Published OnlineFirst February 20, 2018; DOI: 10.1158/1078-0432.CCR-17-2953

Precision Medicine and Imaging

Clinical Cancer Research

Prediction of the Optimal Dosing Regimen Using a Mathematical Model of Tumor Uptake for Immunocytokine-Based Cancer Immunotherapy

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Abstract

Purpose: Optimal dosing is critical for immunocytokine-based cancer immunotherapy to maximize efficacy and minimize toxicity. Cergutuzumab amunaleukin (CEA-IL2v) is a novel CEA-targeted immunocytokine. We set out to develop a mathematical model to predict intratumoral CEA-IL2v concentrations following various systemic dosing intensities.

Experimental Design: Sequential measurements of CEA-IL2v plasma concentrations in 74 patients with solid tumors were applied in a series of differential equations to devise a model that also incorporates the peripheral concentrations of IL2 receptor–positive cell populations (i.e., $CD8^+$, $CD4^+$, NK, and B cells), which affect tumor bioavailability of CEA-IL2v. Imaging data from a subset of 14 patients were subsequently utilized to additionally predict antibody uptake in tumor tissues.

Results: We created a pharmacokinetic/pharmacodynamic mathematical model that incorporates the expansion of IL2R-positive target cells at multiple dose levels and different schedules of CEA-IL2v. Model-based prediction of drug levels correlated with the concentration of IL2R-positive cells in the peripheral blood of patients. The pharmacokinetic model was further refined and extended by adding a model of antibody uptake, which is based on drug dose and the biological properties of the tumor. *In silico* predictions of our model correlated with imaging data and demonstrated that a dose-dense schedule comprising escalating doses and shortened intervals of drug administration can improve intratumoral drug uptake and overcome consumption of CEA-IL2v by the expanding population of IL2R-positive cells.

Conclusions: The model presented here allows simulation of individualized treatment plans for optimal dosing and scheduling of immunocytokines for anticancer immunotherapy. *Clin Cancer Res; 24(14); 3325–33. ©2018 AACR.*

See related commentary by Ruiz-Cerdá et al., p. 3236

Introduction

It is generally appreciated that variables impacting clinical response to cancer immunotherapy are multifactorial. Fundamentally, biological activity is driven by drug exposure in the tumor. Although selecting the optimal biologic dose for an agent with a blocking mode of action (MOA) in phase I studies is relatively simple, and may be extrapolated from peripheral pharmacokinetics, for immunocytokines, with an agonistic MOA, such decisions may be more complex. Here, we present a comprehensive strategy to address the design of a dose-finding clinical study for a novel immunotherapeutic agent with an agonistic MOA and complex target-mediated drug disposition (TMDD).

Several mathematical models have been proposed to describe the pharmacology of immunocytokine-based immunotherapies in preclinical settings and gain insights into optimal dosing regimen in cancer patients. For instance, optimal treatment regimens of IL21 have been proposed by integrating mathematically diseased, pharmacokinetics and pharmacodynamic quantitative considerations (1–3). Mouse tumor volume data also prompted the development of integrated pharmacokinetic/pharmacodynamic models of various immunostimulatory cytokine-based therapies to predict effect of combination treatments (see refs. 4, 5 as examples). So far, most of these mathematical frameworks have been developed on the basis of preclinical data, potentially limiting their application to clinical settings.

IL2 is a potent stimulator of T-cell immunity. The anticancer effects of IL2 were recognized more than 30 years ago, making it one of the first immunotherapeutic agents in clinical oncology (6). Human recombinant IL2, aldesleukin (Proleukin), is indicated for the treatment of adults with metastatic renal cell carcinoma (metastatic RCC) or with metastatic melanoma (http://www.proleukin.com/assets/proleukin.pdf). Aldesleukin

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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doi: 10.1158/1078-0432.CCR-17-2953

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Translational Relevance

The success of immunocytokine-based cancer immunotherapy depends on achieving optimal concentrations of the drugs within the tumor microenvironment. Overly cautious treatment may yield insufficient clinical activity, whereas aggressive treatment can result in undesirable toxicity. The intratumoral immunocytokine concentration is a complex product of administered drug dose, treatment schedule, and anatomic/spatial factors. Cergutuzumab amunaleukin (CEA-IL2v) is a novel tumor-targeted immunocytokine. In this report, we propose a mathematical model that describes its peripheral pharmacokinetics and tumor uptake. The model shows that increasing dose or shortening the time interval leads to higher drug uptake in tumor tissue. Factors such as reduced drug concentration due to expansion of IL2R-positive cells in the periphery were also taken into account. This model allows for the first time the prediction of patient-specific drug uptake into tumors for optimizing the therapeutic efficacy of CEA-IL2v and could serve as a paradigm for the development of immunotherapeutic agents.

is rapidly distributed into the extravascular space and readily permeates tissues, but the short half-life (85 minutes) and rapid clearance necessitate frequent, short infusions (for 15 minutes, every 8 hours; ref. 7). Clinically effective doses of aldesleukin are high (>600.000 IU/kg), and side effects appear to be dose-related and manifest in broad organ toxicity (8). Hence, the use of aldesleukin is mostly restricted to patients with a very good performance status and unimpaired organ function, and treatment should be administered under expert management at specialized centers (9). To overcome these limitations, several efforts are undertaken for developing new IL2-based therapies. NKTR-214 is an engineered IL2 with releasable polyethylene glycol chains that slowly release to generate active IL2 conjugates (10). On the basis of *in vitro* and *in vivo* mice data, a mathematical model has been proposed to gain insights into the mechanism of NKTR-214 pharmacology (11).

Cergutuzumab amunaleukin is a novel immunocytokine that targets carcinoembryonic antigen (CEA) with high affinity (12). The targeting component of the molecule is a high-affinity, bivalent, CEA-specific antibody devoid of Fc-related immune effector functions. The biologically active moiety is a variant of IL2 that is designed to bind IL2Rβγ complex, which is expressed on $CD8^+$ cytotoxic T cells and natural killer (NK) cells but does not bind CD25, which is predominantly expressed on regulatory T cells (Treg) that suppress antitumor immunity (13). Like aldesleukin, upon administration, it effectively drives the activation, proliferation, and expansion of $IL2R^+$ cells (12). The expanded population of immune cells capable of binding IL2 potentially results in faster depletion of available drug due to biological TMDD. To compensate for the increased clearance with time of CEA-IL2v and to optimize tumor drug uptake, we developed a quantitative model to predict the impact of CEA-IL2v dosing regimen on antibody tumor uptake and to identify best schedules for improving tumor uptake.

The serum and intratumoral concentration of any drug is dependent on time and space and also the rate of systemic degradation of the drug. In this study, we utilized sequential pharmacokinetic and imaging data from patients treated with CEA-IL2v to formulate a model that takes into consideration the expanded population of IL2-binding cells, which in turn influence plasma and intratumoral drug concentrations. We used the model to predict antibody tumor uptake in patients after repeated administrations and to identify an optimal dosing regimen.

Materials and Methods

Immunocytokine

CEA-IL2v (molecular weight, ca. 165.5 kDa) is a monomeric targeted immunocytokine composed of a single, engineered IL2 variant fused to the C-terminus of a high-affinity, anti-CEA IgG antibody with a heterodimeric Fc portion. C1q binding is completely abolished by a novel Fc mutation. The IL2 variant lacks the ability to bind to IL2Rα (CD25) but fully retains IL2βγ binding. Therefore, $CD25^+$ Treg cells cannot bind IL2v with their high-affinity trimeric receptor (αβγ) and IL2v preferentially acts on NK, CD4, and CD8 T cells (βγ; ref. 12). CEA is used as an anchor to target the immunocytokine directly to CEA-expressing tumor cells. Early clinical studies with CEA-IL2v demonstrated improved pharmacokinetic and safety profile as compared with aldesleukin. Superior expansion of immune effector NK and CD8 T cells in blood and tumor tissue without expansion of suppressive T cells with CEA- IL2v as compared with aldesleukin led to better tolerability and CEA-specific tumor targeting (14, 15).

Patients

The study was approved by the Institutional Review Boards or independent ethics committees of the participating centers and followed the Declaration of Helsinki and International Conference on Harmonisation Good Clinical Practice guidelines. All patients provided informed consent to participate in the study.

Patient data and samples were collected with informed consent, and all subjects were treated according to the Declaration of Helsinki. A total of 74 patients with advanced and/or metastatic solid CEA^+ tumors, with comprehensive systemic pharmacokinetic and pharmacodynamic data and undetectable antidrug antibodies, were included in the current analysis. These patients were accrued as a part of a clinical study with the primary objective of evaluating the clinical safety of CEA-IL2v. Study details are accessible through the ClinicalTrials.gov (Identifier: NCT02004106). Pharmacokinetics was available in all patients, from which the model was constructed. Doses from 0.1 to 40 mg biweekly and from 6 to up to 30 mg weekly were administered. Peripheral immune cell counts at different time points were measured in 50 patients. Sampling schedule was day 1 (predose), day 2 (24 hours), and day 5 (96 hours) after first and fourth or after first and fifth dose following administration once every 2 weeks or once a week, respectively, and predose before second, third, and fourth (weekly only) administrations. Variations from this sampling design were captured, and our analysis used the information of actual sampling times for each individual patient. Twenty-four patients were subjected to an imaging substudy during the first cycle of treatment to evaluate drug localization in different tissues including their tumor. For the purpose of assessing the model, the pharmacokinetic database was divided into "learning" and "validation" datasets at a 2:1 ratio, that is, 2/3 of the data were used to develop the model and 1/3 to check its ability to reproduce and predict data that were not used for

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Table 1. Patients and their dosing characteristics used for the training and validation dataset

Patients used for CEA-IL2v PK model development		
	Training dataset	**Validation dataset**
Number of patients included	$n = 50$	$n = 24$
Patients with more than one cycle	$n = 44$	$n = 22$
With QW regimen	19 (43%)	8 (36%)
With Q2W regimen	25 (57%)	14 (64%)
Median number of cycles received	6	4.5
Median cumulated dose received	71 mg	60 mg

Abbreviations: PK, pharmacokinetic; QW, weekly; Q2W, once every 2 weeks.

modelling. Table 1 summarizes the numbers, regimen, and dosing characteristics of the patients included in the analysis.

Complete imaging datasets were available from 14 patients, all of whom were used to calibrate the tumor uptake model. For validation of the model, data from 3 additional patients not included in the safety study were used, among whom one has also had drug uptake data at cycle 4 (see below).

Peripheral pharmacokinetic and pharmacodynamic measurements

Pharmacokinetic data were assessed according to a rich sample design for all patients. A total of 803 longitudinal observations were available for analysis (mean 10.9 measurements/patient).

The dose level ranged from 0.1 to 40 mg. Average number of doses received was 6.4 (min 1 and max 44 doses), and average cumulative dose received was 95.6 mg (min 0.1 mg, max 370 mg). Peripheral pharmacodynamics were evaluated by quantifying immune cell counts (NK cells, B cells, CD4, CD8 T cells) in the blood using flow cytometry. Overall, 49 patients (66%) had pharmacodynamic assessments, and a total of 273 assessments were used in the current analysis. Soluble CD25 was measured in plasma samples using ELISA with an assay range of 78 to 5,000 pg/mL (R&D Systems).

Imaging data of antibody uptake in tissues

Fourteen patients within the cohort with CEA^+ or CEA^- tumors were imaged using PET following administration of ^{89}Zr-labeled CEA-IL2v. This radiolabelled compound can easily be used to confirm tumor targeting quantitatively in first-in-human study (16). CEA-IL2v was administered intravenously at a total dose of 6, 20, or 30 mg (including ~50 MBq of 89Zr-CEA-IL2v). All patients underwent up to three ^{89}Zr-PET assessments during cycle 1 (days 1, 4, and 8 after administration). In addition to longitudinal uptake values, pharmacokinetic data were also obtained for these patients. Supplementary Table S1 shows characteristics of the patients from the imaging studies.

Mixed-effect modeling techniques

In the context of longitudinal data analysis, mixed-effect modeling techniques allow for estimation of kinetic parameters from a model, taking advantage of all information collected from several individuals (17). A structural deterministic model is formulated with structural parameters whose values follow a statistical distribution (often assumed to be log-normal to avoid negative values). This variability is called the interindividual variability as it describes the variation of the parameter values to best describe the entire population data. A second level of variability is the intraindividual or residual variability as it represents the error of measurements. In the current studies, pharmacokinetic and imaging uptake data were analyzed by means of this technique. First, a pharmacokinetic model was developed following the concept of TMDD (see Supplementary Material, Model 1). This model was subsequently coupled to a tumor uptake model (Model 2) to formulate a complete model (Model 3) used to simulate the impact of different dosing strategies on CEA-IL2v tumor uptake. A schematic view of the overall model is presented in Fig. 1.

Modeling TMDD pharmacokinetics

TMDD models have been utilized recently to best describe the pharmacokinetics of therapeutic antibodies in blood, taking into account the potential binding of the antibody to a membrane-bound target. The original TMDD model assumed that the two main mechanisms modulating the time course of antibody concentration in blood are target-mediated clearance and linear elimination (18). When the drug amount is low, time course of drug concentration will be principally driven by the linear elimination component. As the drug amount increases, the TMDD process will increasingly impact the pharmacokinetic profile, until target saturation is achieved. At this point, TMDD plateau and further increasing the doses has minimal effect on the TMDD profile over the linear elimination component. Several models of this type have been published to describe this process (see refs. 18, 19 as examples). More detailed information on the developed model can be found in Supplementary Material.

Modeling antibody tumor uptake

The modeling of antibody uptake into a tumor has been described previously. Most notably, Schmidt and Wittrup proposed a theoretical framework with parameters derived from *in vivo* experiments (20). The framework was also validated for human antibodies with CEA binding. According to this model, there are four main processes leading to the uptake of an antibody in tissues: first, the extravasation from blood vasculature; second, diffusion of the antibody into the tumor interstitial tissue; third, the binding of the antibody to the tumor antigen; and fourth, the internalization or degradation of the antibody (see refs. 20, 21 for further details).

This model relies on several assumptions. First, it is assumed that antibody extravasation across the vasculature is slow due to the low permeability of the vasculature and therefore is the rate limiting process. In effect, this assumption has important implications as it simplifies the model from a spatial, 3-dimensional concept (accounting for the geometrical distribution of the vessels, antigen patterns, and tumor cells) to a paradigm without spatial dimensions assuming the extravasation and diffusion could be modeled by using Krogh cylinder (20).

Second, it is assumed that antibody binding occurs in seconds; thus, a local equilibrium between free and bound antibody is rapidly reached in the tissue. It is also assumed that internalization occurs on a slower time scale (minutes to hours). The final assumption is that the tumor is not saturated; therefore, the concentration of antigen in the tumor is greater than the concentration of antibody and that uptake is low enough to not affect peripheral pharmacokinetics (no TMDD in tissue). More detailed information including equation used to model our data according to these processes can be found in Supplementary Material.

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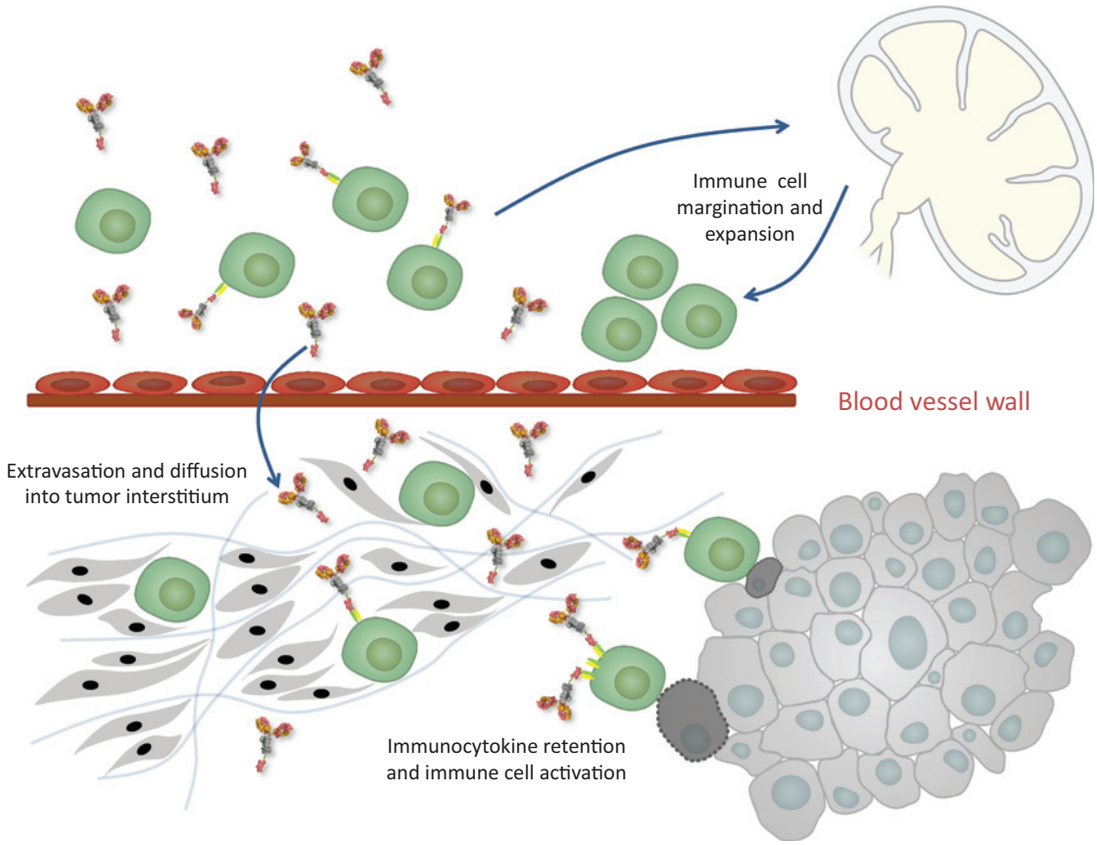

Figure 1.
Schematic view of the model developed to simultaneously integrate CEA-IL2v peripheral pharmacokinetic and tumor uptake data. The mathematical model is written as ordinary differential equations and describes the two main simultaneous occurring processes. Top, binding of the therapeutic antibody to immune cells in the periphery with subsequent cell margination, resulting in expansion of drug target; bottom, drug extravasation, diffusion, and binding to tumor CEA to mediate T-cell cytotoxicity.

Results

CEA-IL2v pharmacokinetic data are well described by a TMDD model with target expansion

Pharmacokinetic data of normalized drug exposure at multiple dose levels are presented in Fig. 2 as first cycle plasma concentrations (mg/mL) versus time in days. The results depicted are for patients administered CEA-IL2v at doses of $\leq$6 mg (top left), 20 mg (top middle), and $\geq$30 mg (top right). Twenty-seven patients dosed weekly and 15 patients dosed biweekly, from whom extended pharmacokinetic data were also available at cycle 4 and 5 in addition to cycle 1, were used to assess variation of drug exposure during the dosing cycles. Changes in drug exposure from cycle 1 onwards were calculated in all individuals and are presented in Fig. 2 (bottom left). Only 3 patients out of 27 did not demonstrate the typical pattern of reduction in exposure noted with the other patients (data not shown). The reduction in drug exposure could potentially be attributed to an expansion of $IL2R^+$ immune cells in the peripheral circulation mediated by CEA-IL2v. If this assumption was true, a more intense dosing regimen would result in higher T-cell activation and more rapid expansion in blood. Figure 2 substantiates this assumption as the reduction in drug exposure at each cycle was significantly more pronounced in patients on the weekly regimen than in patients receiving the drug once every 2 weeks (mean reduction −57% versus −37%, respectively, $P < 0.05$).

Antibody uptake in the tumor tissue of CEA-positive patients was also assessed by PET imaging after administration of ^{89}Zr-labeled CEA-IL2v. Figure 2 (bottom right) depicts the time kinetics of antibody uptake by tumor tissue of 8 CEA^+ patients. Of them, 4 patients received 6 mg and 4 patients received 30 mg of CEA-IL2v. Uptake is quantified as $\mu g/cm^3$ of tumor tissue. Results from CEA^- patients have not been depicted for clarity (please refer to Supplementary Table S1)

The previous observations of two target-mediated events on drug clearance at different doses and dosing schedules provided the foundation of a TMDD model in which the drug targets expanded with time. This initial "classical" model was subsequently extended to incorporate the aspect of the drug interaction with the target to form the drug–target complex. Corresponding equations of the final model selected are shown in Supplementary Material. This model was then coupled to a model of tumor uptake so that the two biological processes, namely drug target

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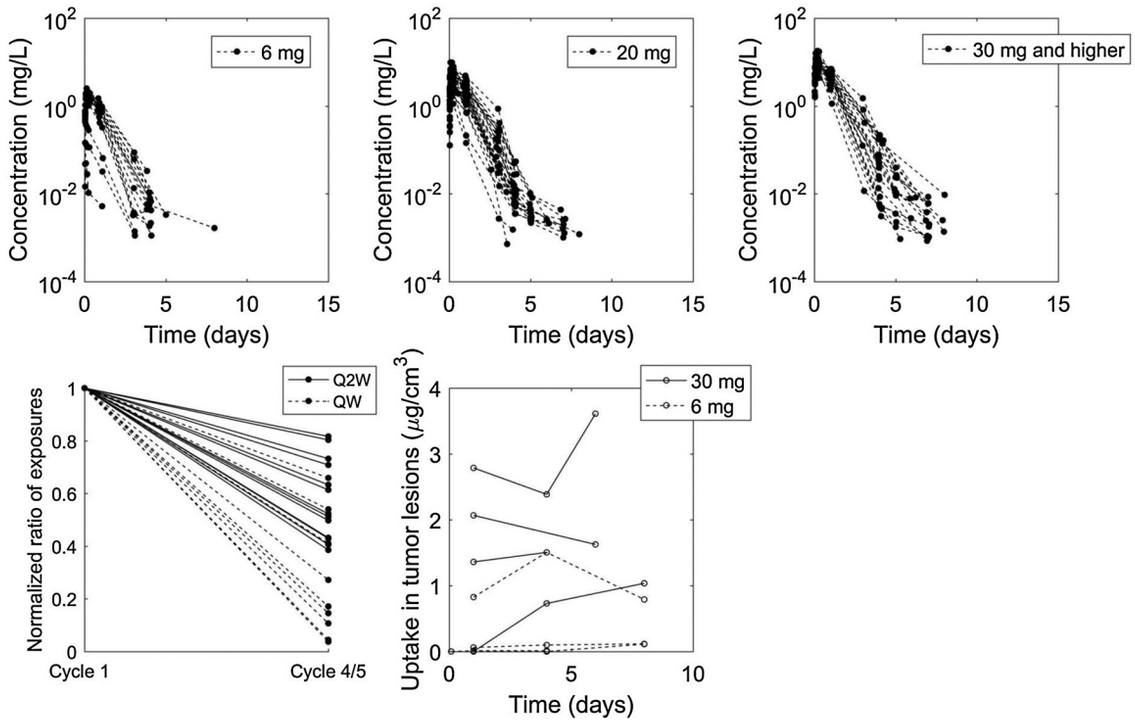

Figure 2.

Summary of CEA-IL2v pharmacokinetic and uptake imaging data. Top, cycle 1 pharmacokinetic profiles in patients receiving different doses of CEA-IL2v: 6 mg (left, $n = 18$); 20 mg (middle, $n = 33$); 30 mg and higher (right, $n = 23$); bottom, left, change in exposure across first 4 or 5 cycles. Weekly (QW) regimen (dashed line) and once every 2 weeks (Q2W) regimen (solid line). Right, uptake of CEA-IL2v in CEA^+ tumor lesions at cycle 1 in patients with dose 6 mg ($n = 4$, dashed lines) and with dose 30 mg ($n = 4$, solid line).

expansion and antibody tissue penetration, were modeled as simultaneously occurring with time.

Model-predicted CEA-IL2v targets correlate with $IL2R^{\pm}$ cell count and soluble sCD25 concentration in blood

Figure 3 presents some validation elements of the model. Visual predictive checks are shown in Fig. 3 (top and middle row, left) for the internal dataset (data used to build the model: top row, 50 patients) and the external dataset (data not used to build the model: middle row, 24 patients). Graphics show predicted 90, 10, and 50 percentiles of simulations together with data empirical percentiles (red lines). The model showed good adequacy with a tendency to underestimate exposure at a later time, although this tendency has to be balanced by the lack of pharmacokinetic data at these later time points. Normalized prediction distribution errors plotted against time (top, middle) and prediction (top, right) show a good fit at the population level. Parameter estimates are reported in Supplementary Table S2 and model equations in Supplementary Material.

Predicted target concentrations of individual patients correlated strongly with the clinically observed sum of $IL2R^+$ cells, that is, $CD4^+$, $CD8^+$, B, and NK cells ($r = 0.9$, $P < 0.0001$) even if these observations were not used for model development (see middle row, middle panel). A significant correlation was also found between predicted AUC of the drug and the observed exposure of soluble CD25 ($r = 0.8$, $P < 0.0001$; middle row, right). The actual values of soluble CD25 however were deviant ($r = 0.05$), which suggested that the kinetic profile of sCD25 does not correlate with the predicted profile of CEA-IL2v targets in the blood.

CEA-IL2v imaging data enable the modeling of antibody uptake by the tumor as a function of dose, tumor type, and CEA expression intensity

Imaging data were utilized to calibrate a model of antibody uptake by the tumor using parameters initially proposed by Schmidt and Wittrup (20) and estimated on the basis of murine experiments. A scaling factor ξ was applied to the extravasation factor. Figure 3 (bottom line) shows the observed versus predicted antibody uptake in tumors of 4 CEA^+ patients treated with 30 mg of CEA-IL2v. The model accurately describes the data from 2 colorectal cancer and 2 NSCLC CEA^+ patients. We subsequently utilized the model to extrapolate tumor uptake of CEA-IL2v in colorectal cancer patients dosed at 20 mg, together with two additional uptake points assessed in 2 new colorectal cancer patients treated with 20 mg of CEA-IL2v whose data were not used for model parameter calibration. Results are presented in Fig. 3 (bottom middle). Finally, superimposition of predicted and observed tumor uptake data demonstrated a reduction of the uptake as predicted by the model. As a comparison, the dashed

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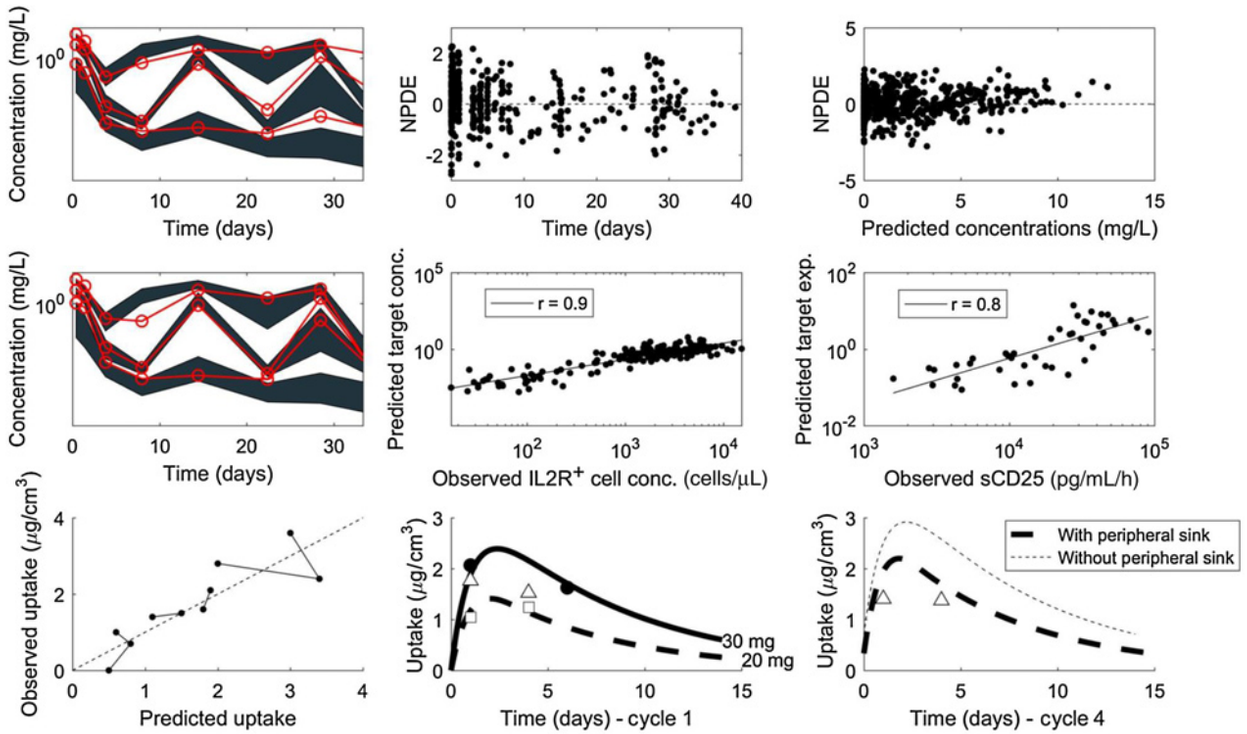

Figure 3.

Model performance and validation. Top, visual predictive check (VPC) of pharmacokinetic profiles in 50 patients used for model construction. Black areas show the 10th, 50th, and 90th percentiles of model-predicted values. Red lines show empirical percentiles of observed data (left); normalized prediction distribution errors (NPDE) versus time (middle); NPDE versus predicted concentrations (right). Middle, VPC of pharmacokinetic profiles in 24 patients whose data were not used to build the model (left); predicted CEA-IL2v target cell concentration versus observed concentration of $IL2R^+$ cells (B, $CD4^+$, $CD8^+$, and NK cells) in blood (middle); predicted CEA-IL2v target cell exposure versus observed sCD25 exposure (right). Bottom, observed versus predicted uptake in tumor lesions in four CEA^+ patients treated with 30 mg of CEA-IL2v (left); predicted uptake in colorectal cancer patients with 30 mg (solid line), including observations used to calibrate the model (dots) and extrapolation to 20 mg (dashed line) together with uptake data from 2 patients (squares and triangles) at 20 mg whose data were not used to build the model (middle); Predicted tumor uptake at cycle 4 with (dashed bold line) or without (dashed thin line) correction of prediction with expansion of target in the periphery together with uptake data from 1 patient who received 20 mg at cycle 1 and 30 mg at cycles 2 to 4, whose data were not used to build the model (right).

line shows the prediction if not accounting for target expansion in peripheral blood and shows that integration of the peripheral sink was needed to correctly predict uptake tumor uptake at successive cycles (bottom right). Model equations can be found in Supplementary Material and parameter estimates are reported in Supplementary Table S2.

Among the 14 patients for whom tumor imaging data had been analyzed, the actual versus predicted percentage as per the theoretical estimation in (value of the scaling factor ξ; ref. 19) was a mean of 18.6% (min 2.5%, max 86.6%). For the CEA^+ patients ($n = 8$), the mean percentage was 28.5% of the theoretical estimation (min 3.4%, max 86.6%). This reduction of uptake with respect to theoretical predictions could be attributed to several factors such as differences between the experimental LST174T mouse model utilized by Schmidt and Wittrup (20) versus our clinical data from human patients. Of note, CEA expression is very different between the two studies as shown in Supplementary Fig. S1. Furthermore, the compounds utilized by Schmidt and Wittrup (20) in their studies could potentially have had a different selectivity for uptake in malignant versus nonmalignant tissue.

The percentage of actual uptake versus theoretical estimation was a mean of 5.5% (min 2.5%, max 13%) for CEA^- patients ($n = 6$). Among the CEA^+ patients, uptake was higher in colorectal tumors with 22.2% of theoretical estimation (min 3.4%, max 38%) than in NSCLC patients (mean 15.3%, min 6.5%, max 24.3). Supplementary Figure S2 shows values of uptake parameters with respect to theoretical estimation from LST174 murine model.

Intensifying the CEA-IL2v regimen is predicted to significantly improve tumor uptake despite peripheral target expansion

Figure 4 shows simulation of the complete model incorporating both TMDD and tumor uptake for a weekly regimen. Simulations for dosing once every 2 weeks are presented in Supplementary Fig. S3. The simulation demonstrates that pharmacokinetic exposure (AUC of the predicted drug concentration) is predicted to continuously decrease over time. Antibody uptake into the tumor is shown for weekly (top row, right). We also show prediction of uptake when the regimen is intensified, either through increasing the dose at each cycle (bottom row, left, dose is increased by 5 mg each cycle starting from 20 mg), or reducing

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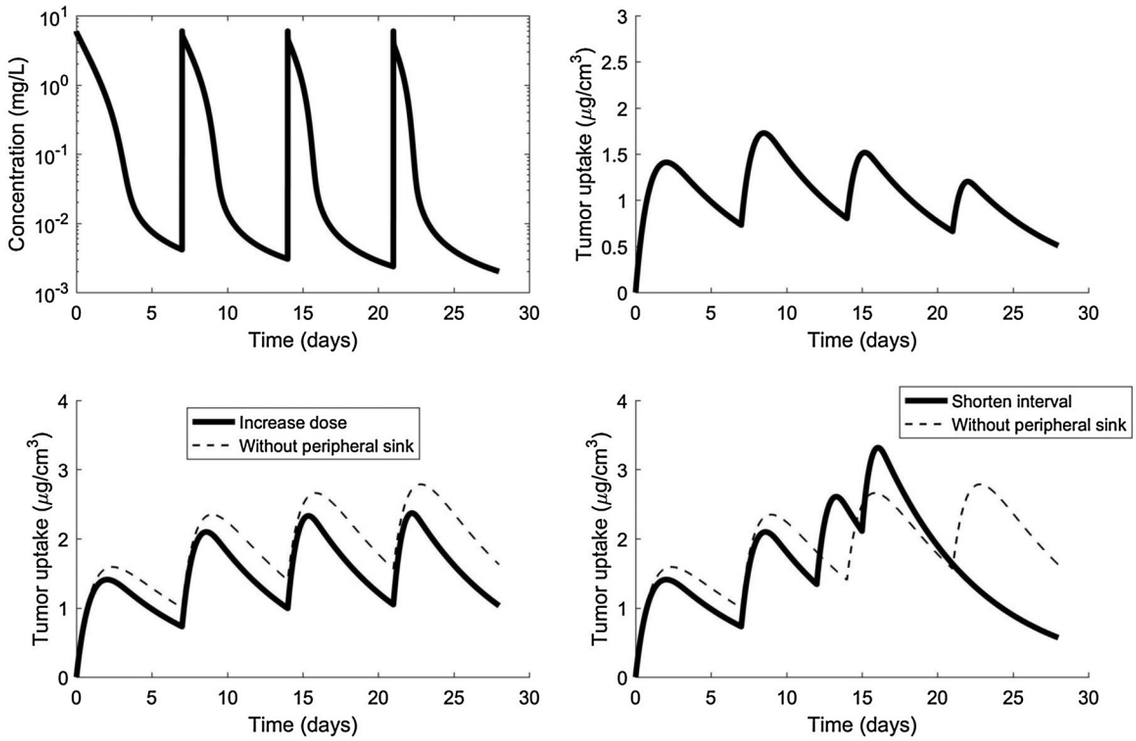

Figure 4.

Exploration of the effect of dosing regimen on CEA-IL2v tumor uptake according to model simulations. Top, predicted peripheral pharmacokinetic population profile for cycle 1 to 4 at 20 mg weekly (left); predicted corresponding tumor uptake (right); bottom, predicted tumor uptake in weekly dosing when the drug dose is increased by 5 mg each cycle (cycle 1, 20 mg; cycle 2, 25 mg; cycle 3, 30 mg; and cycle 4, 35 mg; left). The dashed line is the reference uptake for 20 mg weekly dosing without applying correction for target expansion. Predicted tumor uptake for 4 cycles at 20 mg when dosing interval is shortened (7 days between cycles 1 and 2, 5 days between cycles 2 and 3, and 3 days between cycles 3 and 4). The dashed line is the reference uptake for 20 mg weekly dosing without applying correction for target expansion.

the time interval in between dosing (bottom row, right, 7 days between the first and the second cycle, 5 days between the second and the third and 3 days between the third and the fourth). This illustrates that the predicted outcome of intensified dosing regimen is able to compensate for the peripheral sink, which attenuates the peripheral drug concentration. In particular, simulations indicate that the weekly regimen can improve absolute drug tumor uptake compared with once every 2 weeks despite the accompanying expansion of IL2R-expressing cells in the circulation.

CEA-IL2v early pharmacokinetic data can be leveraged to prospectively predict time course of immune cells in blood and drug uptake in tumor tissue in a given patient

To illustrate the potential application of this approach in clinical settings, we selected a single patient in the dataset that was not used to build the model. For this exercise, we selected the patient in the weekly regimen and for whom we have the most extensive collection of longitudinal pharmacokinetic information. The pharmacokinetic information collected during the first cycle (7 points in total) was used to estimate parameters using a Bayesian approach (see Supplementary Material). Follow-up prediction of individual pharmacokinetics at subsequent dosing cycles is shown in Fig. 5 (left) in dashed line and matches reasonably well with the actual observations. From the estimated individual pharmacokinetic parameters and estimated uptake parameters, we can simulate the prediction of antibody uptake across multiple cycles (middle and right). We could also predict the expected uptake with or without expansion of target cells and the peripheral sink effect. Based upon individual parameters, different dosing regimens can be simulated to select the optimum regimen, which maximizes antibody uptake in the tumor and be the closest match to the pharmacokinetics if there was no expansion of target in peripheral blood. For example, we expect that reducing the time interval to 5 days and dose escalation from 20 to 40 mg in increments of each 5 mg would lead to an uptake of 50 $\mu g/cm^3$/day in a given patient. Compared with this value, the predicted uptake with no expansion in periphery would be estimated to be 48 $\mu g/cm^3$/day (Fig. 5, right). Tumor uptake of CEA-IL-2v in the weekly regimen was however estimated to be 28 $\mu g/cm^3$/day (Fig. 5, middle). Therefore, in this particular example, optimizing the dosing regimen allowed for almost doubling of the antibody uptake in the tumor, which was similar to the predicted absolute uptake in the absence of a peripheral sink.

Discussion

The current study describes the development of a comprehensive model of drug uptake by tumor tissue that assimilates TMDD with aspects of target expansion and the gradual magnification of

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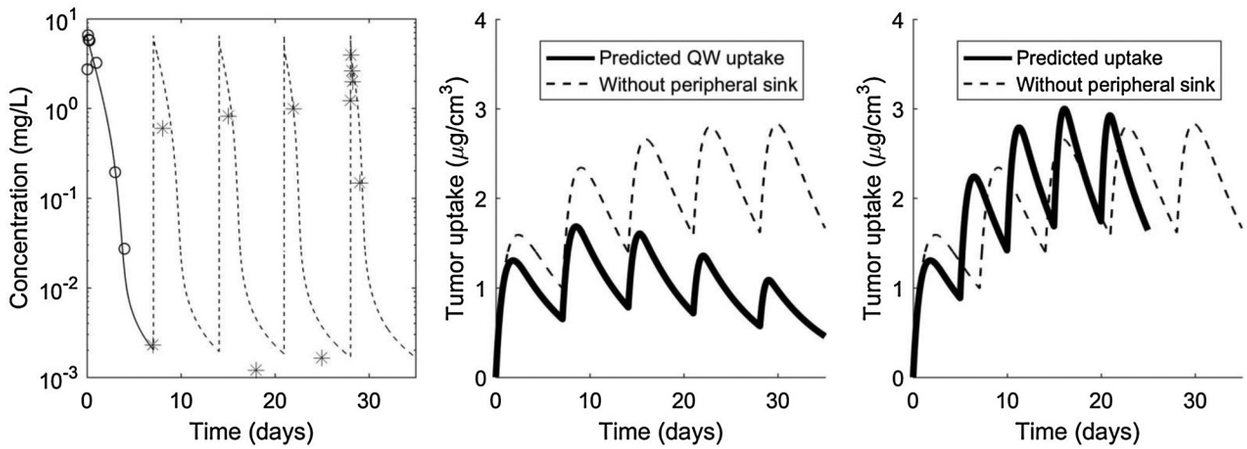

Figure 5.

Personalized treatment. Individual prediction of pharmacokinetic profile in a given patient when only data from cycle 1 is available (circle). Prediction at further cycles is shown (dashed line) together with observations (not used to calibrate the model) for the same individual (stars; left); predicted tumor uptake for the same individual. The dashed line represents the reference uptake for 20 mg weekly dosing without applying correction for target expansion (middle). Predicted uptake with drug given every 5 days, starting at 20 mg and increasing by 5-mg increments at each cycle. The resulting uptake is comparable with the theoretical uptake without expansion (dashed line; see text for details).

a peripheral drug sink. Our simulations indicate that increasing the dose or shortening time interval between doses leads to higher uptake in tumor tissue. We demonstrated that the concentration of CEA-IL2v receptors predicted by the model correlated with measured IL2R$^+$ cell concentrations (CD4$^+$, CD8$^+$, B, and NK cells) and soluble CD25, supporting the hypothesis that the immunocytokine leads to immune cell activation, which in turn acts as a peripheral sink.

This computational exercise relies on the assumption that drug exposure within the tumor will correlate with an increase in response rate. This assumption is currently being tested in clinical trials. The model was used in the design of a dose escalation study of a sister IL2 cytokine targeting the fibroblast activation protein (https://clinicaltrials.gov/ NCT03063762). The study design utilizes the intrapatient uptitration approach to compensate for TMDD-mediated reduction of drug exposure and uses a dose-dense schedule (weekly) to optimize tumor uptake. Although TMDD in the periphery had a significant impact on the pharmacokinetics of CEA-IL2v, the TMDD within tumoral and nontumoral tissue was not incorporated in our modeling. Similar assumptions about TMDD in tissues following antibody uptake in tissues have been made in previously published models, which have also disregarded TMDD in tissues (20).

In the current study, the model developed by Schmidt and Wittrup (20) was used to provide baseline values of kinetic parameters for antibody uptake in tumor tissues. To fit the CEA-IL2v imaging data, a scaling factor was introduced at the level of the extravasation processes, highlighted by Schmidt and Wittrup to be the rate-limiting process of the uptake. Other parameters, except those strictly related to the compound such as molecular weight or binding affinity to CEA, were fixed to the values reported in the original publications (20). Across the 14 patients from which antibody uptake imaging data were analyzed, we found that this scaling parameter was significantly higher for CEA$^+$ patients than for CEA$^-$ patients, as would have been expected given the mechanism of action of the targeting antibody. Moreover, for CEA$^+$ patients, colorectal tumors were characterized by a higher uptake factor than NSCLC tumors (see Supplementary Fig. S2). This is in agreement with the fact that colorectal tumors are known to express a higher concentration of CEA antigen than NSCLC tumor cells. The low scaling factor values are also in agreement with the fact that IL2v binding to immune cells alone happens to a larger extent than tumor target binding and immune cell binding at the same time.

The analysis was performed retrospectively with a small sample size and sparse longitudinal assessments of drug uptake in tumor tissue. For these reasons, additional efforts must be undertaken to further validate the proposed model. Also, it should be noted that high variability in tumor uptake data resulted in large variability of parameter estimates, and simulations integrating this variability would show a strong overlap between uptake projections whether taking into account, or not, the peripheral sink. Finally, long-term extrapolation of the modeling results should be taken with caution given the model's loss of performance at times beyond 3 weeks (Fig. 3, top left and middle). This, however, should not change the proposed rationale for exploration of increasing the dose or the shortening time interval between doses to increase uptake in tumor tissue and compensate for the potential expansion of IL2R$^+$ target cells in peripheral blood due to T-cell activation in periphery. Although this is an important goal to achieve and quantify, it obviously needs to be carefully evaluated in light of the toxicity profiles of the molecule when intensifying dosing regimen. Preliminary clinical data suggest that increasing dose or reducing dosing interval do not lead to increased toxicity of the molecule (data not shown), but this needs to be confirmed with a larger cohort of patients.

We believe that the major use of this model is in the early clinical development stage and design of dose-finding trials, to provide the drug with the best chance to succeed and reduce the failure rate due to lack of exposure.

It is important to note that the clinical efficacy of cancer immunotherapy is determined by a multitude of factors, among

Published OnlineFirst February 20, 2018; DOI: 10.1158/1078-0432.CCR-17-2953

them antigenicity of tumor, frequency of tumor-specific effector cells, suppression status within tumor environment, and immune cell fitness/grade of dysfunction. In the future, as we obtain more information about the various parameters affecting the antitumor immune response, we will incorporate these into the model.

Immuno-oncology agents, such as direct immune cell engagers and other cytokines that are capable of inducing their target (i.e., certain cell types or receptor densities) and therefore present with TMDD may benefit from our model to inform their scheduling and dosing to improve tumor uptake. We believe mathematical models such as this one can be a useful complement to preclinical *in vivo* models and are valuable for the generation of hypotheses generation that can be tested in early studies, both with respect to the dose and schedule, as well as the timing of biomarker sampling.

Disclosure of Potential Conflicts of Interest

T. Nayak and C. Klein hold ownership interest (including patents) in Roche. No potential conflicts of interest were disclosed by the other authors.

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Acknowledgments

The authors acknowledge Eva Rossmann, Ana Silva, Marta Canamero, Claire Petry, and Jonathan Wagg for helpful discussion and review of the manuscript. They also acknowledge Hans Peter Grimm and Hanna Silber Baumann for establishing the translational PK model forming the basis for the current work.

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Received October 5, 2017; revised December 5, 2017; accepted February 14, 2018; published first February 20, 2018.

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Published OnlineFirst February 20, 2018; DOI: 10.1158/1078-0432.CCR-17-2953

Clinical Cancer Research

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Clin Cancer Res 2018;24:3325-3333. Published OnlineFirst February 20, 2018.