Position Paper

Modelling the genesis and treatment of cancer: The potential role of physiologically based pharmacodynamics

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ABSTRACT

Physiologically based modelling of pharmacodynamics/toxicodynamics requires an a priori knowledge on the underlying mechanisms causing toxicity or causing the disease. In the context of cancer, the objective of the expert meeting was to discuss the molecular understanding of the disease, modelling approaches used so far to describe the process, preclinical models of cancer treatment and to evaluate modelling approaches developed based on improved knowledge.

This paper is based in part on discussions at a COST B25 expert meeting held in Prague, Czech Republic, on 20–21st September 2007. Participating experts were Leon Aarons (UK), Hans-Jürgen Ahr (Germany), Jean Clairambault (France), Svein G. Dahl (Norway), Dinesh De Alwis (UK), Gilles Freyer (France), Lena Friberg (Sweden), Ursula Gundert Remy (Germany), Steve Kern (US), Anette Kopp-Schneider (Germany), Wolf-Dieter Ludwig (Germany), Jirina Martinkova (Czech Republic), Maurizio Rocchetti (Italy), Giuseppe De Nicolao (Italy), Jean-Louis Steimer (Switzerland), Iñaki F. Troconiz (Spain).

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1. Introduction

Physiologically based pharmacokinetic models are designed to represent the human body and its physiology. The models allow the implementation of age- and disease-dependent pharmacological changes enabling simulations and predictions of the kinetics under special circumstances which are experimentally hardly accessible, e.g. in internal organs such as the liver, and in special ages such as preterm babies. Up to now, there are not many examples of a similar approach used to describe the dynamic part of the relationship between dose and observed effects. Models for pharmacodynamic/toxicodynamic effect require a priori knowledge of the mechanistic basis of the disease process and at which step in the chain of events the drug acts. The objective of the expert meeting was, in the context of cancer, to discuss the developments in: the molecular understanding of the disease process in the modelling approaches used so far to describe the disease processes; preclinical models of cancer treatment; and therapeutic options which have been recently developed based on the improved understanding of the underlying processes. Thus, the meeting brought together experts involved in evaluating and modelling steps in carcinogenesis, in the pharmaceutical development of new oncology agents or in the therapy of cancer. The discussions among the participants provided new insight into the contribution of physiologically based modelling of pharmacodynamics to current and future cancer treatment.

The manuscript is organised as follows. In the first two sections the molecular basis of cancerogenesis, diagnosis and treatment intervention together with their respective modelling approaches are presented. Section 4 describes how modelling and simulation is currently contributing to many stages and aspects in anticancer drug development: preclinical tumour growth models; management of drug (haemo-) toxicity; intra-tumour pharmacokinetics; physiological aspects of tumour cells and predicting outcomes from drug combinations.

Molecular events in cancerogenesis can be detected using 'omics' technology, a tool applied in experimental carcinogenesis, but also for diagnostics and prognosis. The molecular understanding forms the basis for new drugs, for example targeting protein kinases specifically expressed in cancer. At present, empirical preclinical models of tumour growth are in great use as the development of physiological models is cost and resource intensive. Although a major challenge in PKPD modelling in oncology patients is the complexity of the system, based in part on preclinical models, successful models have been constructed describing the mechanism of action and providing a tool to establish levels of biomarker associated with efficacy and assisting in defining biologically effective dose range selection for first dose in man. To follow the concentration in the tumour compartment enables to link kinetics and dynamics. In order to obtain a reliable model of tumour growth dynamics and drug effects, specific aspects of the modelling of the concentration–effect relationship in cancer treatment that need to be accounted for include: the physiological/circadian rhythms of the cell cycle, the treatment with combinations and the need to optimally choose appropriate combinations of the multiple agents to study; and the schedule dependence of the response in the clinical situation.

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2. Biological mechanisms of cancerogenesis

2.1. Molecular events in cancerogenesis

In the traditional approach, the evaluation of the toxic properties of substances is based on phenotypic pathological and histopathological characterisation of responses after exposure which does not allow insight into the underlying toxic mechanism. In recent years, toxicogenomic techniques have been used to support the interpretation of the phenotypic results by mode of action considerations. Furthermore, gene expression techniques using microarrays have been applied in toxicological studies with the aim of classifying chemicals using biomarkers based on gene expression changes. If the mode of action is understood and can be captured in a set of biomarkers, the detection of molecular events would allow the prediction of selected toxic effects. Currently, the carcinogenic potential of chemicals is evaluated with rodent life time bioassays, which are time consuming and expensive with respect to cost, number of animals and amount of compound required. Since the results of these 2-year bioassays are not known until late during development of new chemical entities, including drugs, and since the short time test battery to assess genotoxicity, a characteristic of genotoxic carcinogens, is hampered by low specificity, the identification of early biomarkers would be a big step forward.

Gene expression profiling on Affymetrix arrays has been used to investigate the molecular events leading to a carcinogenic response in rats. In a first study male rats were dosed with the genotoxic hepatocarcinogen N-nitrosomorpholine for 7 weeks followed by a treatment-free observation period of up to 50 weeks. Already shortly after start of the treatment, significant alterations of various genes were observed, which belong to reasonable mechanistic pathways. Extension of the observation period did not add much further information. Therefore, the study duration was limited to 14 d for further mechanistic studies and for the selection of early biomarkers. In a series of short-term in vivo studies, it was inves-
tigated whether carcinogens at doses known to induce liver tumours in the 2-year rat bioassay alter the expression of characteristic sets of genes and whether these genes represent defined biological pathways. Male rats were dosed with five genotoxic and five non-genotoxic hepatocarcinogens. Three non-carcinogenic compounds were used as control profiles. The expression profiles in the liver indicated that distinct cellular pathways were affected by non-genotoxic carcinogens as compared to genotoxic carcinogens and non-carcinogenic control profiles (Fig. 1). Characteristic early molecular events for genotoxic carcinogens were DNA damage response and the activation of proliferative and survival signalling. Non-genotoxic carcinogens induced responses to oxidative DNA or protein damage as well as cell-cycle progression and signs of regeneration. Although neither a single gene nor a single pathway was sufficient to discriminate the two classes of hepatocarcinogens, it became evident that a combination of pathway associated gene expression profiles may be used to predict a genotoxic or non-genotoxic carcinogenic potential of a chemical in short-term studies. Several statistical methods were then applied to extract biomarkers from these expression profiles. Very low cross-validation errors were obtained for all biomarkers, but, surprisingly, the biomarkers greatly differed in the number of genes, the genes themselves and their mechanistic foundation. Nevertheless, all multigene biomarkers showed a good predictivity for a set of independent validation compound profiles with up to 88% accuracy. This may be considered as a proof of the concept that a classification of carcinogens based on short-term studies is feasible. The usefulness of this approach has also been demonstrated by other authors to model the mode of action in cancerogenesis and make predictions on the properties of chemicals.

In order to get more understanding of the carcinogenic stress response and to possibly overcome the observed instability of biomarker selection, the utility of systems biology approaches were further explored. The gene expression network is interactive and highly correlated. By explicit modelling of the gene–gene interactions in this network a promising alternative description of the global stress response following treatment with carcinogens was obtained. This may point to a future role that systems biology may play in analysing such toxicogenomic data and reducing their complexity.

2.2. Modelling stochastic processes in carcinogenesis

The process of carcinogenesis is inherently a stochastic process, at least as long as it is not known why certain individuals get cancer under conditions where others are unaffected. Therefore the models of carcinogenesis may be formulated in the framework of stochastic processes. The most prominent stochastic models of carcinogenesis are the multistage mod-

![Fig. 1 – Induction of pathway-specific expression profiles in liver tissue by non-genotoxic and genotoxic carcinogens as opposed to non-carcinogens.](image-url)
Cancer is caused by alterations in oncogenes, tumor-suppressor genes, and microRNA genes. These alterations are usually somatic events resulting in changes in growth regulation and thus transform a normal cell into a cancer cell. In the last decade, the functional properties of cancer cells have been linked to underlying mutations through a better understanding of the growth factors and their receptors, signal transducers and apoptosis regulators that control cell proliferation, apoptosis or both. These findings have led to the definition of the so-called hallmarks of cancer including the acquisition of self-sufficient signals for growth, the capacity for extended proliferation, resistance to growth-inhibiting signals, the ability to evade cell death signals, the potential for tissue invasion and metastasis, and the power to induce angiogenesis. Some of these traits are the properties of the cancer cells themselves, but others depend on communication between the cancer cells and their cellular and macro-molecular environments. In cancer cells, not only can the expression levels of proteins be altered but also the stoichiometry of the interaction network can be altered. Hence, our understanding of complex networks has increased our understanding of how oncogenes and tumor-suppressor genes interact and how the networks are modified by gene alterations to result in proliferation or apoptosis of cells. The advances in knowledge have made possible a description of cancer in molecular terms that is now likely to improve the ways in which human cancers are diagnosed, classified, monitored and treated. Each property constitutes a vulnerability in a tumour, to be exploited by new targeted therapies, especially when the underlying mutations and signalling alterations are known. The fundamental challenge of anticancer therapy is the need for agents that eliminate cancer cells at a dose which is tolerated by the patient. Most cytotoxic anticancer drugs inhibit essential functions that are present in both normal and cancer cells. A new generation of cancer drugs has been designed to interfere with a specific molecular target, typically a protein, that is believed to have a critical role in tumour growth or progression. In recent years, by the identification of oncogenes involved in the initiation and progression of tumours, a new generation of drugs has been developed targeting specific proteins (growth factor receptors) on the cell membranes (e.g. monoclonal antibodies) or oncogenic proteins (tyrosine or serine and threonine kinases) in cancer cells (e.g. small molecules) encoding members of signal-transduction pathways. However, contrary to initial expectations, these targeted therapeutics can also cause new drug-related toxicities in normal cells which result from the disturbance of growth factor signalling pathways that are important for maintaining cellular homeostasis. As these new agents will also be given in prolonged treatment strategies, possible long-term toxicities should be anticipated and further studies are needed to explore the biological mechanisms of this toxicity.

Protein tyrosine kinases are essential enzymes in cellular signalling processes and have been identified as regulators of tumour or tumour vessel growth in human cancer. One of the landmark events in the 'targeted-therapy revolution' has been the development of imatinib, an inhibitor of multiple tyrosine kinases, including ABL, BCR-ABL, platelet-derived growth factor receptor and c-kit. The success of imatinib as a treatment for chronic myelogenous leukaemia (CML) can be attributed to the critical role of the BCR-ABL tyrosine kinase in causing the disease and the specificity of imatinib as an ABL kinase inhibitor. Clinical studies conducted over the last years have established that imatinib is nowadays the first-line treatment for CML. Nearly all patients in early chronic phase treated with imatinib achieve a complete haematological response, with 80-90% achieving a complete cytogenetic response. However, BCR-ABL transcripts at very low level are still detectable by reverse transcriptase PCR (RT-PCR) in

3. Molecular basis for diagnosis and therapeutic intervention

3.1. Molecular origins of cancer as a basis for new therapeutic targets – a clinical view

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approx. 96% of responding patients with CML, suggesting that this could be a potential pool from which resistance emerges. Furthermore, there is a high relapse rate among advanced- and blast-crisis-phase patients owing to the development of mutations in the ABL kinase domain that cause drug resistance. Recent strategies circumventing resistance to kinase-inhibitor therapy include the development of second-generation inhibitors (e.g. dasatinib and nilotinib) targeting the integrity and/or stability of the BCR-ABL BCR-ABL itself as well as signalling pathways downstream of BCR-ABL that are necessary for malignant transformation. The activity of imatinib in CML and gastrointestinal stromal tumours (GISTs), as well as of monoclonal antibodies against receptor tyrosine kinase signalling (e.g. trastuzumab against the human epidermal growth factor receptor-2, HER-2) or of small-molecule inhibitors of epidermal growth factor receptor (EGFR; e.g. gefitinib or erlotinib) has validated the concept of targeted cancer therapy. Antisense technology has quickly moved from gene, the prototype of oncogenes with a direct antiapoptotic function. Antisense inhibition of relevant genes involved in cancer progression, especially those that are not amenable to small-molecule or antibody inhibition. The most prominent example is the ASO oblimersen (G3139 or genasense) which targets the BCL-2 gene, the prototype of oncogenes with a direct antiapoptotic function. Antisense technology has quickly moved from preclinical models to testing in the clinic. However, challenges remain to optimise tissue exposure, cellular uptake, and demonstration of mechanism as well as clinically relevant antitumour activity. As is true for the clinical development of all targeted therapies in cancer, crucial issues include the early determination of the optimal biological dose and the need to define the relevant patient population for clinical trials and therapy better through molecular characterisation of somatically acquired mutations in cancer cells and/or biomarkers which predict significant clinical responses to the drug. Furthermore, in order to address the goal of maximising tumour cell kill, rational combinations of conventional cancer therapies (e.g. chemo- and radiotherapy) with targeted therapy have still to be defined.

3.2. PKPD modelling in oncology patients: the challenge of complexity

Daily clinical practice for oncology patients has to be as simple as possible. However, the need to individualise therapy is of utmost concern, perhaps more than in any other therapeutic area. A major challenge in PKPD modelling in oncology patients is the inherent complexity of the system. Despite some successes, the strict application of pharmacokinetic principles to target blood or plasma concentrations should not be overemphasised. As an example, twenty years of PK studies have failed to improve cancer treatment for epirubicin, indicating the limited utility of plasma concentration of these compounds as a surrogate for clinical effectiveness. In contrast, focusing on the pharmacodynamic aspects has been shown to provide multiple examples of success with increasing application of clinical relevance, e.g.:

- protecting patients receiving docetaxel/epirubicin from neutropaenia by proper administration of GSCF at the second cycle, after having ‘learned’ the individual’s concentration time-course in the first cycle,
- diminishing the incidence of hand-foot syndrome by proper adjustment of the dose of capecitabine.

4. Models in drug development

4.1. Tumour growth models in preclinical drug development

The in vivo evaluation of the antitumour effect is a fundamental step in the preclinical development of drugs in oncology. The first in vivo tumour models were developed in the mid-1960s. These models were mouse leukaemia models where the cells were grown in the ascites fluid and, for this reason, not suitable to represent the growth of solid tumours. The evaluation of the antitumoural activity of the compounds tested using these systems was very limited, and generally based on the estimation of the increase in life span, cell doubling time and log 10 cell kill.

Subsequently, with the development of in vivo solid tumour models such as the syngeneic mouse tumours and human tumour xenographs grown as subcutaneous nodules or in orthotopic sites, the complete growth time-course was made available through measurements of tumour volume. More recently, the development of ‘labelled’ tumour models, based on fluorescence proteins or firefly luciferase, enables tumour volume assessment by imaging techniques. The response to treatment can be evaluated by directly comparing the observed tumour growth in the treated animals with that observed in the control group. For this purpose, different metrics have been proposed for assessing efficacy of the tested compounds, for instance, the distances between the tumour growth curves in control and treated animals, mea-
sured either at specific times and expressed as tumour growth delay (TGD) or tumour growth inhibition (TGI). However, these metrics, and other related tumour response endpoints, depend on the doses, dose schedules and the design of the experiments. For this reason, the biological interpretation of the results is limited, making it difficult to obtain a definite evaluation of the activity of anticancer compounds, and this is likely the most important cause of the questionable relevance of these animal models to predict therapeutic efficacy that generates a continuous debate and controversy about their usefulness.

With the aim of fully exploiting the capabilities of these animal models and circumventing the difficulties mentioned above, a number of mathematical tumour growth models have been reported in the literature, reflecting different paradigms. Empirical models use the well-known mathematical equations (e.g. sigmoid functions, such as the logistic, Verhulst, Gompertz, von Bertalanffy) but are unable to correctly describe the underlying pathophysiological processes. For this reason, the effect of a drug can be evaluated only in terms of changes of the parameter values describing the tumour growth and it is uncertain whether the model can be used as a predictive tool outside the tested dose regimens.

Physiological models, conversely, are based on mechanistic descriptions of biological processes underlying tumour growth. These models, partially based on system biology techniques, are constructed by making assumptions about tumour growth, involving cell-cycle kinetics and biochemical processes, such as those related to antiangiogenic and/or immunological responses. Because of the biological complexity they try to capture, the development is time consuming and a much larger number of parameters are necessary compared to the empirical models. Hence, in addition to the standard tumour growth measurements, further data are needed such as flow cytometry analyses and measurements of biochemical and immunological markers in order to avoid identifiability problems due to the over-parametrisation. As a consequence, these models are mainly used as simulation tools to obtain qualitative descriptions of the tumour response to changes in biological pathways within the tumour cells.

In view of the pros and cons of the different approaches, achieving a correct compromise between empirical and mechanism-based models is a real need in preclinical drug development. Along this line, a new simple and effective PKPD Tumour Growth Inhibition (TGI) model, linking the plasma concentrations of anticancer compounds to the effect on tumour growth in xenograft mice (the most common animal model used) has been developed recently. The TGI model was shown to successfully describe the inhibition of tumour growth observed at different dose levels and schedules, independently of the mechanism of action and the therapeutic indications of the compounds. On this basis, from a single experiment it is possible to derive a quantitative evaluation of anticancer activity through the estimate of biologically meaningful pharmacodynamic parameters. In particular, two drug-specific parameters may be obtained: \( k_1 \) is related to how rapidly the tumour cells are killed by the drug action, while \( k_2 \) reflects the antitumour potency of the compound. The TGI model is recognised as a useful tool for supporting the lead optimisation and candidate selection phases. Some extensions of the model including also biomarker evaluations have been presented.

![Fig. 2 – Prediction of human dose by preclinical model estimates. Left panel: data from tumour growth inhibition experiments in rodents are used to estimate the parameters of the so-called Tumour Growth Inhibition (TGI) model. These parameters characterise the unperturbed tumour growth as well as the potency of the antitumour drug (\( k_2 \) parameter). The model is able to predict tumour growth under different drug dosages and schedules and can therefore be used for experiment design optimization. The model provides also an estimate of the concentration threshold in plasma (CT) to be maintained for obtaining tumour regression in these animal models. Right panel: applying the TGI model to known anticancer drugs it has been established that the clinical doses in humans may be predicted by a log-log regression on the estimated threshold concentration parameter. Based on this, an estimate of the expected therapeutically active doses in humans of new compounds may be derived directly from the first preclinical studies in rodents.](image-url)
Recently, predictivity in humans was investigated by applying the TGI model to experiments with anticancer drugs already in therapeutic use and estimating their corresponding potency. Exploiting the regression between the potency parameters estimated in the animal model and the doses commonly used to treat patients is a means to derive an estimate for the expected therapeutically active dose of a new compound (Fig. 2). This estimate, even if approximate, provides a rational basis for the decision-making process in drug development, allowing inappropriate compounds to be discarded earlier, reducing the number of dose escalations in Phase I trials and consequently exposing fewer patients to ineffective treatments. Moreover, in general, the full exploitation of this kind of PKPD models is expected to substantially reduce the number of failures in the clinical phase with an outstanding impact on the costs of late failures.

4.2. The potential of PKPD modelling in early phase oncology drug development

If one considers the approach to the development of cytotoxics and targeted therapies, clear differences are apparent. Conventional cytotoxic chemotherapies follow a well-worn empirical course: dose escalation followed a fixed or semi-fixed scheme from a starting dose based on some multiple of a preclinical toxicity dose. Escalation continues until the maximally tolerated dose (MTD) is found and this single dose is carried forward into later phase clinical trials. Toxicity such as myelosuppression, is often used as a biomarker of the desired cytotoxic effect in the tumour. Pharmacokinetics are largely descriptive, with body surface area dosing a substitute to individualised therapy, but without adequate understanding of the covariates. In contrast, the early clinical development of targeted agents is driven by the need to assess the impact on the target which may occur in the absence of a clinically definable effect on the tumour or other tissues. Translation to clinical efficacy is more remote and hence more difficult to illicit in Phase 1 studies. Dose escalation is more amenable to adaptive study design in which pharmacokinetics and pharmacodynamics play an increasingly important role. Biological effect rather than toxicity determines the subsequent range of doses which may be taken into Phase 2 studies. New considerations have been introduced such as the degree and duration of target inhibition. Complete (100%) inhibition indefinitely may adversely affect the margin of safety and may not be desirable for efficacy.

This is illustrated by the recent development of a cytotoxic and a targeted agent (TGF-β type 1 receptor antagonist). The cytotoxic agent exhibited a very high saturable protein binding, gender difference within a species and an additional species difference in toxicity. Total dose and total drug concentrations failed to explain a 30% mortality in male rats compared to a 3% mortality in female rats given the same dose. However, incorporating protein binding and protein levels in a semi-mechanistic PK model based on unbound concentrations explained these differences. This model also incorporated in vitro bone marrow assay data to explain neutropaenia differences between rat and dog. The model was further used to for designing a safer, more effective phase 1 dose escalation study incorporating a PK and toxicity driven dose escalation scheme. This example also illustrated that carrying out a standard MTD approach to Phase I dose escalation using a Fibonacci or incremental dose escalation could potentially select sub-therapeutic doses. In another example, the construction of preclinical models integrating PK, biomarkers and tumour growth delay data satisfactorily described the mechanism of action of a TGF-β signal-transduction inhibitor and provided a tool to investigate different experimental scenarios to establish levels of biomarker inhibition associated with efficacy and to assist the design and help define biologically effective dose range selection for the first study in man. This model also characterised the signal-transduction processes which could help design the dosing schedule and simulate out the preclinical efficacy outcomes between chronic and intermittent dosing schedules.

4.3. PBPK in tumour compartment – example of capecitabine

Capecitabine is an orally administered precursor of the anticancer agent 5-fluorouracil (5-FU), designed to exploit differences in metabolic enzyme activities between the tumour and healthy tissue. The demonstrated increased conversion to the cytotoxic agent 5-FU in the tumour is intended to enhance safety and efficacy of the treatment of breast and colorectal cancer. Before being converted to 5-FU, capecitabine undergoes extensive sequential metabolism in multiple physiological compartments, and thus presents particular challenges for predicting PK and PD activity in humans. In order to cope with these complexities, several modelling approaches were used during the development of capecitabine, which were ultimately influential on decision-making as well as labelling. These modelling approaches are summarised in the paper of Blesch et al.

An important aspect was the development of a physiologically based pharmacokinetic (PBPK) model, which was used for the prediction of tissue-specific exposure in humans. The model integrated the tissue-specific differences in metabolic enzyme activity between tumour and normal tissues determined on in vitro preclinical data for the metabolites. In particular, metabolic enzyme activity, characterised in vitro by the parameters V_max and K_m, or protein binding in the different species could be integrated into the model directly. The model relied on some key assumptions, including:

- a rapid equilibrium distribution of capecitabine and its metabolites between blood and tissue,
- the sequential metabolism within a tissue according to a (non-linear) Michaelis-Menten process representing the time-varying intrinsic metabolic clearance from one metabolite to the next.

Ultimately, the complete model was composed of four PBPK sub-models that addressed the important pharmacological characteristics of this agent. This ‘mechanistic’ model described the PK of capecitabine and its metabolites (primarily 5-FU), including the (saturable) enzyme transformations. Exploring factors that influenced exposure could be evaluated through a sensitivity analysis of the model (e.g. change in
blood flow rate to the tumour, or change in metabolic enzyme activity). The PBPK model allowed the prediction of a therapeutic index based on exposure to 5-FU of target organs for efficacy (tumour) versus toxicity (GI tract). This provided a better understanding of the therapeutic advantage of oral capecitabine compared to other modes of 5-FU administration (intravenous). This was confirmed in terms of adequate efficacy and improved safety in two Phase 3 trials comparing oral capecitabine to intravenous (i.v.) 5-FU/leucovorin in metastatic colorectal cancer. Patients in the capecitabine arm had an improved overall safety profile with one exception, the frequency of hand-and-foot syndrome. This phenomenon, related to the accumulation of capecitabine, is not addressed in the PBPK model. It has been addressed recently by developing a longitudinal PKPD model for predicting a score for risk with the goal to enhance individual treatment adaptation (see Section 3.2). Other successful examples of adequate therapy including capecitabine have been published since the registration of the compound, including the use of capecitabine in advanced breast cancer in older women or in combination with indisulam in patients with solid tumours. It represents the classical phases G1, S, G2, M, with the possibility to add a quiescent phase G0, and it is used to analyse physiological and pharmacological controls on tumour and on healthy tissue proliferation. The targets for control inputs are the death rates inside phases and, more importantly, the transition rates between phases, particularly at the G1/S and G2/M checkpoints. The growth behaviour of the population is determined by a Malthus exponent, positive for exponential growth and negative for exponential decay in the simple case of a linear model. When a G0 phase is added, with exchanges of cells between G0 and G1 phases (non-linear model), other types of behaviours – linear or polynomial growth – can be observed. The controlling inputs to this model at the checkpoint level are Cyclin-Cdk dimers, which are themselves outputs of: (a) a system of ordinary differential equations (ODEs), the ‘mitotic oscillator’ involving in its minimal form a Cyclin, a Cdk and a Cyclin-degrading protease that is controlled by physiological circadian inputs exerting their influence on proteins Wee1, p21, p53; and (b) pharmacological inputs resulting from cytotoxic drug dynamics on DNA and the subsequent triggering of p53 and Cdk blockade.

The intracellular pharmacokinetics of these drugs is under the dependence of: (a) their corresponding blood kinetics; (b) ABC transporters that ensure their active efflux; (c) detoxication enzymatic mechanisms that may be subject to both circadian modulation and genetic polymorphism. For each cytotoxic drug considered (until now oxaliplatin and irinotecan), a resulting PKPD model based on ODEs, describing the evolution of the average drug concentration in the proliferating cell population, was achieved. The input to this molecular PKPD model is drug infusion flow from the blood compartment. This flow is in its turn amenable to computer delivery time schedules, stored in programmable portable pumps, and based on mathematical optimisation algorithms designed after the model equations.

A circadian clock model has been developed, that is a network of circadian oscillators, each of which is the FRQ oscillator designed for Neurospora. This simple ODE oscillator can be replaced by a more physiological one if necessary. It consists of a central hypothalamic conductor (representing coupled neurons in the suprachiasmatic nuclei), a pathway from the centre to the periphery, and uncoupled similar circadian oscillators in the peripheral cells. This structure has been designed to take into account at the central locus the synchronising effects of light and the desynchronising effects of circulating cytokines, both of which are known to exert influences on the central circadian clock, and on peripheral cell proliferation.

The complete model is still under construction. The building blocks that constitute it, and will be presented in the talk, make it a modular model easily amenable to improvements. The final goal is the optimisation of anticancer multiple drug delivery time schedules, with the constraint to protect healthy tissues from unwanted side-effects. It is clear that such a model, that involves both intracellular drug metabolism and whole body pharmacokinetics (blood and tissues, with enzymatic activity determination), as exemplified in the previous paragraph of this article for capecitabine, with the added difficulty that it involves circadian dependencies both at the cell and at the whole body levels, raises non-negligible parameter identification issues. Such questions must be settled by both pharmacokinetic–pharmacodynamic measurements in cell cultures and in different tissue samples in whole animals. It is noteworthy that to this purpose, a pioneering study of intracellular metabolism and transmembrane transport, with identification of parameters by high performance liquid chromatography (HPLC) and mathematical optimisation techniques, is under way in a European network (TEMPO) for the anticancer drug Irinotecan. Other such studies should be undertaken for oxaliplatin and for 5-fluorouracil, thus making progress towards a complete identification of the model, which will take a long time.

4.5. Modelling schedule dependence of therapy

Many anticancer drugs exhibit schedule dependence where the grade of tumour response and/or toxicity are dependent on the rate of drug administration and a changed administration schedule can have a larger impact on the outcome than increasing the dose. For example, the tumour response of etoposide in small-cell lung cancer was much increased following 1-h infusions on 5–8 d compared with a 24-h infusion despite that the total exposure (AUC) was the same.
The grade of neutropaenia is lower following weekly administration than after once-every-3-weeks administration without compromising efficacy.\textsuperscript{67} The maximum tolerated dose (MTD) intensity for a drug given by bolus injection can differ by several orders of magnitude compared to MTD intensity when the drug is administered by slow infusion (Fig. 3). Schedule dependence of anticancer drugs can be difficult to predict since drug administration and observed effects generally are dissociated in time. Schedule-dependent effects may be due to cell-cycle specificity, saturable drug transport, time-dependent repair mechanisms, co-factor depletion, drug resistance development or multiple mechanisms of action. For example, metronomic dosing, where low drug concentrations are retained during a long time period, may result in pronounced antiangiogenetic effects that are negligible with short exposure times of high concentrations.\textsuperscript{68} Historically the optimal schedule has been searched for by performing numerous clinical trials where most often a single schedule has been tested at a time making comparisons to other schedules ambiguous. Among other advantages, population pharmacokinetic–pharmacodynamic modelling offers a rational approach to analyse several studies simultaneously and thereby a good understanding of the dose–concentration–effect relationships following different dosing regimens can be acquired.

To be able to predict the effect of a changed schedule it is of importance to characterise the pharmacokinetics because for drugs with non-linear pharmacokinetics the dose–response relationship will appear to be schedule dependent. The optimal schedule of 5-fluorouracil (5-FU) has been debated for long and the fact that 5-FU has capacity-limited elimination is often neglected, i.e. the AUC is dependent on the dosing regimen. When the response rates in 39 study arms on different schedules of 5-FU in colorectal cancer were analysed\textsuperscript{69} and the non-linear pharmacokinetics were considered,\textsuperscript{70} it was found that for exposures with a large AUC (>0.2 mg week/l) continuous administration was superior over shorter exposure times while for lower AUC values the exposure time was less important.\textsuperscript{71} The concentration–response relationship was characterised with a model where the drug concentration is related to a direct effect and the cumulative direct effect is related to the observed response.\textsuperscript{72} The relationship between the drug concentration and direct effect was shown to neither be linear (AUC-dependent) nor described by a step-function (time-above-threshold-dependent).

It is of importance to clarify the end-point when classifying if there is schedule dependence or not. For example, two different dosing regimens may result in a similar neutrophil nadir although the duration of neutropaenia differs. Models which describe the full time-course of effect are therefore preferable as well as models which are built on the essential mechanisms of the affected system as such models potentially have the capability of predicting different dosing regimens. Several semi-physiological models have been developed to describe the time-course of myelosuppression\textsuperscript{73–75} where different schedules of administration were well characterised. Such models have potential to investigate schedule dependence in different measures of neutropaenia and may be used in an efficient model-based search for optimal schedules of therapy.

Models describing the time-course of tumour response following different schedules of chemotherapy are rare. For the investigational anticancer agent CHS 828 the effect in a rat hollow fibre model was larger when the same total oral dose was administered once-daily for 5 d compared with when the whole dose was given on a single occasion.\textsuperscript{76} Experiments that followed the time-course of effect showed that this was not due to re-growth following the single dose regimen, but the AUC after a single dose was lower because of dose-dependent bioavailability. However a relatively simple pharmacokinetic–pharmacodynamic model of cancer cell response over time revealed that not only the pharmacokinetics, but also the pharmacodynamics were schedule dependent, i.e. a prolonged administration schedule of CHS 828 is preferred.

To predict schedule dependence of the response in the clinical situation resistance and metastasis development may also need to be accounted for to obtain a reliable model of tumour growth dynamics and drug effects. A developed longitudinal dose-tumour size model, in combination with patient characteristics, may predict survival time, as previously shown.\textsuperscript{77} Finally, simulations from developed effect and toxicity models can be performed to find the schedule that maximises the response while limiting the toxicity to an acceptable degree.

4.6. **Drug combination modelling beyond isoboles**

Pharmacological agents are given in combination either simultaneously or in sequence to produce a greater desired effect with less risk for side-effect than equi-effective amounts of the individual agents when given alone. To optimise these drug combinations for an application, design techniques were explored that compared two traditional ways of choosing drug combinations for clinical studies with a novel approach. The standard paradigms for choosing drug combinations and assessing the interaction are referred to as mesh and radial designs.\textsuperscript{78,79} For two drug combinations, these methods essentially plot the minimum and maximum dose

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**Fig. 3 – Logarithm of ratio of maximum tolerated dose (MTD) intensity (mg/m²/week) when cytotoxic drugs are given as bolus injections compared to when given as infusions. A value of 1 indicates the same dose intensity can be administered and there is therefore no schedule dependence in MTD for these drugs.**
of each agent on an X-Y axis like a grid and then combinations are chosen at points on the grid that are expected to cover the range of effects from the combinations. The mesh approach uses combinations at the grid points while the radial approaches pick combinations along radii extending out from the grid origin. These qualitative graphical approaches were compared to a novel D-optimal design technique based on methodology adapted from Duffull et al. The comparison explored the number of subjects and dosing groups needed to obtain reasonably precise estimates of interaction model parameters that create a surface that describes the interaction.

By simulating an optimal design experiment, it was estimated that a standard mesh design will produce large estimation errors for both the fixed and variance terms of the interaction model parameters. Using the optimal design significantly reduces these error estimates. While both the standard and optimal designs performed worse at estimating synergism when the transition from no effect to maximum drug effect occurred over a narrow concentration range or when the degree of interaction was small, the optimal design, however, handled such situations better, producing interaction model parameter estimates with lower levels of uncertainty.

These analyses suggest that the D-optimal design allows the quantification of drug synergy much better than the standard design. When assumptions about the likely degree of interaction can be made a priori and the individual dose/response relationship is known for each drug, the D-optimal method predicts the dose combination pairs to study clinically that minimise the expected interaction model parameter variability and simplifies the experimental design. This method provides a basis for developing clinical interaction studies for oncological applications where it is desired to determine pharmacodynamic synergy.

### 5. Conclusions

The presentations and discussions from the COST meeting illustrated the significant progress in understanding the molecular basis and the mode of action of the development of cancer in experimental animal models and in humans. Progress has also been made in the area of mathematical modelling of carcinogenesis and in the application of these approaches in anticancer drug development over the last few years. The presented models ranged from the knowledge-based, systems biology type of models capturing the circadian and pharmacological control of the cell cycle to data driven PKPD or PBPK models such as those used in the drug development. The presentation on preclinical models in tumour growth clearly illustrated the ability of linking well-designed biological models and semi-mechanistic models. The predictive ability of this approach to define the efficacious dose range in the clinic for cytotoxic agents is significantly better than a purely empirical approach. These types of models then can be used in preclinical stages of drug development supporting the lead optimisation and candidate selection phases. The presentation on modelling schedule dependence of therapy highlighted the value of carrying out retrospective modelling using semi-physiological models of myelosuppression on established drugs such as 5-FU and paclitaxel to identify schedule dependency and how this could be used to search for optimal schedules of therapy of old and new agents. Combination therapy in cancer is a common therapeutic approach. The use of D-optimal techniques is a helpful tool to guide combination therapy in cancer.

The simulation of future experiments based on the types of preclinical or clinical models discussed in this paper, may aid the decision-making processes saving resources and expense by carrying out optimised clinical studies crucial in the fight against cancer. These modelling efforts overall illustrated the work done to understand the mechanistic nature of the relationship between dose and effects and its variability and uncertainty. The efforts are directed to elucidate the situation with the aim to be able to quantify these aspects to the advantage of the patient.

### Conflict of interest statement

None declared.

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