A ‘Dual’ Cell-Level Systems PK-PD Model to Characterize the Bystander Effect of ADC

Aman P. Singh[1] and Dhaval K. Shah[1*]

```
; A Dual In-Vitro PK Model for T-vc-MMAE in Cocultures of GFP-MCF7 and N87 Cells

Method STIFF

STARTTIME = 0                          ; hrs
STOPTIME = 120                         ; hrs
DT=1e-3                                ; Integration time step (only Euler, RK2, RK4)
DTMAX=0.001                            ; Maximum DT (only Auto stepsize and Stiff)
TOLERANCE=0.00001                      ; Relative accuracy for Auto and Stiff integration
methods
DTOUT = 1.0                            ; Output time interval (0, store every step)

;%%%%%%ADC Pharmacokinetics States %%%%%%%%%%%%

init ADC_M = Conc ; T-vc-MMAE in media in nM
init D_M = 0.1*MV; Amount of MMAE in Media in Nano Moles

init NC_N87 = (Percent_N87/100)*Total_init_cells
init NC_MCF7 = (1-Percent_N87/100)*Total_init_cells

init DAR = 4.8; Initial DAR value

init ADC_b_N87 = 0; Number of ADC-receptor complexes per cell (#/cell)
init ADC_lyso_N87 = 0; Number of ADC-receptor complexes in lysosomal space per cell (#/cell)
init D_f_N87 = 0; Number of Drug Molecules inside cytosol (#/cell)
init D_b_N87 = 0; Number of Drug Molecules bound to tubulin (#/cell)

init ADC_b_MCF7 = 0; Number of ADC-receptor complexes per cell (#/cell)
init ADC_lyso_MCF7 = 0; Number of ADC-receptor complexes in lysosomal space per cell
(#/cell)
init D_f_MCF7 = 0; Number of Drug Molecules inside cytosol (#/cell)
init D_b_MCF7 = 0; Number of Drug Molecules bound to tubulin (#/cell)

;%%%%%%ADC Pharmacokinetics Equations%%%%%%%%%%%%

SF = (1/6.023E+23)*1E+9 ; Scaling Factor to convert # molecules into nMoles
Conc = 75 ; Incubating Concentration of ADC (in nM)
Total_init_cells = 10000; total cell seeding density
Percent_N87 = 10; %
```

```
;%%%%%%%%%% MEDIA EQUATIONS %%%%%%%%%%%%%%%

d/dt(ADC_M) = (- Kon*ADC_M*(Agex_N87-ADC_b_N87) +
Koff*ADC_b_N87)*NC_N87*SF/MV+
(- Kon*ADC_M*(Agex_MCF7-ADC_b_MCF7) + Koff*ADC_b_MCF7)*NC_MCF7*SF/MV -
Kdec*ADC_M

d/dt(D_M) = Kdec*ADC_M*DAR*MV + (Kdec*ADC_b_N87*DAR +
Keff_N87*D_f_N87)*NC_N87*SF +(Kdec*ADC_b_MCF7*DAR +
Keff_MCF7*D_f_MCF7)*NC_MCF7*SF - Kdiff_N87*(Vcell_N87/MV)*D_M -
Kdiff_MCF7*(Vcell_MCF7/MV)*D_M

;%%%%%%%%%% CELL EQUATIONS in NUMBER N87 %%%%%%%%%%%

d/dt(ADC_b_N87) = Kon*ADC_M*(Agex_N87-ADC_b_N87) - Koff*ADC_b_N87 -
Kdec*ADC_b_N87 - Kint*ADC_b_N87

d/dt(ADC_lyso_N87) = Kint*ADC_b_N87 - Kdeg*ADC_lyso_N87

d/dt(D_f_N87) = Kdeg*ADC_lyso_N87*DAR - Keff_N87*D_f_N87  -
Kon_tub_N87*D_f_N87*(Tub_N87-D_b_N87) + Koff_tub*D_b_N87
+Kdiff_N87*(Vcell_N87/MV)*(D_M/SF)

d/dt(D_b_N87) = Kon_tub_N87*D_f_N87*(Tub_N87-D_b_N87) - Koff_tub*D_b_N87

;%%%%%%%%%% CELL EQUATIONS in NUMBER N87 (END) %%%%%%%%%%%

;%%%%%%%%%% CELL EQUATIONS in NUMBER MCF7 %%%%%%%%%%%

d/dt(ADC_b_MCF7) = Kon*ADC_M*(Agex_MCF7-ADC_b_MCF7) - Koff*ADC_b_MCF7 -
Kdec*ADC_b_MCF7 - Kint*ADC_b_MCF7

d/dt(ADC_lyso_MCF7) = Kint*ADC_b_MCF7 - Kdeg*ADC_lyso_MCF7

d/dt(D_f_MCF7) = Kdeg*ADC_lyso_MCF7*DAR - Keff_MCF7*D_f_MCF7  -
Kon_tub_MCF7*D_f_MCF7*(Tub_MCF7-D_b_MCF7) + Koff_tub*D_b_MCF7
+Kdiff_MCF7*(Vcell_MCF7/MV)*(D_M/SF)

d/dt(D_b_MCF7) = Kon_tub_MCF7*D_f_MCF7*(Tub_MCF7-D_b_MCF7) - Koff_tub*D_b_MCF7

;%%%%%%%%%% CELL EQUATIONS in NUMBER MCF7  (END) %%%%%%%%%%%

DT_MCF7 =35.0; hours
DT_N87 = 40.10; hours

MCF7_max = 100; 000
N87_max = 80.5


Kg_MCF7 = (0.693/DT_MCF7 )*(1-(NC_MCF7)/(MCF7_max *1e+3));
```

```
Kg_N87 = (0.693/DT_N87 )*(1-(NC_N87)/(N87_max *1e+3))

d/dt(NC_N87) = Kg_N87*NC_N87
d/dt(NC_MCF7) = Kg_MCF7*NC_MCF7

;%%%% ADC Deconjugation Rate %%%%%%%%%%

d/dt(DAR) = -Kdec*DAR

;%%%%%%%%Parameters%%%%%%%%%%%%%%%%%%%%%%%%%

Vcell_MCF7 =  8*1E-12; L volume of MCF7 cells
Vcell_N87 =  4.12*1E-12; L volume of N87 cells
MV = 0.0001; L (media volume)
Kdiff_N87 = 2.41; 1/h (estimated)
Kdiff_MCF7 = 7.72; 1/h (estimated)
Keff_MCF7 = 0.131
Keff_N87 = 0.156
Tub_N87 = (65*Vcell_N87)/SF; #/cell of tubulin in a Cell
Tub_MCF7 = (65*Vcell_MCF7)/SF; #/cell of tubulin in a Cell
Kon_tub_N87 = 0.44*SF*Vcell_N87/24; 1/#/Cell/hr
Kon_tub_MCF7 = 0.44*SF*Vcell_MCF7/24; 1/#/Cell/hr
Koff_tub = 13.1/24; 1/hr
Kon = 0.03; 1/nM/hr
Koff = 0.014; 1/hr
Kint = 0.11; 1/hr
Kdeg = 0.3; 1/hr
Kdec = 0.000007; 1/hr

Agex_MCF7 = 0.052*1e+6; # of HER2 receptors on a cell.
Agex_N87 = 0.95*1e+6; # of HER2 receptors on a cell.

;%%%%%%%%%% OUTPUT %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

Media_MMAE_f = D_M/MV ; Concentration of MMAE in Media (nM)
Media_MMAE_T = ((D_M/MV)+ADC_M*DAR) ; Concentration of Total MMAE in Media (nM)
Media_TTmAb = ADC_M ; Concentration of Total Antibody in Media (nM)

Cell_MMAE_f_MCF7= (D_f_MCF7 + D_b_MCF7)*SF/Vcell_MCF7;
Cell_MMAE_f_N87= (D_f_N87 + D_b_N87)*SF/Vcell_N87

Cell_MMAE_T_MCF7= (D_f_MCF7 +
D_b_MCF7+(ADC_b_MCF7+ADC_lyso_MCF7)*DAR)*SF/Vcell_MCF7
Cell_MMAE_T_N87= (D_f_N87 +
D_b_N87+(ADC_b_N87+ADC_lyso_N87)*DAR)*SF/Vcell_N87

Cell_TTmAb_MCF7 = (ADC_lyso_MCF7)*SF/(Vcell_MCF7)  ; Concentration of Total Antibody in
Cell (nM)
Cell_TTmAb_N87 = (ADC_lyso_N87)*SF/(Vcell_N87)  ; Concentration of Total Antibody in Cell
(nM)
```