Towards Development of a Systems PK-PD Model to Characterize Bystander Effect of Antibody-Drug Conjugates (ADCs)

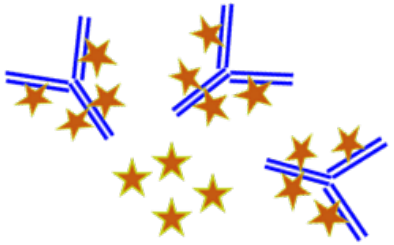
Aman P. Singh

PK-PD Scientist

Translational Research

Biologics Development Sciences

Janssen R&D, PA



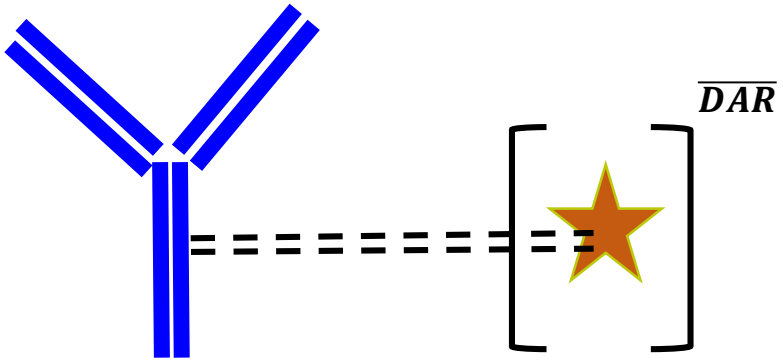
06/13/2018

My Email:

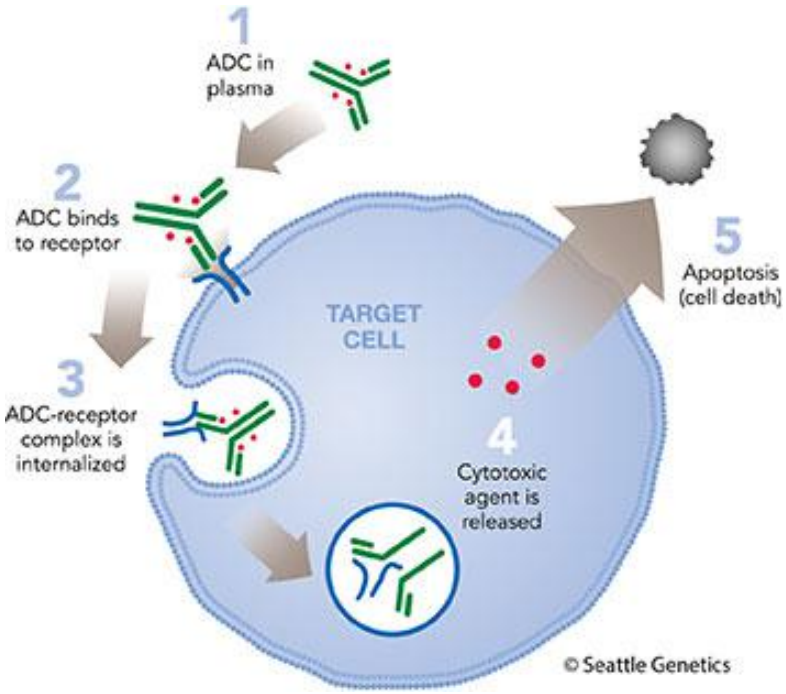
ASing215@its.jnj.com

Background: Antibody Drug Conjugates

~ 75 Antibody Drug Conjugates are in clinical trial



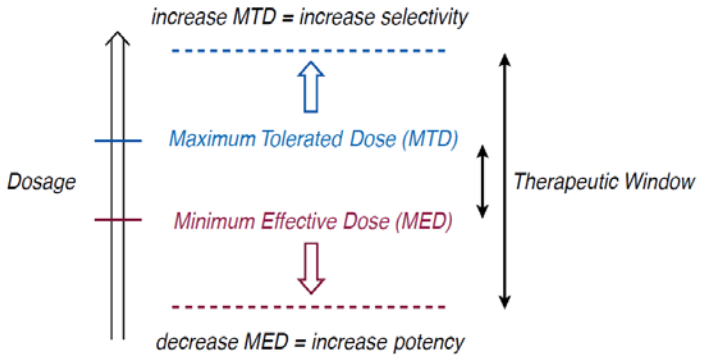
mAb | **Linker** | **Drug**



Humanized mAb construct (*IgG1, IgG2, IgG4)*
Selective for ***tumor antigens.***
Ab-Ag complexes *efficiently internalized*

Cleavable:
Cleaved based on differential extra- and intracellular properties.
e.g. *Enzymatic, Acid-labile, Disulphide*
Non-Cleavable:
Proteolytic Degradation inside a cell
e.g. *SMCC linker.*

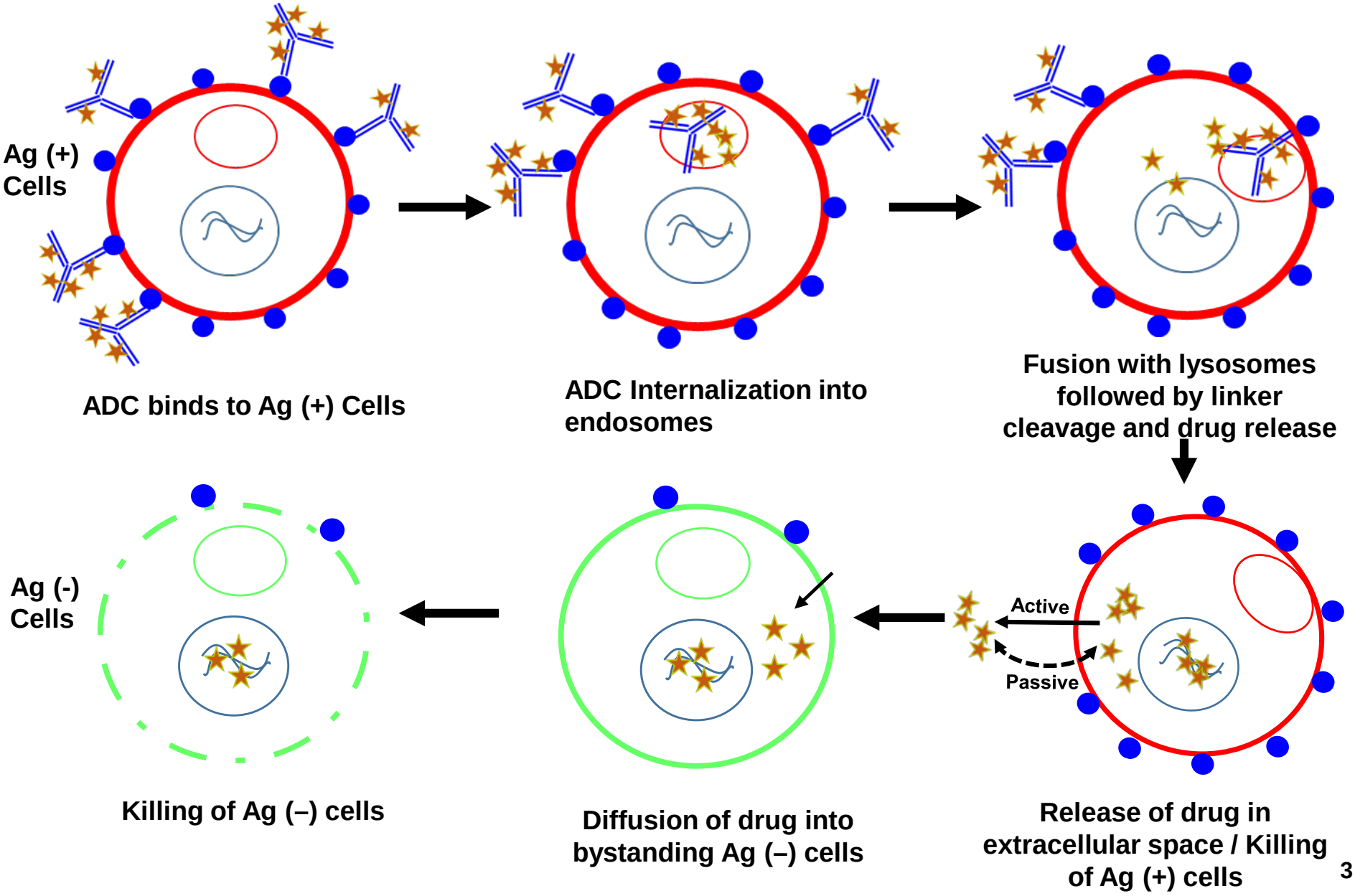
Microtubule Inhibitors:
e.g. *Maytansines (DM1)*
Auristatins (MMAE)
DNA Damaging Agents:
e.g. *Duocarmycins (DC1)*
Calcheamicin



Angew. Chem. Int. Ed. **2014**, *53*, 3796.

Rostami et al. **2014**

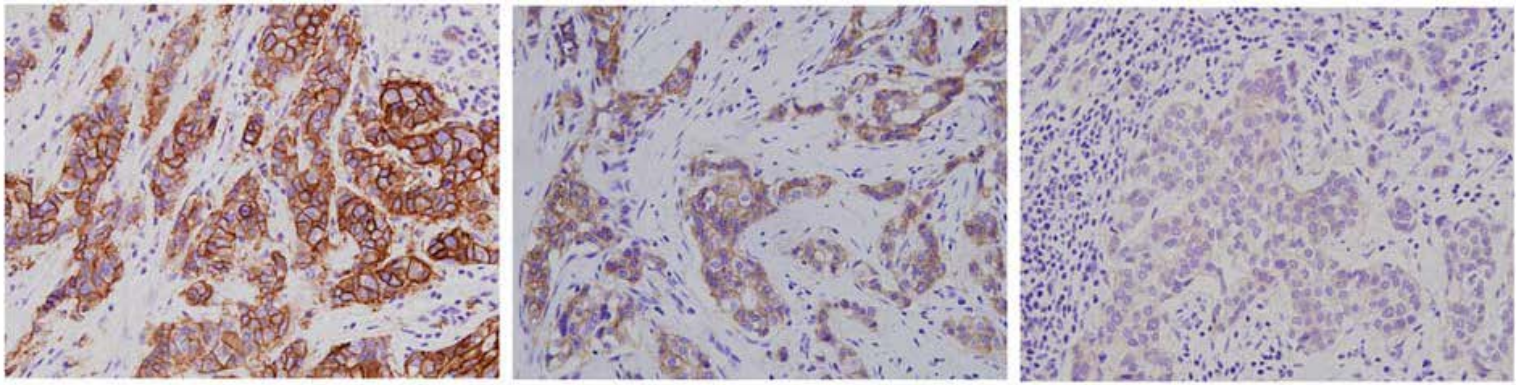
Background: Bystander Effect of ADCs



Our Motivation: Tumor Heterogeneity

Why are we studying Bystander Effect?

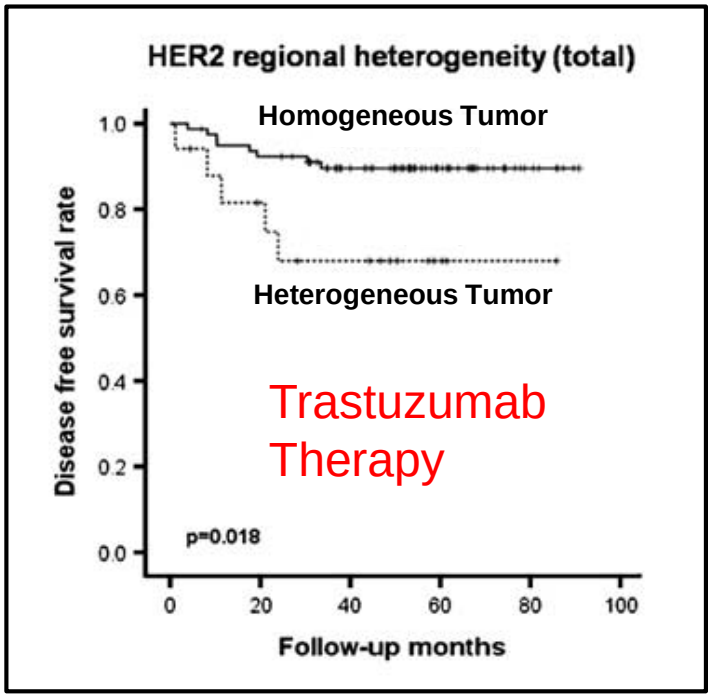
Tumor heterogeneity leads to poor disease free survival rate



TMA Core 1
HER2 3+

TMA Core 2
HER2 2+

TMA Core 3
HER2 1+



- ***Tumor Heterogeneity leads to higher relapse of disease.***
- ***Clinical significance of developing ADCs demonstrating bystander effect***

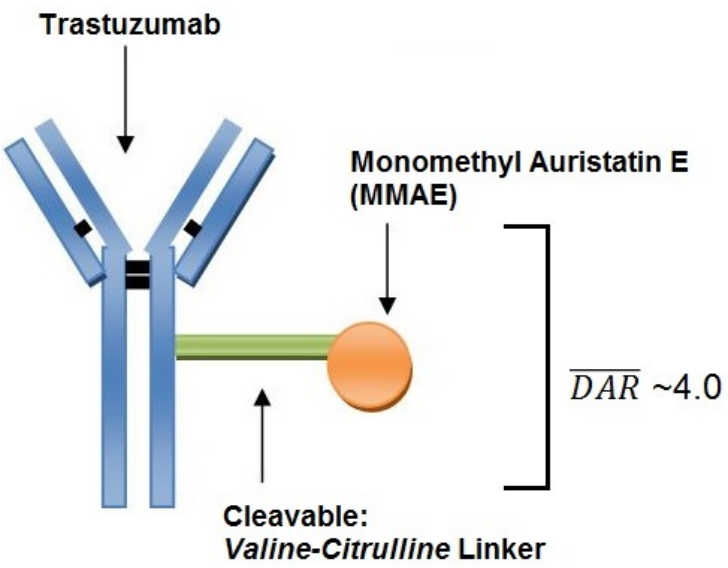
Seol et al. *Modern Pathology* **2012**

Our Hypothesis: Bystander Effect

Quantitatively characterizing ADC disposition at a cellular level will help us understand Bystander Effect *in vivo*. Once validated, our PK-PD model can then:-

I. Identify Prominent Pathways/Parameters in the system to maximize Bystander Effect
II. Identify novel dosing regimens to maximize bystander effect.
III. Inform target selection and ADC design for future ADCs.

Tool ADC: Trastuzumab-vc-MMAE



Trastuzumab [Herceptin®]

- Humanized ***anti-HER2 mAb***
- MW. 148 Kda
- Indicated for HER2-positive metastatic breast Cancer

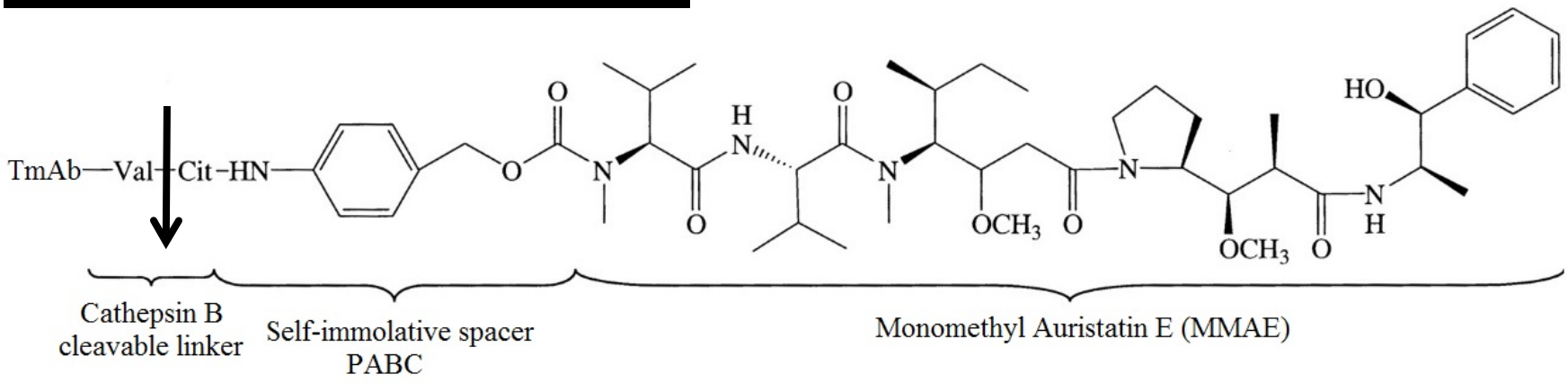
Monomethyl Auristatin E

- Synthetic Derivative of Auristatins (Marine life)
- ***Microtubule Inhibitor***
- Highly potent: IC50 values of 1-10 pM

Valine-Citrulline Linker

- Cleaved by **Cathepsin B** in lysosomes
- Cathepsin B highly expressed in cancer cells
- Self-immolative spacer is attached rendering free MMAE release

Synthesis and Characterization in Shah Lab

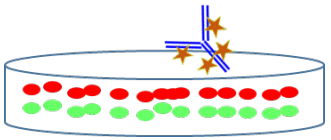


Development of PK-PD model for Bystander Effect

Aim 1

Quantification of Bystander Effect *In Vitro*

Develop a Coculture system of Ag+ and Ag- Cells



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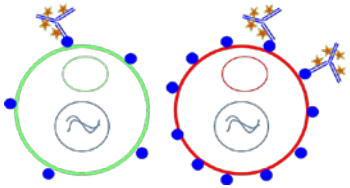
Development of a Single-Cell PK model for ADC

Perform Cellular Disposition Studies in Ag+ and Ag- Cells



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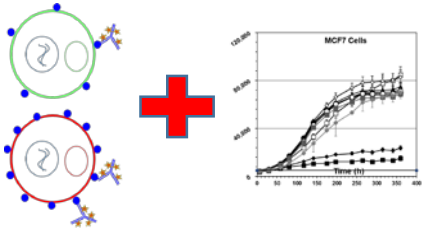
Development of *In Vitro* PK-PD model for Bystander Effect

Linking Intracellular Concentrations to drive Cytotoxicity



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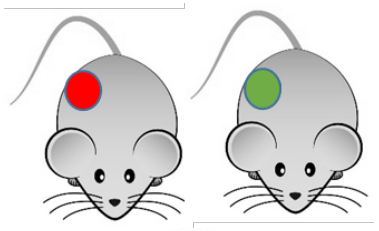
Perform Tumor PK and Tumor Growth Inhibition (TGI) Studies



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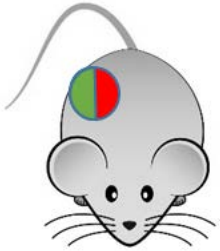

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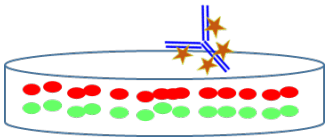

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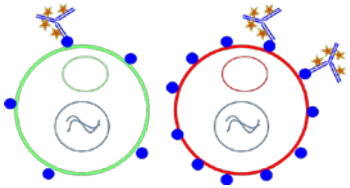

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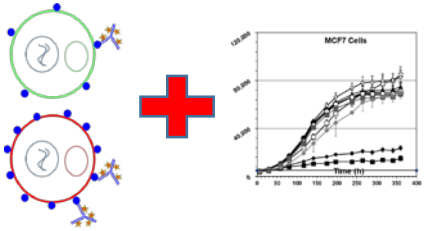

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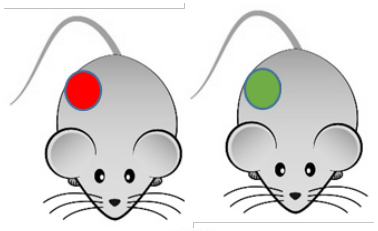

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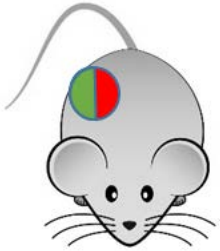
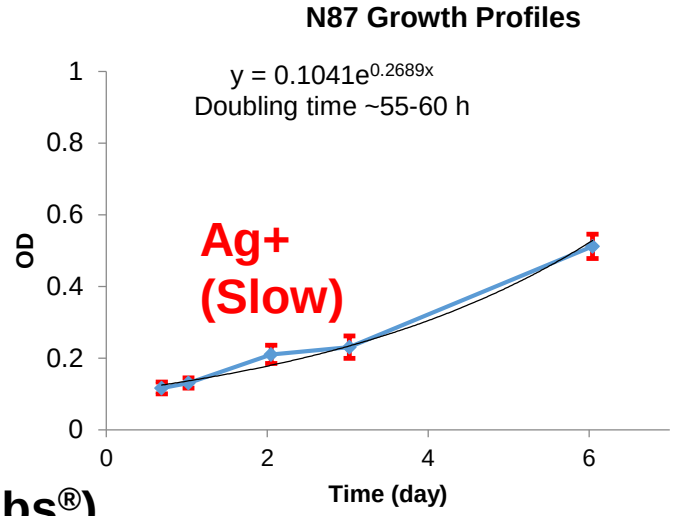


Singh et al. JPKPD 2016

Cell Lines with Different HER2 Expression

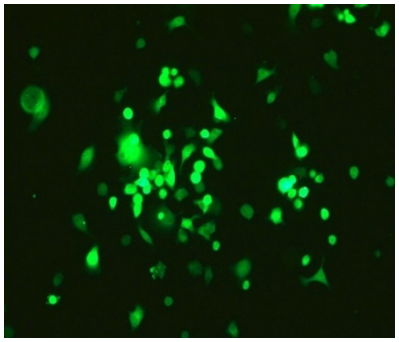
HER2 High (3+) NCI-N87 Cell line (ATCC®)

- Adherent, patchy epithelial cells obtained from human gastric carcinoma
- Over expression of HER2/neu protein
- Doubling time ~**55 h.**

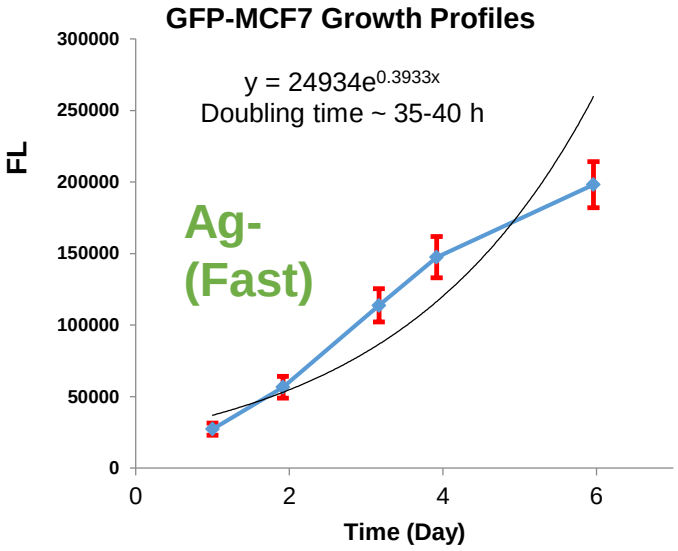


HER2 Low (0/1+) GFP-MCF7 Cell line (Cell Biolabs®)

- Adherent, epithelial cells obtained from human mammary gland
- Low expression of HER2/neu protein
- Doubling time ~***35 hrs***.

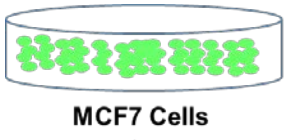


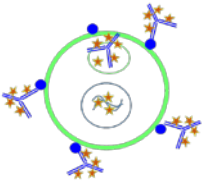
Fluorescence microscopy image at excitation/emission wavelength of 485/535 nm



Baselga et al. *Cancer Res.* **2003**
Spink et al. *Cell Bio Int.* **2003**

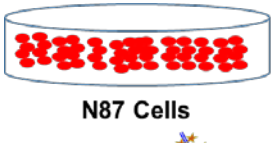
In Vitro Viability with T-vc-MMAE in Each Cell Line

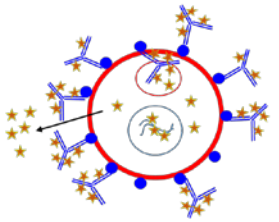




100 nM T-vc-MMAE

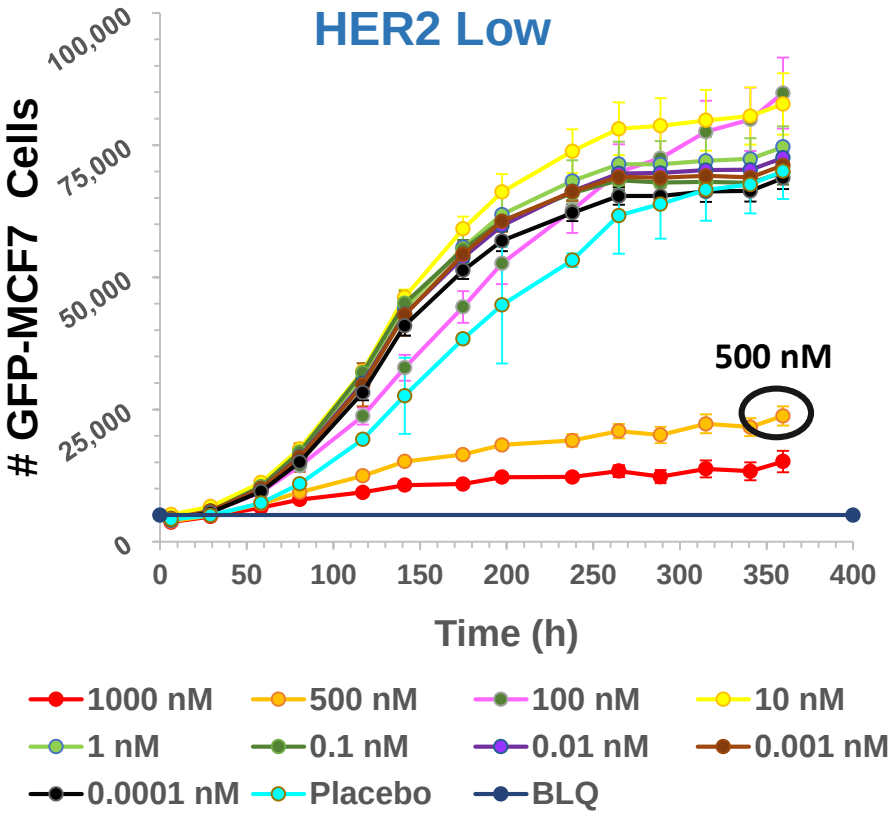
↓ IC_{50} MCF7 ↑ IC_{90} N87





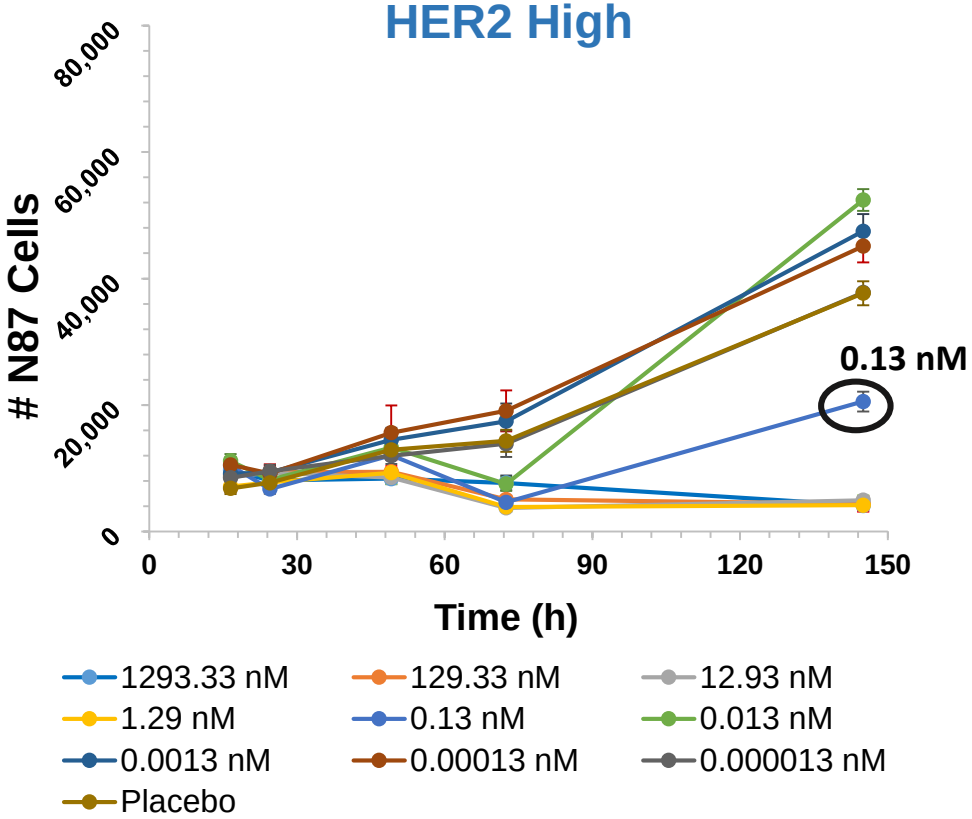
GFP-MCF7 Cell Viability

HER2 Low



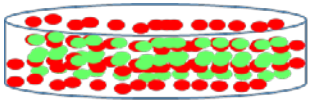
N87 Cell Viability

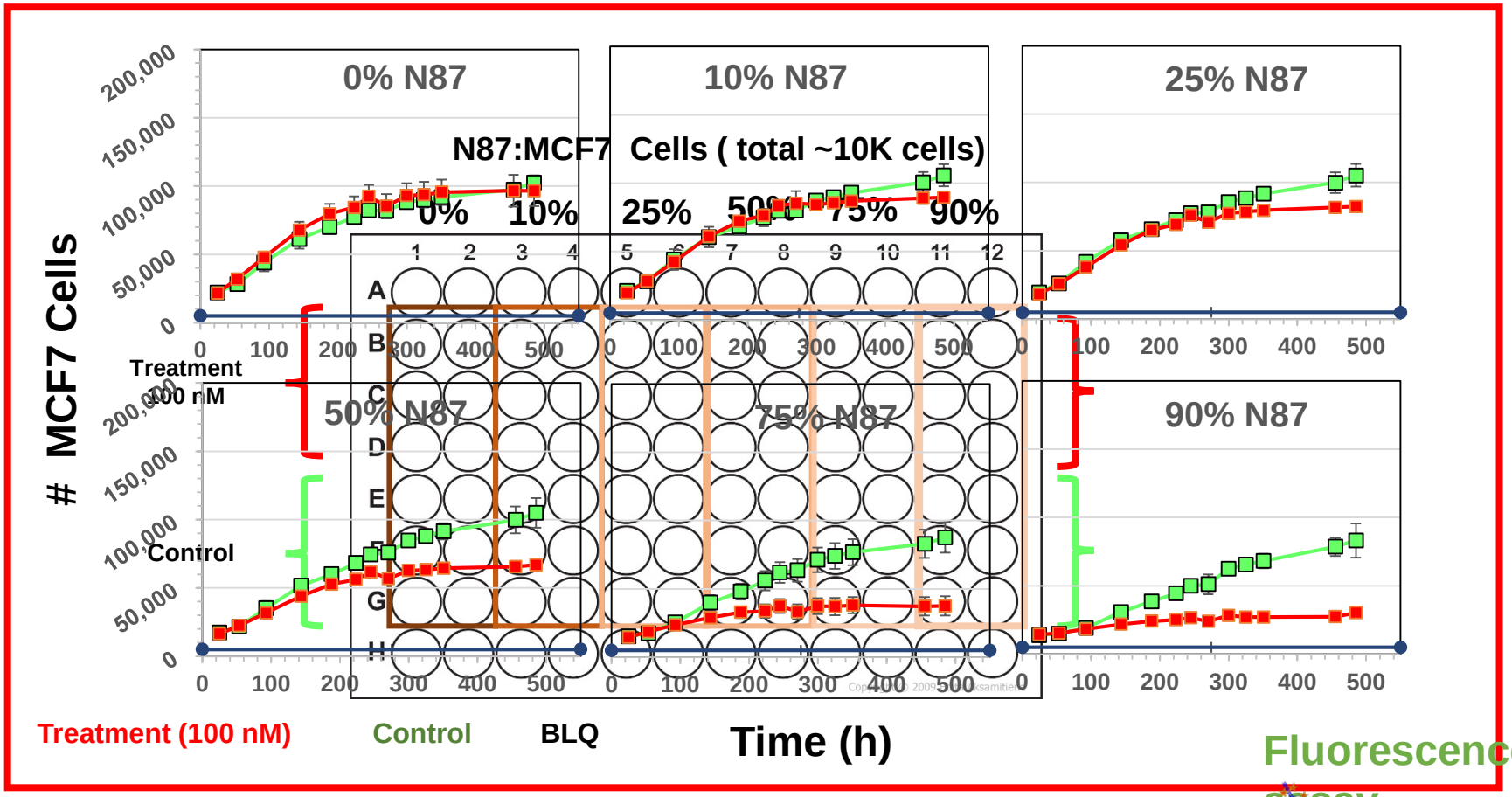
HER2 High



Quantification of Bystander Effect *In Vitro*

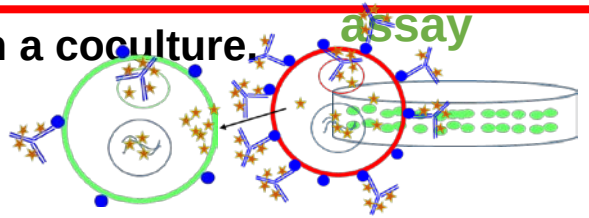
Cocultures with MCF7 and **N87 Cell Line**





Read Fluorescence to quantitate *MCF7 cells* in a coculture.

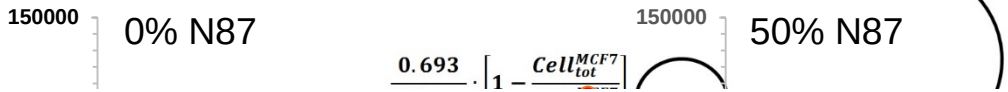
Fluorescence assay



Semi-mechanistic Modeling of Bystander Effect

Model Fittings:

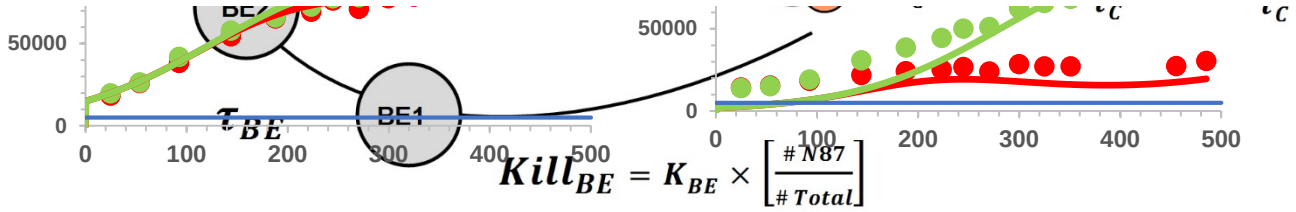
$$K1_{Kill}^{MCF7} = \frac{Kmax_{MCF7} \cdot ADC^{\gamma}}{IC_{50}^{\gamma} + ADC^{\gamma}}$$



$$\frac{0.693}{} \cdot \left[1 - \frac{Cell_{tot}^{MCF7}}{}\right]$$

Table 1 A list of parameters used for the development of the bystander effect PD model and their estimated values

Parameters	Units	Description	Estimate (%CV)
$Kmax_{N87}$, $Kmax_{MCF7}$	1/h	Maximum rate of T-vc-MMAE induced cell killing in the respective cell lines	0.023 (7.8 %), 0.016 (4.1 %)
IC_{50}^{N87}, IC_{50}^{MCF7}	nM	Concentrations of T-vc-MMAE that produces 50 % of maximum killing in the respective cell lines	0.19 (32.7 %), 353.3 (55.1 %)
DT_{N87}, DT_{MCF7}	h	Doubling times for the two cell lines	40.1 (20.1 %), 33.6 (1.5 %)
γ^{N87}, γ^{MCF7}	Unitless	Curve fitting parameter that determines the steepness of the concentration-effect relationship for each cell line	1.1 (15.8 %), 2.48 (18.1 %)
τ_C^{N87}, τ_C^{MCF7}	h	Transit time parameter used for the cell distribution model applied to each cell line	9.1 (34.2 %), 18.9 (25.4 %)
$Cell_{max}^{N87}$, $Cell_{max}^{MCF7}$	Number of cells	Maximum achievable cell number per well for each cell line	80,500 (34.2 %), 75,000 (1.86 %)
K_{BE}	1/h	Bystander killing constant	11.36 (15.2 %)
τ_{BE}	h	Transit time parameter used to capture the delay in the bystander killing	35.8 (13.9 %)



$$Kill_{BE} = K_{BE} \times \left[\frac{\#\,N87}{\#\,Total}\right]$$

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Aim 1

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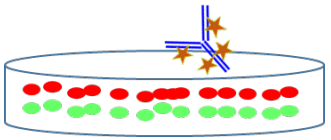

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Development of a Single-Cell PK model for ADC

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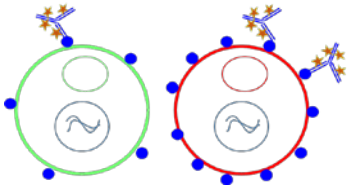

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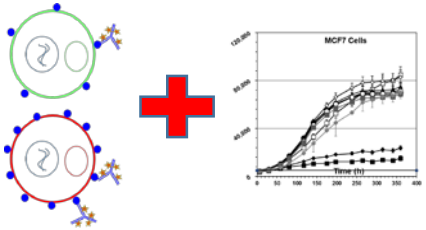

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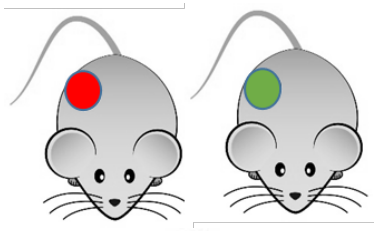

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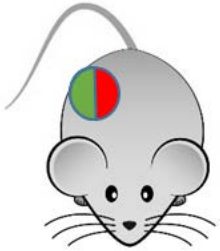
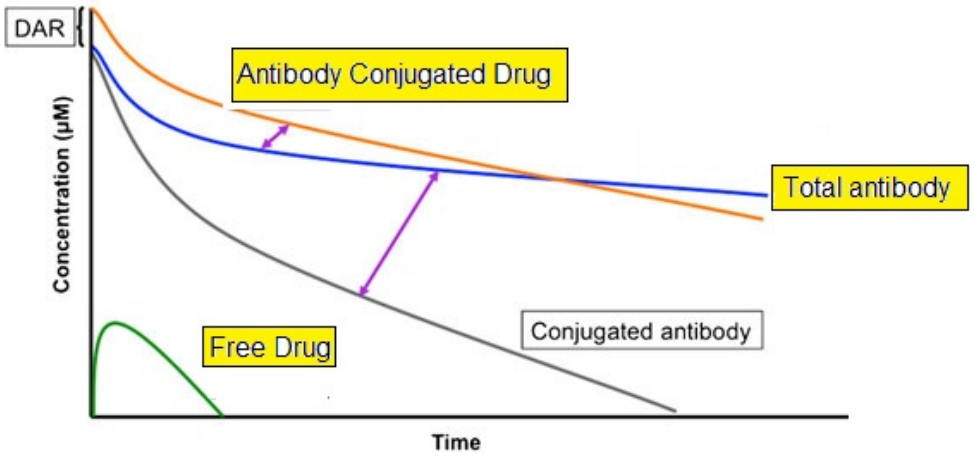
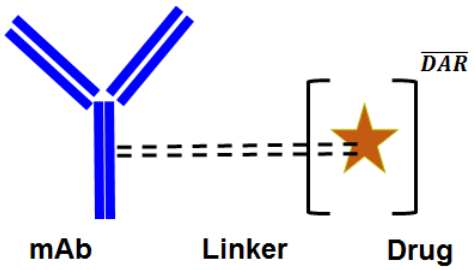
Perform TGI Studies in Heterogeneous Tumor Model with Ag+ and Ag-



Singh and Shah, Drug. Metab. Disp. 2017

Different Analytical Methods for T-vc-MMAE





- Heterogeneous Formulation
- Complex Pharmacokinetics

Development and Method Validation of 3 Analytical Techniques in Lab

Sandwich ELISA Method for
Total Antibody

LC-MS/MS Method for
Unconjugated MMAE

LC-MS/MS Method + Forced Deconjugation using *Papain* Enzyme for
Total MMAE

Single-Cell PK Model for MMAE

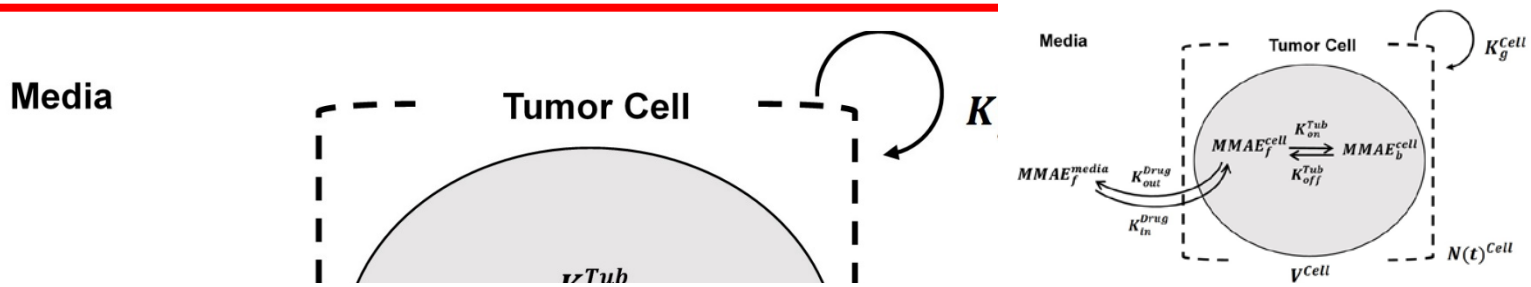
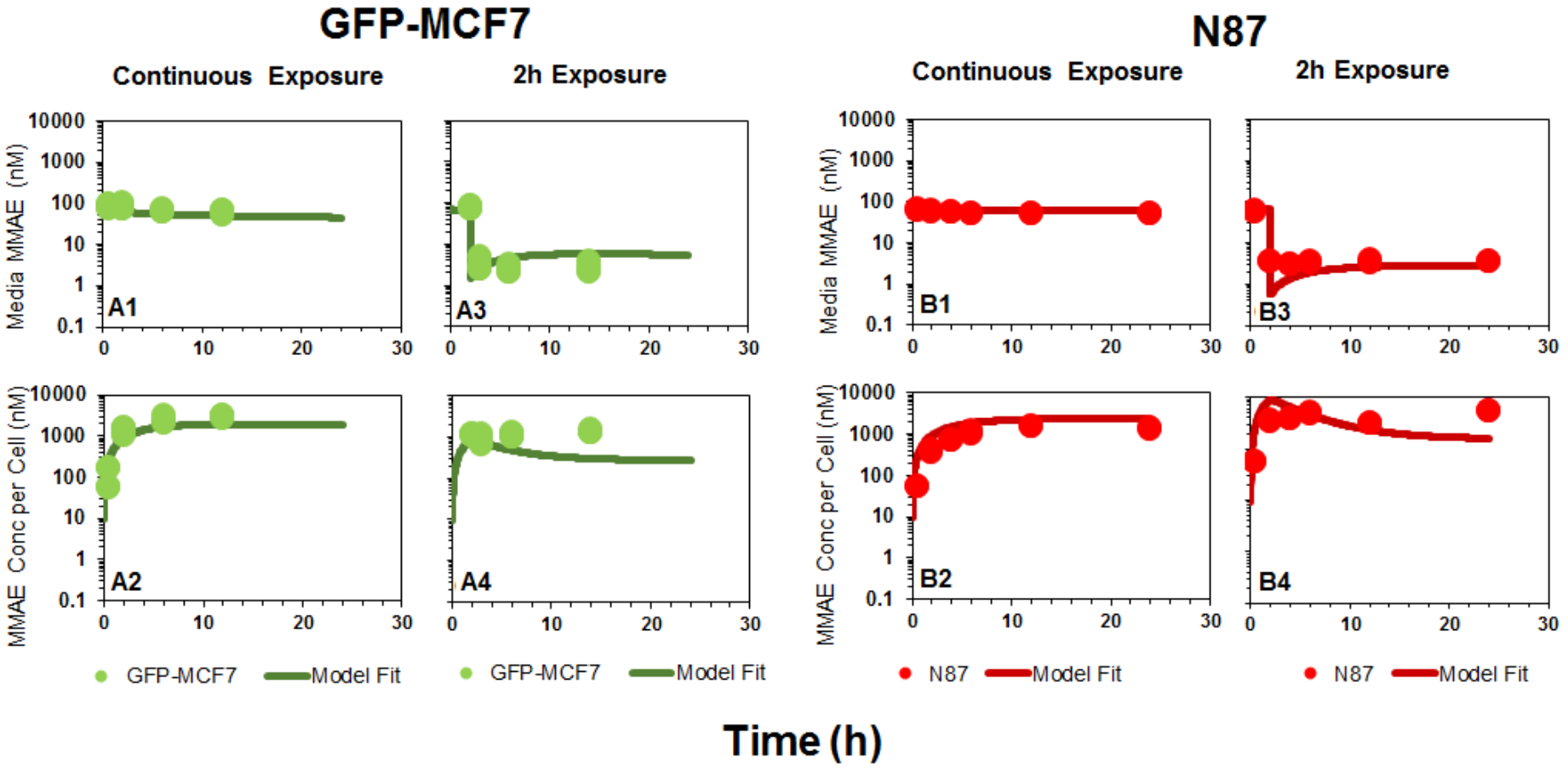
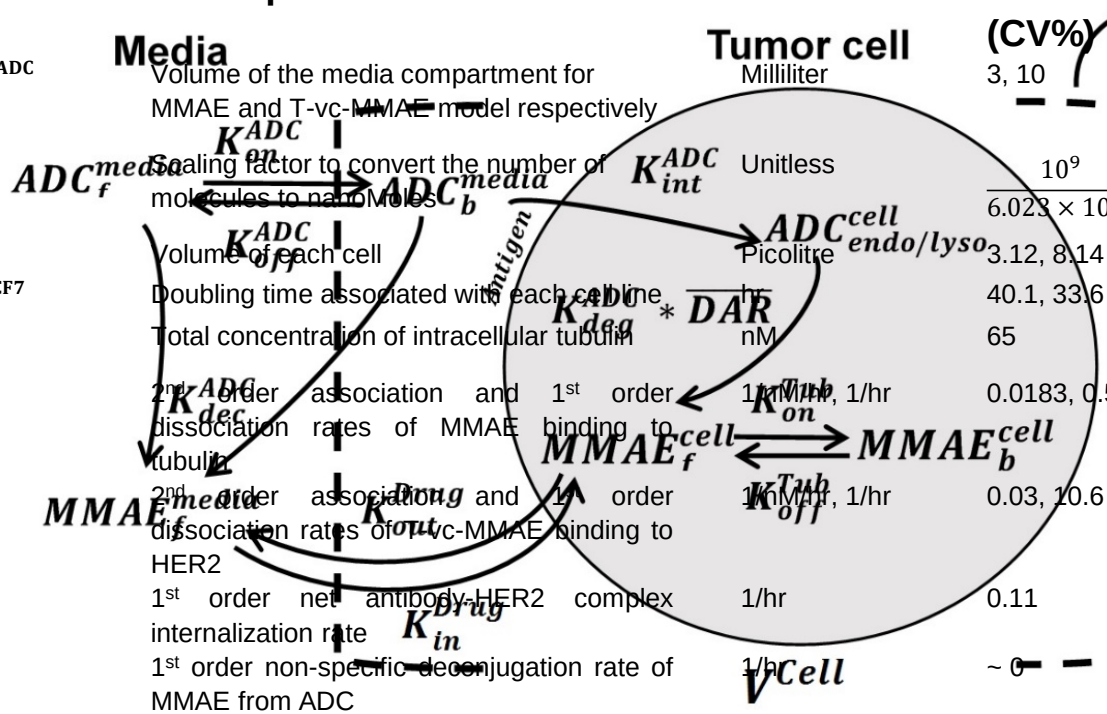


Figure 5



Single-Cell PK model for T-vc-MMAE



Parameters	Description	Units	Value (CV%)	Source
MV^{MMAE}, MV^{ADC}	Volume of the media compartment for MMAE and T-vc-MMAE model respectively	Milliliter	3, 10	Fixed
SF	Scaling factor to convert the number of molecules to nanoMoles	Unitless	$\frac{10^9}{6.023 \times 10^{23}}$	Fixed
V_{Cell}^{N87}, V_{Cell}^{MCF7}	Volume of each cell	Picolitre	3.12, 8.14	Fixed
DT^{N87}, DT^{MCF7}	Doubling time associated with each cell line	hr	40.1, 33.6	(Singh et al., 2016b)
$Tubulin^{Total}$	Total concentration of intracellular tubulin	nM	65	(Shah et al., 2012)
K_{on}^{Tub}, K_{off}^{Tub}	2nd order association and 1st order dissociation rates of MMAE binding to tubulin	1/nM/hr, 1/hr	0.0183, 0.545	(Shah et al., 2012)
K_{on}^{ADC}, K_{off}^{ADC}	2nd order association and 1st order dissociation rates of T-vc-MMAE binding to HER2	1/nM/hr, 1/hr	0.03, 10.6	(Maass et al., 2016)
K_{int}^{ADC}	1st order net antibody-HER2 complex internalization rate	1/hr	0.11	(Maass et al., 2016)
K_{dec}^{ADC}	1st order non-specific deconjugation rate of MMAE from ADC	1/hr	~ 0	Estimated to be very low value
$\overline{DAR}$	Average Drug: Antibody Ratio for the formulation of T-vc-MMAE	Unitless	4.5	Calculated using HIC (Singh et al., 2016b)
Ag_{HER2}^{Cell}	Number of HER2 receptors on N87 and GFP-MCF7 cells respectively	Unitless	950,000, 52,000	In-house
K_{deg}^{N87}, K_{deg}^{MCF7}	1st order rate of proteases-induced intracellular ADC degradation and MMAE release	1/h	0.03 (Maass), 0.353 (9%)	Estimated
K_{in}^{MMAE}	1st order inflow rate constant for MMAE from extracellular space to intracellular space for N87 and MCF7 cells respectively.	1/h	8.33 (8.5%)	Estimated
K_{out}^{MMAE}	1st order efflux rate constants for MMAE from intracellular space to extracellular space for N87 and MCF7 cells respectively	1/h	0.199 (22%)	Estimated

MMAE Modeling

Model Predictions for 3 Analytes of T-vc-MMAE