Where Do New Medicines Come From? Mechanistic Modelling and Simulation for Bispecific Antibodies

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There is high unmet need in every disease area

**Coming up with new drugs is not getting any easier**

– The low hanging fruit have long since been taken
– High expectations regarding efficacy and safety
– Better than the existing alternatives, should these exist

*It’s a bit like having to be “better than The Beatles”, all the time, every time*
The rise of biologics

Small molecule drugs
- Naturally occurring: salicylic acid, penicillin, morphine…
- Synthetic: from modified natural medicines (e.g. aspirin) to fully man-made with no connection to anything found in nature

Protein drugs
- Naturally occurring: insulin, EPO, growth hormones, enzymes…
- Engineered: mAbs mimicking wild-type antibodies and beyond

From small molecule drugs to biologics

Many alternative formats for multispecific mAbs have been proposed

Bispecific vs combination therapy? Which format?

The elephant in the room, occasionally

- One bispecific mAb or two monospecific mAbs: what’s the difference?

- Is the additional time, effort and risk of developing a bispecific mAb justified?

Polypharmacology: when two or more is better than one

- Co-dosing of two or more drugs can be beneficial
  - Infectious: HAART
  - Inflammatory: Antiasthmatics
  - Oncology: NCE and mAbs

- Which target combinations?
  - Experimental insight
  - QSP: in silico modelling
  - Combinatorial screening

Bispecific mAb target space

In clinical trials

- **Oncology** (27/31)
  - T cell engagement (15/31)
  - Immuno-Inflammation (3/31)

- Target expression
  - Both in solution (5/31)
  - One in solution, the other on a cell (2/31)
  - Both on cell surface
    - Same cell (6/31)
    - Different cells (18/31)

Bispecific antibody targets

- One in solution, the other on the cell
- On the same cell
- On two different cells

**In silico insight**

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**Experimental data**

**Mechanistic mathematical model**
- Rate equations

**Parameters, measured or fitted**
- $k_{on}$, $k_{off}$, $K_d$, $D$, $IC_{50}$, $EC_{50}$...

**Predictions**
- New drug formats
- Affinity and dosing
The binding activities of Fab arms are independent

- Both targets are in solution
  - Only mAb binding site concentration matters
  - No difference between a bispecific mAb and combination of monospecific ones is expected

- One of the targets in on cell membrane
  - Monospecific mAb binding to the membrane target benefits from the avidity effect
  - At the same molar dose, the combination can be more efficacious against the membrane target than a bispecific

Mechanistic Modeling and Simulation for Bispecific Antibodies
Both targets on the same cell

A number of different approaches have been used

- Assume the targets to behave as if both were in solution:
  - How to handle the volume?

- Assume that the targets are *immobile* on membrane
  - How about target cross-linking?

- Membrane proteins are *mobile* in lipid bilayer
  - Lateral diffusion is experimentally measurable

For example: at 50000 receptors $A$ and $B$ per cell, the average distance between them is $\approx 60$ nm
Experimental data: Anti-CD4/CD70 bispecific DuetMab

- **T Lymphocytes, targets per cell**
  - $CD4^+CD70^+$ (46000:52000)
  - $CD4^+CD70^-$ (38000:<100)
  - $CD4^-CD70^+$ (<100 :≈31000)

- **CD4$^+$CD70$^+$ cells**
  - All bound DuetMab is ib cross-linking complex with CD4 and CD70
  - No DuetMab is attached monovalently, i.e. to CD4 or CD70 only
  - Strong binding even at DuetMab concentrations below respective Kd values

- **CD4$^+$CD70$^-$ and CD4$^-$CD70$^+$ cells**
  - DuetMab binding is dictated by concentration and Kd

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Kinetic model of DuetMab binding to CD4+CD70+ cells

- Sequential binding of DuetMab to CD4 and CD70
  - Trimolecular reactions are very rare

- Lateral diffusion of proteins in cell membrane
  - At typical $D=10^{-10}$ cm$^2$/s, mean displacement in 1s is $\approx 200$ nm
  - A typical monovalent mAb-target complex dissociation $t_{1/2} \geq 2h$

- Simulate DuetMab binding and compare with experiment
  - Monte Carlo numerical and ODE analytical
Monte Carlo simulation for DuetMab binding

**MCell3** model of a cell with 46000 CD4 and 52000 CD70 molecules

- Virtual cuboid $0.1 \times 0.1 \times H \ \mu m$
  - 31 CD4 and/or 39 CD70 diffusing on bottom surface
  - $\approx 700$ DuetMab diffusing in volume
  - DuetMab monovalent complex with CD4 or CD70
  - DuetMab cross-linking complex with CD4 and CD70

- Reaction-diffusion in volume and on surface
  - Brownian motion
  - 1-100 $\mu s$ time steps, experimental parameters

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DuetMab binding to $\text{CD4}^+$, $\text{CD70}^+$ or $\text{CD4}^+\text{CD70}^+$ cells

Simulation:
1 nM DuetMab on $\text{CD4}^+\text{CD70}^+$ cells

Experiment vs simulation
Total bound DuetMab binding after 1-hour incubation

Only DuetMab cross-linked complex with $\text{CD4}^+$ and $\text{CD70}^+$ accumulates

DuetMab binding dose-response curves are predicted for all cell types

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DuetMab \( K_{d(CD4)} = 0.9, 10, 17, 42, 63, 69 \text{nM} \); \( K_{d(CD70)} = 25 \text{nM} \)

- Surface molecules in surface concentration units
- Surface reaction \( k_{on} \) is surface diffusion limited

Good agreement between experimental data and ODE, Monte Carlo predictions is observed

Mazor, Y. et al. (2015) mAbs 7(3): 461-469,


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**In silico** insight: mAb binding can be kinetically limited

Bimolecular reactions can be diffusion-limited and very slow at low concentrations


A Mechanistic Model for Bispecific Antibody Binding to Cell Surface Targets
In *silico* insight: target cross-linking and mAb dissociation

ODE model: DuetMab $K_d$(CD4) = 0.9, 10, 17, 42, 63, 69 nM; $K_d$(CD70) = 25 nM

- Simulated dissociation from pre-formed complex
  - Monovalent
    - $t_{1/2}$(CD4) = 19 s to 45 min
    - $t_{1/2}$(CD70) = 2.5 min
  - Bivalent cross-linking is effectively irreversible:
    - Terminal half-life: $t_{1/2} = 83$ h to 16 months
    - Target internalization is likely to be *much* faster

- The net result is up to $10^4$-fold more stable binding of DuetMab to CD4$^+$CD70$^+$ cells through *avidity* effect

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Avidity effect can be lower for a normal mAb
- Fab rotation around the hinge may be required for cross-linking
- Membrane distortion or target conformation change
- A suitable combination of epitopes and paratopes in a bispecific format may alleviate these constraints in biparatopic format

Dimeric receptors
- Homodimeric receptor subject to 180° rotational symmetry
- Epitopes are unlikely to be accessible to the same mAb
- Cross-linking of two dimers is more likely
- Linear oligomers on cell surface could form

Beyond bispecifics: Biparatopics and dimeric targets
Reactions on cell surface follow 2D kinetics
- DuetMab on CD4/CD70 cells
- EGF
- INFγ...

Cellular synapse can be considered a 2D space
- TCR-pMHC complex
- *Perhaps all PPIs across cellular synapse*

A bispecific for cell-cell cross-linking
- A bispecific mAb or fragment bound to a receptor cross-links to another on the other cell
Physiologically based pharmacokinetics

- Bispecific vs monospecific target engagement in all organs

Cross-species

- Mouse-rat-monkey-human

Parameters:

- Experimental
  - Organ volumes
  - Blood flow rates
  - Glomerular filtration

- Empirically estimated
  - Lymph flow rates

Integration into PBPK for exposure and dose prediction
Conclusion: Bispecific mAbs can be precision medicines

Bispecific mAbs can be designed to bind predominantly cells simultaneously expressing two different antigens only

- Lateral diffusion allows rapid ternary complex formation on cell surface
- The cross-linked complexes are very stable
- First principles modelling and simulation was possible and can guide
  - No empirical fitting involved, all parameters are measurable
  - Volume reaction constants by SPR
  - Surface association reaction constant calculated from diffusion coefficient

The challenges we have met

- Experimental: protein engineering, linkers, stability, PK
- Modeling: Mechanistic framework for cell-cell cross-linking scenario
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