Application of PKPD Modeling to Guide the Development of New Immunotherapies

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Pharmacokinetics & Pharmacodynamics

Pharmacokinetics: Investigation of the time-course and determinants of drug absorption, distribution and elimination

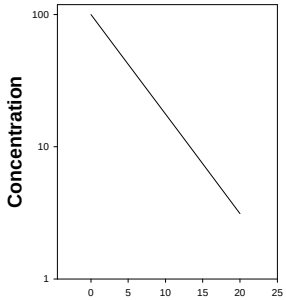
"What the ***body*** does to the drug"

Pharmacodynamics: Investigation of relationships of drug concentration and drug effects

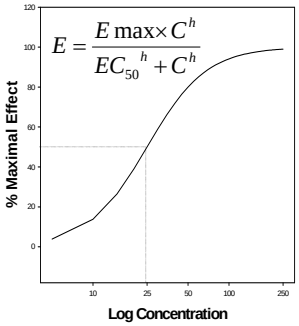
"What the ***drug*** does to the body"

PK/PD Modeling: Application of mathematical models to characterize and predict the time-course of drug effects

PKPD Modeling

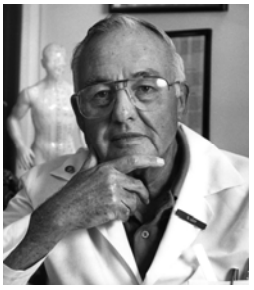


$\log C = \log C_0 - (k \cdot t / 2.303)$



$E = \frac{E\max \times C^h}{EC_{50}{}^h + C^h}$

$E = m \cdot \log C + E_0$



Dr. Gerhard Levy
Father of Pharmacodynamics

$$E = E_0 - m \cdot k \cdot t / 2.303$$

Predicts: time course of drug response, that the rate of decline of effect with time is determined by PK (i.e., k) and PD (i.e., m), greater "efficiency" of divided dosing

PKPD Modeling

Main Applications

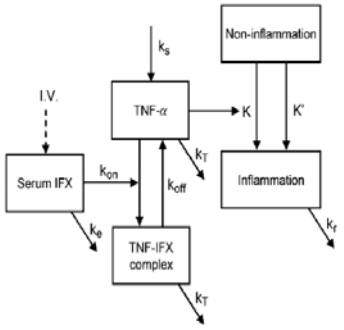
- Prediction of PK, PD in man from preclinical data (FIH studies)

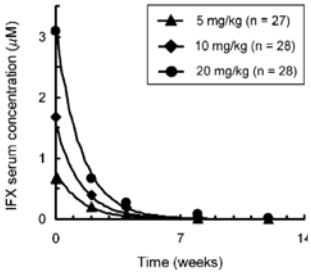
- Phase II/III clinical trial design (modeling used to design trials, assuring adequate statistical power, reducing failure rate)

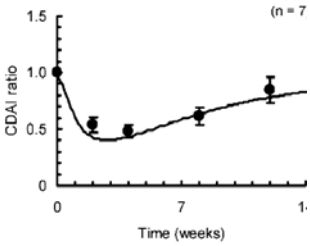
- Development of relationships between patient characteristics and PK, PD (e.g., to facilitate prediction of dosage reduction for renal failure, etc.)

- Characterization of data

PKPD: Science or “Connect the Dots”??







“The estimated half-life value of TNF-a, calculated from [kT], ***was 31.9 and 44.1 days***, respectively, which were both greater than the half-life of serum TNF-a, ***reported to be several minutes***.”

Furuya et al. Drug Metab Pharmacokinet. 2007 Feb 25;22(1):20-5.

Application of PKPD to Inform Discovery

- Improve understanding of the “system” to generate new hypotheses regarding effective therapy of disease (systems biology)
- Probe mechanisms of drug action, potentially assisting in the identification of new targets for therapy
- Assist in the investigation of kinetic determinants of drug action & prediction of optimal therapies for desired effect (e.g., optimal drug-receptor binding kinetics, optimal drug combinations)

Application of PKPD to Inform Discovery

1. Identify problem of interest

2. Obtain drug disposition data, drug efficacy data, and (in some cases) drug toxicity data

3. Create a mechanistic mathematical model to describe kinetic determinants of drug disposition and drug effects; generate new hypotheses

4. Employ simulation to predict effects of new therapies (inform discovery / test hypotheses in silico)

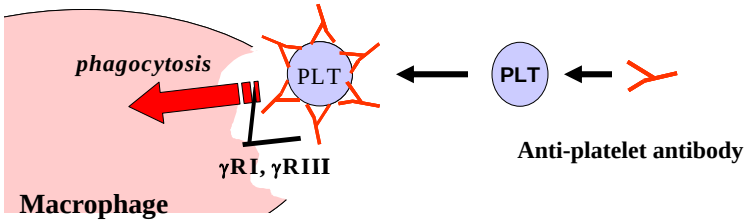
5. Develop new therapies based on model predictions

6. Repeat steps 2- 5

Balthasar Lab: Current Projects

- **R01 HL70227 (PI: Balasubramanian, S.V.; Balthasar Co-I)**
  Development and pharmacology of novel lipidic rAHF
- **R01 HL55461 (PI: Yang, V.C.; Balthasar PI for consortium)**
  Triggered Local Release of Active Thrombolytic Agents
- **R01 CA114612 (PI: Yang, V.C.; Balthasar PI for consortium)**
  PTD-Mediated Protein or Drug Delivery for Cancer Therapy
- **R01 CA118213 (PI: Balthasar, J.P.)**
  Pharmacokinetic Strategies to Optimize IP Chemotherapy
- **R01 AI60687 (PI: Balthasar, J.P.)**
  FcRn Inhibitors for Antibody-Mediated Immune Conditions
- **R01 HL67347 (PI: Balthasar, J.P.)**
  Pharmacology and Bioengineering of New Treatments for ITP

Immune thrombocytopenic purpura (ITP)



- Increased rate of platelet destruction
  - Mediated by anti-platelet antibodies
- Low platelet count
- 25% of chronic ITP patients are refractory to standard therapy
- 16% rate of fatal hemorrhage (intracranial) in refractory patients

IVIG: IntraVenous Immune Globulin

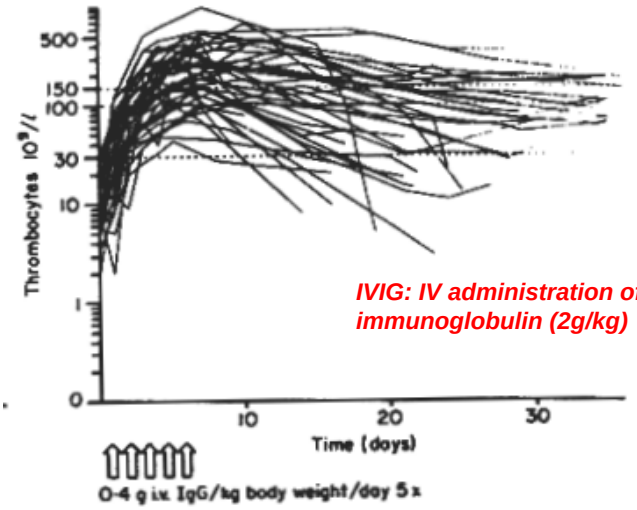
- Immune globulin purified from blood of 'disease-free' donors
- Polyspecific (i.e., directed against a variety of antigens/epitopes)
- Primarily comprised of Immune Gamma Globulin (IgG, ~95%)

Also contains:
- Other immunoglobulin (e.g., IgA, IgM)
- Cytokines
- Soluble cytokine receptors

Initial indication:
- 'Replacement' therapy for agammaglobulinemia

IVIG Therapy for ITP

Results from 42 children with ITP; IVIG effects in ITP first presented by Imbach et al. (1981).



IVIG: IV administration of pooled immunoglobulin (2g/kg)

Proposed Mechanisms of IVIG Action in ITP

Fc receptors
- Blockade of Fc receptors on macrophages and effector cells
- Induction of antibody-dependent cellular cytotoxicity
- Induction of inhibitory Fcγ receptor IIB

Inflammation
- Attenuation of complement-mediated damage
- Decrease in immune-complex–mediated inflammation
- Induction of antiinflammatory cytokines
- Inhibition of activation of endothelial cells
- Neutralization of microbial toxins
- Reduction in corticosteroid requirements

B cells and antibodies
- Control of emergent bone marrow B-cell repertoires
- Negative signaling through Fcγ receptors
- Selective down-regulation and up-regulation of antibody production
- Neutralization of circulating autoantibodies by antiidiotypes

T cells
- Regulation of the production of helper-T-cell cytokines
- Neutralization of T-cell superantigens

Cell growth
- Inhibition of lymphocyte proliferation
- Regulation of apoptosis

Kazatchkine and Kaveri, NEJM 345: 747-755, 2001

Project Plan

1. Identify problem: ***Mechanism of action for IVIG in ITP***

2. Obtain drug disposition data, drug efficacy data, and (in some cases) drug toxicity data

3. Create a mechanistic mathematical model to describe kinetic determinants of drug disposition and drug effects; generate new hypotheses

4. Employ simulation to predict effects of new therapies (inform discovery / test hypotheses in silico)

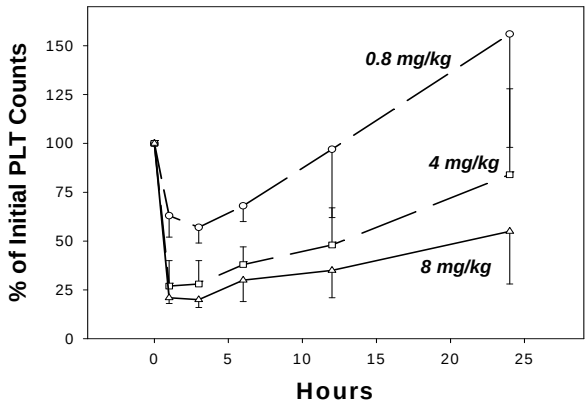
5. Develop new therapies based on model predictions

6. Repeat steps 2- 5

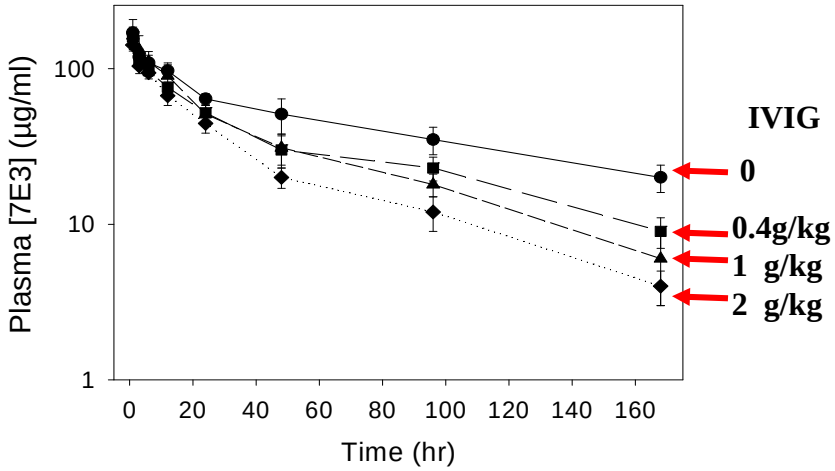
7E3-Induced Thrombocytopenia in Rats

METHODS

- 7E3 administered to SD rats at doses of 0.8, 4 and 8 mg/kg.
- PLT count assayed over 24h



Influence of IVIG on 7E3 PK in Rats



CL values ranged from 0.78±0.09 ml hr^{-1} kg^{-1} (control) to 1.85±0.19 ml hr^{-1} kg^{-1} (2 g/kg IVIG), $p<0.001$

Q: Is this effect mediated by the Brambell Receptor?

- Transient, saturable GI absorption in neonates
- Very long plasma half-life
- Concentration-dependent elimination

Dr. F. W. Rogers Brambell

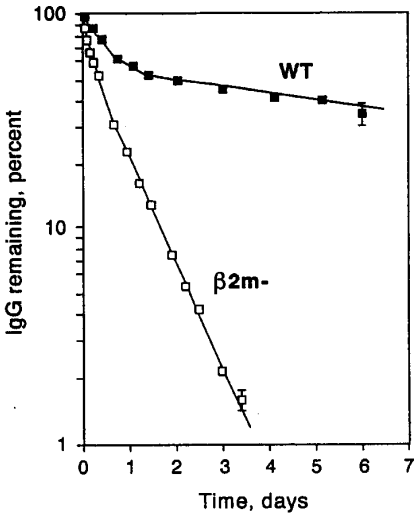
FcRn 'Knockout' Studies

1995. Isreal et al: Neonatal mice lacking expression of FcRn light-chain (β2m) are unable absorb maternal IgG

1996: 3 groups show that FcRn is responsible for 'systemic protection'

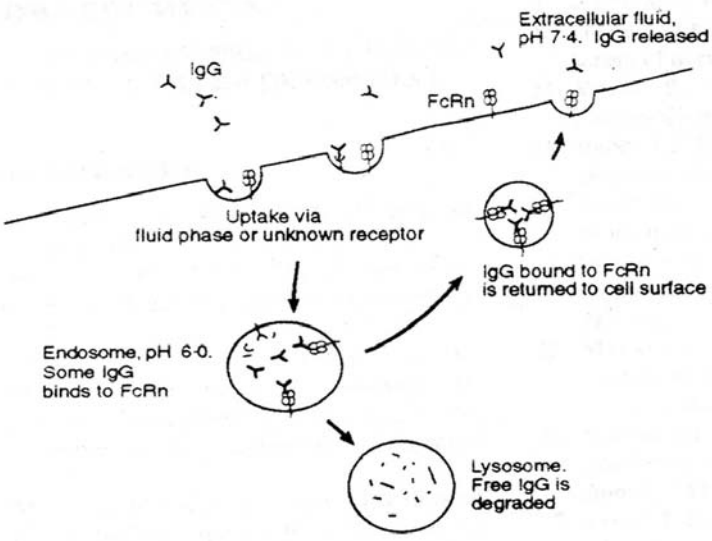
- *Ghetie et al., Eur J Immunol, 26:690-6*
- *Junghans and Anderson, PNAS, 93:5512-6*
- *Israel et al., Immunology, 89:573-578*

β2m knockout animals do not express functional FcRn, and show ~10 fold increase in IgG CL (no change in PK of albumin, IgA)



Junghans and Anderson, PNAS, 93:5512-6

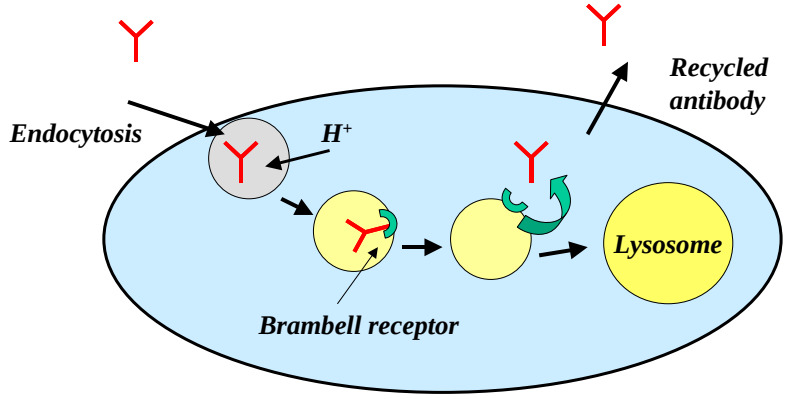
FcRn: Proposed Mechanism for IgG 'Recycling'



•***Israel et al., Immunology, 89:573-578, 1996***

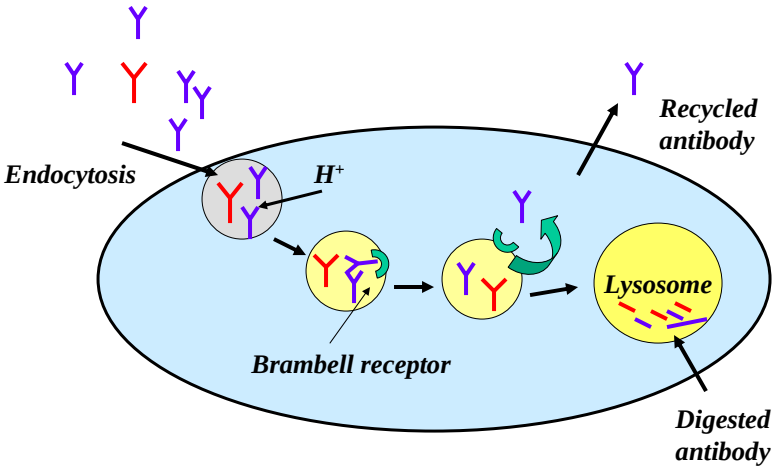
Ho: IVIG increases CL of APAb via saturation of FcRn

In the absence of IVIG, APAb is recycled by the Brambell receptor….



Ho: IVIG increases CL of APAb via saturation of FcRn

Following IVIG, the receptor is saturated, and APAb CL increases



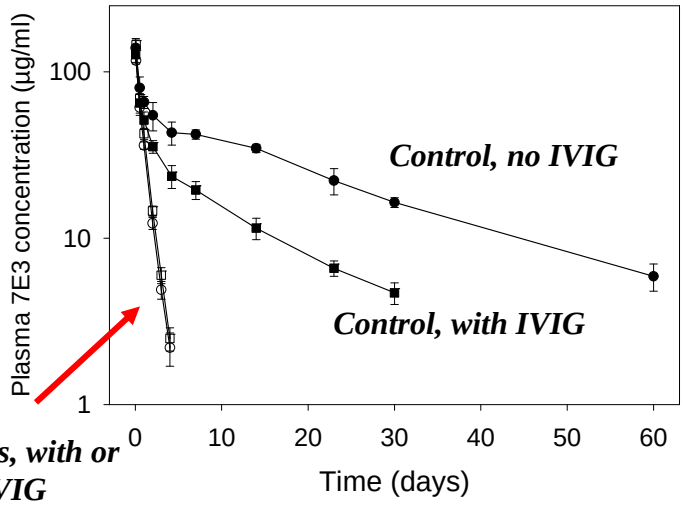
Ho: IVIG increases CL of APAb via saturation of FcRn

Investigation of IVIG effects on 7E3 disposition in FcRn 'knockout' mice and in 'wild-type' controls

METHODS

- β-2 microglobulin knockout mice (lacking FcRn) & C57BL/6 control mice were obtained from Jackson
- JVCs inserted 3-4d prior to experimentation
- Pretreatment with saline or IVIG (1g/kg) via JVC
- 7E3, 8 mg/kg, dosed via JVC
- Blood samples collected from saphenous vein (over 4d for knockout mice, and over 30-60d for controls)
- 7E3 conc determined via ELISA

Ho: IVIG increases CL of APAb via saturation of FcRn



Knockouts, with or without IVIG

Hansen and Balthasar, Thromb Haemostasis, 88:898-899, 2002

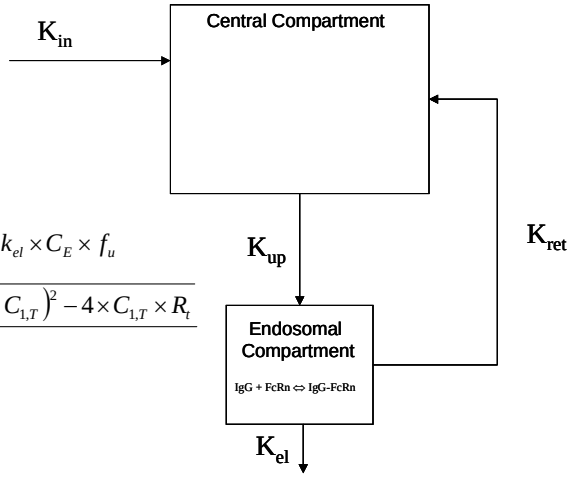
Development of Mechanistic Mathematical Models to Describe the System

PK/PD Modeling: IVIG effects on 7E3 PK

$$\frac{dC_1}{dt} = -k_{up} \times C_1 + k_{ret} \times C_E \times (1 - f_u)$$

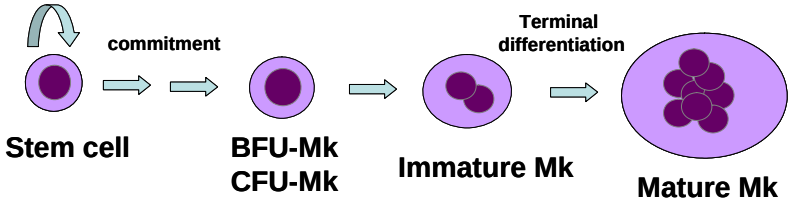
$$\frac{dC_E}{dt} = k_{up} \times C_1 - k_{ret} \times C_E \times (1 - f_u) - k_{el} \times C_E \times f_u$$

$$f_u = 1 - \frac{K_d + R_t + C_{1,T} - \sqrt{(K_d + R_t + C_{1,T})^2 - 4 \times C_{1,T} \times R_t}}{2 \times C_{1,T}}$$



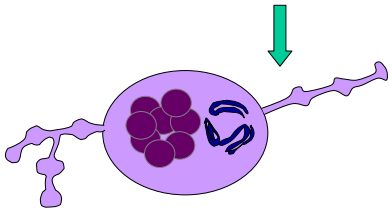
Hansen and Balthasar, J Pharm Sci, 92(6):1206-1215, 2003

Mathematical modeling of thrombopoiesis



➢All stages are driven by thrombopoietin (TPO)

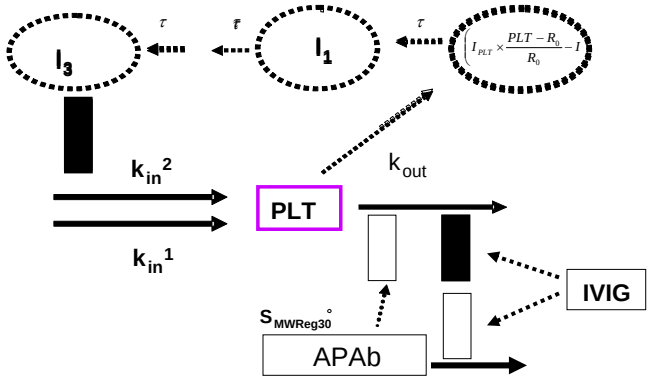
➢Plasma TPO concentration is inversely correlated to the total body quantity of platelets and megakaryocytes



Platelet shedding

http://www.pdsa.org/conference2004/Drachslsh.pps#9
Kaushansky K. New Engl J Med., 1998, 339(11):746-754

IVIG Effects on Ab-induced Thrombocytopenia



- PLT production is inversely related to PLT count
- There is a delay between changes in PLT count and changes in PLT production
- APAb stimulates PLT elimination
- IVIG inhibits the effect of APAb on PLT elimination

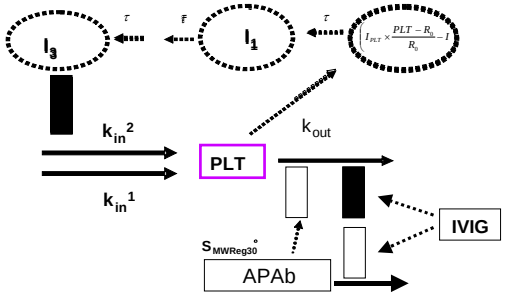
Model fitting of IVIG effects on MWReg-induced thrombocytopenia in mice

$$\frac{dI_1}{dt} = \frac{1}{\tau} \times (I_{PLT} \times \frac{PLT - R_0}{R_0} - I_1)$$

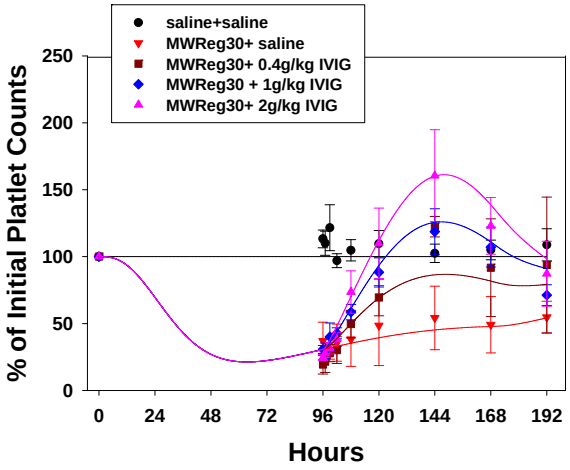
$$\frac{dI_2}{dt} = \frac{1}{\tau} \times (I_1 - I_2)$$

$$\frac{dI_3}{dt} = \frac{1}{\tau} \times (I_2 - I_3)$$

$$\frac{dPLT}{dt} = k_{in}^1 + k_{in}^2 \times (1 - I_3) - \frac{(k_{in}^1 + k_{in}^2)}{R_0} \times PLT \times (1 + S_{MWReg30} \times (1 - \frac{I_{max,IVIG} \times C_{IVIG}}{IC_{50,IVIG} + C_{IVIG}}) \times C_{MWReg30})$$

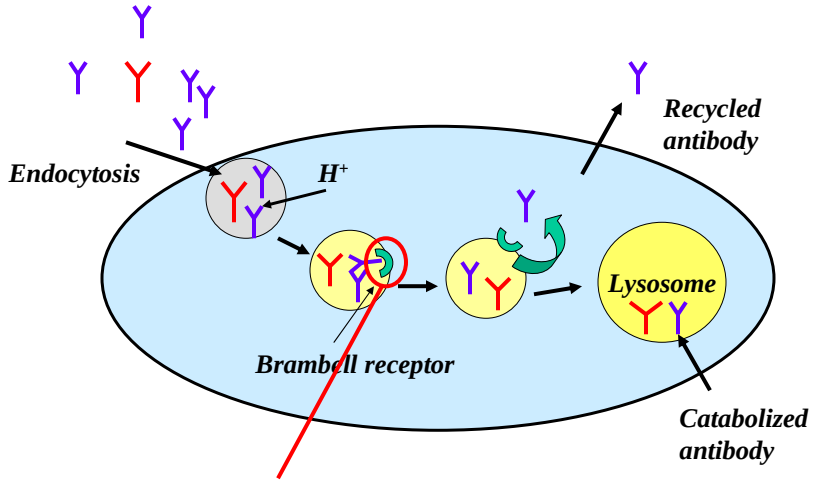


Model fitting of IVIG effects on APAb-induced thrombocytopenia in mice



Simulations with the model indicated that inhibition of FcRn accounted for 45-50% of the effect of IVIG in mouse and rat models of ITP

FcRn: potential target for new therapies



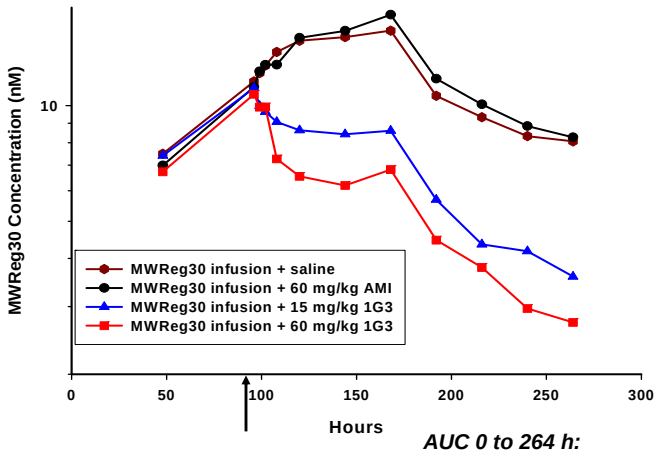
FcRn is a NEW TARGET

Anti-FcRn Mab: Feasible inhibitors of FcRn?

1G3 effects in the mouse model of sustained ITP

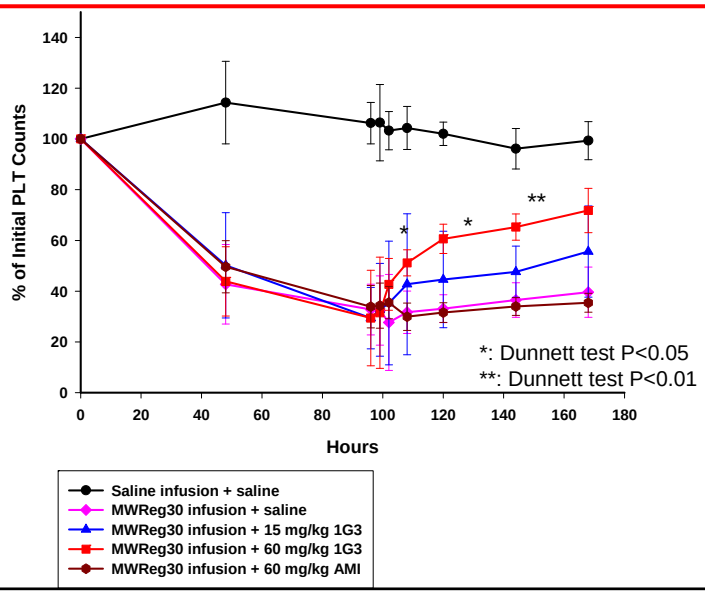
- Animals: ~20g Swiss-Webster mice
- Treatment (n=5/group)
  - 7 days i.p. infusion MWReg30 (anti-platelet antibody)
    - ✓ i.p. 15 or 60 mg/kg 1G3 at day 4
    - ✓ i.p. saline at day 4
- Sample collection: collect blood at 0, 96, 97, 99, 102, 108, 120, 144, 168, 192, 216, 240,264 h
- Measurements:
  - MWReg30 plasma concentrations were assessed by ELISA
  - Platelet counts were assessed by Cell-Dyn

Effect of 1G3 on MWReg30 PK



AUC 0 to 264 h:
saline group: 2723 nM•h
60 mg/kg AMI: 2645 nM•h
15 mg/kg 1G3: 1742 nM•h
60 mg/kg 1G3: 1467 nM•h

Effects of 1G3 on MWReg-induced ITP in mice



Brief Summary, FcRn Project

- IVIG therapy was found to increase the clearance of antiplatelet antibodies in rodent models of ITP
- Work conducted with FcRn-deficient mice suggest that IVIG achieves this effect via saturation of FcRn
- PK/PD modeling suggests that ~50% of the overall effect of IVIG is mediated by alterations in APAb pharmacokinetics
- Specific FcRn inhibitors achieve similar PK effects at doses ~100-fold less than required for IVIG
- Specific FcRn inhibitors increase PLT counts in the mouse model of sustained ITP
- Anti-human FcRn heavy chain antibodies inhibited the binding of human IgG to human FcRn transfected 293 cells *in vitro*.
- Three monoclonal anti-human FcRn heavy chain antibodies have been developed (1D6, 11C1, m1D5)

Balthasar Lab: Current Projects

R01 CA118213 (PI: Balthasar, J.P.; Bernacki PI for consortium)
Pharmacokinetic Strategies to Optimize IP Chemotherapy
9/2006 – 7/2010

•This project attempts to enhance the pharmacokinetic selectivity of IP chemotherapy to improve the safety and efficacy chemotherapy for ovarian cancers

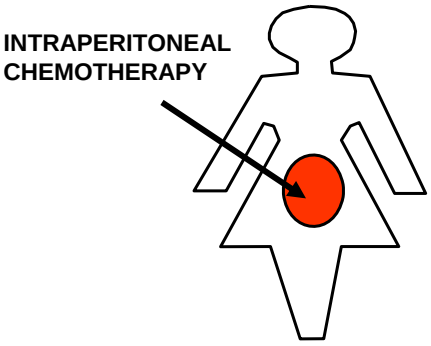
Aims:

1.Evaluation of the use of anti-drug antibodies to optimize IP chemotherapy

2.Evaluation of the use of lipid emulsions to optimize IP chemotherapy

3.Evaluation of the use of anti-angiogenic agents to increase tumor exposure to chemotherapeutics following ip chemotherapy

Regional Chemotherapy of Peritoneal Tumors

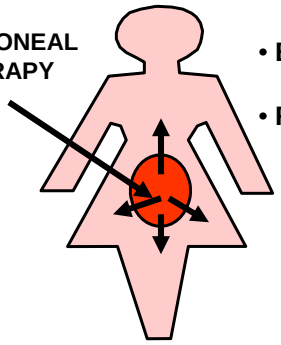


- **Systemic CL >> Peritoneal CL**
- **High peritoneal concentrations relative to blood concentrations**
- **Ho: Increased ratio of drug efficacy to drug toxicity**

R. L. Dedrick et al.
Pharmacokinetic rationale for peritoneal drug administration for the treatment of ovarian cancer. Cancer Treat Rep., 1978

Regional Chemotherapy of Peritoneal Tumors

INTRAPERITONEAL CHEMOTHERAPY



- **Systemic toxicities remain dose-limiting[a]**
- **Early studies failed to show substantial therapeutic benefits[b]**
- **Recent work has led to a renewed interest in IP chemotherapy[c]**

a. Howell, SB et al., Ann Intern Med, 1984
Howell, SB et al., J Clin Invest, 1981
Howell, SB et al., Ann Intern Med, 1982
Speyer, JL et al., Cancer Res., 1990
Pfeiffer, P et al., Gynecol Oncol., 1990

b. Alberts, DS et al An Soc Clin J Oncol., 1995
Kimani, S. et al, Gynecol Oncol., 1994

c. Armstrong et al. N Engl J Med, 2006

It is now clear that IP chemotherapy is superior to IV chemotherapy of optimally-debulked, advanced ovarian cancer

Nonetheless, IP chemotherapy has never delivered the survival increases predicted by the high local concentrations of cytotoxic drugs

Pharmacokinetic Problems in Peritoneal Drug Administration: Tissue Penetration and Surface Exposure

*Robert L. Dedrick, Michael F. Flessner**

Journal of the National Cancer Institute, Vol. 89, No. 7, April 5, 1997

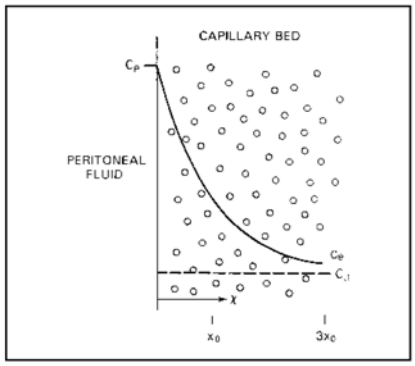
- **Several studies have demonstrated a rapid, exponential decrease in tumor concentration with distance (from the peritoneal surface)**
- **The high concentrations of drug in the peritoneum do not translate to high concentrations of drug in peritoneal tumors (ie., at depths greater than 100-200 µm from the peritoneal surface)**

Capillary Washout Explains Limited Penetration

Determinants of penetration depth:
a. Driving force: = ($C_{peritoneal} - C_{blood}$)
b. Removal of drug by tumor capillaries

Rate constant associated with decreases in tumor concentration with depth into tumor: k = paq/(pa+q)

p is capillary permeability, ***a*** is capillary surface area, and ***q*** is tumor blood flow per unit of tumor volume

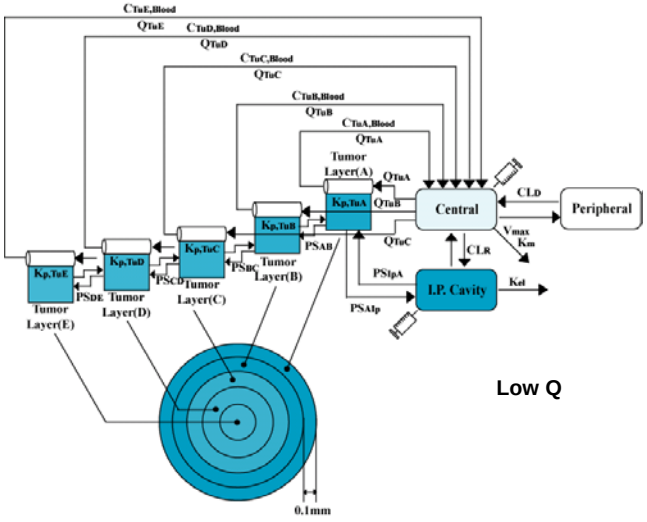


New Hypothesis: Anti-angiogenic therapy will increase the depth of penetration of drug following IP chemotherapy

Gupta, Wientjes, Au. Pharm Res 12:108-112, 1995

Dedrick and Flessner, JNCI 89: 480-487, 1997

"Sphere" Model for Drug Penetration



PS: D_{eff}*A/W
Kp, Q: assumed

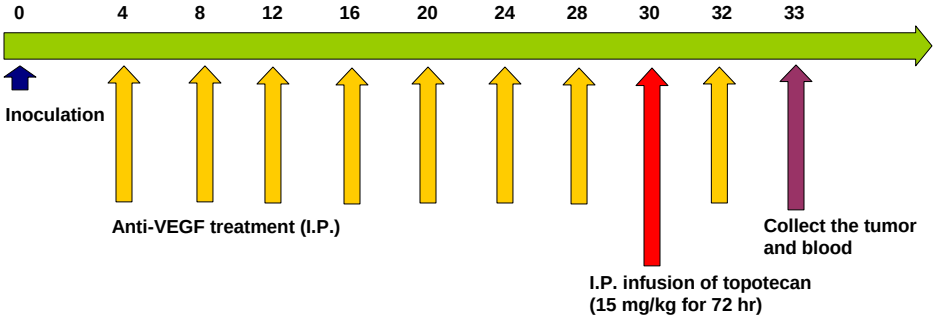
Low Q

Influence of Tumor Blood Flow on Drug Delivery

IP infusion: 0.208 mg/hr/kg for 72 hr	Blood flow			
Blood flow of tumor (mL/min/g)	6E-03	1.5E-02	3E-02	6E-02
IP conc. (ng/mL)	4191	4191	4191	4191
Blood conc. (ng/mL)	24.16	24.16	24.16	24.16
1st tumor layer conc. (ng/mL)	878.4	440.3	249.6	141.9
2nd tumor layer conc. (ng/mL)	208.6	67.92	36.99	27.66
3rd tumor layer conc. (ng/mL)	67.51	29.14	24.93	24.26
4th tumor layer conc. (ng/mL)	36.19	24.79	24.19	24.15
5th tumor layer conc. (ng/mL)	29.11	24.25	24.15	24.15
Tumor Homogenate TPT (ng/g)	503	241	138	83
	TPT in tumor			

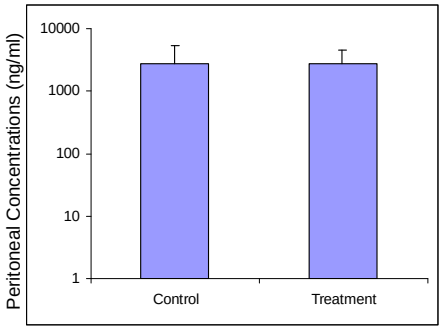
Study Design: *In vivo* investigation

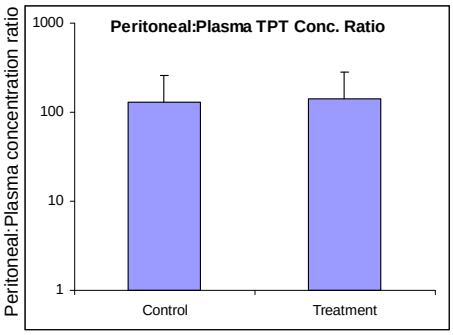
Study with Avastin (5 mg/kg)



- Studies performed in Fox nu/nu mice bearing A2780 xenografts
  - 2M/0.5ml IP
- Evaluation of blood and tumor topotecan concentrations via HPLC

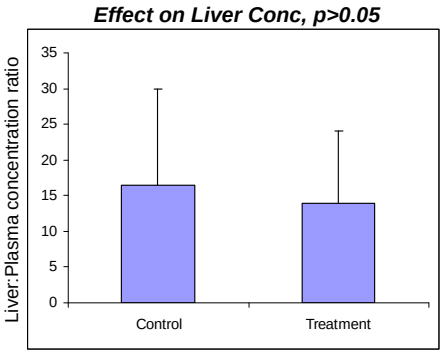
Effect of Anti-VEGF Mab on Peritoneal TPT Conc.

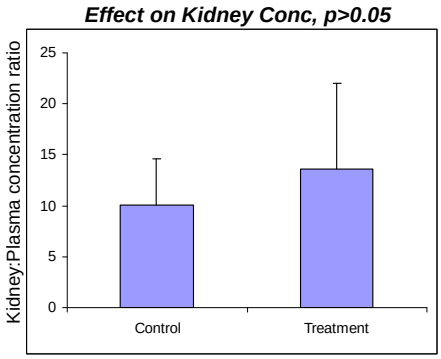




No significant alterations in peritoneal exposure, systemic exposure, or ratio of peritoneal:plasma exposure ($p>0.05$)

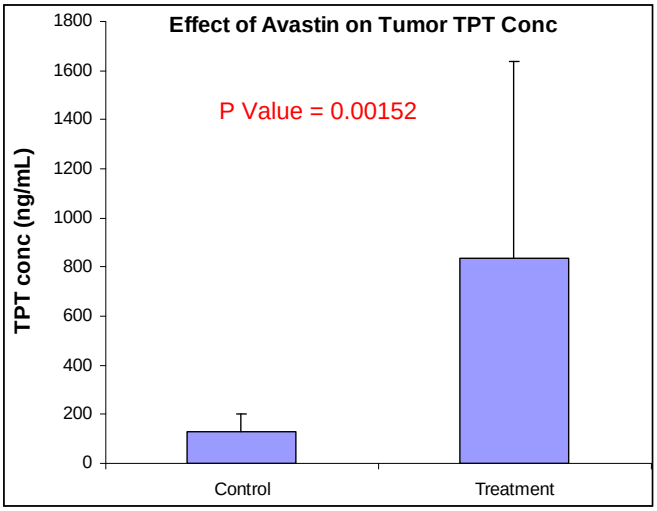
Effect of Anti-VEGF Mab on Kidney & Liver TPT Conc.





No significant alterations in topotecan exposure in systemic tissues (e.g., liver, kidney; p>0.05)

Effect of Anti-VEGF Mab on Tumor TPT Conc.

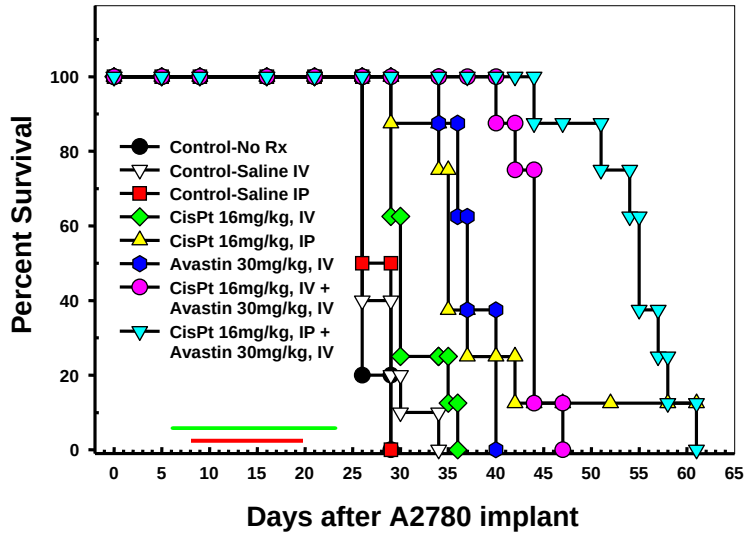


Highly significant (selective!) increase in tumor concentrations (p=0.00152)

Therapeutic Studies in Mice

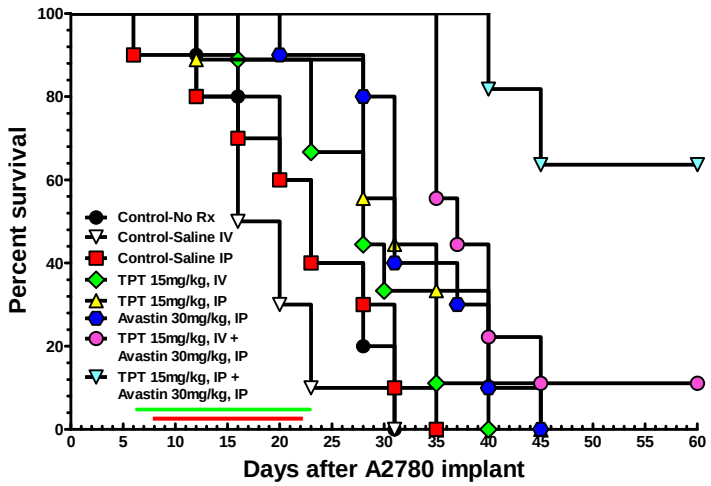
Studies at Roswell:	*Studies at UB:*
Cisplatin IV	Topotecan IV
Cisplatin IP	Topotecan IP
Avastin IV	Avastin IP
Avastin + Cisplatin IV	Avastin + Topotecan IV
Avastin + Cisplatin IP	Avastin + Topotecan IP

Examination of Avastin / DDP Tx of A2780 Xenografts



Avastin (IV) as split doses on days 6, 9,13,16,19 and 23 post tumor implant
CisPt (IV or IP) as split doses on days 8,12,16 and 20 post implant

Examination of Avastin / TPT Tx of A2780 Xenografts



Avastin (IP) as split doses on days 6, 9,13,16,19 and 23 post tumor implant

TPT (IV or IP) as split doses on days 8,15 and 22 post implant

Summary

• Pharmacokinetic theory predicts that combined anti-angiogenic therapy and IP chemotherapy will allow increased drug penetration into peritoneal tumors

• Preliminary pharmacokinetic studies demonstrate significant increases in tumor exposure to drug, with no alteration in the exposure in peritoneal fluid, plasma, or in systemic tissues (liver, kidney)

• Preliminary therapeutic studies in mice bearing human ovarian cancer xenografts indicate that combined IP cisplatin chemotherapy and anti-angiogenic therapy leads to significant improvements in animal survival (TPT work is ongoing)

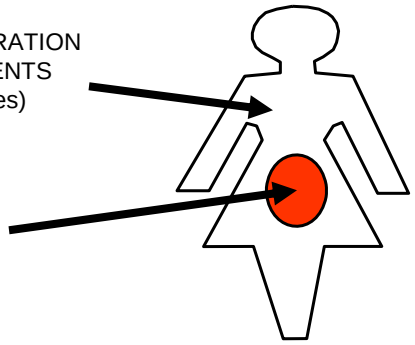
• IP/Avastin combination therapy utilizes FDA-approved treatments; consequently, clinical translation may proceed quickly

Inverse Targeting: Modulation of Systemic PK

Problem: ***Systemic drug toxicity following IP chemotherpay***

SYSTEMIC ADMINISTRATION
OF MODULATING AGENTS
(e.g., anti-drug antibodies)

INTRAPERITONEAL
CHEMOTHERAPY



GOAL

TO ACHIEVE TARGETED DRUG THERAPY BY OBSTRUCTING DRUG DISTRIBUTION TO SITES ASSOCIATED WITH DRUG TOXICITIES

Effects of Anti-TPT Ab on TPT Exposure

In vivo **study**

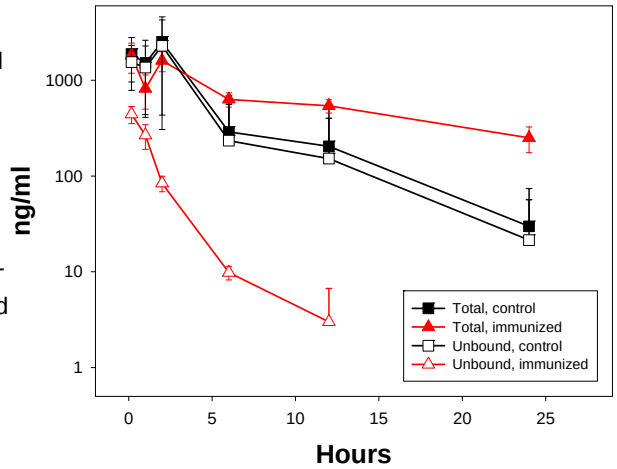
Balb/c mice immunized with TPT-KLH

TPT, 20 mg/kg, administered IP

Blood collected via cardiac puncture

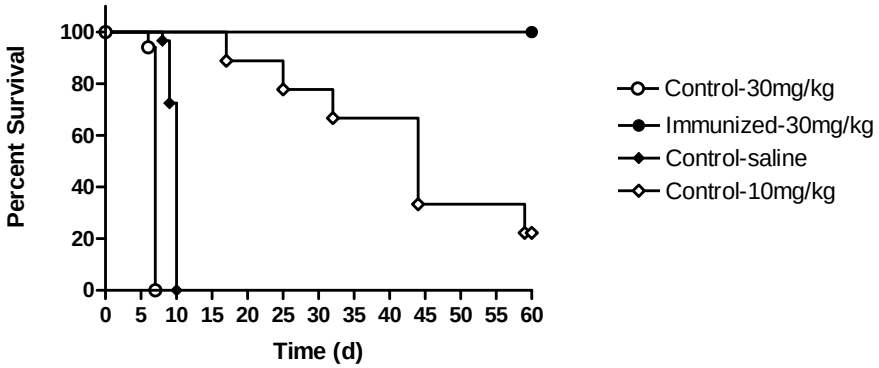
Unbound and total TPT concentrations assayed via HPLC

n=3 mice / time-point



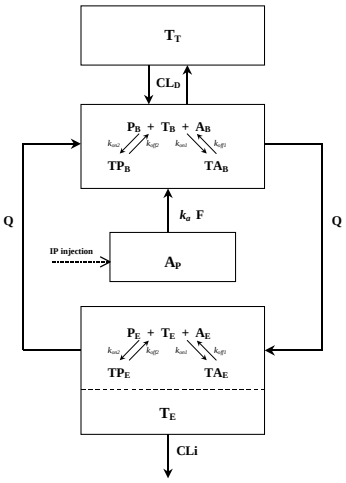
In immunized mice, the AUC of unbound TPT decreased by 92.5% (8.85±2.20 µg/ml•h vs. 0.66±0.09 µg/ml•h, p<0.05, Bailer-Satterthwaite method)

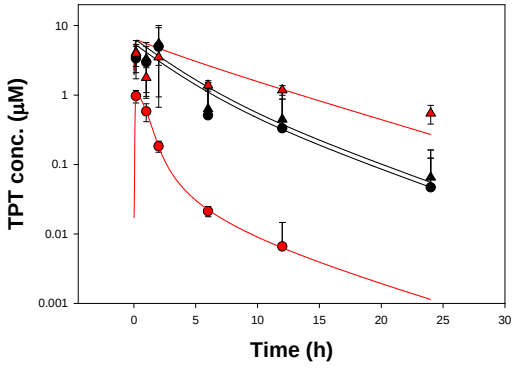
Topotecan Efficacy in S180 Bearing Balb/c Mice



- **Immunized group: tolerated 30 mg/kg IP topotecan with minimal systemic toxicity (~10% weight loss), and *100% of the animals survived through day 60*.**
- **Non-immunized group: MTD = 10 mg/kg (100% mortality by day 7 following 30 mg/kg)**
- **10 mg/kg topotecan (non-immunized) v. 30 mg/kg (immunized): *p= 0.0009***

Pharmacokinetic Modeling





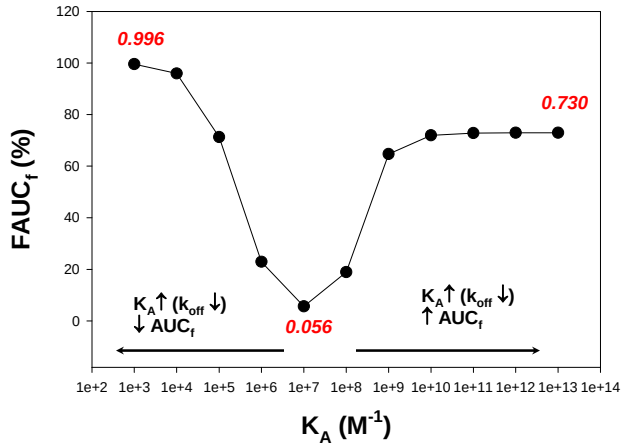
Simulations: Influence of Affinity on Ab Effects

Ho: Decreases in k_{off} may result in increase of "dissociation time" relative to organ transit time, which may shift the effect of antibody on drug elimination from "non-restrictive" to "restrictive" elimination

Simulations

- Increase K_A (i.e., decrease k_{off})
- $FAUC_f = AUC_{f,Ab}/AUC_{f,ctrl}$
- $k_{on} = 3.6 \times 10^3\ \mu M^{-1} \cdot h^{-1}$
- k_{off}: 3×10^{-4} - $3 \times 10^6\ h^{-1}$, K_A: 10^3 - $10^{13}\ M^{-1}$
- TPT IP infusion at 1 μM/h for 72 h, Ab = 5 μM

Influence of K_A (same k_{on}) on $FAUC_f$



Summary for ATAb Modeling & Simulation

- A PK model was developed to describe the effect of endogenous ATAb on TPT pharmacokinetics
- Simulations predicted that k_{off} may be the most critical determinant of the effect of ATAb
- Simulations predicted that an Ab with ***intermediate binding affinity*** (i.e., $K_A = 1 \times 10^7\ M^{-1}$) may be the most efficient in decreasing AUC_f for IP chemotherapy.

Summary

- PKPD analyses & modeling has applications beyond data characterization ("connecting dots")
- PKPD analyses led to the development of specific Mab inhibitors of FcRn, which may find utility in the treatment of a wide range of humoral autoimmune diseases
- PKPD modeling predicted that anti-angiogenic therapy would lead to increased drug penetration into peritoneal tumors following IP chemotherapy
- PKPD modeling predicts that "intermediate affinity" anti-drug antibodies will provide optimal reductions in drug exposure in blood following IP chemotherapy

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