Tumor Penetration Theory
Tumor modeling literature

• Weinstein & coworkers
  – Spheroids w/ no intratumor convection
  – ‘‘Binding site barrier’’
    • High affinity antibodies stuck at periphery
      – Math model (Cancer Res 51:4776 ‘91)
      – Experimentally confirmed (PNAS 92:8999, ‘95)
    • A potential argument against ultrahigh affinity

• Jain & coworkers
  • Bulk vascularized tumors
  • ‘‘Because of uniformly elevated interstitial fluid pressure in solid tumors, convection in the tumor interstitium is negligible, and drug delivery through the extracellular matrix relies on passive diffusive transport.’’ Jain et al., PNAS 98:4628, ‘01
Binding & Diffusion kinetic model

Prevascular spheroid micrometastasis

- Fickian diffusion
- No convection
- Pseudo-homogeneous medium

- Reversible monovalent binding
- Constant Surface Ab concentration
- Antigen turnover possible

< 600 µm
Goals of the modeling exercise

• What antibody dose is necessary to saturate a tumor microspheroid?

• What is the relationship between binding affinity and tumor AUC?

• What kinetic process(es) limit maximum achievable tumor AUC?
Numerical simulation

\[
\frac{\partial Ab}{\partial t} = D \frac{1}{r^2} \frac{\partial}{\partial r} \left( r^2 \frac{\partial Ab}{\partial r} \right) - k_{on} AbAg + k_{off} B
\]

Diffusion \hspace{1cm} \text{Binding} \hspace{1cm} \text{Release}

\[
\frac{\partial Ag}{\partial t} = R_s - k_{on} AbAg + k_{off} C - k_e Ag
\]

Synthesis \hspace{1cm} \text{Binding} \hspace{1cm} \text{Release} \hspace{1cm} \text{Degradation}

\[
\frac{\partial B}{\partial t} = k_{on} AbAg - k_{off} B - k_e B
\]

\hspace{1cm} \text{Binding} \hspace{1cm} \text{Release} \hspace{1cm} \text{Degradation}

BCs: zero gradient at center given surface Ab(t)

Numerical Solution:
Absoft Fortran 77
Mac Powerbook G3 (~ 10 seconds)
IMSL MOLCH (method of lines)
Shell of bound Ab moves inward

Constant $[\text{IgG}]_o = 100 \text{ nM}$
$[\text{Ag}]_o = 800 \text{ nM}$

$K_d$:
- 10 nM
- 1 nM
- 0.1 nM

Lower affinity:
- Penetrates faster
- But binds less
- ∴ not as effective

Distance from center (µm)

Bound antigen fraction
Affinity: Time to load vs. level of loading

Maximum loading @ $K_d < 1 \text{ nM}$

Equilibrium loading ($M \times \mu m^3$)

Faster loading = Lower loading

$[scFv]_0 = 100 \text{ nM}$

$[Ag]_0 = 800 \text{ nM}$
Loading rate vs. affinity

• Catch-22:
  - Low affinity ($K_d > 100$ nM) accelerates loading
    » but
  - Low affinity lowers tumor AUC

∴ Affinity should not be used as a variable to alter loading rate
Shrinking Core Model (SCM)

Assumption: Binding is much more rapid than diffusion

SCM is a good approximation for $K_d < 1$ nM

Classic chemical engineering model of heterogeneous catalysis

$r_c$ from SCM

SCM is a good approximation for $K_d < 1$ nM
What parameters affect SCM-predicted saturation time?

\[ t = t_{sat} \left( 1 - 3 \left( \frac{r_c}{R} \right)^2 + 2 \left( \frac{r_c}{R} \right)^3 \right) \]

\[ t_{sat} = \frac{R^2 ([Ag]_o / \varepsilon)}{6D[Ab]_o} \]

From tumor biology, \( t_{sat} \) increases as:
- square of radius
- proportional to antigen concentration

Accessible to engineering, \( t_{sat} \) decreases as:
- diffusivity increases (scFv fragments vs. IgG)
- antibody concentration increases (maximal dose)

\( t_{sat} \) is independent of binding affinity
SCM predicts numerical simulation

Varied parameters:
- $K_d$: 1 pM - 1 nM
- $[\text{Ab}]_o$: 10-100 nM
- $R$: 50-150 µm

SCM accurately predicts saturation time $t_{sat}$
Loading & retention kinetic phases

Incorporating: antibody pharmacokinetics and antigen turnover

Diffusion-limited  Reaction-limited

Loading  Retention
SCM predicts bolus dosing

Parameter ranges:

1 pM < $K_d$ < 1 nM
1 hr < $\tau_{1/2}^{Ag}$ < 1 day
15 min < $\tau_{1/2}^{Ab}$ < 2 hr
10 \( \mu \text{m}^2/\text{s} \) < $D$ < 80 \( \mu \text{m}^2/\text{s} \)
0.4 \( \mu \text{M} \) < $[Ag]$ < 1.6 \( \mu \text{M} \)
100 \( \mu \text{m} \) < $R$ < 300 \( \mu \text{m} \)
0.1 < vol frac $\epsilon$ < 0.28

\[
AUC_{Ab,\text{plasma}} \geq 1.5 \left( \frac{R^2([Ag]_o/\epsilon)}{6D} \right)
\]

\[
[Ab]_o = 1.5 \left( \frac{(\ln 2)R^2([Ag]_o/\epsilon)}{6D\tau_{1/2}} \right)
\]

For base-case scFv:

$[Ab]_o = 300$ nM
Effect of affinity on tumor dosing

Affinity affects **Retention** but not **Loading** phases

\[ R = 300 \, \mu \text{m} \]
\[ [\text{Ag}]_0 = 800 \, \text{nM} \]
\[ [\text{scFv}]_0 = 300 \, \text{nM} \]
\[ \text{Ag} \tau_{1/2} = 1 \, \text{day} \]
Turnover rate defines limiting affinity

Limiting affinity: \( k_{\text{off}} \sim k_{\text{deg}} \)

i.e., there is no benefit to binding antigen after it’s been internalized, degraded, or shed

Antigen turnover

scFv plasma clearance

Total antibody in tumor

Time (hr)
Model agreement w/ ErbB2 data
(Adams et al., Cancer Res. 61:4750, ’01)

ErbB2 constitutive endocytosis $\tau_{1/2} = 17$ min.
(JBC 271:5251, ’96, JBC 274:8865, ’99)

No adjustable parameters

1: $\tau_{1/2} \sim 5$ sec
2: $\tau_{1/2} \sim 110$ sec
3: $\tau_{1/2} \sim 15$ min.
4: $\tau_{1/2} \sim 3$ hr
5: $\tau_{1/2} \sim 19$ hr
Testable model predictions, ErbB2

• Tumor localization maximum @ ~ 25 min.

• Tumor can be homogeneously penetrated by increasing the bolus dose

• $^{125}\text{I}$-labeled scFv ($K_d \leq 1 \text{ nM}$) internalized and degraded in SK-OV-3 cell culture w/ $\tau_{1/2} \sim 15 \text{ min.}$
Model agreement w/ EGFRvIII data

(Bigner et al., Int. J. Cancer 88:962, '00)

Fraction of saturation

Time (hr)

K_d: 23 nM vs. 1.5 nM
Model agreement w/ CEA data
(Williams et al., Cancer Biother. & Radiopharm. 16:25, '01)
Antigen turnover determines impact of affinity

![Graph showing the relationship between tumor AUC and antigen off-rate (k_{off})](image)

- **Antigen τ_{1/2}**
- **K_d (pM)**
- **1 day**
- **10 days**
- **2.4 hrs**

**Tumor AUC**

**k_{off} (s^{-1})**
Model results summary

- Key variables for tumor **loading:**
  - **Diffusivity** of antibody (favors scFv)
  - **Antibody AUC** (dose x time)
  - *Affinity is irrelevant for $K_d < 1 \text{ nM}$*

- Key variables for maximal tumor **retention:**
  - **Dissociation rate** of antibody/antigen
  - Point of diminishing returns:
    - *dissociation rate ~ antigen turnover rate*
Optimal targeting agent

- Small (high diffusivity): $\text{scFv}$
- Dosed at minimum $\text{AUC}_{\text{Ab,plasma}}$ required to achieve tumor saturation
- Targeted to stable antigen
- High affinity ($k_{\text{off}} < k_{\text{deg,Ag}}$)
### Time scales

**scFv & microspheroid**

- **Binding equilibration:**
  \[
  \frac{1}{k_{on} Ab + k_{off}} \approx \text{min - hr}
  \]

- **Ab/Ag complex dissociation:**
  \[
  \frac{1}{k_{off}} \approx \text{sec - days}
  \]

- **Diffusion:**
  \[
  \frac{R^2([Ag]_o / \epsilon)}{6D[Ab]_o} \approx \text{min-hrs}
  \]

- **Ab plasma clearance:**
  \[
  \tau_{1/2} \approx \text{hr}
  \]

- **Ag cellular turnover:**
  \[
  \tau_{\text{deg}} \approx \text{min - days}
  \]
Take home messages

• Antibody AUC in plasma, and not binding affinity, determines tumor saturation.

• Antigen degradation kinetics determine the limiting affinity for tumor retention.
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