Spatial Modeling of Drug Delivery Routes for Treatment of Disseminated Ovarian Cancer

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Abstract

In ovarian cancer, metastasis is typically confined to the peritoneum. Surgical removal of the primary tumor and macroscopic secondary tumors is a common practice, but more effective strategies are needed to target microscopic spheroids persisting in the peritoneal fluid after debulking surgery. To treat this residual disease, therapeutic agents can be administered by either intravenous or intraperitoneal infusion. Here, we describe the use of a cellular Potts model to compare tumor penetration of two classes of drugs (cisplatin and pertuzumab) when delivered by these two alternative routes. The model considers the primary route when the drug is administered either intravenously or intraperitoneally, as well as the subsequent exchange into the other delivery volume as a secondary route. By accounting for these dynamics, the model revealed that intraperitoneal infusion is the markedly superior route for delivery of both small-molecule and antibody therapies into microscopic, avascular tumors typical of patients with ascites. Small tumors attached to peritoneal organs, with vascularity ranging from 2% to 10%, also show enhanced drug delivery via the intraperitoneal route, even though tumor vessels can act as sinks during the dissemination of small molecules. Furthermore, we assessed the ability of the antibody to enter the tumor by in silico and in vivo methods and suggest that optimization of antibody delivery is an important criterion underlying the efficacy of these and other biologics. The use of both delivery routes may provide the best total coverage of tumors, depending on their size and vascularity.

Introduction

Ovarian cancer is the seventeenth most common and twelfth most deadly cancer in the United States (6). Because it is largely asymptomatic during the early stages of disease, 61% of patients present with cancer already disseminated throughout the abdominal cavity. As a consequence of late-stage diagnosis, the 5-year survival rate is only 44% (7). Intraperitoneal administration of cisplatin has been shown to correlate with improved overall survival with the greatest survival effect seen in patients surgically debulked to no gross residual disease (8–10). Combined intravenous/intraperitoneal chemotherapy has been recommended by the NCI (Bethesda, MD) as the standard-of-care for optimally debulked, FIGO stage III ovarian cancer patients (11). In this study, we explore the effectiveness of intraperitoneal versus intravenous therapy for residual disease as a function of tumor attachment and vascularity. We predict that improved outcomes may result from pathologic assessment of peritoneal tumor characteristics after cytoreduction, potentially leading to individualized decisions on the routes of drug administration. We tested this hypothesis using a hybrid agent–based computational model to simulate delivery of both small-molecule and antibody therapies.

Disseminated ovarian tumors often exist as two distinct types: (i) avascular cell aggregates ("spheroids") that are loosely attached to organs within the peritoneal cavity or free floating in the ascites fluid, and (ii) vascularized tumors colonizing peritoneal organs. For optimal treatment, drugs must be effective against both types of tumors. Furthermore, the route of drug delivery will likely have an effect on tumor drug penetration, binding, and accumulation. We focus here on strategies for the postcytoresection treatment of microscopic residual tumors, where debulking has removed both the primary tumor and larger secondary tumors (>1 cm in diameter).
Quick Guide to Model and Major Assumptions

We assume that during the time scale of drug penetration (2–9 hours), cancer cells neither grow nor migrate. This is a reasonable assumption as in vitro studies suggest that ovarian cancer cells grown as spheroids have a reduced proliferation rate (1). Each cell is considered a single agent, occupying one voxel on a 3-dimensional lattice in the CompuCell3D simulation environment. Chemical dynamics are described in the following reaction–diffusion equation:

\[ \frac{\partial C_i}{\partial t} = D_i \nabla^2 C_i - \gamma C_i + \alpha_i [1 - \delta(t(S), \text{vessel})] - \xi_i [1 - \delta(t(S), \text{tumor})], \]  

where \( C_i \) is the chemical concentration, \( D_i \) is the effective diffusion coefficient, \( \gamma \) is the decay rate, \( \alpha_i \) is the chemical output at the vessel, \( \delta \) is the Kronecker delta function that equals 0 when its variables are the same and equals 1 when they differ, \( S \) is the cell ID, \( \tau \) is the time for molecules to diffuse the distance of one cell diameter, which is equivalent to \( 1/1207.183 \) minutes for cisplatin and \( 1/25.011 \) minutes for pertuzumab. Each vascular tumor contains a simulated vascular meshwork generated in Matlab by randomly selecting vessel voxels. The primary tumor is a single agent, occupying one voxel on a 3-dimensional lattice in the CompuCell3D simulation environment. Chemical dynamics are described in the following reaction–diffusion equation:

\[ \frac{\partial C_{\text{VesselSurface}}}{\partial t} = C_{\text{Plasma}}(t) \times B, \]  

where \( C_{\text{VesselSurface}}(t) \) and \( C_{\text{Plasma}}(t) \) is the concentration in blood and at the vessel surface, respectively, and \( B \) is the Biot number. The Biot number is the ratio of capillary extravasation to the free diffusion coefficient in tumor tissue, an approach pioneered by Thurber and colleagues (2–4) to quantify passage of proteins across the vascular wall as the rate-limiting step of delivery.

Our simulation environment represents small tumors of approximately 30 cells in diameter with a total of 13,997 cells. Tumors of this size should be well oxygenated with no necrotic core (5). The spherical tumor surface is completely exposed to the peritoneal fluid at each time step, we use constant concentrations determined by fits to patient data and rat data (Supplementary Table S1). Vessel voxels are reset to a new constant concentration at each time step; therefore, only voxels comprising the tumor surface contribute drug to nonvessel neighbor voxels, as in real vessels. Peritoneal fluid voxels are treated similarly. After intravenous delivery, small-molecule drug has the same concentration at the vessel surface as in the primary compartment. In contrast, antibody concentration at the vessel surface is inhibited by the vascular wall, and concentration at the vessel surface is described by

\[ C_{\text{VesselSurface}}(t) = C_{\text{Plasma}}(t) \times B, \]  

where \( C_{\text{Plasma}}(t) \) and \( C_{\text{VesselSurface}}(t) \) is the concentration in blood and at the vessel surface, respectively, and \( B \) is the Biot number. The Biot number is the ratio of capillary extravasation to the free diffusion coefficient in tumor tissue, an approach pioneered by Thurber and colleagues (2–4) to quantify passage of proteins across the vascular wall as the rate-limiting step of delivery.

Drug Modeling Assumptions

We consider only the primary rate-limiting step for drug diffusion in tumor tissue as determined by the molecular weight, shape, and lipophilicity of a drug (4). In the model, for low molecular weight cisplatin, we assume no explicit barriers within blood or tissue. For large molecular weight, cell-binding antibody, we consider the penetration from the intraperitoneal fluid into tumor tissues as a passive process, and we parameterize it from our own fluorescence recovery after photobleaching (FRAP) measurements.

We consider drug distributions in two compartments, the blood (intravenous) and the peritoneal fluid (intraperitoneal). The primary delivery compartment is the first compartment into which drug is injected (either intravenously or intraperitoneally); the secondary compartment receives a wave of drug during distribution throughout the body. We fit drug concentrations as polynomial functions of time. All cisplatin compartment concentrations and pertuzumab primary compartment concentrations are fitted using patient data from the literature. Because we do not have simultaneous intraperitoneal and intravenous data for pertuzumab in patients, we use intraperitoneal/intravenous and intravenous/intraperitoneal ratios from antibodies delivered intravenously and intraperitoneally to rats (see Supplementary Table S1). We apply those ratios to patient data for primary intraperitoneal or intravenous delivery, assuming that ratios of drug in the secondary compartment to the primary compartment are dose-independent. Secondary compartment pertuzumab concentrations are calculated as the current concentration in the primary compartment times the ratio of the current concentration in the secondary compartment to that in the primary compartment.

Explicit equations of primary and secondary fits are provided in Supplementary Table S1. Primary and secondary concentrations are set as constant concentrations in the compartment fluids for the duration of the time step, creating constant boundary conditions for the vessels and tumor surface.

Drug movement between the peritoneal and the blood plasma compartments is a key feature of the pharmacokinetics affecting abdominal tumors. In healthy individuals, the volume of peritoneal fluid is small (10–30 mL). This fluid resides in the interstitial spaces and is secreted by mesothelial cells (reviewed in 12). The fluid circulates through the cavity and enters the blood circulation via adjacent capillaries (40%–50%) or stomata on the underside of the diaphragm. The stomata can admit particles.
up to 25 μm in diameter into the diaphragmatic lymphatics, which connect to the greater lymphatic system. Fluid ultimately drains back into the venous circulation. Because the blood and intraperitoneal compartments are intimately connected, our model accounts for the initial drug infusion into the primary peritoneal or blood compartment as well as the delayed appearance of drug in the secondary compartment.

Mathematical modeling of drug delivery was pioneered in the 1960s and has progressed to current models that incorporate tumor-induced angiogenesis (13, 14). Recent reviews have summarized current strategies used to model anticancer drug penetration at different temporal and spatial scales (15, 16). Frieboes and colleagues incorporated experimental data into their 3-dimensional computational model of lymphoma growth (17) and have also modeled nanoparticle delivery and accumulation in developing tumors (18). Of particular note, Sinek and colleagues created a 2-dimensional multiscale model of cisplatin and doxorubicin intravascular delivery (19). For ovarian cancer, previous mathematical models have addressed the penetration of doxorubicin through multiple cell layers (20) the effectiveness of new or established treatments (21) and predicted survival rates of patients after surgery or drug treatment (22). The metabolic and spatial characteristics of ovarian cancer cell spheroids have also been considered (23). Few spatially explicit models of ovarian cancer exist aside from our own (5). Giverso and colleagues built a 2-dimensional cellular Potts model of ovarian cancer that explores the interaction of ovarian cancer cells with the mesothelial layer and underlying extracellular matrix during invasion (24). El-Kareh and colleagues modeled the penetration distance of cisplatin into the rat peritoneum with and without hyperthermia (25). This model and others, as well as experimental measures for penetration into tissues in the peritoneal cavity, suggest absorption of small-molecule drugs by vessels can be a barrier to drug delivery (2).

Here, we report results of a 3-dimensional spatial model of drug delivery using OvTM, our ovarian tumor model (5) parameterized for ovarian tumor spheroids in the peritoneum with and without hyperthermia. This model and others, as well as experimental measures for penetration into tissues in the peritoneal cavity, suggest absorption of small-molecule drugs by vessels can be a barrier to drug delivery (2).

Materials and Methods

Drug penetration into spheroids

SKOV3.ip-GFP-stable transfectants were a kind gift from Laurie Hudson and Angela Wandinger-Ness (University of New Mexico,

### Table 1. Modeling parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Model value</th>
<th>Model units</th>
<th>Values in common units</th>
<th>Source</th>
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<tr>
<td>Time step for cisplatin</td>
<td>$\Delta T_C$</td>
<td>Min</td>
<td>6.40E–6 cm$^2$/s</td>
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<tr>
<td>Cisplatin effective diffusion coefficient in tumor tissue</td>
<td>1</td>
<td>Cell diameter$^2$/m</td>
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<td></td>
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<td>Platinum (Pt) accumulation at current cisplatin concentration in SKOV3 cell (R$^2 = 0.99$) per MCS</td>
<td>(0.4735 + cisplatin$^{2.29}$) $\Delta T_C$/120</td>
<td>Accumulated per cell per $\Delta T_C$</td>
<td>25% pmol/mg protein</td>
<td>40</td>
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<tr>
<td>Cisplatin accumulated at IC$50$ (50% viability of control) for SKOV3, 2-hour exposure</td>
<td>52.71</td>
<td>pmol/L (pmol per liter of cells)</td>
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<td></td>
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<tr>
<td>Time step for pertuzumab</td>
<td>$\Delta T_P$</td>
<td>Hour</td>
<td>1.35E–7 cm$^2$/s</td>
<td>m</td>
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<td>Nonspecific IgG FRAP-derived effective diffusion coefficient in SKOV3.ip1 spheroids</td>
<td>981.68</td>
<td>Molecules/cell volume</td>
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<td></td>
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<tr>
<td>Pertuzumab Kd (binding percentage)</td>
<td>5.57</td>
<td>Cell diameter$^2$/hour</td>
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<td>Pertuzumab binding reversal half-life</td>
<td>=</td>
<td>Hour$^{-1}$</td>
<td>m, 59</td>
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<td>Pertuzumab decay rate*</td>
<td>0</td>
<td>Hour$^{-1}$</td>
<td>60</td>
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<td>Biot number for pertuzumab in ovarian tumor*</td>
<td>0.0225</td>
<td>Ratio of plasma conc. to vessel surface conc.</td>
<td>30</td>
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<td>ERBB2 receptor count on SKOV3.ip1</td>
<td>2,100,000</td>
<td>Receptors</td>
<td>m</td>
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<tr>
<td>Radius of SKOV3.ip1 cell</td>
<td>3.5</td>
<td>μm</td>
<td>22</td>
<td></td>
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<tr>
<td>Vascular area in human secondary tumors from bowel and omentum</td>
<td>2–10</td>
<td>% Area of central slice</td>
<td>m</td>
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<tr>
<td>Mean length of tumor vessel segment in breast cancer xenograft</td>
<td>40 ± 5</td>
<td>μm</td>
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<tr>
<td>Mean diameter of tumor vessel segment in breast cancer xenograft</td>
<td>18.46 ± 0.54</td>
<td>μm</td>
<td>Fig. 5; 61</td>
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<tr>
<td>Mean diameter of tumor vessel segment in human ovarian tumors</td>
<td>19.2 ± 16</td>
<td>μm</td>
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<td>mg protein per SKOV3 cell</td>
<td>0.21</td>
<td>mg protein/μL</td>
<td>40</td>
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</tr>
</tbody>
</table>

Abbreviation: MCS, Monte Carlo step; m, measured.

*Value estimated based on data obtained with other IgGs.
Recently conjugated pertuzumab at a 1-hour with 350 μmol/L nonbinding Alexa488-labeled IgG to allow complete penetration by diffusion. Images were collected on a Zeiss Axioplan 200M META using an LD LCI Plan-NeoFluar 3× objective. A 14-μm circular region of interest was photobleached using an Argon laser at full power with maximum pinhole. Images were corrected for background fluorescence and for photobleaching during acquisition. Fluorescence recovery over time was plotted and fit to a single exponential to determine the recovery half-time. The diffusion coefficient was estimated as shown by Axelrod and colleagues (27). Average values were obtained from analyzing a total of 34 regions of interest from two separate experiments.

Ovarian cancer xenograft model

Details of the human xenograft model in nude mice were previously described (5). In brief, 6-to 8-week-old BALB/c athymic nu/nu female mice purchased from the NCI-Frederick were engrafted by IP injection with 100 μL of a single-cell suspension containing 5 × 10⁶ SKOV3.ip-GFP cells. Tumors develop in the peritoneal cavity within 1 to 3 weeks. Where specified, recipient mice were injected with 20 mg/kg pertuzumab-Pacific Blue either by intraperitoneal injection (i.p.) or by tail-vein injection (intravenous). Mice were humanely sacrificed after specified time intervals. Tumors were excised and imaged on a Zeiss 510 confocal microscope equipped with a Coherent Chameleon Ultra II IR laser for two-photon imaging. All experiments using mice were approved by the UNM Animal Care and Use Committee, in accordance with NIH (Bethesda, MD) guidelines for the Care and Use of Experimental Animals.

Quantification of vascular density and vessel diameter in patient tumors

Specimens from 9 ovarian cancer patients were obtained from the UNM Human Tissue Repository. Patient samples were identified for which metastases to both bowel and omentum were available. Adjacent sections were stained with hematoxylin/eosin (H&E) to identify higher-chromatin tumor cells or processed for IHC with anti-CD31 antibodies (BD Biosciences) to identify endothelial cells. Brightfield montages of each specimen were generated using Stereo Investigator image analysis software (MBF Bioscience) at 4× magnification on an Olympus IX-81 spinning disk confocal microscope. A board-certified pathologist distinguished tumor tissue from normal tissue by visual assessment of H&E-stained samples. CD31-labeled sections were imaged at ×20 magnification on the Olympus IX-81, and contours...
delineating the tumor area were drawn using Stereo Investigator software. Vessel area density within these tumor regions was calculated using the Stereo Investigator Area Fraction Option. Each tumor contour was systematically, randomly sampled with a rectangular region (counting frame) containing a lattice of grid points. Grid points were spaced 10 μm apart. A grid point was marked as vessel if the triangle defined by the upper right-hand quadrant of the grid point “cross-hair” contained pixels darkly stained for CD31. Structure of the overall tissue and relative darkness of stain were taken into account in marking CD31-labeled points. Counting frame size and spacing varied from tumor to tumor to mark enough points to obtain good vessel area density estimates (at least 200 points/contour). Stereo Investigator software estimated the fractional area of each tumor covered by CD31-labeled vessels using the number of marked grid points, the size of the sampled area, and the total area of the tumor contour. Vessel diameter was calculated using Stereo Investigator Line Measure or Circle Measure tools. Each tumor section was divided roughly into quadrants, and 3 to 5 vessels were measured per quadrant, with multiple vessels (10–20) chosen per tumor (236 vessels measured in 18 tumors).

Results

Schematic of the modeling approach

The integration of experimental and modeling results is outlined in Fig. 1. A critical aspect of our OtVM model is that, once drugs are administered by the intravenous or intraperitoneal route, there is transient delivery to the other compartment. Drug exchange between the two compartments is based on published studies as described in Supplementary Data. Fits for drug concentrations in the primary and secondary compartments over time, which are used as parameters in our model, are listed in Supplementary Table S1. Time courses of drug exchange used in the model are plotted in the pharmacokinetics box (Fig. 1). The model also explicitly integrates experimentally measured levels of tumor vascularity, with values discretized to fit voxel dimensions. Parameters for penetration, binding, and cellular accumulation are listed in Table 1. Further explanation of model assumptions can be found in the Supplementary Material.

In vitro spheroids serve as a model for drug penetration in avascular tumors

In Fig. 2, we first experimentally evaluate drug delivery to avascular, 3-dimensional cancer cell aggregates by passive penetration. SKOV3.ip1 ovarian cancer cells were grown as spheroids (~2,000 cells), incubated for defined intervals with fluorescent small molecules or antibodies and then imaged by confocal microscopy. Naturally, fluorescent doxorubicin (MW 543 Da) was used to represent the class of low molecular weight, highly lipophilic chemotherapeutic compounds; it is used here as a surrogate for the behavior of cisplatin (MW 300 Da), a first-line therapy for ovarian cancer. Results for doxorubicin are shown in Fig. 2A, where the fluorescence intensity plot below the 10-minute image shows the drug well distributed across the spheroid. Note that the fluorescence intensity continued to rise over the incubation period of 90 minutes, attributed to drug accumulation in cell nuclei as doxorubicin intercalated into DNA. The data are consistent with the fast penetration of small–molecular weight drugs in tumors (28). These results validate the use of a fast diffusion rate for cisplatin (640 μm²/second; ref. 29) in our simulations.

We next experimentally evaluated the uptake of therapeutic antibodies into cultured spheroids. The passive diffusion coefficient for these large proteins (~150 kDa) was estimated by FRAP. The spheroids were incubated with fluorescently tagged, nonbinding IgG for 1 hour. As shown in Fig. 2B, this is a sufficient period for the IgG to diffuse through the intercellular spaces to the spheroid interior. A 28-μm diameter spot was photobleached, and diffusion of fluorescent antibodies within the interstitial space in the absence of binding was estimated based upon fluorescence recovery. As shown in Fig. 2C, an estimated diffusion coefficient of 4.5 μm²/second for nonspecific IgG was similar to that of control (70 kDa dextran; 4.8 μm²/second). We interpret this as evidence that the intercellular junctions in these spheroids are insufficient barriers to strongly limit protein penetration. Interestingly, average diffusion within in vitro spheroids was three times slower than estimates of in vivo nonbinding antibody diffusion by Berk and colleagues, indicating that other factors play an important role in vivo (30).

Next, the penetration of targeted antibody was evaluated by incubating spheroids with subclinical levels (3.5 μg/mL) of Alexa 488-conjugated pertuzumab, which binds the ERBB2 receptor and is abundantly expressed on the surface of SKOV3.ip1 cells. Note that ERBB2 expression is a feature of only approximately 35% of ovarian tumors (31); it is reasonable to assume that our results should apply to antibodies directed at other surface receptors on ovarian tumor cells, such as ERBB3 and MET (31). Images in Fig. 2D and accompanying intensity plots show that there is a "wave front" of antibody binding as it penetrates into the tumor spheroid. Under these conditions, saturation of antibody binding is not achieved within the center of the spheroids even following an incubation period of 24 hours. The depth of fluorescent antibody penetration into spheroids was measured at each time point and is plotted in Fig. 2E. On the basis of these data, the effective penetration rate of antibodies with high affinity for tumor surface antigens is three orders of magnitude slower than predicted based upon nonspecific antibody diffusion. One goal of our model is to explore the relationship between binding rates, antibody concentration, and tumor penetration.

Incorporating vessel density from human ovarian tumors into the mathematical model

Vascularization of tumors will also affect drug penetration. The range of vascular densities was estimated based on the analysis of disseminated ovarian tumors collected from 9 ovarian cancer patients. Formalin-fixed paraffin-embedded sections from these tumors were labeled with anti-CD31 antibodies as a vascular endothelial cell marker. For each patient, paired samples of omentum and bowel metastases were evaluated to examine whether the site of metastasis affected vascular density. Vascular density was measured and reported as percent of total area analyzed (Fig. 3). Samples from each site showed no statistical difference with respect to distribution of vascular density, and there was no statistical correlation between percent vascular areas at the two sites in each patient (Supplementary Fig. S1). Mean percent vascular area was 4.8% with SD of 2.47% and a 95% confidence interval from 3.57% to 6.03%. In our models, we consider vascular areas of 2% and 10%, which represent the extremes of observations from the 18 tumors. Supplementary Figure S2
Figure 2. Experimental measures of diffusion across spheroids in vitro. A, SKOV3.ip1-GFP spheroids of ~2,000 cells were incubated with 3.5 mg/mL doxorubicin (red) for 10–90 minutes as shown and imaged on a confocal microscope. Scale bar, 100 μm. Graphs below images indicate fluorescent intensity measured across the spheroid along the specified line (white arrow). B, SKOV3.ip1-RFP spheroids were incubated with 350 mg/mL nonbinding FITC-conjugated IgG for 1 hour to allow homogeneous distribution of the IgG throughout interstitial spaces in the spheroid. (left; scale bar, 20 μm). Regions of interest (red circles) were bleached and fluorescence recovery was measured over time (middle and right; scale bar, 10 μm). C, diffusion was estimated from the rate of recovery of fluorescence (r) based on the radius of the circular region of interest. Diffusion of nonbinding IgG in the spheroids was comparable with that of 70 kDa FITC-Dextran. Black lines mark the mean diffusion rate ± SEM. D, SKOV3.ip1-RFP spheroids were incubated with 3.5 mg/mL Alexa 488-conjugated pertuzumab for 1–24 hours, washed, and imaged live on a confocal microscope. Graphs indicate fluorescence intensity across the spheroid along the specified line (white arrow). Scale bar, 50 μm. E, average depth of penetration over time of Alexa 488-conjugated pertuzumab into SKOV3.ip spheroids in vitro. Two to three spheroids were measured per time point. Error bars represent SD.
shows box plots with the means, quartiles, and farthest outliers for bowel and omentum samples.

As a first step for integrating these values into our cellular Potts model, we measured vessel diameters in tumor samples and showed that they predominantly fit a truncated Gaussian distribution ranging from 1 to 48 μm. Vessel diameters exceeding 48 μm were omitted, including longitudinal sections. Using Matlab, a randomized distribution of unconnected vessels with diameters representing the calculated distribution was generated within spheroids of 13,997 cells, such that the vascular area varied from 2% to 10% in the central slice of the simulated tumor. Figure 3H and I illustrate this important feature of our simulation strategy.

**Figure 3.**
Vascular density was measured in ovarian cancer patient metastases to parameterize simulations of vascularized tumors. A–F, four-micron sections of matched bowel and omentum tumors from 9 ovarian cancer patients (UNM Human Tissue Repository) were labeled with anti-CD31 antibody (DAB, brown) to detect vascular endothelial cells. Vascular density (% area) is reported at the bottom right of each image. Metastases showed within- and between-patient variability in vascular density, with some patients having metastases with low vascular density (patient 7, A and B), some patients having tumor-dependent variability in vascular density (patient 9, C and D), and other patients having tumors with uniformly high vascular density (patient 4, E and F). G, average vascular density is comparable in omentum (4.85%) and bowel (4.75%). H and I, Matlab-based simulation of vascular trees that yield 1% and 7% vascular area within the center slice of a spheroid (H and I; radius, 65 cells).

**Intraperitoneal delivery of cisplatin to avascular tumors is superior to intravenous delivery**

In Fig. 4, we show cisplatin uptake in the OvTM model. We compare cellular accumulation of drug given different routes of delivery and tumor vascularity. Results are expressed in molecules cisplatin/cell. Note that prior work estimated the IC$_{50}$ for...
Intraperitoneal versus Intravenous Drug Delivery for Ovarian Cancer

Intraperitoneal delivery offers advantages for therapeutic antibodies

Therapeutic antibodies have evolved as critical options for the targeted treatment of cancer (33), motivating our inclusion of these high–molecular weight biologics in our model. In vivo, passage of antibody across the vascular endothelium is a critical step in intravenous delivery of antibody to the tumor. In the model, this process is represented by the Biot value (see the Quick Guide). Effectively, for normal vasculature, this translates to an available pool of IgG that is only 2% of that inside the vessel. This applies equally to circulating antibody that is introduced by primary infusion or enters the blood by secondary exchange from the peritoneum. Simulations described below use Biot numbers reflecting both normal vasculature and 'leaky' tumor vasculature. Other important considerations include expression levels of target receptors in the tumor tissue and the affinity of the antibody. SKOV3.ip1 cells express more than 2 million ERBB2/HER2 receptors per cell; the affinity of pertuzumab for ERBB2 is 9.1 nmol/L (Table 1).

Results in Fig. 5 show predictions from the model for clinically relevant doses of pertuzumab (5 mg/kg), when administered by either intraperitoneal (top) or intravenous (bottom) routes, assuming normal vasculature. For the case of intraperitoneally delivered antibody, simulation parameters were fit to the data of Pai and colleagues (34), where infusion of 1 L saline was followed by a 50 mL injection of antibody and a second infusion of saline. For the case of intravenously delivered antibody, parameters were fit to data in the FDA bulletin 125409, with an infusion period of 3 hours (35). Simulation results indicate the early appearance of a wave front of antibody binding (red). Peritoneal delivery to both the avascular tumor in Fig. 5A and the 2% vascular tumor in Fig. 5B results in a rapidly advancing front and saturation of receptors within 0.4 hour. Note that the wave front is faster than intratreatment results in Fig. 1 with subclinical doses of antibody, as expected for the higher clinical dose. For the 10% vascular tumor, some heterogeneity is seen near the center of the tumor which is attributed to physical barriers of vessels that block antibody penetration within the tumor geometry. The median values for each of these simulations are plotted at right.

As shown in Fig. 5D–F, simulation results for intravenous infusion of antibody are particularly striking and highlight the limitations of antibody transport across normal endothelium, represented by the low Biot number (4). Under these stringent conditions, there is a marked delay in antibody binding, with the wave front initiating at the border of the spheroid. This indicates that most of the antibody entering the tumor comes from the intraperitoneal fluid, having arrived there after exchange from blood. Half-maximal saturation is seen many hours after intravenous introduction of drug. Even in the tumors with 2% and 10% vascularity, there is negligible contribution from antibody entering the tissue via vessels. Plots to the right demonstrate that vascularized tumors have much greater heterogeneity in antibody binding.

As vessels in tumors are often described as ‘leaky’ (36), we considered it important to report results of simulations using higher Biot values to reflect a greater range of drug transport out of vessels within tumors (37, 38). For intravenous delivery of antibody, increasing the Biot number 3.6–(Biot, 0.08) or 8.9-fold (Biot, 0.20) resulted in a proportional improvement in accumulation rate (Fig. 6A and B). With the highest Biot value, simulations predict that cells could be nearly saturated within 5.5 hours, compared with less than 6 hours with the transport conditions of normal vasculature. As seen in the plots in Fig. 6A and B, leaky vessels also reduce the heterogeneity in drug accumulation.

Images in Fig. 6C–F provide experimental validation of our predictions that direct delivery of antibody to the peritoneum is...
the superior route. Here, we took advantage of our human xenograft animal model. GFP-expressing SKOV3.ip1 cells were injected intraperitoneally into nude mice. After 2 weeks of tumor growth, fluorescently tagged pertuzumab was injected either intravenously or intraperitoneally, followed by humane sacrifice of recipient mice at time points indicated. Tumors were excised from the mesentery or omentum and imaged by two-photon microscopy. As shown in Fig. 6C, intraperitoneal injection resulted in a near uniform binding of antibody to ERBB2 on tumor cells within 3 hours. In contrast, intravenous injection resulted in variable levels of drug binding within the first 3 hours after administration (Fig. 6D). As shown in Fig. 6E, there was little improvement in drug binding even at 24 hours after intravenous delivery, particularly in tumors on the mesentery. At 72 hours after intravenous injection, binding of tagged antibody to cells in the tumor is markedly diminished, possibly due to internalization and degradation. Antibody at the tumor periphery at late time points after intravenous injection may be principally due to delayed secondary delivery from the peritoneal compartment. On the basis of the incomplete antibody penetration seen in vivo after intravenous delivery, we conclude that drug transport across tumor vessels in the SKOV3.ip1 xenograft model is close to that of normal vasculature but acknowledge that this may be highly variable among patients.

**Discussion**

Here, we use a spatially explicit model to represent the delivery of soluble drug and antibody-based therapies to microscopic ovarian tumors disseminated in the abdominal cavity (Fig. 1). In silico models have been useful for predicting drug response in ovarian cancer spheroids (23) and for examining the distribution and uptake of therapeutic antibodies during treatment (39). We incorporate pharmacokinetic parameters from in vitro studies along with vascular densities derived from human patients to help us understand how the interplay of drug dynamics at the molecular, cellular, and tissue scales impacts delivery to tumor cells. Our model is well integrated with experimental data, including measurements in cultured spheroids and xenograft models.

In our simulations, we chose to focus on treatment strategies for microscopic residual disease after debulking surgery. Clinical evidence suggests a survival advantage for intraperitoneal over intravenous chemotherapy in optimally debulked patients with no remaining gross residual disease (R0) showing the highest survival advantage (8, 9, 40, 41). However, there is still reluctance in many hospitals to administer intraperitoneal therapy, due to the expertise required to implant peritoneal ports and to monitor for the potential complications of cytokine storms with intraperitoneal antibody delivery and other toxicities. A recent study reports that less than half of eligible patients receive intraperitoneal chemotherapy (40). In addition, neoadjuvant chemotherapy followed by interval cytoreductive surgery is gaining more popularity based on data suggesting equivalent survival in patients with any visible residual disease (42). Our model was able to assess the effect of delivery route on drug penetration into tumors, showing that the impact of the intraperitoneal route on efficacy may be underappreciated. Results emphasize the importance of direct drug delivery to the peritoneum for superior penetration into microscopic avascular tumors, which likely comprise the majority of microscopic residual tumors in optimally debulked patients. We show that the same dose of cisplatin delivered intraperitoneally leads to uniformly higher accumulation within tumor cells. Although we do not include cell death in this model, the platinum accumulation levels modeled throughout a small tumor exceed accumulation levels determined in vitro under incubation conditions at the IC50 concentration of cisplatin established for cytotoxicity (32). Thus, the model predicts highest efficiency killing after intraperitoneal cisplatin treatment. Intravenous delivery leads to much less accumulation of cisplatin, with levels far below accumulation associated with effective killing. Because drug must reach avascular tumors from the peritoneal fluid, intravenous delivery increases the time it takes for the drug to enter the peritoneal cavity. Intravenous delivery also limits the total concentration of cisplatin achieved in the peritoneal cavity. Therefore, this secondary delivery markedly reduces accumulation in tumor cells.

Ovarian cancer cells also grow as vascularized tumors attached to peritoneal organs, and our previous studies in the SKOV3.ip1 xenograft model indicate that even small tumors (30–40 cells in diameter) may be well vascularized. Therefore, we went on to test how vascular density will alter drug penetration using our model. As the tumor vascular densities were variable among patients and between omentum and bowel tumors from the same patient (Supplementary Figs. S1 and S2), we tested drug penetration in tumors at the extremes of the vascular density range (2% and 10% vascular density). Surprisingly, the advantage of intraperitoneal delivery persists for small vascularized tumors. Even in tumors with 10% vascular density, intraperitoneal delivery improved the accumulation of cisplatin in small tumors by 100-fold (Fig. 4) providing one explanation for improved survival after intraperitoneal treatment. This underscores the need for intraperitoneal chemotherapy in cases where patients have been optimally debulked to R0. An interesting finding from the simulations is that, as vascular density of the tumor increases, the drug accumulation after intraperitoneal delivery becomes much less uniform. After intraperitoneal delivery of cisplatin, drug concentration in the vessels is always lower than in the peritoneal fluid. Therefore, the vessels act as a sink, absorbing small molecular weight drugs and reducing cisplatin accumulation in a small number of cells in proximity to the vessels. These cells

Figure 4.
Simulation of cisplatin accumulation in avascular and vascular spheroids of ~14,000 cells. Central cross-sections of 3-D simulations after delivery of 60 mg/m² cisplatin to 0.01 mm tumors (30 cells in diameter) show marked differences in accumulation based upon vascularity (0, 2% or 10%) and route of delivery (intravenous (IV) or intraperitoneal (IP)). Images on the left represent drug accumulation in central cross-sections of 3-D simulations after intraperitoneal or intravenous delivery. The color bar on the right indicates the scale in molecules cisplatin/cell. A, C, and E, intraperitoneal delivery of cisplatin (diluted in ~1,060 mL of saline); B, D, and F, intravenous delivery of cisplatin (diluted in the blood volume, ~5L per patient). Plots report minimum/maximum (green area) and median (blue line) accumulation of drug over time, expressed in molecules per cell. Plots on the right show histograms of the distribution of accumulated cisplatin, showing heterogeneous accumulation across cells in the vascularized tumors at 180 minutes.
Figure 5.
Simulations of pertuzumab delivery to spheroids show that antibody binding exhibits "wave front" behavior when administered by either intravenous (IV) or intraperitoneal (IP) route. Binding of antibody within 0.01 mm tumors (avascular, 2% or 10% vascular density) is compared with intraperitoneal and intravenous delivery of 5 mg/kg pertuzumab. A–C, simulation results reporting pertuzumab accumulation in spheroids after intraperitoneal delivery. Because of fast antibody penetration after intraperitoneal delivery, pertuzumab accumulation is shown for multiples of 4.2 minutes in a 0.8-hour simulation. D–F, simulation results reporting pertuzumab accumulation after intravenous delivery (pertuzumab diluted in the blood volume, ~5 L per patient). For simulations of intravenous delivery where antibody penetration is slower, pertuzumab accumulation is shown over 9 hours. The simulations predict at least a 2-fold improvement with intraperitoneal delivery over intravenous delivery. Plots on the right report the minimum/maximum (green area) and median (blue line) accumulation over time, expressed as molecules of antibody (each binding two receptors) per cell.
Figure 6. A and B, simulated intravenous (IV) delivery of pertuzumab in tumors with leaky vessels. The Biot number of the vessels was increased to 0.08 (A; 3.6 times that of normal vasculature) and 0.2 (B; 8.9 times higher). Images show pertuzumab accumulation at 2.5 and 3.5 hours. Plots on the right report minimum/maximum (green area) and median (blue line) accumulation of pertuzumab over time expressed as receptors bound bivalently to antibody per cell. C–F, in vivo confirmation that intraperitoneal (IP) delivery of Pacific Blue 410–conjugated pertuzumab (blue) shows more complete tumor penetration compared with intravenous delivery. Nude mice were engrafted with human SKOV3.ip1-GFP cells. Two weeks after engraftment, mice were treated intraperitoneally or intravenously with 0.4 mg Pacific Blue–conjugated pertuzumab (20 mg/kg, injected in 50 μL saline). Images were acquired 3, 24, or 72 hours after intraperitoneal or intravenous delivery. Scale bar, 50 μm. C and D, comparison of fluorescent antibody binding in tumors on the mesentery (C) or omentum (D) 3 hours after intraperitoneal or intravenous injection. After delivery by either route, antibody binding is equivalent on the tumor surface (top). Antibody penetration to the center of the tumor is only seen after intraperitoneal delivery (bottom). E and F, limited binding of fluorescent antibody in tumors 24 (E) or 72 (F) hours after intravenous injection of pertuzumab-Pacific Blue. Red arrows designate areas within the tumor mass with no detectable antibody binding.
could be those that survive therapy and lead to relapse. Taken together, our model data strongly suggest that intraperitoneal therapy has biologic advantages in eradicating microscopic residual disease in the peritoneal cavity after complete cytoreductive surgery and underscores the need for prompt treatment postoperatively before residual cells and spheroids have an opportunity to establish macroscopic implants.

Although patients initially respond well to platinum/taxol regimens, many will relapse. Novel treatment strategies, including therapeutic antibodies, are being considered to limit recurrence (43). Previous trials with anti-EGFR and anti-HER2 antibodies had limited success (44). These disappointing results may be due to two factors: (i) ineffective delivery and (ii) poor stratification of patients based upon receptor expression patterns. On the basis of IHC results for a tumor microarray representing 200 ovarian tumor patients, we recently determined that only a limited subset of ovarian tumors express significant levels of either ERBB1/EGFR or ERBB2/HER2 (31). The SKOV3.ip cell line used in our studies here is an example of this ERBB2+ subset, as they express more than 2 million ERBB2 per cell. With this as a model system, we examined the penetration of the anti-ERBB2 therapeutic antibody, pertuzumab, to determine whether the advantage of intraperitoneal delivery extends to this class of therapies. Antibodies, being larger molecules than chemotherapeutic agents, have more limited exchange between compartments. Simulations predicted a significant difference in intraperitoneal versus intravenous delivery, again attributed to lower penetration from the blood into tumors. Our results suggest that intraperitoneal delivery, possibly in combination with delivery through the intravenous route, will yield optimal results for this class of therapeutics.

Computer simulations provide powerful tools to investigate questions that cannot be answered with experiments alone. For example, our model predicts the distribution of cisplatin and pertuzumab accumulation with a spatial and temporal resolution that would be impractical to produce experimentally. The model additionally allows us to consider hypothetical tumor vascular densities and leakiness, which are difficult parameters to manipulate experimentally. Where possible, we validate model predictions experimentally. In this model, we made the assumption that the vasculature is spatially homogeneous throughout the tumor, with the cross-sectional density reflecting the 3-dimensional network density. We recognize that the model could be further refined to include a connected network of vessels with varying permeability and greater vessel heterogeneity.

Because this study focused on early treatment time points, we did not include cell proliferation or cell death in our model. This is an acknowledged limitation of our model as, at later time points, apoptosis of cancer cells will likely increase the permeability of tumor tissue and influence drug penetration (45). Future additions to the OvTM could include explicit determination of pharmacodynamic outcomes such as tumor cell proliferation, migration, and viability in response to drug accumulation. For example, recent work by Powathil and colleagues examined the effect of hypoxia and cell-cycle dynamics on the efficacy of chemotherapy (46). Tumor heterogeneity, as well as mutations and phenotypic changes that occur over the course of treatment, could be included in the model as data become available (47).

Our model is a powerful predictive tool that can be used to test optimal dosing of both small-molecule drugs and therapeutic antibodies. The benefits of peritoneal delivery likely applies to antibodies now in the pharmaceutical pipeline or in clinical trials that target other surface receptors more commonly expressed on ovarian tumor cells. Examples include intact antibodies and small-molecule inhibitors targeting ERBB3 and MET, as well as antibody–toxin conjugates, where low doses may be required to limit toxicity (48–50). In addition to new receptor targets, antibody affinity, receptor expression levels, and vascular leakiness will be factors in the success of the antibodies in the pipeline for this disease. The OvTM model may be useful to predict outcomes for patients with advanced stage ovarian cancer, offering a new tool for optimizing personalized therapy decisions regarding drug choice and mode of delivery. Continued integration of simulations and experiment would maximize impact on future clinical practice.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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