiels

KU Leuven, Belgium

Bacterial persistence plays a crucial role in determining the number of surviving cells after antibiotic exposure, thereby having an important effect on the effectiveness of antibiotic treatment. Recent evidence suggests that the persister phenotype may also influence the evolvability of antibiotic resistance. The most common hypothesis to explain this link is that persistence leads to a larger reservoir of viable cells, thus 'buying the population time' to generate resistance mutations. However, persistence has the potential to affect the evolutionary process in many other ways that have so far remained underexplored. Using an evolutionary simulation approach, we here investigate how persister frequency and lag time influence the probability and speed of fixation of resistance mutations depending on the antibiotic treatment regime. With a simple model we show that lower persister frequency is associated with higher extinction, but a comparatively faster fixation of these mutations if they do fixate. We then present a framework to study the interplay between the antibiotic driven bottleneck size (determined by persistence level) and the lag time of these surviving cells (based on empirical lag time distributions) on the predictability of resistance evolution. More generally, our work stresses the importance of considering the impact of phenotypic heterogeneity on the evolutionary process.

Mathematical models of collective antibiotic tolerance

Pierre Lafont, and Helen Alexander

School of Biological Science, University of Edinburgh, UK

We are interested in modelling Collective antibiotic tolerance (CAT). CAT occurs any time a bacterial population of sufficiently high density survives an antibiotic dose or treatment that a smaller population would succumb to. Various mechanisms have been identified, including cell-to-cell signalling and antibiotic degradation. A known manifestation of CAT is through occurrence of inoculum effect (IE), most often shown through minimum inhibitory concentration (MIC) assays. Definitions of IE and more largely of CAT are slightly inconsistent across the literature and models looking into this effect do so in different ways. Collective responses such as CAT are important to take into account as accurately as possible when designing treatment strategies/regimens. We undertook a systematic review of mathematical models to have a more complete picture of how CAT has been modelled in the literature. To be selected, models should describes population dynamics of one or more bacterial populations facing antibiotic treatment or analogues such as anti-microbial peptides (AMPs). It must include some density-dependent response of bacteria to antibiotic concentration. This effect can be built-in, where response to antibiotic is a function of the MIC, for instance. Or it can be emergent, by including antibiotic removal by bacteria or bacterial product (e.g. enzymes such as beta-lactams). This work gives us metrics to depict the landscape of mathematical modelling associated to CAT. Although those numbers are still preliminary, 47 modelling papers were selected, 85% were published in the past 12 years. ODE systems are over-represented (37). While built-in and emergent implementation of CAT is equally represented, many of the models considered do not explicitly mention CAT, meaning that consequences of CAT are not explored in these. We also observe that very few modelling papers make qualitative predictions on IE, but those that do, do not capture the range of experimental observations. This is combined with a mismatch when comparing model outputs to experimental data. These finding demonstrate a still incomplete understanding of the implications of CAT in bacterial population dynamics. Understanding the consequences of CAT, and how to model these collective effects are very relevant to the way design treatment strategies and understand complex interactions between drugs and bacteria. How, for example, to better link model outputs to reproduce common experimental assays. I am also interested in discussing critically MIC assays as a proxy for measuring IE, in particular in the context of emergence and establishment of resistance. Reliable models of CAT are key to produced testable predictions of how these collective effect impact evolution of resistance.

Evolution of multidrug resistance from plasmid-mediated heterozygosity

Ian Dewan, and Hildegard Uecker

Department of Evolutionary Theory, Max Planck Institute for Evolutionary Biology, Germany

Antibiotic resistance genes are frequently carried on bacterial plasmids. Because plasmids exist in multiple copies in the host bacterial cell, distinct plasmid copies can carry distinct alleles, allowing for heterozygosity not possible for loci on haploid bacterial chromosomes. This plasmid-mediated heterozygosity of antibiotic resistance alleles can produce multidrug resistance, in which a single bacterial strain is resistant to multiple antibiotics, which is a serious problem in the clinical context. However, the contribution of plasmid-mediated heterozygosity to resistance evolution is limited by the fact that it is subject to constant loss due to random segregation of plasmids on cell division: each division has some probability of producing a homozygous daughter cell. We present a model of the rapid evolution of multidrug resistance in a bacterial population due to plasmid-mediated heterozygosity, focusing on the establishment of a novel resistance allele on a plasmid in a bacterial population already adapted to one antibiotic but undergoing demographic decline due to simultaneous treatment with multiple antibiotics (an evolutionary rescue scenario). We show the probability of population persistence (rescue) of the bacterial population is largely determined by the selective advantage of the heterozygote (multidrug resistant) bacteria and the plasmid copy number. In particular, we determine the threshold on the selective advantage of heterozygotes required to overcome segregative loss and make population persistence possible at all; this threshold decreases with increasing copy number of the plasmid. We further show that the possibility of the formation of plasmid cointegrates from the fusion of plasmids increases the probability of rescue, as cointegrated plasmids which carry both resistance alleles are no longer subject to stochastic loss. These results contribute to our understanding the evolution of antibiotic resistance in complex selective environments and the contribution of plasmid traits, such as copy number to bacterial evolution; future work will include extending the models to the emergence of multidrug resistance by multiple mutations from entirely non-resistant cells, and the incorporation of horizontal gene transfer.

Memory and Hysteresis in the adaptive evolution of bacterial resistance in environments of varying antibiotic concentration

Suman G. Das, Joachim Krug, and Muhittin Mungan

Institute of Biological Physics, University of Cologne, Germany

Evolution in changing environments is still poorly understood. We analyze a recently introduced and empirically well-grounded model for antibiotic resistance evolution in bacteria [1]. In this model the corresponding fitness landscape changes with the antibiotic concentration, thereby giving rise to tradeoffs between adaptation to low and high antibiotic concentrations. We show that the adaptive evolution under slowly changing antibiotic concentration exhibits hysteresis loops and memory formation: the selection of a fit genotype not only depends on the current concentration of the antibiotic, but also on the history of concentration changes. Our method of analysis borrows ideas and techniques that were developed for the study of the dynamics driven disordered condensed-matter systems [3].

References

[1] Suman G. Das, Susana O. L. Direito, Bartlomiej Waclaw, Rosalind J. Allen, Joachim Krug, "Predictable properties of fitness landscapes induced by adaptational tradeoffs," eLife 9 (2020) e55155.
[2] Suman G. Das, Joachim Krug, and Muhittin Mungan, "Driven Disordered Systems Approach to Biological Evolution in Changing Environments," Phys. Rev. X 12 (2022) 031040.
[3] M. Mungan, "Putting Memories on Paper," PNAS 119 (2022) e2208743119.

Talk Session 2

Multi-step Resistance Evolution in Compact Populations

Serhii Aif, Oskar Hallatschek, and Jona Kayser

[1] Max-Planck-Zentrum für Physik und Medizin, Germany
[2] Max Planck Institute for the Science of Light, Germany

Many pathogenic cellular populations, such as microbial biofilms or solid tumours, are densely packed. However, little is known about how growth-induced collective dynamics - an inherent feature of these systems - reshape the evolution of resistance against antibiotic or anti-cancer therapy. Modelling such emergent phenomena, coupling the mechanical interactions of individual cells to evolutionary outcomes on the population level, is inherently challenging. In my presentation, I will discuss an integrated modelling approach that combines concepts from active granular matter physics and stochastic numerical models with agent-based simulations and data from genetically tailored microbial experiments. Using this strategy, I will show how spatial population expansion and a density-mediated alteration of selection conspire to create an "inflation-selection balance". The resulting stabilization of less-fit resistant mutants facilitates their continued evolution, including evolutionary rescue via subsequent cost-compensatory mutations. Finally, I will give a brief outlook on how these physical effects could be integrated with other models to inform evolution-based therapy strategies.

What to target in evolving populations - population size, growth or survival?

Michael Raatz, and Arne Traulsen

Department of Evolutionary Theory, Max Planck Institute for Evolutionary Biology, Germany

Populations of microbial pathogens or cancer cells possess enormous adaptive potential. Such adaptations regularly lead to the failure of treatment, with drastic consequences for individual and public health. From a reductionistic viewpoint, the fundamental processes in such microbial populations are replication, mutation and death. Characterizing these processes by traits allows us to understand adaptation as an uphill walk on a fitness landscape spanned by replication rate and death rate, with mutation rate dictating the walking speed in this picture. Different treatment types exist to tamper with any of these fundamental processes. How such treatment types affect the trajectory of adaptation in trait space is not clear. In this contribution, I will tackle this question and present i) which exact trajectory a population takes in a trait space spanned by replication rate and death rate, ii) how this trajectory is affected by treatment, and iii) how treatments that target either the population size via bottlenecking or the traits via static and toxic drugs differ. Further, I will discuss the fitness gradient(s) that prescribe the adaptation and show that the information on such fitness gradients can guide effective treatment strategies.

Method Session: Opening

Ecological vs. game theoretical models for interaction

Arne Traulsen, and Corina Tarnita

Department of Evolutionary Theory, Max Planck Institute for Evolutionary Biology, Germany

Ecological vs. game theoretical models for interaction Both ecologists and evolutionary game theorists study the dynamics in populations of interacting types. Ecologists prefer to use the Lotka-Volterra equations (and non linear generalizations of it), while evolutionary game theorists use the replicator dynamics instead. In their book, Josef Hofbauer and Karl Sigmund have shown that these two approaches are mathematically closely related and lead to the same orbits. Unfortunately, this relation between the two approaches seems less appreciated by experimentalists. On the other hand, a deeper understanding of interactions may emerge from a better understanding of the relationship between the two approaches.

Thursday 27th

Talk Session 3

Model-based design of innovative treatment strategies to suppress antimicrobial resistance using collateral sensitivity

Linda B. S. Aulin[1,2], **Apostolos Liakopoulos**[3], **Daniel E. Rozen**[3], **J. G. Coen van Hasselt**[1]

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To alleviate the threat of antimicrobial resistance (AMR), innovative treatment strategies are urgently needed. The phenomenon of collateral sensitivity (CS) may be exploited to achieve this goal using existing antibiotics. CS occurs when resistance to one antibiotic increases the sensitivity to another antibiotic. CS-based combination treatments could potentially suppress resistance, but it remains unclear how to design such treatments. To this end, we use a model-based approach to assess the potential of CS-based treatments to suppress AMR, integrating pharmacokinetics-pharmacodynamics (PK-PD) and principles of evolutionary dynamics. A modelling framework was developed including components accounting for antibiotic PK-PD and population dynamics of bacterial growth and evolution of resistance. Bacterial population dynamics was described using a four-state stochastic hybrid ordinary differential equation model, where each state represents a bacterial subpopulation, including a wild type (WT) population, two single mutant subpopulations, and a double mutant subpopulation. Resistance evolution was modelled according to a stochastic process based on a binomial distribution informed by a mutation rate. Each subpopulation had unique antibiotic sensitivity based on the minimum inhibitory concentration (MIC). Antibiotic sensitivities were incorporated in sigmoidal concentration-effect relationships. The framework was implemented using the RxODE package in R. We used the framework to systematically study how certain pathogen- and drug-specific parameters influence AMR evolution. We simulated different combination dosing regimens with sequential, cyclic, or simultaneous administration using two antibiotics, ABA and ABB, which were assumed to have identical PK and additive bacterial killing effects. Here, in the bacterial model, the two single mutant subpopulations represent resistance to ABA and ABB, respectively, while the double mutant subpopulation was resistant to both ABA and ABB. To understand how antibiotic with different types of PD impact AMR evolution we performed simulations varying the drug-specific parameters relating to the maximal effect and shape of the concentration-effect relationship. We studied the importance of CS reciprocity, effect magnitude, and how pathogen-specific factors including fitness cost and mutation rate, influence the ability of CS to suppress the resistance for different dosing regimens. We find that the impact of reciprocal CS relationships on the probability of resistance at end of treatment is dependent on the drug PD type and dosing regimen. Simultaneous or one-day cycling treatment schedules were most effective dosing regimens to suppress resistance. For these treatments, a CS effect

of 50% fully suppressed the resistance when concentration-dependent antibiotics were used. The effect of antibiotic concentration shows that CS-based treatments are most clinically relevant for antibiotics with a narrow therapeutic window. One-directional CS relationships, and not only reciprocal relationships, can be utilized in the design of CS-based treatment schedules. In this analysis, we used modelling and simulation to systematically unravel drug- and pathogen-specific factors influencing optimal design of resistance-pressing CS-based treatment strategies. Our modelling approach addresses important open questions around the topic of CS that are not easily tested experimentally, and provides new insights regarding key design aspects of CS-based treatments, contributing to the unmet need toward innovative strategies to alleviate the threat of AMR.

Fitness seascapes reveal heterogeneous mutant selection windows in clinically-relevant pharmacokinetic models

Eshan S. King, Beck Pierce, Michael Hinczewski, and Jacob G. Scott

Case Western Reserve University School of Medicine, USA

The evolution of drug resistance in infectious disease and cancer is a serious threat to public health. The mutant selection window (MSW), defined as the range of drug concentrations that selects for a drug resistant strain, has previously been used as a model to predict and avoid resistance. Under the MSW paradigm, drug regimens should be designed to minimize time spent in the MSW. A limitation of the MSW model is that it only offers comparisons between two strains at a time– i.e. between drug sensitive and drug resistant strains. In contrast, fitness seascapes, which we model as collections of genotype-specific dose-response curves, provide comparisons between many genotypes simultaneously. Furthermore, previous work has shown that MSW comparisons are intrinsically embedded in fitness seascapes. Here, we explore the consequences of modeling evolution with fitness seascapes through the lens of the MSW framework. First, we show how an N-allele fitness seascape embeds N*2N mutant selection window comparisons. Then, we develop mathematical models for drug pharmacokinetics in three clinically-relevant scenarios: serum drug concentration during a daily dosing regimen and drug diffusion in tissue in 1- and 2-dimensions. Each scenario reveals the presence of heterogeneous mutant selection windows. Importantly, we find that different MSWs arise at different times in a treatment regimen, and multiple MSWs appear simultaneously at different points in space. While prior work has analyzed the importance of time spent in a MSW, we argue that both time and space occupied by a MSW impact the probability of drug resistance. This work further explores the connection between mutant selection windows and fitness seascapes using realistic pharmacokinetic and drug diffusion models. Our results highlight the importance of fitness seascapes in modeling evolution when drug concentration varies in time and space. Furthermore, because of the multiplicity of mutant selection window comparisons in a single fitness seascape, this work suggests that two-state mutant selection window models may not be sufficient to predict or control the evolution of drug resistance.

Modeling Stress-Induced Responses in Bacterial and Cancer Therapeutic Resistance – Plasticity and Genetic Evolution

Anuraag Bukkuri

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[2] Lund University, Sweden

Mathematical models of cancer and bacterial evolution have generally stemmed from a gene-centric framework, assuming clonal evolution via acquisition of resistance-conferring mutations and selection of their corresponding subpopulations. More recently, the role of phenotypic plasticity has been recognized and models accounting for phenotypic switching between discrete states have been developed. However, seldom do models incorporate both plasticity and mutationally-driven resistance. In this talk, we will use evolutionary game theory, matrix population models, and integral projection models to develop a framework that can incorporate plastic and mutational mechanisms of acquiring resistance in a continuous/discrete and gradualistic fashion, respectively. We use this framework to examine ways in which populations can respond to stress (focusing on polyaneuploid cancer cells, neuroblastoma, and bacteria) and consider implications for therapeutic strategies. Although we primarily discuss our framework in the context of cancer and bacteria, it applies broadly to any system capable of evolving via plasticity and genetic evolution.

Mechanisms of non-genetic resistance to cancer therapy

Irina Kareva

Northeastern University, USA

Development of therapeutic resistance in cancer is typically attributed to natural selection, where a cytotoxic agent eliminates sensitive cells in the population, leaving behind only the resistant ones. However, it appears that non-genetic mechanisms of therapeutic resistance exist as well, and as such they may be reversible through better understanding of underlying biology. Here we discuss two examples of non-genetic resistance to cancer therapy: resistance to PI3K inhibitors that can be reversed through combination therapy that targets metabolism, and resistance to checkpoint inhibitors, which may be addressed through modifying the dosage of drug administration. We discuss existing evidence for these mechanisms, and possible modeling approaches that may be applied to help mitigate non-genetic resistance to cancer therapy.

Friday 28th

Talk Session 4

Competing effects of mutation bias and selection on resistance evolution

Su-Chan Park, and Joachim Krug

Institute for Biological Physics, University of Cologne, Germany

Recent experiments on the evolution of drug resistance in bacteria have identified a transition from the preferred substitution of high-rate, low-effect mutations to low-rate, high-effect mutations with increasing population size [1]. The greater mutation supply in large populations increases the probability for rare high-effect mutations to arise, which subsequently outcompete the more frequent low-effect mutations through intensified clonal interference. In this way, the balance between mutation bias and selective differences in their effects on mutation choice is increasingly skewed in favor of selection in large populations. A minimal setting in which the interplay between mutation bias, selection and population size can be quantified is provided by the Yampolsky-Stoltzfus model, which considers the competitive fixation of two mutations, one of which is favored by mutation bias and the other by selection [2]. Using a suite of approximations based on a detailed analysis of the fixation process, we derive accurate expressions for the relative fixation probability of the two mutations that cover all adaptive regimes of interest. This allows us to precisely pinpoint the critical population size beyond which mutation bias is superseded by selection for any choice of mutation rates and selection coefficients.

References

[1] M.F. Schenk et al., Population size mediates the contribution of high-rate and large-benefit mutations to parallel evolution. Nature Ecology & Evolution 6:439-447 (2022).
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Learning and predicting the pathways of AMR evolution with hypercubic inference

Jessica Renz

University of Bergen, Norway

Understanding the evolution of antimicrobial resistance is central for their treatment. In this talk, I want to show a possible way to address this problem from a statistical point of view, namely the hypercubic inference, which we developed and introduced during the last years at the University of Bergen. The basis of this model is a hypercubic transition graph, whose nodes represent possible resistance states and the edges between correspond to the different evolutionary steps. This new approach allows us to efficiently make predictions about the most likely evolutionary pathways leading to AMR and learn their structure and variability. For this we can either use Bayesian inference via Monte Carlo Markov Chain methods or a frequentist approach for the estimation of likelihoods, whereby we only need cross-sectional datasets. The focus of the talk will be the introduction and explanation of the methods themselves, whereby I will address both the advantages and strengths of using a hypercubic structure, but also open problems and ongoing work. In addition, I will also present the results of concrete current applications to real AMR datasets from Klebsiella pneumoniae and Escherichia coli and discuss some biological insights that can be derived from them.

Modeling selection for evolvability in the evolution of cancer therapy resistance

Malgorzata Tyczynska Weh, Andriy Marusyk, and David Basanta

[1] H. Lee Moffitt Cancer Center, USA
[2] University of South Florida, USA

Despite rapid initial responses and low toxicity, targeted therapies commonly fail to provide long-term benefits to cancer patients due to the development of therapy resistance. In multiple solid tumors, this resistance emerges due to gradual, multifactorial adaptation, i.e., a selective process combining genetic and non-genetic methods of cell diversification. This suggests a significant link between the evolution of cancer treatment resistance and evolvability – a selective trait of generating heritable phenotypic variation. However, the interplay between selection, evolvability, and resistance has not yet been fully investigated. We addressed this problem by studying the selection for mutator phenotype. The mutator phenotype is common in many cancers and results from errors in DNA repair mechanisms. This phenotype generates mutations at a higher frequency than other phenotypes. Since mutations can both benefit or reduce cell viability, we hypothesized that the selection for a mutator phenotype changes during the evolution of resistance to cancer targeted therapies. We tested this hypothesis by developing a 2D on-lattice Agent-Based Model (ABM). In the model, a cell can die, divide and mutate, yet mutations have a stochastic impact that can be beneficial, neutral, or deleterious for the individual cell fitness. Consequently, the resistance emerges as a stochastic event depending on the mutation frequency. Our results demonstrate that 1) the mutator phenotype initially accelerates adaptation to treatment, but 2) only intermediate mutation frequencies can sustain high fitness long-term. This work provides a versatile experimental platform that can be adjusted to study the evolution of resistance in other cancers beyond NSCLC and treatments. Moreover, our results challenge the commonly held assumption that resistance develops only due to pre-existing driver mutations and provide an opportunity to integrate evolutionary theory and oncology to improve treatment in cancer patients.

Repeatability of antibiotic resistance evolution for heavy-tailed distributions of fitness effects

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The repeatability of evolution depends strongly on the distribution of fitness effects (DFE) of beneficial mutations. While theoretical modeling has focused mainly on light-tailed DFEs, experiments on antibiotic resistance evolution have also uncovered signatures of heavy-tailed DFEs. We show that in the latter case the repeatability behaves in counter-intuitive ways. Firstly, the evolutionary process is dominated by only a few mutations even in the limit of an infinite number of available beneficial mutations. This enhances the repeatability, but it also implies that the degree of repeatability is less predictable from the DFE. Secondly, the measure of repeatability becomes a non-self-averaging variable which does not converge to its mean. This necessitates a careful conceptual distinction between typical and mean values of the repeatability measure, with important consequences for the quantification of the repeatability of antibiotic resistance evolution from empirical data. I will discuss the theoretical results and illustrate them with experimental data on the DFE of mutations in an antibiotic resistance enzyme.

Mechanistic pharmacokinetic/pharmacodynamic understanding of the antibiotic therapy of piperacillin and tazobactam and its role in resistance development – combining in vitro and in silico approaches

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Worldwide, an increase in spread of extended spectrum β-lactamases (ESBL) producing Escherichia coli, is threating the public health. Piperacillin/tazobactam (PIP/TAZ) is a widely used antibacterial combination therapy and could be a valuable alternative to the carbapenems, which are the first line therapeutic option against ESBL producing Enterobacteriaceae today. PIP is a β-lactam antibiotic and TAZ acts as a β-lactamase inhibitor capable of inhibiting ESBL β-lactamases belonging to the group of CTX-M [1]. PIP/TAZ is today administrated in a fixed 8:1 dose ratio, although there is no stated scientific rationale for the 8:1 dose ratio [2]. In addition, according to the guideline of European Committee on Antimicrobial Susceptibility Testing, the minimum inhibitory concentration (MIC) of PIP in vitro is determined using a fixed TAZ concentration of 4 mg/L [3]. However, the rational underlying the fixed TAZ concentration is not specified nor is how the resulting MIC is related to the 8:1 dose ratio used in vivo. To establish a rational dosing strategy, a quantitative understanding of the pharmacokinetics (PK) and the pharmacodynamics (PD) is essential. For antibiotics, the MIC is often used as a PD metric and in conjunction with the PK to derive PK/PD indices that are related to e.g. treatment outcome. However, the MIC is a summary metric with the limitation of being based on a single time point visual read out, and does not accurately reflect kill or (re)-growth dynamics. Furthermore, there is no established PK/PD index for β-lactam-β-lactamase inhibitor combinations [4]. Thus, further investigation into the PD interaction of PIP/TAZ and its role in resistance evolution is warranted. To this end, this project aims to mechanistically elucidate the PK/PD of the PIP/TAZ combination in clinical E. coli isolates to enable the characterisation of in vitro resistance development. To achieve the objective, we here present a workflow how assessment of in vitro resistance development can be incorporated in a modelling framework. Time-kill experiments will be performed to characterise bacterial (re-)growth and kill dynamics during PIP/TAZ exposure for different concentration combinations, thus going beyond the current 8:1 ratio. Furthermore, experiments designed to identify the acquisition of resistance mechanisms over time will be performed. In parallel, a mechanistic PD model describing the PD interaction between the drugs as well as the drug-pathogen interaction will be developed in silico. The mechanistic model will enable us to describe the dynamics in the system, including the evolution of resistance development. As a next step, a new PD metric shall be derived that includes the consideration of resistance evolution. Moving beyond the MIC, a quantitative mechanistic understanding of the PK/PD of PIP/TAZ and resistance development would enable us to apply a translational approach facilitating the design of optimised dosing regimens.

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Stochastic modelling of lineage correlations in glioblastoma cells to capture non-genetic heterogeneity

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Glioblastoma is one of the most aggressive and difficult to treat cancers. One obstacle to developing effective and widely applicable treatments is the high level of heterogeneity both between patients and within a single tumour. The observed variability in treatment response even in genetically identical cells could be random due to the inherent stochasticity of gene regulation or alternatively an unrecognised deterministic process. We propose several candidate models for cell proliferation and death following radiotherapy that aim to capture this heterogeneity and the non-trivial correlation structure of genealogically related cells. We use live-cell imaging data on different patient-derived cell lines with stably expressed H2B reporters to identify and track their nuclei. A computational image analysis pipeline extracts the cell fates and genealogical information to automatically construct lineage trees from the microscopy data. The mathematical models for the proliferation dynamics are formulated within the Bayesian framework to capture the high levels of stochasticity present in the data and allow for a probabilistic interpretation of the results. They incorporate different hypotheses for the inheritance mechanics, such as deterministic inheritance, single-factor inheritance from mother cells and effects due to the circadian rhythm. Model parameters describing proliferation and mortality rates as well as the correlation structure are extracted using Bayesian inference techniques and a model comparison is carried out to quantify the goodness of fit of the individual models for the various cell-lines. Once parameter settings are extracted, our mathematical models can be used to simulate the responses of the cancer cells to different treatment approaches. A better understanding of the mechanisms governing the observed heterogeneity can inform the design of novel treatment strategies, such as new dosing schedules or drug-radiation combination therapies. This is work in progress.

Role of bacterial filamentation in population dynamics at sub-MIC concentrations of cefotaxime

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Treating Escherichia coli with the antibiotic cefotaxime at sub-MIC concentrations leads to complex responses: (i) filamentation of cells, which is known to be related to delayed lysis and enhanced antibiotic tolerance, (ii) higher biomass growth rates at intermediate antibiotic concentrations, and (iii) increased stochastic variation of the growth rate with growing antibiotic concentrations. Moreover, we find that the filamentation displays complex time-dependent dynamics, with a crossover from filamented to normal-sized cells at long times near the MIC. We explore the relationship between filamentation, growth rates, and the time-dependent drug concentration in the medium through experiments and modeling, and discuss possible consequences for the evolution of drug resistance.

Modelling growth of pancreatic cancer cell lines and unveiling treatment effects

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In vitro experiments are an important tool in cancer research to understand the growth of cancer cells and their response to chemotherapeutic treatment. Mathematical models facilitate the analysis of the obtained experimental data and can increase our understanding of cancer cell dynamics.

Using confluence time series capturing the in vitro growth of pancreatic cancer cell lines treated with different doses of Gemcitabine, we have defined a system of ordinary differential equations to describe the logistic growth over time. Distinguishing cell growth into birth and death processes and applying Bayesian inference, we have estimated the birth and death rates and their variability across different cell lines and drug concentrations. We derived dose-response curves for the birth and death rates and found that Gemcitabine has a mainly cytostatic effect on Panc1 Parental cell lines, while it acts both cytostatic and cytotoxic in Panc89 Parental cell lines.

Our mathematical approach thus helps to analyze the experimental data, widens the understanding of how chemotherapeutic drugs affect the growth of different cancer cell lines, and also deepens our knowledge of the role of drug concentration.

Establishment threshold and the inference of establishment rates along a concentration gradient

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Evolution of drug resistance is contingent on sufficient mutational supply as well as successful establishment of the initially rare resistant cells that are subject to stochastic extinction. Importantly, the potential for resistance evolution strongly depends on the drug concentration that not only directly affects the strength of selection, but also impacts the required mutational targets, probability of establishment as well as the mutational processes that generate genetic and phenotypic variability. Quantifying the risk of drug resistance along a concentration gradient thus represents a highly important but challenging task, because this requires factoring in the various complex dose-dependencies none of which are easily empirically investigable. In this contribution we present a fitting method which allows the inference of dose-dependent establishment rates from evolutionary rescue data and present theory which can then be used to compute optimal treatment strategies. We also refine the concept of establishment threshold and discuss its implications for resistance evolution.

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Persistence and resistance evolution in weeds with complex life cycle

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Weeds are a major threat to crop production, causing the highest potential yield losses. Already since the late 1960s conventional agriculture has primarily relied on the application of herbicides for controlling weeds. However, the number of available herbicides is limited and they often share the same mode of action. The overuse of those active ingredients has led to the widespread evolution of herbicide resistance in weeds. Weedy traits that increase the persistence, like seed banks and asexual propagation, impose further challenges to weed management. Effective and sustainable weed management strategies are urgently needed. Mathematical modelling can contribute to our understanding of the processes that drive the evolution of resistance in weeds and inform the development of management tactics. In this talk, I present a population-based model of seed and rhizome propagated perennial weeds, specifically Sorghum halepense, incorporating the complete complex life cycle and control measures of herbicide application and tillage. I show that the natural frequency of target-site resistance mainly depends on the resistance cost and less on its dominance. I highlight the pivotal role of the sexual phase of the life cycle, including self-pollination and seed bank dynamics, in the persistence and rapid herbicide resistance adaptation of Sorghum halepense. In particular, I illustrate how the seed bank helps preserve genetic diversity under recurrent selective pressure imposed by herbicides. Moreover, I indicate the potential of the seed bank to ensure population survival under control by delaying extinction and increasing the probability that resistant mutants establish. I show that the speed of resistance evolution in self-pollinated plants increases in such seed and rhizome-propagated weeds.

A network model for the growth of a bacterial population in adverse environments

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Bacterial populations can consist of several isogenic subpopulations known as phenotypes. Individuals in a bacterial population can switch from one phenotype to another in order to adapt to changing environments. Phenotypic switching can thus confer survival benefits to a bacterial population and may be a mechanism for the development of antibiotic resistance. Kussell and Leibler in "Phenotypic diversity, population growth, and information in fluctuating environments" ("Science", 2005), studied a model of a bacterial population consisting of “n” phenotypes and “n” environments. The population is subjected to these “n” different environments, which occur in a random sequence. In a given environment, a single phenotype is the “fittest", which implies that individuals of that phenotype have the largest growth rate. Kussell and Leibler derived an analytical expression for the Lyapunov exponent, which is a measure of the asymptotic growth rate of the total population. We study a modified version of their model by constructing a network model of the bacterial population, such that each environment can be thought of as a specific antibiotic where the corresponding “fittest” phenotype is resistant and drives the population growth. In our network model, each node represents a single phenotype, and each directed edge represents the ability of one phenotype to switch to some other phenotype. Kussell and Leibler derived an analytical expression for the Lyapunov exponent under the assumption that all the inter-phenotypic switching pathways are available. We compute the Lyapunov exponent of bacterial populations corresponding to different network models experimentally and analytically, using some approximations. We find that our approaches can be used to compute the Lyapunov exponent when some of these switching pathways are restricted, i.e. some edges are removed from the network. We also derive some results about the growth-maximizing inter-phenotypic switching rates, encoded as the network edge weights, for sparse networks where multiple edges are removed.

Pharmacodynamics of inter-species interactions in polymicrobial infections

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The antibiotic response of bacterial pathogens can be altered by interactions with other species of bacteria which have been suggested as a cause for treatment failure and resistance development in polymicrobial infections. This study aims to investigate the influence of interspecies interactions on the pharmacodynamics of antibiotics which may guide design of treatment strategies. We constructed an individual-based model of an interactive bacterial community consisting of multiple species. Two types of interspecies interactions are incorporated which can negatively or positively affect either the bacteria's susceptibility to drugs or their growth potential. The modelled interactions reflect mechanisms of collective resistance, growth enhancement and impairment mediated via an exchange of signals or nutrients. The in silico bacterial population was simulated with either type of interaction in the presence of different types of antibiotic drugs at constant concentration. Therefor the in silico bacterial population is placed on a two-dimensional grid where individual bacteria can move around, interact with neighboring bacteria, replicate, die and are exposed to antibiotics. Sensitivity changes in single cells as a result of interactions are not heritable, however the presence of interactions influences the selective pressure of the drug locally, which can lead to an optimized spatial arrangement of the population over time. Analysis of the focal pathogen's sensitivity to the drugs showed that both the type of interaction and the type of drug determine how the pharmacodynamics of the focal pathogen are impacted which can be predicted qualitatively. Furthermore, we found that changes in the movement speed and the interaction distance influence the magnitude of the impact of interactions on bacterial sensitivity for some drugs. When bacteria move more slowly, the bacterial population benefits more from beneficial interactions and is more resilient against harmful interactions in the presence of bactericidal drugs. Similarly, when interactions have a larger interaction distance, beneficial interactions affecting the bacteria's susceptibility are less beneficial while harmful interactions of any type are more harmful in the presence of any type of drug than when they have a short interaction distance. We think these results reflect changes in the spatial arrangement of the population over time which optimize the number of interactions between the species to their benefit. While a slower movement speed supports the development of an optimized spatial arrangement of the bacteria on the grid, a larger interaction distance hinders it. In conclusion, we can identify types of drugs most resilient or sensitive to interaction effects for each interaction type. We suggest the movement speed of bacteria and the maximal interaction distance, which both alter the magnitude of the impact of interactions, as potential treatment targets for polymicrobial infections. Additionally, the developed single cell level model is a flexible framework which is extendable and can be used in future research of polymicrobial infections. We are planning to incorporate random resistance mutations in single cells to investigate how interspecies interaction as well as population heterogeneity influence the evolution of antimicrobial resistance during treatment.

Towards rational design of antibiotic therapies: a mechanistic approach

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Antimicrobial resistance is on the rise globally. Increased levels of resistance have been reported across bacterial strains and antibiotic compounds. In order to better target resistant bacteria, understanding the relationship between drug susceptibility and bacterial physiology is key. Here we present a mechanistic modelling approach to quantitatively predict antibiotic effect on bacterial growth dynamics under different environmental conditions. We focus on ribosome-targeting antibiotics, which constitute more than half of drugs used to treat infections and are among the most successful antimicrobials. We model the uptake of antibiotics and their dynamic interplay with ribosomes within an established model of bacterial growth physiology [2]. Integrating literature data on growth responses to four ribosome-targeting antibiotics (chloramphenicol, kanamycin, tetracycline and streptomycin [3]), we infer drug-associated parameters and obtain estimates that are consistent with reported literature values. Our model displays growth bistability: two possible growth states can be reached by isogenic cells experiencing the same antibiotic dose. This observation holds true for drugs that irreversibly bind the ribosomes but not for those that bind reversibly. Understanding the underlaying mechanisms of this behavior might help us gain insight on how tolerant or persistent populations of bacteria emerge. Currently, we are working on expanding our framework to predict the levels of phenotypic heterogeneity in isogenic populations of bacteria caused by the bistable growth response. By integrating theoretical knowledge and data on growth responses, we expect to identify crucial interactions and gain further mechanistic understanding of drug action. This will bring us closer to a predictive theory of bacterial responses to antibiotics, and thus on a path to rational design of antibiotic therapy.

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On the role of deleterious mutant regime in steering long-term evolution

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Evolutionary Graph Theory (EGT) aims to understand the interplay of natural selection and genetic drift in spatial structures. A spatial structure is modelled as a graph with nodes representing asexually reproducing individuals, and edges dictate the interaction among these individuals. Based on the fixation probabilities of mutants on graphs, graphs are mainly categorised as amplifiers of selection and suppressors of selection. Studying fixation-related properties of various graphs has been the focus of EGT. In our work, we study Moran origin fixation dynamics on graphs by allowing mutations to appear continuously with mutants fitnesses sampled from a continuous fitness space. As expected, structures that amplify selection attain higher steady-state average fitness in the mutation-selection balance than the complete graph. Interestingly, we also found that a suppressor of fixation, a structure having lower probabilities for fixing mutants regardless of their fitness values, beats the complete graph in the long-term mutation-selection dynamics by attaining higher average fitness. This happens because of the suppressor's ability to reject deleterious mutants more efficiently, thus compensating for its lower fixation probability to fix beneficial mutants. Similarly, an amplifier of fixation, a structure with a higher probability of fixing mutants regardless of their fitness values, attains lower steady-state average fitness than the well-mixed population. This happens because of the amplifier's poor ability to reject deleterious mutants. Moreover, by randomly generating graphs, the amplifiers and suppressors of fixation are found to be in abundance. And interestingly, all the found amplifiers (suppressors) of fixation attain lower (higher) steady-state average fitness than the complete graph. These results illustrate the importance of the deleterious mutant regime in steering long-term evolution, which, to our knowledge, has been overlooked in the literature on adaptive evolution.

Predicting the time to relapse for individual patients with glioblastoma for optimising the second line treatment

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Glioblastoma (GBM) is a malignant diffusive brain tumor with a poor prognosis and a low survival rate despite conventional cancer treatments. In this research, we aim to estimate the time to relapse for the proliferation of marginal cells after resection at the tumor edge until they reach the maximum density detection threshold of MR images. We also considered the role of tumor-associated macrophages (TAMs) by considering their polarization to see how they influence tumor growth. Monocyte-derived TAMs are predominant in cases of recurrent tumor growth. They can not only affect the speed of tumor growth after resection but they could be also considered potential targets for immunotherapy. We developed a temporal model, to first investigate TAMs and tumor cell interaction and then use it as an index to estimate the probability of relapse for each patient. The model-based predictions of time to relapse will be then compared to the clinical follow-up of patients. The results of this study can be then used for medical decision-making and optimizing the timing of second-line treatment interventions.

Modeling collateral sensitivity-based treatments to suppress antibiotic resistance in Streptococcus pneumoniae

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Introduction Collateral sensitivity (CS) offers a strategy to counter antibiotic resistance, where resistance to one antibiotic collaterally increases the sensitivity towards another antibiotic agent. The clinical relevance and applicability of CS-based treatments remains unclear. Key factors which could determine the clinical relevance and application of CS-based treatments include: (i) heterogeneity in the collateral effects observed across resistant mutants, (ii) inter-individual variability in pharmacokinetics (PK), and (iii) the (combination) dosing schedule applied. Here, we aimed to compare the impact of CS-based clinical treatment scenarios using a mathematical pharmacokinetic-pharmacodynamic (PKPD) modeling approach, with Streptococcus pneumoniae bacteremia infections as proof-of-concept. Methods We generated resistant strains of S. pneumoniae R6 for 13 antibiotics. For each antibiotic, 10 mutant strains were selected. Antibiotic sensitivity assays were performed on all mutants to quantify collateral effects, which revealed reciprocal CS between linezolid (LNZ) and fusidic acid (FUS)-resistant strains. The pharmacodynamics (PD) of LNZ and FUS on parental and resistant strains were characterized using static time-kill assays. A mathematical PKPD modeling framework was implemented using a set of ordinary differential equations. The model included a total of 21 bacterial sub-populations, including the wildtype (LNZ and FUS-susceptible) sub-population, 10 LNZ-resistant mutant sub-populations, and 10 FUS-resistant mutant sub-populations. The model included both a stochastic component to account for mutational events leading to resistance and a deterministic component for the PK and bacterial growth/kill dynamics. The model was parametrized to reflect a condition that is expected during S. pneumoniae bacteremia cases. Simulation scenarios included the initial development of infection from a cell density of 10 colony-forming units (CFU)/mL to a cell density of 10,000 CFU/mL was reached before antibiotic treatment was initiated. This was performed to recapitulate the spontaneous emergence of resistant bacterial sub-population seen in our experiments and to represent a realistic bacterial density at start of clinical treatment. The PKPD model was used to estimate the probability of resistance establishment, which was defined as the number of times in which the sum of the resistant sub-populations was able to reach a density of at least 10,000 CFU/mL at the end of the treatment period from 1,000 replicate stochastic simulations. Result We estimated the probability of resistance establishment at the end of standard LNZ and FUS treatment over the course of 14 days for different patient groups characterized by their intrinsic differences in PK exposure to the treatment. While the standard LNZ treatment was not expected to cause resistance for a typical individual, the probability of resistance may increase to 3.6% and 98.4% in patient groups which LNZ exposure were at the lower 5.0 and 2.5 percentile. On the other hand, the standard FUS treatment was expected to consistently lead to resistance even for individuals with a higher PK exposure. Further exploratory simulations showed how a CS-based LNZ-FUS combination treatment can be used to suppress the establishment of resistant bacterial sub-population during a treatment. By accounting for toxicity limits of LNZ and FUS, future studies may use our PKPD model as a framework to develop an optimal CS-based treatment to suppress resistance, especially for patient groups with a lower exposure

to LNZ and/or FUS. Conclusion This study demonstrated the utility of PKPD models to explore the potential application of a CS-based treatment to suppress the establishment of resistant bacterial sub-population(s) during an antibiotic treatment.

Mathematical modelling of effector T cell stimulation, elimination, and binding with target cells

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In immune-oncology, the lifecycle, engagement, and cytotoxicity of natural or engineered T cells are important dynamical processes that need to be quantified to better understand cancer resistance and susceptibility to immunotherapy. We investigate the process of naïve and memory T cells engaging antigen-presenting cells to be activated to proliferate and differentiate to effector cells. In addition, the model considers the formation of conjugates of effector T cells and target (cancer) cells, resulting in either target cell death or effector T cell inactivation. To capture the dynamics in both small and large populations we apply a stochastic and a deterministic approach. We also aim to characterize the resistance of the target cell population by focusing on the mutated target cells. The assumptions enable that mutated target cells are more likely to prevent from forming a conjugation with effector cells and subsequently avoid being lethally hit. Our model will help to illustrate whether the mutation of target cells can result in failure of immunotherapy.

Integrated in vitro/in silico approach towards a quantitative understanding of killing behaviour and resistance evolution dynamics of antibiotic combinations

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Background: Fosfomycin is an old "forgotten" antibiotic that is effective against both multidrug resistant Gram-positive and Gram-negative bacteria. In order to prevent the emergence and spread of resistances, fosfomycin is often used in combination with other antibiotics, e.g. amikacin. However, the design of effective combination therapies requires a comprehensive understanding of the underlying pharmacodynamics (PD) as well as the pharmacoresistance dynamics. Deriving such an understanding necessitates the integration of experimental and modelling approaches. Here, we aim to quantitatively characterise the growth, kill and resistance evolution dynamics of Escherichia coli under fosfomycin and amikacin exposure, as mono-treatment and in combination. Furthermore we aim to elucidate and incorporate the relationship between genomic and phenotypic resistance mechanisms. Materials/methods: Determination of the minimum inhibitory concentration (MIC), checkerboard experiments and static time-kill experiments with fosfomycin and amikacin are performed in two E. coli isolates and one reference strain. Leveraging these data, a semi-mechanistic pharmacokinetic/PD (PK/PD) model will be developed, aiming to characterise the growth, kill and regrowth behaviour, with its corresponding resistance evolution, in E. coli under fosfomycin and amikacin exposure. Results: Both clinical isolates were classified as fosfomycin resistant. The checkerboard experiments indicated the enhancement of fosfomycin's potency by the addition of amikacin as a left shift of the bacterial concentration – fosfomycin concentration curve was observed with increasing amikacin concentration. Regrowth was observed for all fosfomycin monotherapy time-kill experiments. Mathematical modelling will be used to elucidate the phenomenon underlying the regrowth behaviour, e.g. heteroresistance, persister formation or de novo resistance evolution. These phenomena will be represented in the model as bacterial subpopulation with certain phenotypical qualities. Furthermore, as a step towards characterising the PK/PD of the combination therapy, the fosfomycin and amikacin checkerboard assay data will be used to quantitatively characterise their PD interaction using a general pharmacodynamic interaction model (GPDI) [1]. Conclusions: In this ongoing work, a semi-mechanistic PK/PD model will be developed based on the amalgamated in vitro data generated with different experimental approaches. The GPDI model will be included to capture the interaction between fosfomycin and amikacin. The developed model will serve as a tool to investigate the underlying mechanisms leading to regrowth under fosfomycin monotherapy. Using the model, we will assess the feasibility of combining fosfomycin with amikacin as a strategy to suppress regrowth and potential resistance evolution. The model derived knowledge could act as a primer to build towards mechanistically informed fosfomycin-amikacin combination treatments, optimised to supress resistance development.

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