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Accelerating the Development of Personalized Cancer Immunotherapy by Integrating Molecular Patients' Profiles with Dynamic Mathematical Models

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Abstract

We review the evolution, achievements, and limitations of the current paradigm shift in medicine, from the "one-size-fits-all" model to "Precision Medicine." Precision, or personalized, medicine – tailoring the medical treatment to the personal characteristics of each patient – engages advanced statistical methods to evaluate the relationships between *static* patient profiling, e.g., genomic and proteomic, and a simple clinically-motivated output, e.g., yes/no responder. Today, precision medicine technologies that have facilitated groundbreaking advances in oncology, notably in cancer immunotherapy, are approaching the limits of their potential. A different approach to treatment personalization involves methodologies focusing on the *dynamic* interactions in the patient-disease-drug system, as portrayed in mathematical modeling. Achievements of this scientific approach, in the form of algorithms for predicting personal disease dynamics and in individual patients under immunotherapeutic drugs, are reviewed as well. The contribution of the dynamic approaches to precision medicine is limited, at present, due to insufficient applicability and validation. Yet, the time is ripe for amalgamating together these two approaches, for maximizing their joint potential to personalize and improve cancer immunotherapy. We suggest the roadmaps towards achieving this goal, technologically, and urge clinicians, pharmacologists and computational biologists to join forces along the pharmaco-clinical track of this development.

1. Historical background: a paradigm shift in medicine

In the last decade, we have witnessed a paradigm shift in medicine, from the **one-size-fits-all** concept to **Precision Medicine**. The one-size-fits-all paradigm, applying the same treatment to all patients of a specific disease, embodied the rationale of therapy in the 20th century. However, the unavoidable low response rates to most medical therapies, which help only a relatively small subset of the patients, hampers the success of the one-size-fits-all approach. The response to one of the most efficacious chemotherapeutic drugs, docetaxel, ranging from 6 to 38%, [1-6] illustrates this problem. Thus, thirty years after the declaration of "the War on Cancer" by the USA Federal Government (1971), it was sadly acknowledged as a failure: "while there have been substantial achievements since the crusade began with the National Cancer Act in 1971, we are far from winning the war. So far away, in fact, that it looks like losing". [7]

The Genomic revolution. Today we know that the efficacy of a particular therapy depends on the specific physiological and disease attributes of the individual patient. This recognition is grounded on the achievements of the Genome Project (1990), whose underlying premise was that identifying human genetic variation would allow clinicians to sub-classify patient populations and personalize medical treatmentIn 2003, the achievements of the Genome Project drove the director of the National Cancer Institute (NCI), Andrew von Eschenbach, to prophesy that by 2015 suffering and death due to cancer would end. "Cancer will become a chronic disease that we will manage much the same way we manage high blood pressure or diabetes." von Eschenbach projected that this would be accomplished by the tools of genomics, identifying mutations that affect response to drugs, and using this knowledge to validate biomarkers for distinguishing patients likely to benefit from new treatments. [8]

The "Precision Medicine Initiative" of President Obama (2015) was initiated to leverage advances in the Genome Project for accelerating biomedical discoveries, fueling the development of new treatments, and catalyzing a new era of databased and more precise medical treatment. Essentially, precision medicine is the view that incorporating information encoded in the human genome as the dominant factor in the prediction, diagnosis, and treatment of human disease will improve human health. The first precision medicine drug approved for the treatment of people with advanced non-small cell lung cancer (NSCLC), bearing mutations of the epidermal growth factor receptor (EGFR), was erlotinib -- an oral epidermal growth factor receptor tyrosine kinase inhibitor (EGFR TKI). However, the advantage of erlotinib, shown in terms of progression-free survival (PFS), did not translate to an advantage in overall survival, implying a possible limitation of this molecularly targeted drug. [9] Notwithstanding such hindrances, the precision medicine Initiative has generated new successful targeted drugs, new research areas and a rich repertoire of new technologies, including genome sequencing, metabolomics, pharmacogenomics, proteomics, magnetic resonance imaging, bioinformatics, machine learning (ML), and the electronic record of personal or familial medical history. These are innovative approaches now available for advancing the aims of precision medicine. [10]

Achievements of precision medicine. In recent years, there has been remarkable progress in medicine, based on the achievements of precision medicine. Improved understanding of the underlying genomics allows identification of inherited syndromes, early detection and prevention of diseases, and prescription of molecularly targeted treatments that are most likely to benefit the individual patient. Numerous actionable molecular targets have been found due to next-generation genomic sequencing, transcriptomics, and proteomics, and currently over seventy targeted agents have already been approved by the Food and Drug Administration (FDA) for the treatment of solid and hematologic malignancies. Concurrently, genomic markers such as BCR-ABL, KIT, BRAF, ALK, and EGFR gene aberrations were most efficiently used to identify individuals who will benefit from associated targeted inhibitors.[11] These new advances already begin to show fruits. A meta-analysis of 346 clinical trials (phase I) studied the impact of a biomarker-based oncological treatment strategy by comparing response rates and PFS in arms that used biomarker selection to those that did not. Results show a significant association between the use of a biomarker-based approach and improved outcomes, vis-à-vis the response rate and PFS. Studies that used targeted agents without a biomarker had negligible response rates.[12]

Challenges of precision medicine. Nevertheless, some experts questioned the success of precision medicine, suggesting that it is less successful than usually advocated. This view rests on the doubtful validation of both gene expression profiles and response biomarkers, as well as on failed proof-of-concept trials. A prominent example of the latter is the SHIVA open-label clinical trial, measuring PFS in patients with solid tumor cancers, who had already undergone all conventional treatments to prolong survival. In this trial, the patients were treated by pathway-directed therapy (denoted Precision Oncology), or by treatment of choice, as a control. The results of the SHIVA trial failed to show improvement of the PFS in the Precision Oncology arm of the trial. Experts believe that for becoming more successful, precision medicine needs to undergo substantial adjustments, principally, more rigorous testing, for ensuring a significant clinical benefit over the standard unguided treatment. [13]

Big data. The use of genomics, transcriptomics, proteomics and metabolomics technologies, and large sample sizes, has generated massive amounts of data, collectively known as "Big Data." The avalanche in the volume, velocity, and variety of the information available today has become a major bottleneck in the progress of precision medicine, requiring the implementation of new and more sophisticated computational and statistical technologies. Indeed, artificial intelligence (AI) and ML algorithms, computational biology methods, and digital biomarkers are developed at present for translating the accumulating data into actionable information.[14] In particular, a growing range of ML methods allows the extraction of hidden patterns, or trends, in the patient populations, directly from the databases themselves.[15]

However, at present, there are several challenges, which make healthcare data difficult to be fully beneficial. Data retrieval is complicated, and in the medical institutions, it is usually segmented, or siloed, in a controlled

departmental repository, which is isolated from the rest of the organization. No wonder, then, that retrieval of patients' data from conventional medical registries is not a cost-effective labor for healthcare providers, who show reluctance to perform this task. Protection of the patient's privacy is another obstacle, hampering the efficient extraction of knowledge from healthcare data, and obstructing the useful exploitation of healthcare data for advancing precision medicine.[15-16] The solution of the technological, legal, administrative and conceptual challenges in the retrieval of big data will clear the scene for answering the main question, namely, how to use the analysis of healthcare big data for improving the efficiency of care delivery.[17] At present, the contribution of methods of data analytics, such as new data mining technologies, predictive modeling, population health, and quality measurement to healthcare has been rather limited. The big ascent in clinical care, thus far achieved by precision medicine, employing targeted therapy and response biomarkers, is reaching a plateau.

Medical Biomathematics. Since the 1980s, the concept and the technology of personalized medicine has been developed in the field of biomathematics, independently of the molecular approaches to precision medicine. In the preliminary stage of the scientific development, it was necessary to prove that relatively simple mathematical models could offer medically relevant predictions, which would be validated experimentally. Thus, Agur and colleagues suggested an improved strategy of oncology drug application, based on the analysis of a mathematical model. *In vitro* and *in vivo* experiments of the theory followed thereafter, proving that simple mathematical models could identify better chemotherapy regimens which prolong the survival of cancer-bearing animals.[18-20] This first, albeit modest, success motivated the scientists to introduce the Virtual Patient concept and computer methodology,[21 22]and confirm it experimentally in mice receiving supportive treatment.[23] A heuristic optimization method was then developed, for identifying improved drug regimens,[22] which was tested in mice and in Rhesus monkeys for the chemotherapy-induced thrombocytopenia drug, Thrombopoietin. The model's predictions of individual monkey responses to new protocols of Thrombopoietin were validated, proving sufficient robustness in providing high prediction accuracy with limited input data. Scientific development of the Virtual Patient Population approach followed thereafter (Fig. 1). According to this approach, a collection of Virtual Patients is created, each characterized by a set of model parameters drawn from the distributions of these parameters in the real patient-population. Virtual Patient populations can undergo virtual clinical trials, endpoints of which are those employed in research, for example, for analyzing properties of individual patients, which may affect phenomena on the level of patient population (see below),[24] or in drug development projects, e.g., for examining how shelved drugs can be rescued.[25]

FIG. 1

Personalized models. The next step in the biomathematical effort to establish a computational personalization methodology was to turn the Virtual Patient, until now representing nonspecific members of the population, to representing specific patients. To develop this methodology, Agur and her colleagues introduced the concept of

heuristic treatment personalization, whereby clinicians could individualize the treatment regimen based on predictions of a model, which was personalized in conjunction with the patient's clinical characteristics and metrics [22,26-27] (Fig. 1).

A case study, identifying an improved treatment schedule for a patient having a rare cancer disease, mesenchymal chondrosarcoma (MCS), provided a proof-of-concept of the Virtual Patient idea. This was an original project investigating how personalization of oncological treatments can be done by integrating computational work with gene expression analysis, experiments in mice, xenografted with the patient's tumor, and clinical work. Thus, growth curves and gene expression analysis of xenografts, derived from a patient's lung metastasis, served for creating a mathematical model of xenograft progression. The pharmacokinetics (PK) and pharmacodynamics (PD) of several chemotherapeutic and antiangiogenic drugs were modeled, model parameters being adjusted by patient-specific chemosensitivity tests. The xenografted animals were treated by various monotherapy and combination drug schedules, and the mathematical xenograft model was simulated under the same treatment scenarios. Model-simulated results of tumor growth inhibition were compared to the experimentally observed results, showing good predictability. The computational xenograft growth model was then up-scaled to retrieve the patient's tumor progression under different treatment schedules; up-scaling was done using gene expression analysis of several key proteins, such as Angiopoietin, Vascular Endothelial Growth Factor, etc., in the patient biopsied lung metastasis. Subsequently, the personalized MCS patient model was simulated assuming the application of a docetaxel and bevacizumab combination in different schedules. The potentially optimal schedule was administered to the real patient, resulting in the stabilization of his galloping metastatic disease, relief of his life-risking pancytopenia and extension of his life span. Yet, this case study was unique in the richness of the patient's molecular and clinical information, becoming available through an extraordinary experimental effort to evaluate the patient-specific cytokine excretion rates, tumor cell growth rates, gene expression analysis, etc. Since in the daily clinical routine one cannot rely on the accessibility of similarly rich data, the model personalization methodology developed in [28] is not operational in the current clinical reality.

In general, the efforts to identify routinely accessible clinical or molecular measures to aid in the personalization of the mathematical model have not been fruitful until recently. This is because the analyses of the patients' measured clinical parameters, retrieved from the few accessible clinical trial datasets, did not show any statistical correlation to the patient's response. Moreover, big clinical databases were scarce and intensive data mining was not an option. Additionally, massive computations, which were necessary for simulating large virtual clinical trials, were hard to perform, due to the relatively weak computer capacity of the early 21st century. Therefore, despite the progress in the understanding that personal dynamic differences between patients can affect their responses, and that mathematical models can retrieve those and use them for tailoring drug

regimens to individual patients' data, the development of these concepts has come to a quasi-standstill in the first decade of the 2000s.

Lately, these problems have largely dissipated. This has been mainly due to the US health authorities acknowledging the necessity to render big clinical databases approachable to professionals, as an important means to improve healthcare. In 2010, leaders from the U.S. government, federal agencies, health care delivery systems, and others, directed to catalyze the formation of a new "Community Health Data Initiative," by harnessing the power of information to improve health. HealthData.gov was activated, and today it includes thousands of health-related datasets, motivating the authorities in many other countries around the world to create their open data sites. The accessibility of a growing number of globally collected public and private databases emphasizes the need for much-increased computing power to facilitate the analysis of the accumulating data. The new cloud computing technology satisfied this need by providing an internet-based platform with myriads of services and system resources and allowing large storage and computation capacity with no information technology infrastructure costs for the end-users.

This breakthrough in the availability of health and computing resources allows precision medicine and medical biomathematics to converge. As precision medicine matures, a growing body of personal clinical information, such as molecular biomarkers, circulating tumor DNA, etc., are regularly evaluated. The improved information on the patient provides biomathematicians with better means to liaise the mathematical models to real-life patients, create personalized models, and use them to individualize medical treatments. This review aims to describe this evolution in the field of cancer immunotherapy, where treatment personalization is most necessary and where signs of a breakthrough already appear.

2. A paradigm shift in cancer immunotherapy

Precision Cancer Medicine. Advances in cancer genomics and molecular profiling have shown that the same mutations or signaling pathways can drive different cancers, and treatment based on the molecular abnormality rather than on the anatomical origin can be efficacious. Such molecularly targeted strategies have been termed Precision Cancer Medicine.

Personalizing cancer immunotherapy. Immunotherapy by checkpoint blockers (ICBs) has emerged as a successful targeted therapeutic modality, reactivating effector T lymphocytes, which were previously blocked by cancer.[29-30] This underlines the fundamental distinction between cytotoxic chemotherapy, attacking generic cell-cycle mechanisms, hence befitting the “one-size-fits-all” paradigm in medicine, and targeted drugs, interfering with specific aberrant biologic pathways in cancer cells, or boosting specific immune capabilities, which befits the precision medicine approach.

Complex dynamics characterize the interactions between the patient's immune system, the growing tumor, and the immunotherapeutic drug. In the most successful example to date – cancer therapy by ICBs – the immuno-inhibitory receptor expressed on T and B cells, programmed cell death protein 1 (PD-1), and its ligand, PD-L1, are key players in the regulation of adaptive cellular immunity. Cancer cells "piggyback" on this natural immune regulation, by expressing PD-L1 molecules that bind to PD-1 receptors on effector T lymphocytes, pushing these cells into apoptosis. This weakens the immune response prematurely and hampers cancer cell clearance.[31] The ICBs pembrolizumab, atezolizumab, and others disrupt this cancer-induced evasion of immunity, by blocking this receptor-ligand binding, thereby reactivating exhausted effector T lymphocytes.[32]

Immunotherapy by ICBs is an exemplar case for the need to replace the “one-size-fits-all” paradigm by precision medicine. The efficacy of the patient's response to ICBs depends largely on the vigor of the patient-specific cellular immune arm. The latter depends on personal immune parameters, such as immune cell infiltration and functionality within the tumor microenvironment, etc.[33] Tumor immunogenicity -- which determines the intrinsic ability to induce adaptive immunity -- depends on the frequency of neoantigens present on the tumor surface following somatic mutations, a patient-specific and versatile process in itself.[33] For these reasons and more, the response to ICBs is highly variable among patients, substantiating the personalization requirement for the treatment by ICBs. It is not surprising that biomarkers, such as high microsatellite instability (MSI-H), deficient MisMatch Repair (dMMR), or tumor mutational burden (TMB), prove efficient in classifying potential responders to ICBs.[11]

In the case of ICBs, treatment personalization is essential, also considering the unusual related phenomena, such as hyper-progressive disease (HPD), manifested in 9-16% of patients with different cancer types, and in 13-37% of patients with NSCLC, depending on the definition of HPD.[34-35] The occurrence of patients with HPD, experiencing accelerated tumor growth and clinical deterioration after the onset of ICB administration, raises serious concerns about the use of these drugs and emphasizes the critical need to predict the patient response before treatment. Another hurdle is the high cost of the drug, imposing an unacceptable burden on the patients. This cost is even less justified if the patient is a non-responder. Taken together, the necessity to personalize interventions with ICBs is compelling. Yet, to date, this important requirement remains with no satisfactory answers.

In this review we make a clear distinction between two developmental tracks - scientific and pharmaco-clinical. Along the scientific track, scientists develop personalization concepts and predictive technology, and retrospectively validate them by patients’ information. Along the pharmaco-clinical track, the journey only begins after reaching the final destination along the scientific track. It involves the implementation of the scientifically developed technology in a diagnostic or prognostic medical decision support tools and their testing in prospective clinical trials for accuracy and medical benefits.

The scientific development track of computational therapy personalization methods consists of constructing mathematical models that reflect the complex biology (Milestone S1), showing that the predictions of the mathematical models can be supported experimentally (Milestone S2), showing that mathematical models can be clinically relevant (Milestone S3), and have shown clinical pragmatism and sufficient accuracy in retrospective clinical trials (Milestone S4). The pharmaco-clinical track involves translation of the model or computational tool developed along the scientific track into a predictive technology to be employed as a decision support tool for the treating oncologists (Milestone PM1). The next milestones comprise prospective clinical trials to validate the accuracy of the developed technology in predicting patients' response to treatment (Milestone PM2) and in improving desired clinical measures, such as time to progression (TTP) and overall survival (Milestone PM3). The point of view of the present paper is that, conceptually, most of the long and rocky scientific track has been trodden already. To complete the process successfully, the development along the pharmaco-clinical track must be initiated and fully executed. Below, we will glimpse into exemplary works along the scientific track and suggest where we stand today and how to accelerate the development along the pharmaco-clinical track, namely, the clinical implementation of the computational-based personalization technology.

3. Current personalization approaches in cancer immunotherapy: predicting the patient's response by analysis of molecular profiles

The limitations of currently approved diagnostics in cancer immunotherapy hold back the institutionalization of precision oncology. At present, the only companion or complementary diagnostics approved by the FDA for use in cancer immunotherapy, are based on the assessments of PD-L1 expression. These markers employ simple cutoff models, dichotomizing assay outcomes according to a pre-defined threshold, e.g., 1% or 50%. They generally show low accuracy, allow for a large proportion of false negatives and false positives,[36-37] and show little success in classifying good responders that have low PD-L1 expression, or non-responders that have a high PD-L1 expression.[38] This inaccuracy, possibly due to the variability in the biomarker assays, points to the insufficiency of PD-L1 expression as a sole predictor of patient response.[38-39] Indeed, the mechanisms determining the efficacy of ICB treatments are intricate, encompassing the timing and extent of the effector T cell response, the expression of related cytokines, signaling pathways associated with the PD1 receptors in T cells, various evasion strategies used by cancer cells, and perturbations which may be caused by ICBs to all these factors. This suggests that more extensive and detailed personal information, beyond the expression level of a single receptor, may be required to personalize the treatment selection faithfully and accurately.[40]

Current attempts to improve the stratification of cancer patients considered for immunotherapy mostly focus on developing biomarkers that are more precise. These efforts capitalize on various experimental technologies for retrieving multi-dimensional patient information. Examples include transcriptome analysis,[41] or genetic

sequencing methods, such as whole genome sequencing (WGS), whole exome sequencing (WES) and next generation sequencing (NGS).[42] These technologies create high-dimensional datasets, whose interpretation requires more advanced modeling and computational approaches; simple statistical tests and linear regression models are not sufficiently powerful and are prone to bias and overfitting. Additionally, the clinical validity of a new biomarker must be demonstrated before its introduction to the clinical market. The success of this elaborate task depends, among others, on the ability of the associated computational algorithm to interpret the results of the experimental assays with respect to the patient's clinical response.[40,42] This task becomes even more intricate when the information on the individual patient is diverse. In such a case, it is essential to apply computational models that can process high-dimensional inputs effectively. Despite the awareness of biomarker developers to this important need, no general unified methodology exists for the specific task of building such predictive algorithms. Recently, this requirement motivated the introduction of various ML methodologies, which allow robust identification of statistical correlations between the extensive input variables and the patient's response to the ICB drug. However, the multi-dimensional data analyses are yet to be verified by yielding improved predictive biomarkers for the application in clinical immuno-oncology. Below we outline several developments in this direction. In Table 1 we list biomarkers under development and their associated computational methodologies.

Table 1

A straightforward way to interpret multi-variate data is by processing multidimensional input to yield a simple index, indicative of the expected treatment efficacy in a given patient. The best established among these newly developed indices are TMB and dMMR estimates[43-44], where computationally straightforward algorithms use quantile-based thresholds of genome-wide mutational load to classify patients, demonstrating significant enrichment for responding patients in several cancer indications.[45-47] Another method is based on the evaluation of immune infiltration into cancer tissue, such as a semi-qualitative Immunoscore, based on the assessment of immune cell subsets, and shown to be associated with disease prognosis.[48-49] The recently developed Immunophenoscore method employs ML to find the major determinants of cancer immunogenicity in genome data, mined from the cancer genome atlas (TCGA). This score evaluates immune infiltration and cancer-related neoantigens, having a high predictive value for melanoma patients treated with ICBs.[50] Another recently developed method employs data from over 100 studies to identify a gene signature of T cell dysfunction, applying statistical interaction test with a proportional hazards Cox model to evaluate the effect of different genes on T cell activity in the tumor. The results in patients with melanoma suggest that the scores of tumor immune dysfunction and exclusion (TIDE) are associated with the rate of infiltration of cytotoxic T cells into the tumor, immune evasion by the tumors, and eventually, response to ICBs.[51] Another work uses

multivariate regression models to predict the patient's response to immunotherapy.[52] Other examples for the application of ML to multi-dimensional genetic and transcriptomic profiles, in the aim of producing predictive response signatures to ICBs, are listed in Table 1.[53-54]

Awareness of the complexity of the interactions between tumor genomics, cell signaling, chemokines and immunosuppressive molecule expression, motivated Brogden and colleagues to apply an ordinary differential equations (ODE) model of an extensive intracellular signaling network to personal mutation profiles in a dataset of 34 NSCLC patients. Taking the patient's reported mutations as an input, the model simulations enabled to predict the personal changes in the levels of 24 proteins, including PD-L1. The authors used ML to develop and validate a decision tree model for predicting the patients' responses to pembrolizumab from the simulated personal protein levels, generated by the individual mathematical pathway models.[55] Other scientists used image analysis, based on AI methodologies and the patients' computed tomography scans, to define radiomic biomarkers for predicting the response to ICBs. Retrospective analysis of patient data showed significant accuracy of this method in NSCLC patients.[56]

The main limitation of the approaches involving static molecular profiles is their construction from single snapshots that are processed by statistical methods. This enables to evaluate the relationships between the feature extracted from the input data, e.g., TMB, or gene expression pattern, and a simple, clinically motivated, strictly categorized, output, such as "yes/no responder". However, even though the implicated assays focus on hand-picked components of the system, which are known to be highly relevant to the response to immunotherapy, this is insufficient. The current methodologies mostly ignore the rich interactions between different parts of the system, and the computational models that are used are oblivious to the vast biological knowledge on the involved processes. Instead, purely statistical, essentially, correlation-based analysis is applied. While the results of this analysis can be statistically significant, and can even yield an acceptable level of accuracy, most of these approaches have not been translated to development along the pharmaco-clinical track, and are likely nearing their maximum potential. The main reason for this may be the exclusive reliance on single-type features for response prediction, perhaps resulting from technological incapacity to integrate information from different sources and scales.

To improve the performance of the available technologies, it will be necessary to develop new models for integrating genomic, proteomic, metabolomic and other relevant information on patients. To do this one should employ methods that take into account what, owing to their generic nature, the statistical approaches neglect -- the dynamic interactions between the main forces in the system. We discuss such methods in the next section.

4. Current computational approaches in cancer immunotherapy: allowing for system's complexity by modeling disease dynamics

Mathematical modeling is a powerful tool for succinctly describing complex biological systems and for examining the relative influence of various, sometimes contradicting, biological forces on the overall dynamics. Being an intricate balance of several elements, the immune system has provided a perfect arena for mathematical modeling to do its thing. In cancerous conditions, to achieve effective immunity the immune system must be steered towards productive, but not excessive, cellular effector-based immunity, while keeping the other arms of immunity – humoral and regulatory – at bay. Such natural balances of the system's activity provides a fertile ground for the development of dynamics-based models for immunotherapy. Mathematical analysis is especially essential for scrutinizing the variation between patients in the response to immunotherapy, and for suggesting new avenues for biomarker development. In this section, we glimpse into the evolution of mathematical modelling in cancer immunotherapy, from the first theoretical explorations until now, that the scientific development is almost ripe for the transition to the pharmaco-clinical development track.

4.1 Milestone S1: theoretical mathematical models of cancer immunotherapy

Dynamic mathematical immunotherapy models have come a long way in the last decades. Early models were fully theoretical, toying with immune-modulating cancer treatment on several levels, but providing insight into the parameters governing the coevolution of the immune system, the tumor, and the particular treatment. We will show that even simple theoretical models, examples of which are discussed herein, can have some power to uncover unknown relationships that more faithfully reflect the real clinical scenario.

One of the early models describing important features of the immune response to cancer is due to Kuznetsov et al.[57] This work proposes a simple model for the growth and regression of B cell lymphoma 1 (BCL1) in the spleen of chimeric mice. The model reproduces major tumor-immune interactions, such as immuno-stimulation, tumor dormancy, and tumor evasion. For evaluating model parameters, Kuznetsov and colleagues relied on published experimental data of BALB/c mice, bearing different loads of BCL1.[58-59] Model analysis identified important system parameters and showed a region in the space of parameters, within which any external stimulation towards increased numbers of cytotoxic T cells can be detrimental. This result was initially counter-intuitive, as it was expected that increased numbers of effector T cells always reinforce the immune response. However, a more elaborate mathematical scrutiny of Kuznetsov's model predicts no cycles in the number of lymphocytes, contrary to observations in certain leukemias,[60-61] hence questioning the model's suitability to describe the dynamics of this disease. Other simple theoretical models for tumor-immune interactions have also provided interesting insights.[62]

After approval of the first cancer immunotherapy -- the cytokine interleukin (IL-) 2 -- for the treatment of renal cell carcinoma and melanoma in the 1990s, biomathematicians began raising questions concerning the action mechanisms of cytokines, optimal regimens to administer cytokine drugs, etc.[63-65] In one of the first models, Kirschner and Panetta focused on the impact of IL-2 on the immune reaction to cancer.[66] Optimal IL-2 treatment, maximizing the sum of effector T cells and IL-2, while minimizing the tumor load and cost of treatment, was also studied based on the latter Kirschner and Panetta's model.[67] Analysis shows that the optimal treatment schedule of IL-2 is always "bang-bang," namely, an intermittent application of the maximal admissible IL-2 dose. As this strategy relies on a much-simplified model, where each model parameter stands for a combination of several other more "natural" parameters, it was not clinically applicable since there was no easy way for parameter estimation.

A more complex model for adoptive T cell transfer in high-grade malignant glioma was suggested, comprising six coupled ODEs, which describe the interactive dynamics of tumor cells, T cells, and their respective secreted cytokines and immune mediating receptors.[68] Theoretical analysis of the model, supported by results of murine experiments and by clinical information on glioma case-reports, suggests that in untreated patients, the physiological system always converges to a steady-state of a large tumor mass. An increase in the patient's pro-inflammatory activity only marginally reduces tumor load at the steady-state, suggesting that the patient's natural immune system is never sufficient for eliminating glioma. In contrast, infusion rates above a certain computable threshold value, guarantee a cure from any initial state of the system. This work provided insight into practical guidelines for improving glioma immunotherapy by T cell infusion, and into the necessity to personalize the application regimens of this immunotherapy modality. Notwithstanding the support by experimental and clinical data to the qualitative results of the model simulations, at its current state the model is still an intellectual exercise. It has yet to undergo retrospective validation by patient information before moving to the pharmaco-clinical developmental track for its implementation.

Mathematical modeling enables to compare the benefits and drawbacks of different cancer immunotherapeutics. In 2003, Szymanska proposed models for two cancer immunotherapy modalities: adoptive immunotherapy, involving transfer of T helper cells, and active immunotherapy, administering attenuated cancer cells or their antigens. Theoretical model analysis suggests that adoptive immunotherapy is safer for the patient. Specifically in the case of active vaccination, the model predicts a massive increase in the number of T helper cells, which may critically boost in the levels of various cytokines, potentially causing a life-threatening cytokine storm.[69]

The exposure of such findings to the medical community should have motivated studies of potential adverse consequences of immuno-stimulation. However, in the early 2000s, hardly any biomathematical research news could transcend the walls of the isolated small interdisciplinary community. Thirteen years later, the public was shocked to learn of six healthy volunteers, enrolled in a Phase I trial of a novel immunomodulatory drug, who suffered unexpected severe systemic inflammatory response. They became critically ill and were admitted to an intensive care unit in a London hospital within minutes of receiving a single intravenous drug, theralizumab, an antibody activating T helper cells. These events shed some light on the immune-mediated cytokine storm, which leads to multi-organ failure in the absence of infection.[70] Even though there may have been several reasons for this mishap, we dare speculate that much of the drama could have been avoided by timely consulting the mathematical model and joining forces for its validation.

We can already see that mathematical models of immune interactions in cancer describe processes occurring on different scales and organization levels - from the molecular to the macroscopic level. Until recently, this caused difficulties in estimating values of parameters. However, today, with increasing access to clinical data, and advanced statistical and numerical methods, like non-linear mixed effects modeling (NLMEM),[71] intensively developed in the context of pharmacokinetics,[72] model parameters referring to different organization levels of the biological system can be jointly estimated from the experimental and clinical data.

4.2. Milestone S2: experimentally-supported mathematical models for cancer immunotherapy

Until recently, to obtain substantial experimental support for a mathematical model was a tedious task, due to the little flow of data between experimental and theoretical disciplines. Therefore, only a few mathematical immunotherapy models were corroborated by sufficient real-life data. In one of the first instances, de Pillis et al.,[73] proposed a model accounting for different roles of natural killer (NK) cells and tumor-specific CD8+ T cells, for learning how the immune system interacts with a growing tumor. The aim of this work was to learn how the immune system interacts with a growing tumor, and which components of the immune system play a role in responding to cellular immunotherapy. Model simulations were compared to experimental studies in which murine tumor cell lines were modified to express higher levels of immune-stimulating ligands. Fits to data of two patients with metastatic melanoma, treated by tumor-reactive T cells, support the generality of the tumor growth model, and its relevance to human cancer patients.

Immunotherapy of solid cancers by the cytokine IL-21 was similarly modeled as an ODE system, representing stimulation of innate immunity, followed by adaptive, tumor-specific, immunity. The model was initially fitted using mouse experiments in melanoma and renal cell carcinoma, and collaboration with the drug developers resulted in model expansion to include PK/PD data, and subsequent model validation by independent murine experiments. The model predicted that a fractionated or low-dose regimen would reduce the tumor mass as

efficiently or even better than the original regimen - predictions that were corroborated experimentally. This joint effort, although realized pre-clinically, demonstrated the potential of employing *in silico*-guided design and rationalization of cancer immunotherapy in the clinic.[74-76]

4.3 Milestone S3: clinically-corroborated personalization models for cancer immunotherapy

The above biomathematical efforts have mostly remained in the domain of theory, as they still require showing precision in human subjects. For models to make a meaningful contribution, particularly in onco-immunology, they should be able to represent actual patients, and be implemented in simple-to-execute algorithms. Recent progress in precision medicine warrants the transition from theoretical analyses and basic dynamic modeling to complex statistical, mathematical, or computational modeling to guide clinicians towards improved cancer care. The overflow of heterogenic patient data from clinical trials and hospital registries, as well as the availability of powerful computational resources, represent a unique opportunity for the development of effective immunotherapy personalization technologies.

One way to address patient variability issues is to develop individual patient-tailored models. The model of Kronik et al.,[77] designed for vaccination immunotherapy of prostate cancer patients, was one of the first examples of personalized model development for a clinical cohort, with the patient's tumor dynamics serving for both training and retrospective model validation. Thus, with prostate-specific antigen (PSA) as a surrogate marker for tumor burden, pre-treatment and initial in-treatment PSA data of a given patient served for model personalization (training set), and subsequent PSA measurements of that same patient served for retrospective model validation (validation set). The personalized models accurately predicted PSA dynamics over the entire measured period (ca. one year) in 12 of the 15 responsive patients. This was a creative new approach to validating personalized models (in contrast to population-based model training and testing). Model analysis further illuminated the futility of single vaccination schemes and supported periodic treatment in such patients.[78]

A novel interactive modeling approach, proposed by Kogan et al., took the *in-treatment personalization* concept one step further, offering real-time design and validation of a patient-specific model.[79] Kogan and his colleagues used the above vaccination model[77] as a basis for developing a clinically applicable algorithm that builds the patient's own model *ad hoc*, to the level of a sufficiently good personal model obtained as early as possible after treatment onset.[79] This *in-treatment personalization/validation* methodology involves three major steps (Fig. 2): iterative integration of longitudinally measured patient data into the mathematical model, until the point of sufficient personal model validation; application of the validated personal model to predict the patient's response to the currently applied treatment regimen; if necessary, simulation of the validated personal model to identify regimen that is more beneficial. The major drawback of this approach is the scarcity of situations in which regimen personalization of a given treatment can be clinically feasible after treatment onset. Yet, below

we suggest why this approach can be of value in immunotherapy drug development and how it can be effectuated in clinical trials of immunotherapeutic molecules.

FIG. 2

What is the benefit of personal models for the specific challenges of ICB therapy? A mechanistic model that formalizes the interactions between cellular immunity, advanced melanoma , and the ICB, pembrolizumab, was recently put forward by Perlstein and colleagues for studying this question. The model (Fig. 3)[80] is new in considering adaptive immunity as a developing tissue, as suggested by Gattinoni et al,[81] and incorporates both senescence and exhaustion – two dominant cellular immunity mechanisms in the context of PD-L1/PD-1 blockade. Model simulations successfully capture the atypical disease dynamics in an individual patient from a hospital cohort (box in Fig. 3). Computational analysis of the model shed light on the predictive power of existing and potential response markers. The inter-patient variation in the values of the T cell toxicity parameter explain the rich variation in response, including a pattern roughly resembling HPD. Virtual clinical trials (see above) in the Perlstein and colleagues' model[80] successfully retrieve real-life clinical trial results, showing that the ratio of reinvigoration rate to baseline tumor load can serve to cluster patients according to the predicted quality of their response.[82] Such endeavors demonstrate how multiple personal model parameters, which are impossible to estimate in the clinical setting, can be combined into one quantifiable measure helping to classify responsive/non-responsive patients. Pending validation, implementation of such models in the clinic can help in developing efficient predictive biomarkers.

FIG. 3

4.4 Milestone S4: clinical pragmatism and sufficient accuracy in retrospective clinical trials

Ultimately, only a minority of models contain the clinical orientation and personalization attributes to make them medically-geared. Inspired by the personalization approach developed hitherto, Tsur and her colleagues developed a medically feasible and easily implementable innovative algorithm for navigating ICB treatment of advanced melanoma.[83] The goal was to use a mathematical model that could input molecular and clinical metrics available before treatment, of the nature and sampling frequency used in the clinic, and be able to generate personal predictions interpretable by known clinical criteria, such as Response Evaluation Criteria in Solid Tumors (RECIST 1.1). Obviously, this meant devising a new methodology for integrating information on different organization levels of the biological system within PK/PD and dynamical disease modeling. In tandem with collecting hospital data of 54 melanoma patients given pembrolizumab, a mechanistic model for the interactions of the ICB drug with the tumor and immune system was developed. Analysis of correlations between personal pretreatment metrics of the 54 patients and the model parameters showed that the baseline tumor load, the Breslow tumor thickness, and the status of nodular melanoma was significantly correlated with

the model parameter for activation rate of CD8+ T cells and the net tumor growth rate, which was in line with biological findings[84-85].

Embedding the discovered correlation functions and personal measurements of the correlates in the model, the resulting personalization algorithm enabled prediction of the TTP for every individual patient in the hospital cohort (Table 2). The predictive ability of the algorithm was checked by Leave-One-Out cross-validation; moderate predictive accuracy was obtained at this first step, with increased accuracy expected upon further validation in a larger group of patients. This is the first algorithm, which enables to predict an important clinical outcome - TTP, by combining clinical metrics collected before treatment with a simple mechanistic model for the cancer-immune system network affected by ICB. The methodology suggested by Tsur and colleagues is scale-independent, allowing integration of information collected on different organization levels for predicting the response of individual patients to ICBs. Following retrospective clinical testing for completing the algorithm's initial validation (Milestone S4), this algorithm, and other like it, are ready to move from the scientific development track into the pharmaco-clinical track to undergo implementation as medical software devices (Milestone PM1), and prospective clinical trials to prove their prediction capacity (Milestone PM2) and medical impact (Milestone PM3).

Table 2

5. Future directions

How to personalize immunotherapy by integrating complex genomic, transcriptomic, metabolomic or other patients' data with mechanistic PK/PD models, is a critical question at present. The plethora of available new technologies does not offer effective personalization solutions, mainly because these technologies cannot efficiently integrate information from different profiling scales. The road to successful personalization of cancer immunotherapy is the one that merges two different tracks, which call for a new impetus to their further progress: the analysis of clinical and molecular profiles**,** and the mathematical modelling of the complex interactive dynamics in the host-disease-drug system. Studies like Tsur et al., 2019, demonstrate the promise, as they integrate massive information from different organization levels in one framework, facilitating the personalization process in a scale-insensitive way, to ultimately determine personal drug-disease-host dynamics and predict personal responses to ICBs.

The understanding, emerging from the analysis of the mathematical models, that relatively small changes in personal parameters can significantly affect a patient's response, as suggested, for example, for T cell functionality,[80] forces one to revisit the basics of drug development. The "one-size-fits-all" paradigm is still the central pillar in drug development, where the performance of an investigational new drug is evaluated by the response of hundreds, or even thousands, of patients to a specific treatment protocol. However, today, the

large variation in the response to ICBs is evident, and the necessity and feasibility in the adjustment of therapy to the individual patient under ICBs, have also been elucidated. Therefore, one cannot use the population response to a single treatment protocol as a criterion for evaluating the efficacy of a drug, whose application to the patient needs to be attuned on a personal basis. In cancer immunotherapy, this "one-size-fits-all" strategy would result in underestimation of the efficacy of the developed drug.

How can we introduce a personalization scheme during clinical trials of immunotherapeutic drugs? Agur and Vuk-Pavlovic addressed this question, calling for a conceptual change in the design of clinical trials for immunotherapy drugs, and suggesting a new approach for clinical testing of drugs whose schedules are to be personalized (denoted P-trials).[86] Based on the immunotherapy personalization concepts discussed in this review, the authors. In P-trials, the regulatory authorities should replace the state-of-the-art procedure, by allowing testing of a few, *a priori* selected, dosing schedules that vary within a certain range, restricted by e.g., drug toxicity. Within the allowed range, the selection of the individual regimen will be left to the discretion of the treating physician, based on the clinical parameters of the particular patient.[87] Tools like the recently developed personalization algorithm predicting TTP,[83] would be instrumental in such a clinical decision-making process once it is soundly validated. This will, hopefully, lead to improved individual responses and, collectively, increase the response rates in studies of new immunotherapeutic agents.

In the targeted therapy realm, molecular response biomarkers enrich patient enrollment, and their use increases the likelihood rate of regulatory drug approval from 55% to 76%.[88] However, the lack of efficient methods to identify and validate potential biomarkers creates a shortage of approved diagnostics, holding back the institutionalization of precision oncology. Particularly in cancer immunotherapy, the limited predictive value of the first response biomarkers associated with PD-1/PD-L1 expression illustrates the urgent need for more reliable immunotherapy biomarkers, but the search for these is limited by our incomplete understanding of how immunotherapies modify the already complex immune response to cancer. As outlined in this review, the gap can be bridged by computational analyses of mathematical models, employed for analyzing the sensitivity of decisive system parameters. We expect this will highlight new, potentially efficient, simple or composite, response predictors.

In conclusion, technologies that process patient data from hospital registries, along with mathematical models for the underlying interactive dynamics, can help creating personalization algorithms for facilitating prediction of the patient's response. Such efforts could only be a product of a fruitful collaboration between clinicians divulging real-world data, and experienced mathematicians formalizing the pertinent pharmacological-biological interactions and working out the model personalization methodology. We believe that clinicians, pharmacists, and biomathematicians should join forces to accelerate the maturation of precision medicine.

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Author Contributions

ZA, ME, UF, YK wrote the manuscript.

Figure 1. The Virtual Patient – design and application. The Virtual Patient concept states that a multi-module model can efficiently describe a patient, by integrating pathophysiological processes with drug-related efficacy and toxicity, including the pharmacokinetics (PK) and pharmacodynamics (PD). By incorporating diagnostic intelligence and patient data from various databases, the virtual patient model can be personalized, and a collection of Virtual Patients can be formed, each characterized by a set of model parameters drawn from the distributions of these parameters in the real patient-population. Virtual Patients can be simulated to pinpoint improved general and personal treatment schemes and predict clinical outcomes. Additionally, virtual clinical trials could be simulated for designing trials and rescuing drugs.

Figure 2. An interactive modeling approach for personalization of a patient-specific model and its clinical application in real-time. A novel interactive modeling approach, proposed by Kogan et al., for patient-specific real-time modeling,[79] developed based on the whole-cell vaccination prostate cancer model in.[77] The operation of the algorithm begins by model training on the initial data of the patient, followed by iterative integration of subsequent data until reaching model validation at sufficient accuracy. Once a validated personal model is reached, it may be simulated for predicting patient outcomes and improving treatment regimens. The timeline exemplifies how, for a given patient, a validated model may not be reached in time to predict initial patient outcomes (such as the response rate or time of progressive disease (PD) to the first-line treatment), but maybe available for simulating such outcomes to further treatment by the same drug or by second- or subsequent-line treatment. Blue circles mark patient data; the red line portrays model simulation.

Figure 3. A model for cancer immunotherapy by the ICB pembrolizumab, and its simulation of tumor load in an advanced melanoma patient from a hospital registry[80]. Immunity is represented by T cell subsets (circles; increasing shading represents increased maturity). Naïve T cells (*TN*) differentiate (solid arrow) into stem cell memory cells (*TSCM*), then into central memory cells (*TCM*), then into effector memory cells (*TEM*). Those differentiate into effector cells (*TEFF*), which gradually differentiate into fully exhausted cells (*TEXH*). Self-renewal occurs in gradually decreasing rates (curled arrows). Cell death of gradually increasing rates (diagonal dashed arrow) is assumed for all T cell compartments. *TEFF* and *TEM* target the proliferating cancer cells causing them to die, but this activity is inhibited by the binding of cancer-expressed PD-L1 to PD-1 receptors on the effector cells. Cancer cells also act to inhibit proliferation and functionality of *TEFF* and *TEM* cells via PD-1/PD-L1 ligation. Pembrolizumab, a PD-1 antibody, is injected (freehand arrow) and blocks the PD-1/PD-L1 pathway, preventing the inhibition of *TEM* and *TEFF*. Dendritic cells are activated by cancer antigens and stimulate the proliferation of *TSCM* and *TCM* cells. Lower box shows model simulations of disease dynamics in an individual patient from a hospital cohort (solid line), where the patient's complex clinical response to pembrolizumab was retrieved (empty circles; bars represent standard deviation).

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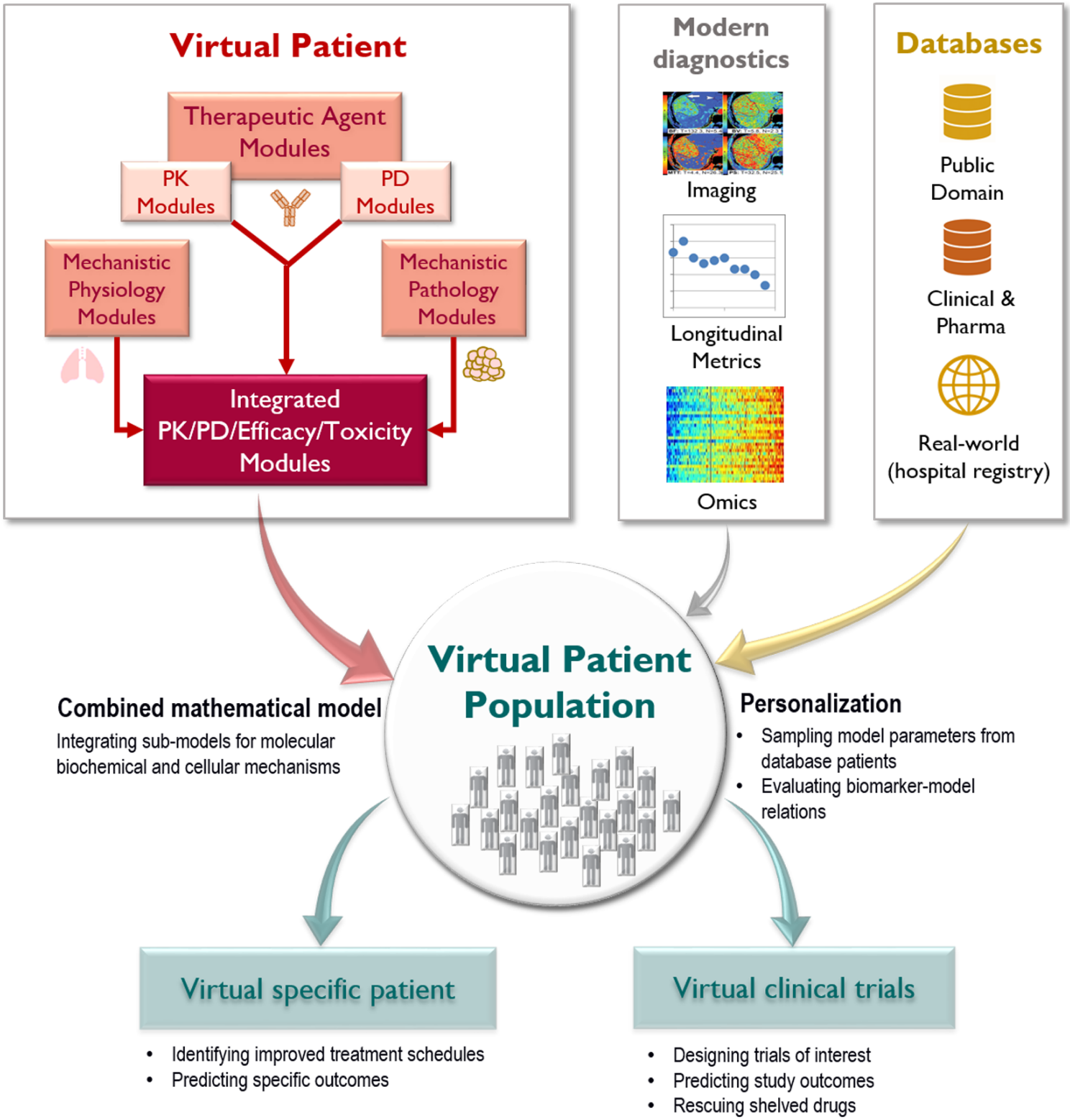
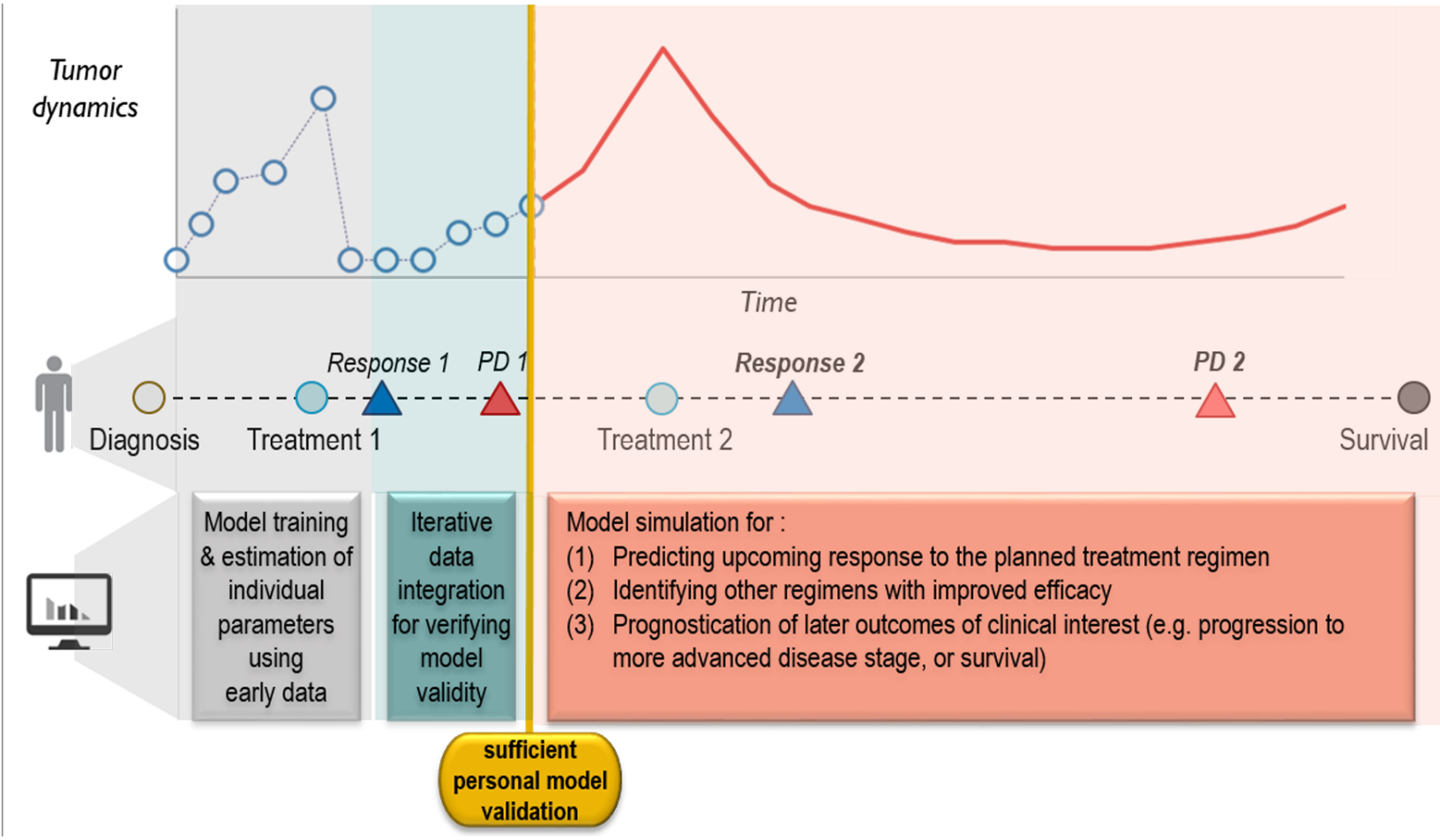
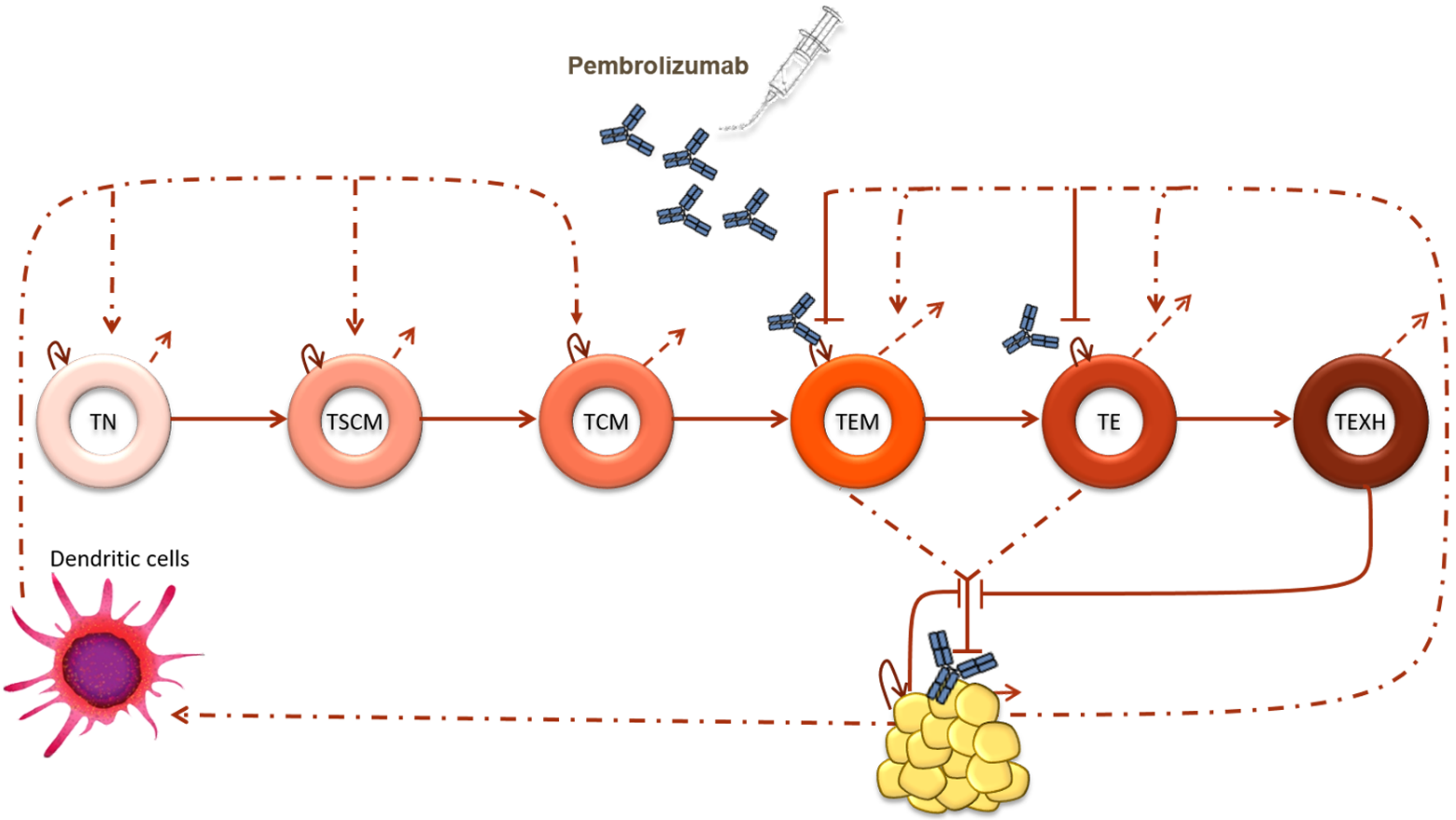
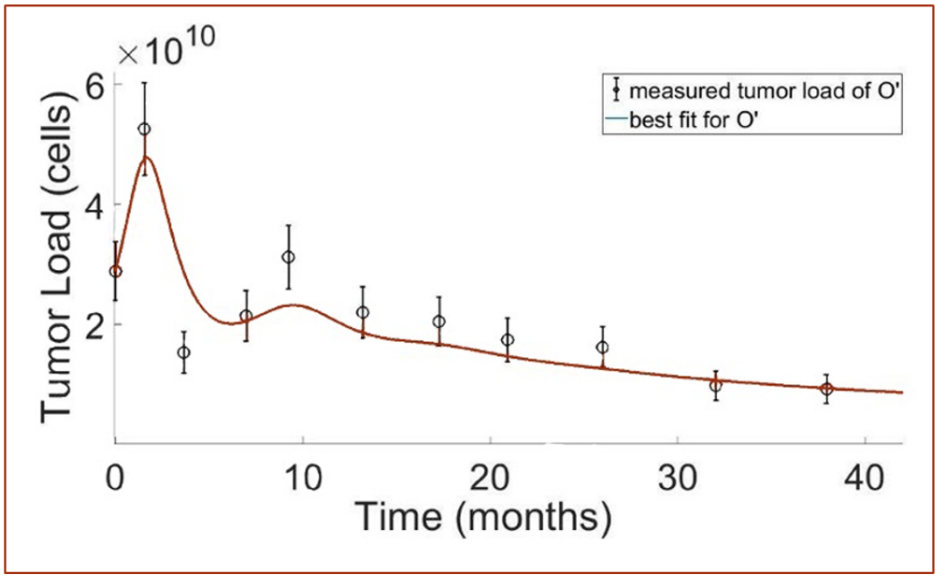

Virtual Patient
Therapeutic Agent Modules
PK Modules
PD Modules
Mechanistic Physiology Modules
Mechanistic Pathology Modules
Integrated PK/PD/Efficacy/Toxicity Modules
Modern diagnostics
Imaging
Longitudinal Metrics
Omics
Databases
Public Domain
Clinical & Pharma
Real-world (hospital registry)
Virtual Patient Population
Combined mathematical model
Integrating sub-models for molecular biochemical and cellular mechanisms
Personalization
• Sampling model parameters from database patients
• Evaluating biomarker-model relations
Virtual specific patient
• Identifying improved treatment schedules
• Predicting specific outcomes
Virtual clinical trials
• Designing trials of interest
• Predicting study outcomes
• Rescuing shelved drugs


Tumor dynamics
Time
Response 1
PD 1
Response 2
PD 2
Diagnosis
Treatment 1
Treatment 2
Survival
Model training & estimation of individual parameters using early data
Iterative data integration for verifying model validity
Model simulation for :
(1) Predicting upcoming response to the planned treatment regimen
(2) Identifying other regimens with improved efficacy
(3) Prognostication of later outcomes of clinical interest (e.g. progression to more advanced disease stage, or survival)
sufficient personal model validation


Pembrolizumab
TN
TSCM
TCM
TEM
TE
TEXH
Dendritic cells


×10^10
6
4
2
Tumor Load (cells)
measured tumor load of O'
best fit for O'
0
10
20
30
40
Time (months)

Types of individual patient data	Computational approaches for personalization	References
Expression of single receptors	IHC measurement cutoff value	36-38
Tumor mutational burden	Mutations Score + cutoff value	43-47
Pathology – immune infiltration	Semi-qualitative score	48-50
Genetic/ transcriptomic signatures	Multivariate regression and ML	51-54
Cell signaling data	ODE model and ML	55
CT scans	AI (radiomics, feature selection, ML)	56

Table 1. Molecular and clinical information in recent development in precision oncology and the corresponding computational approaches used for their employment in therapy personalization.

Clinic. TTP / Pred. TTP	0 – 90 days	90 – 150 days	150 – 365 days	No PD during follow-up
0 – 90 days	**6** (11.1 %)	0 (0 %)	2 (3.7 %)	2 (3.7 %)
90 – 150 days	0 (0 %)	**2** (3.7%)	0 (0 %)	3 (5.6 %)
150 – 365 days	0 (0 %)	0 (0 %)	**1** (1.8 %)	0 (0 %)
No PD during follow-up	4 (7.4 %)	2 (3.7 %)	2 (3.7 %)	**30** (55.6 %)

Table 2. Comparison between the Time to Progression (TTP) derived from model predictions of the personalization algorithm, and the clinically measured TTP.[83] Each cell includes the number of cases and percentage from the total number of patients in the cohort (in brackets; N=54).[83] The bold numbers represent the number of cases for which the algorithm correctly predicted whether disease progression will occur during a one year follow-up, and correctly predicted the time interval in which it occurred. Note that the algorithm predicted no progression during the 1-year follow-up period for 30 out of the 35 patients who had not shown clinical progression during that period (bottom right cell). Cohen's κ=0.489.