Spatial Modeling of Drug Delivery Routes for Treatment of Disseminated Ovarian Cancer
Kimberly R. Kanigel Winner1,2, Mara P. Steinkamp3,4, Rebecca J. Lee4, Maciej Swat5, Carolyn Y. Muller6,7, Melanie E. Moses1,2, Yi Jiang7, and Bridget S. Wilson3,4

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 postsurgery 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:

$$\frac{dC_i}{dt} = D_{C_i} \nabla^2 C_i - \gamma C_i + \alpha_i [1 - \delta(\tau(S), \text{vessel})] - \zeta_i [1 - \delta(\tau(S), \text{tumor})],$$

where $C_i$ is the chemical concentration, $D_{C_i}$ is the effective diffusion coefficient, $\gamma_i$ 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 cell type, and $\zeta_i$ is the chemical uptake by the tumor cells. We use a forward Euler method to solve this diffusion equation.

For drug concentrations in blood plasma and 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 peritoneal 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 plasma. In contrast, antibody concentration at the vessel surface is inhibited by the vascular wall, and concentration at the vessel surface is described by

$$C_{\text{VesselSurface}}(t) = C_{\text{Plasma}}(t) \ast B,$$

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.

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 fluid or attached to the mesentery. Drug is delivered simultaneously from tumor vessels and the peritoneal cavity. Simulation volume is 33$^3$ voxels. Voxels have a cube edge of 5.6 $\mu$m. The volume of each voxel is equivalent to the volume of an SKOV3.ip1 cancer cell, or 179.4 $\mu$m$^3$ (5). For each drug, we define each Monte Carlo step as 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 placing unconnected cylinders of specified radii and lengths drawn from distributions corresponding to experimental observations.

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 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 tissue.

Explicit equations of primary and secondary compartments 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 through the peritoneal 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. OvTM was based on the cellular Potts model using the CompuCell3D simulation environment (26). We extend the OvTM to study drug dynamics for two delivery compartments, the blood and peritoneal fluid, respectively. Results show that, for avascular spheroids and small-vascularized tumors in the process of seeding peritoneal organs, the intraperitoneal route is superior for drug delivery.

**Materials and Methods**

**Drug penetration into spheroids**

SKOV3.ip-GFP-stable transfecteds were a kind gift from Laurie Hudson and Angela Wandinger-Ness (University of New Mexico, New Mexico Cancer Research Institute, Albuquerque, NM).
Recently conjugated pertuzumab at a 0.5 μmol/L concentration was used to image vascular densities and in vivo pharmacokinetic data from clinical and experimental studies. The pharmacokinetic data shown are fits of cisplatin concentrations in the serum and peritoneal compartments (top) during and after intraperitoneal (IP) dosing from the peritoneal fluid (left) or intravenous (IV) infusion (right) and fits of pertuzumab concentrations (bottom) after intraperitoneal (left) or intravenous infusion (right). Available pertuzumab is much lower than the pertuzumab concentration in the blood due to the vascular endothelial barrier. (In the model, concentration is scaled with the Biot number; see Materials and Methods). Intraperitoneal and intravenous doses are 5 mg/kg. The intraperitoneal antibody concentration time course was calculated as 1,000 times the fit for an immunotoxin conjugate administered at 5 μg/kg i.p.

Albuquerque, NM) and were obtained in 2010. The parental SKOV3.ip cell line was authenticated in November 2014 by ATCC using short tandem repeat (STR) analysis by the Wandinger-Ness lab. For spheroid assays, 2,000 SKOV3.ip-GFP cells were seeded into each well of a Lipidure-coated 96-well plate (NOF America Corporation) and incubated for 48 hours to allow the formation of spheroids. Pertuzumab (Perjeta, Genentech) was purchased from the UNM Pharmacy and conjugated to Alexa Fluor 488 succinimidyl ester dyes per the manufacturer’s guidelines (Life Technologies). Spheroids were incubated in 2,000 SKOV3.ip-GFP cells were seeded using short tandem repeat (STR) analysis. The parental SKOV3.ip cell line was authenticated in November 2014 by ATCC (In the model, concentration is scaled with the Biot number; see Materials and Methods). Intraperitoneal and intravenous doses are 5 mg/kg. The intraperitoneal antibody concentration time course was calculated as 1,000 times the fit for an immunotoxin conjugate administered at 5 μg/kg i.p.

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

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.
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 Fractionator Probe. 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 so that enough points obtained 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 (256 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 OvTM 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 cell–cell 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 vitro (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 μg/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 μg/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 (t) 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 μg/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...
cellular cytotoxicity to be approximately $5.6 \times 10^6$ cisplatin molecules per cell (32). To reach this level, cultured cells were exposed to 38 µmol/L cisplatin for 2 hours. Importantly, this provides a target level of drug accumulation to be achieved in our cell-based tumor model.

Simulation conditions began with infusion of cisplatin by either route for 15 minutes (total 60 mg/m²), followed by tracking of drug accumulation until cisplatin levels in both compartments dropped to negligible levels (~180 minutes). Figure 4A and B reports results for avascular spheroids with a 30 cell diameter (~14,000 cells). Results show that, for the same drug dose, the intraperitoneal route is clearly superior. Direct infusion of cisplatin in the peritoneum at this dose results in substantial and uniform drug levels across the avascular tumor (Fig. 4A; plot at right), reaching a predicted level of almost $5.7 \times 10^6$ molecules per cell. There is again little cell–cell variability in drug uptake in this avascular setting. Importantly, cisplatin delivery to avascular tumors via the intravenous route results in accumulation far below the IC50 value.

Cisplatin accumulation in tumor cells after intraperitoneal or intravenous delivery is influenced by vascular density

We next report results for cisplatin accumulation in the same tumor volume, but with vascular densities of either 2% or 10%. Despite direct delivery of drug from the blood through vessels, the intraperitoneal route is again superior for these small tumors (Fig. 4C–F). The cell-to-cell variability of cisplatin accumulation in vascularized ovarian tumors is one of the most striking results of these simulations. The uptake of drug is highly heterogeneous, as indicated by the large spread around the mean values plotted in each case. The marked spatial gradients in drug accumulation particularly following intraperitoneal delivery to vascularized tumors (Fig. 4C and E) is notable. As accompanying plots show, accumulation across the tumor is much less uniform than for the same size of avascular tumor (Fig. 4A). This result indicates that vessels are a sink for drug when cisplatin is infused directly into the peritoneum. The inverse relationship between the vascular density of the tumor and drug accumulation after intraperitoneal delivery may be an unperturbable factor in chemotherapy regimens.

As shown in Fig. 4D, intravenous drug delivery to the 2% vascular tumor accumulates a median value (solid blue line) of only approximately 40,000 molecules per cell. Accumulation can reach a maximum of 140,000 molecules per cell in the cells nearest the vessels (green shaded area). For the 10% vascular tumor, these values reach a median of approximately 90,000 molecules per cell, with a range of 50,000 to 180,000. This represents only a small improvement in delivery to the tumors over the avascular tumor (compare plots in Fig. 4B and F).

**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 intratatal 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 vitro 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 underestimated. 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 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 on 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.

Authors’ Contributions
Conception and design: K.R. Kanigel Winner, M.P. Steinkamp, C.Y. Muller, Y. Jiang, B.S. Wilson
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K.R. Kanigel Winner, M.P. Steinkamp, R.J. Lee
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K.R. Kanigel Winner, M.P. Steinkamp, R.J. Lee, M. Swat, C.Y. Muller, M.E. Moses, Y. Jiang
Writing, review, and/or revision of the manuscript: K.R. Kanigel Winner, M.P. Steinkamp, R.J. Lee, C.Y. Muller, M.E. Moses, Y. Jiang, B.S. Wilson
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): K.R. Kanigel Winner
Study supervision: M.E. Moses, Y. Jiang, B.S. Wilson

Acknowledgments
The authors thank Dr. Michael Wester for algorithm development and run-time estimates; Scalenet Laboratory members Neal Holtschulte and Joshua Hecker for help with Python and Git; Dr. Tim Thomas for offering a computing “cluster in a box” to run simulations; the CCSD development group including Julio Belmonte; Dr. Susan Atlas and the Center for Advanced Research Computing (CARC); pathologist Dr. Lesley Lomo for scoring human ovarian tumor sections; Dr. Diane Lidke and Samantha Schwartz for help with FRAP analysis; Drs. Ashput Rajut, Meghan Pryor, and Francois Asperti-Boursin for valuable discussion; Dr. Sarah Adams for conceptual contributions to OvTM model development; The Human Tissue Repository, Flow Cytometry and Microscopy Core Facilities at the UNM Cancer Center, and Anna Holmes for technical assistance with IHC.

Grant Support
This study was supported by P50GM085273 (B.S. Wilson), NIH-CA119232 (B.S. Wilson), NSF grant EF-1038682 (M.E. Moses), a James S. McDonnell Foundation Complex Systems Scholar Award (M.E. Moses and K.R. Kanigel Winner) and NIH grant 2T15-LM009451 (K.R. Kanigel Winner). Y. Jiang is partially supported by NIH/NCI: 1U01CA143069.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received June 12, 2015; revised October 23, 2015; accepted December 18, 2015; published OnlineFirst December 30, 2015.


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

Kimberly R. Kanigel Winner, Mara P. Steinkamp, Rebecca J. Lee, et al.


Updated version

Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-15-1620

Supplementary Material

Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2015/12/30/0008-5472.CAN-15-1620.DC1

Cited articles

This article cites 44 articles, 11 of which you can access for free at:
http://cancerres.aacrjournals.org/content/76/6/1320.full#ref-list-1

E-mail alerts

Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.