

Unraveling killing kinetics: real-time cell analysis in oncology

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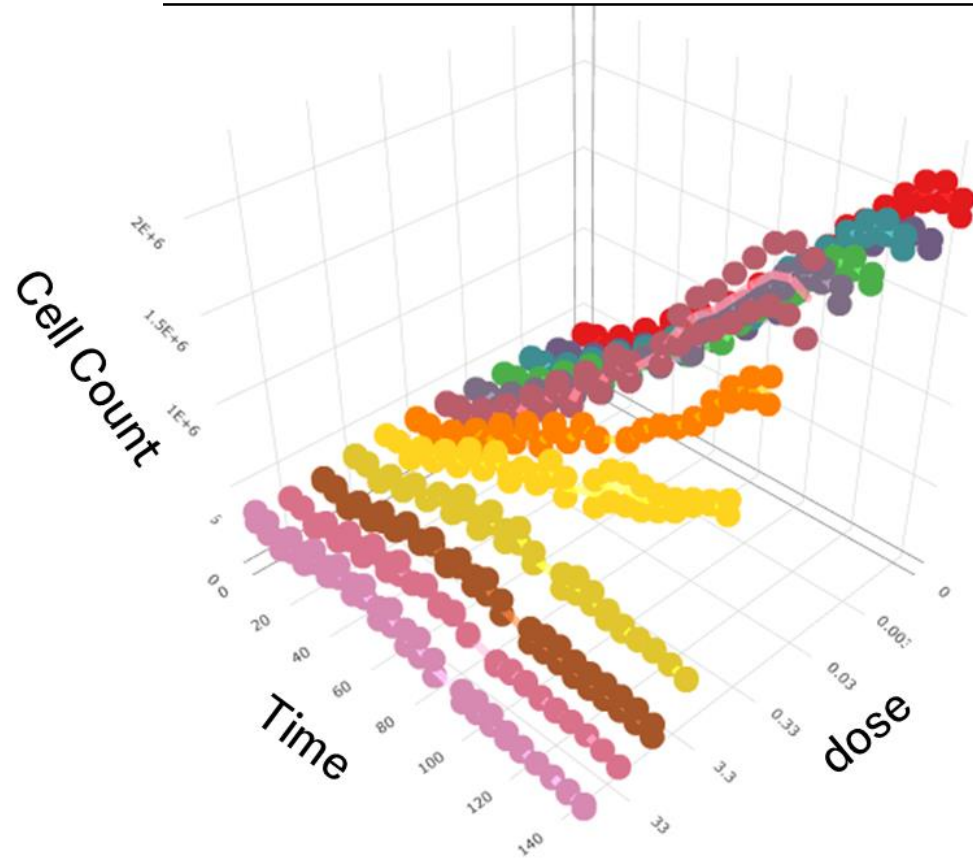
Johnson & Johnson
Innovative Medicine

Introduction: cell counts in real-time

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×CELLigence

= Real-time cell analysis assays

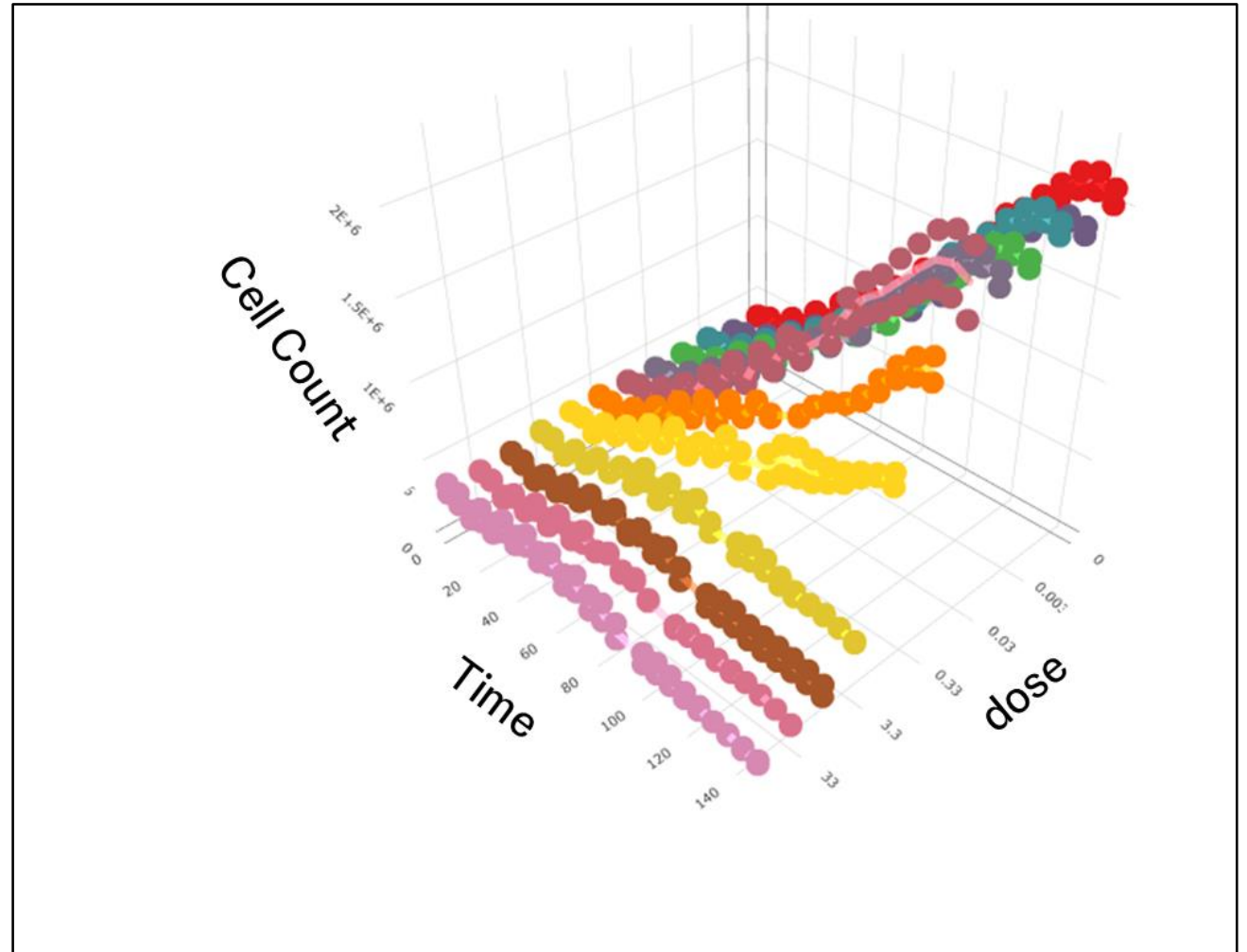


In oncology:
Measuring cell proliferation to understand impact of treatment on cell count.

Introduction: cell counts in real-time

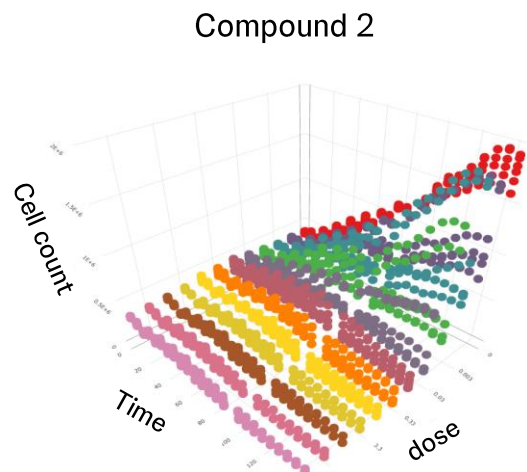
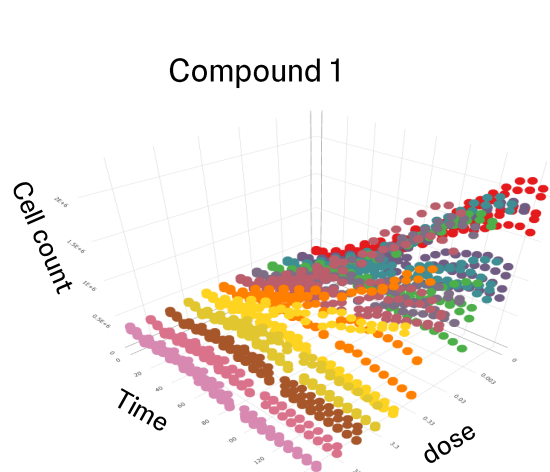
Experimental setup:

- Read-out: Cell Count
- Variables:
 - Time (every x hours, for period t)
 - Dose levels (at least 6)
- Replicates:
 - Technical
 - Biological (e.g. donors)



Motivating example

Compare compounds based on **maximum killing capacity** and **killing kinetics**.



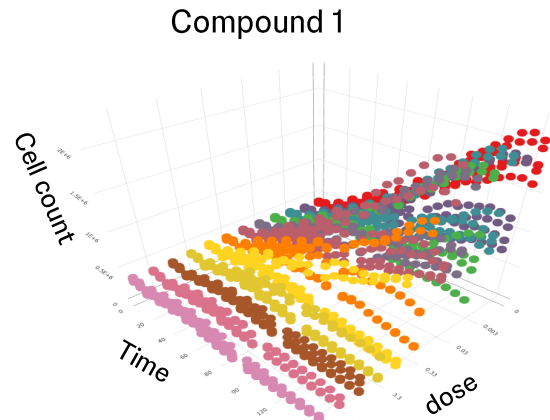
- Which compound kills the quickest?
- Which compounds kill most cells?
- Which compound kills at lower concentrations?



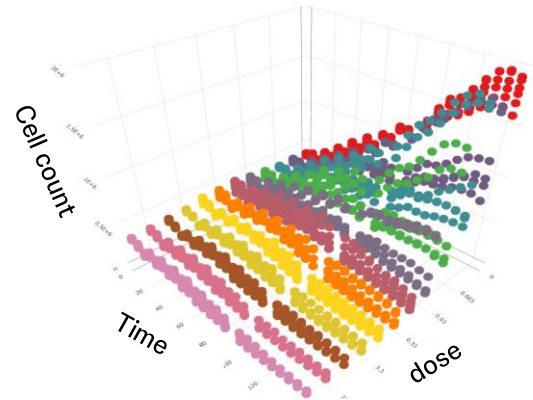
Which compound has best efficacy?

Typical analysis: focus on dose response

3D data

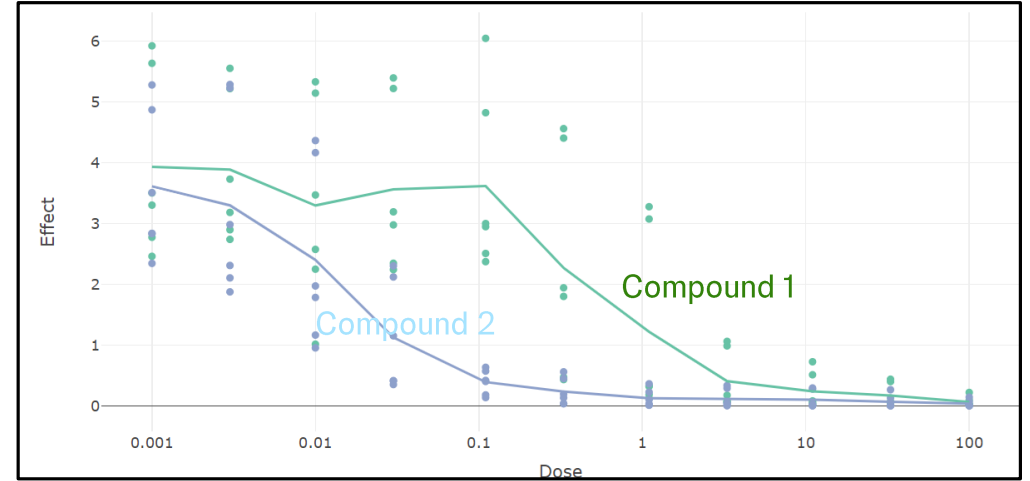


Compound 2

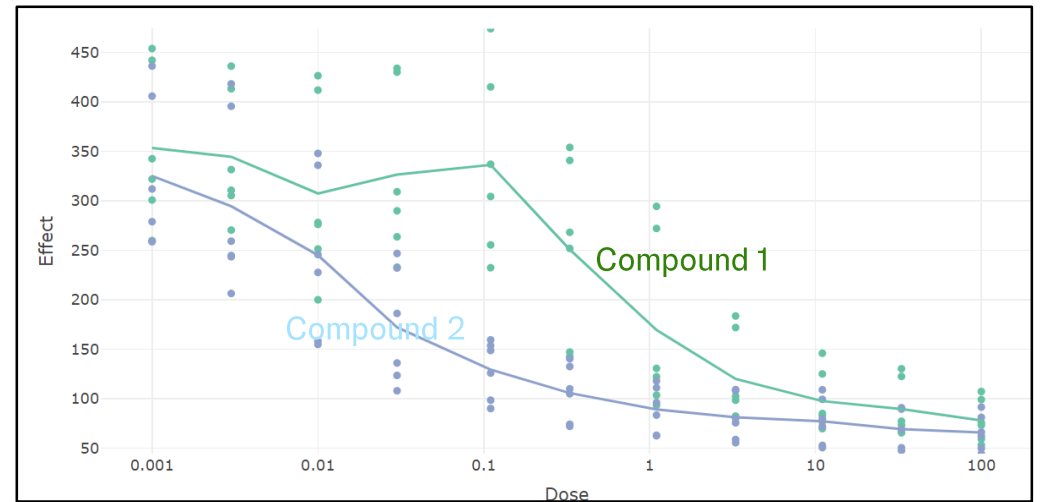


2D data

Cross-sectional at time= t (e.g., t= 144 h)

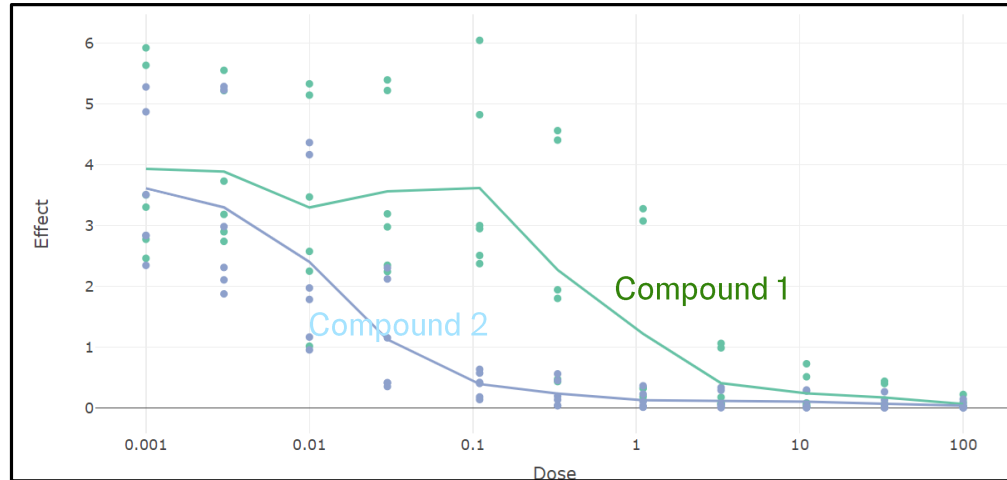


AUC-based (summarized over time)

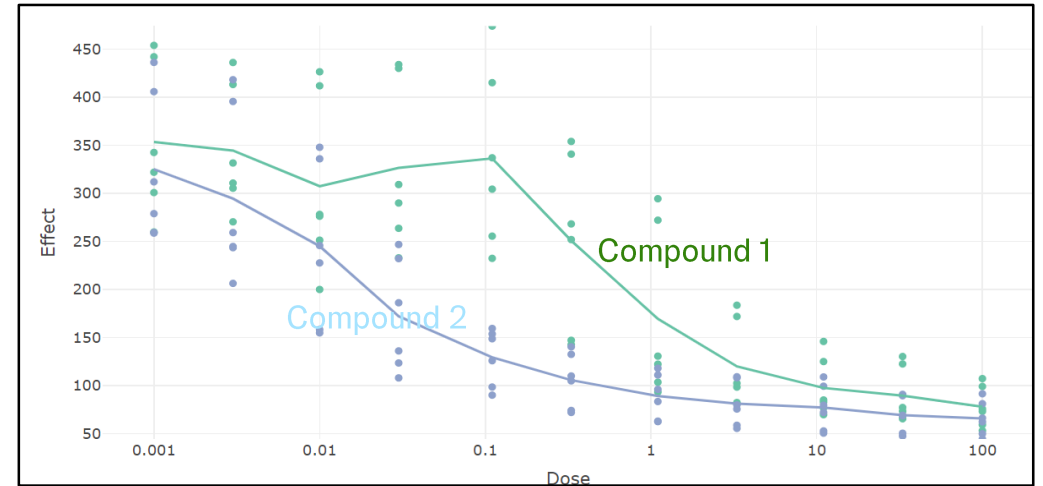


Dose response analysis results

T= 144



AUC based

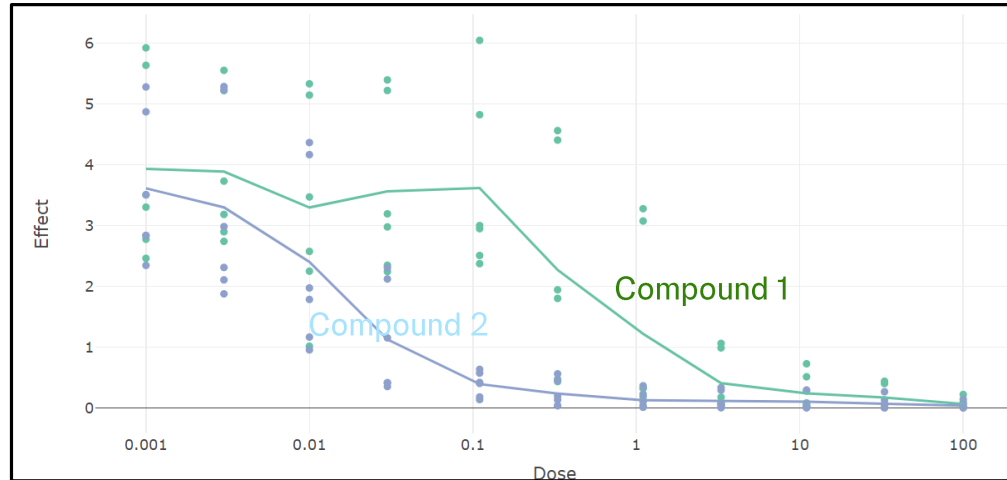


	EC50 [95% CI]	EC50 [95% CI]
Compound 1	0.47 [0.18, 1.21]	0.57 [0.27, 1.20]
Compound 2	0.014 [0.0068, 0.028]	0.014 [0.0049, 0.04]

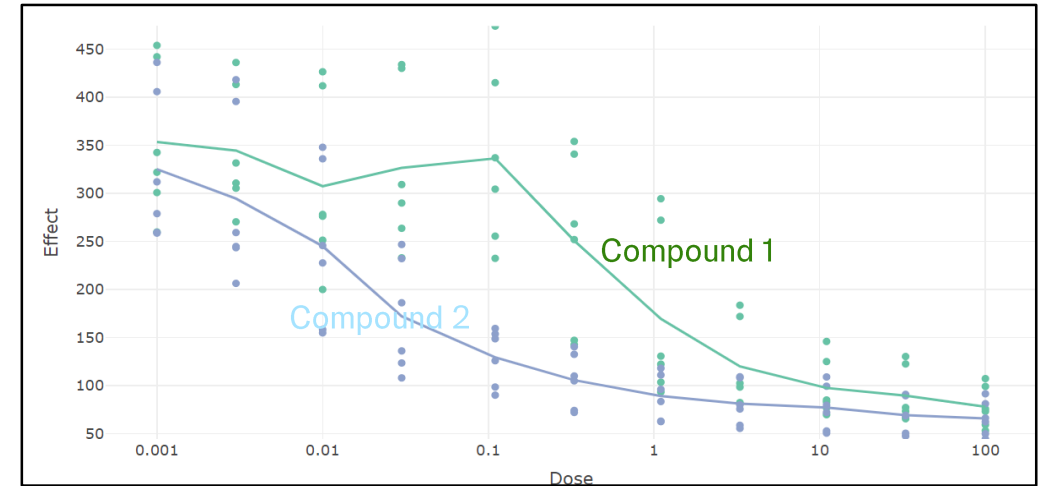
- Both compounds are able to kill all cancer cells (same lower asymptote)
- Compound 2 more potent (start killing at lower concentration)

Dose response analysis results

T= 144



AUC based



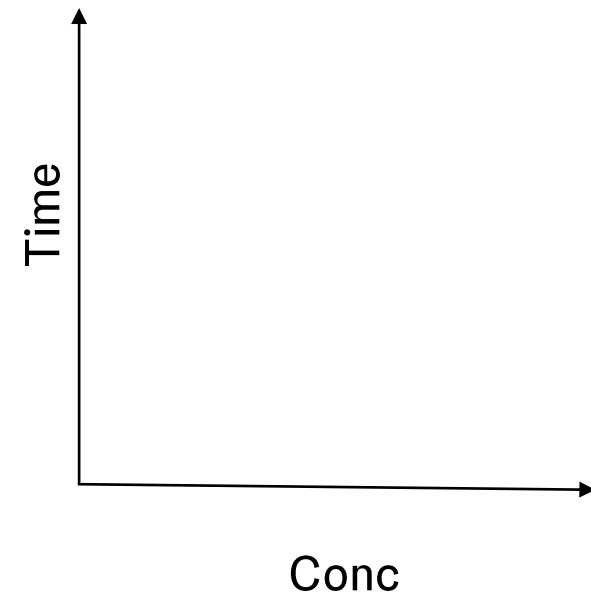
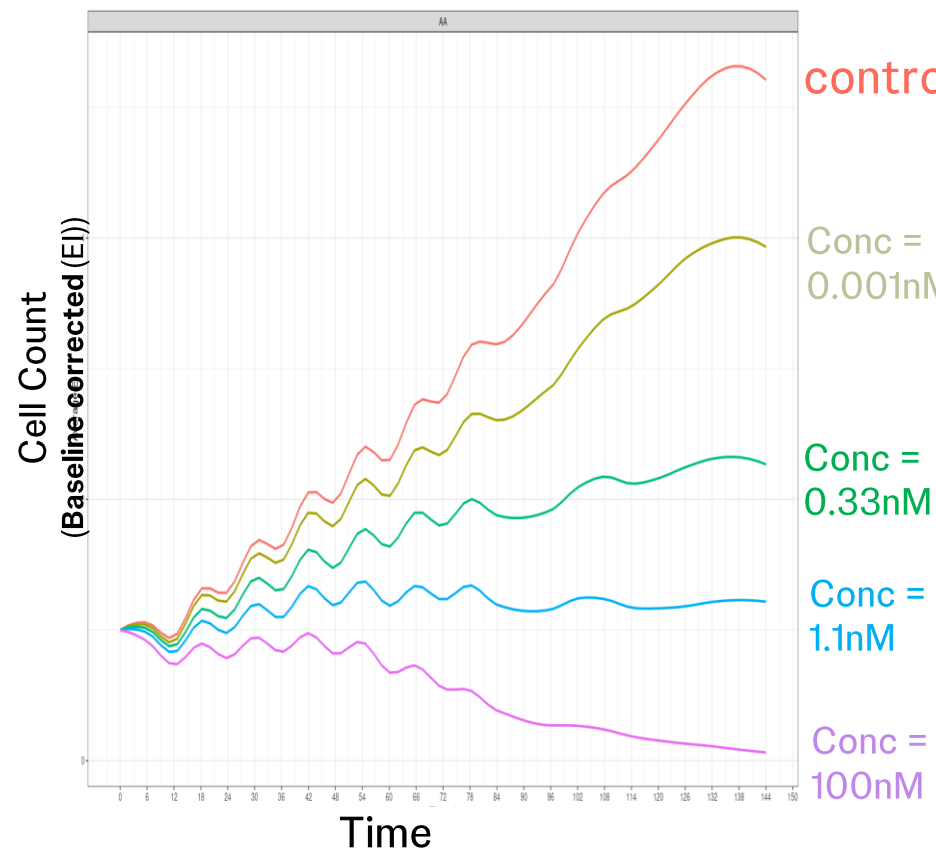
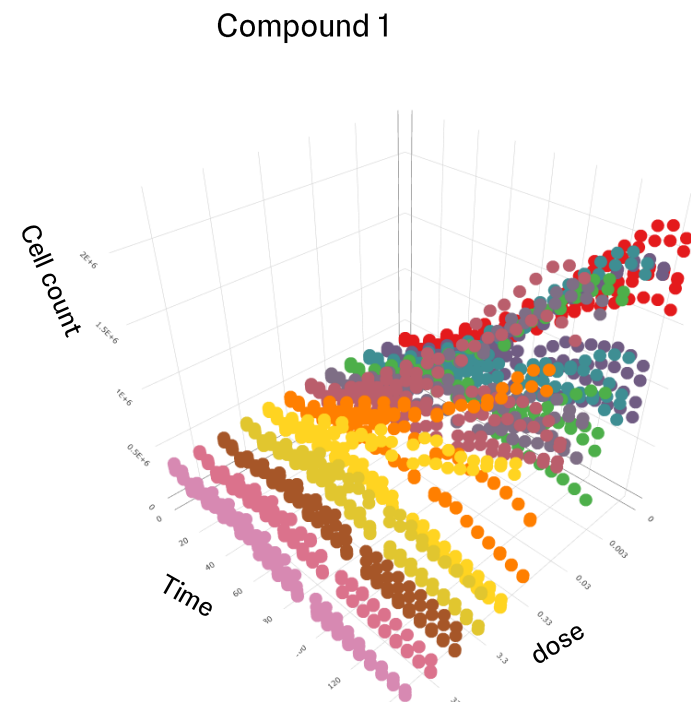
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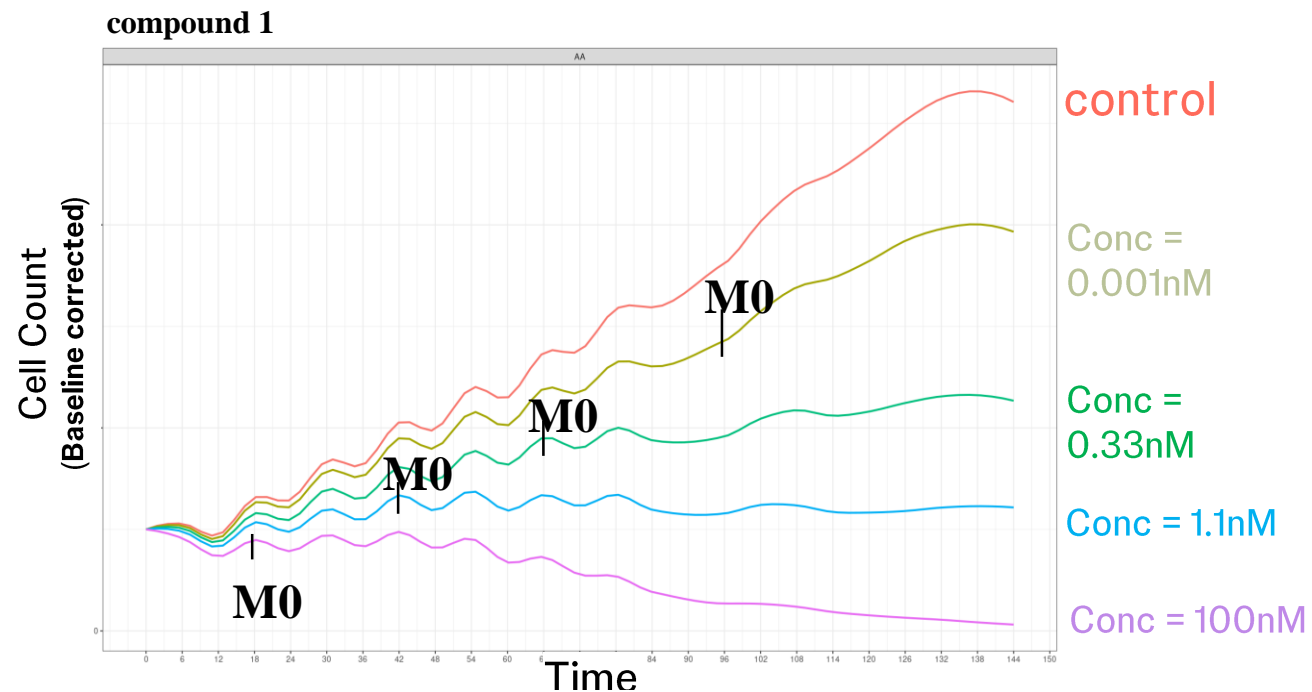
- Both compounds are able to kill all cancer cells (same lower asymptote)
- Compound 2 more potent (start killing at lower concentration)
- Does Compound 2 also start killing at earlier timepoint ??

Understanding killing kinetic

How to bring in time aspect?



Understanding killing kinetic



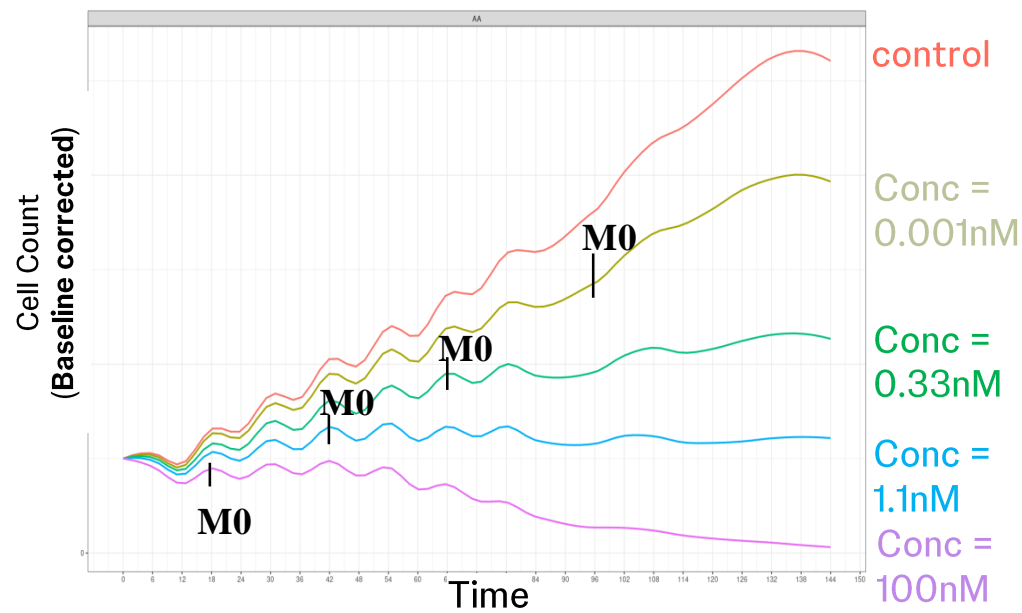
At each concentration extract:

MO = Earliest time at which we can statistically detect difference in cell count compared to control

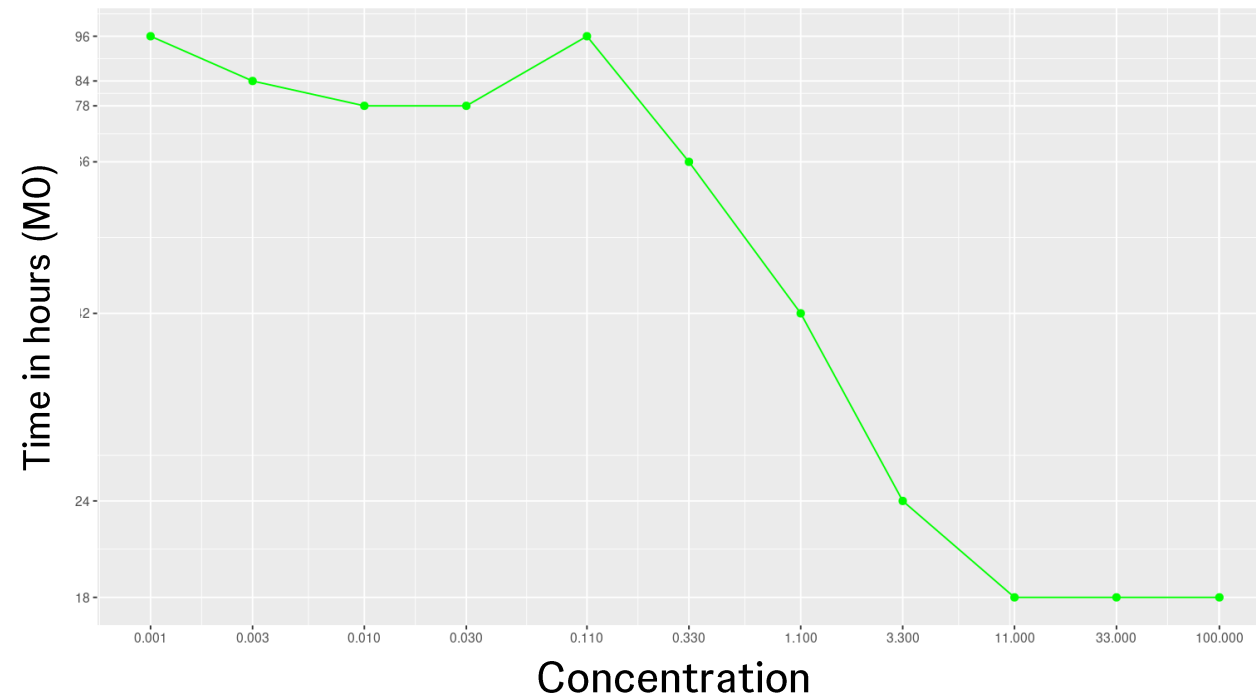
If no difference: **MO** = > last timepoint **t**

$$EI_{i,j} = \alpha + \beta Treatment_i + \delta_j factor(Time_{i,j}) + \gamma_j Treatment_i \times factor(Time_{i,j}) + \epsilon_{i,j}$$

Understanding killing kinetic: results



M0 time-concentration profile: compound 1

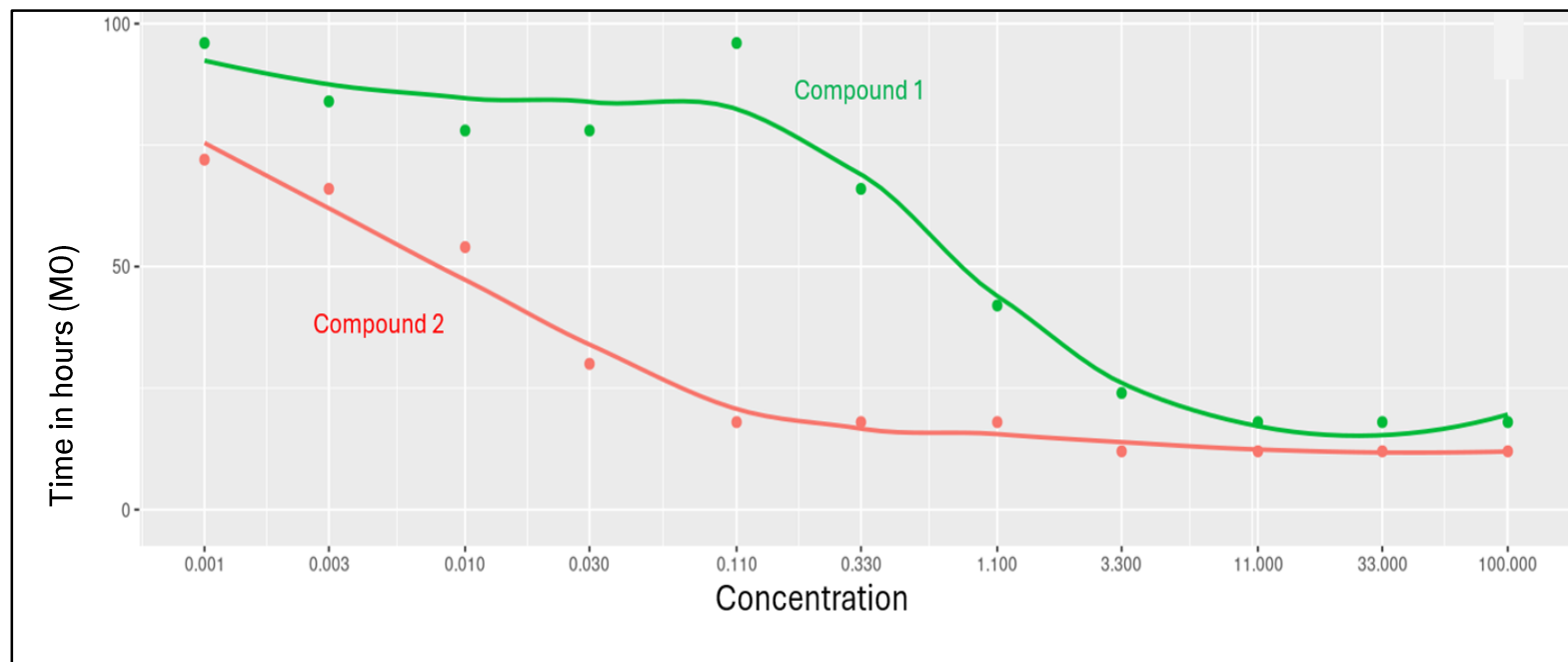


Min M0 = 18h; earliest timepoint where at least one concentration show difference from control

Max M0 = 96h; earliest timepoint at which all concentrations show difference from control

Understanding killing kinetic: results

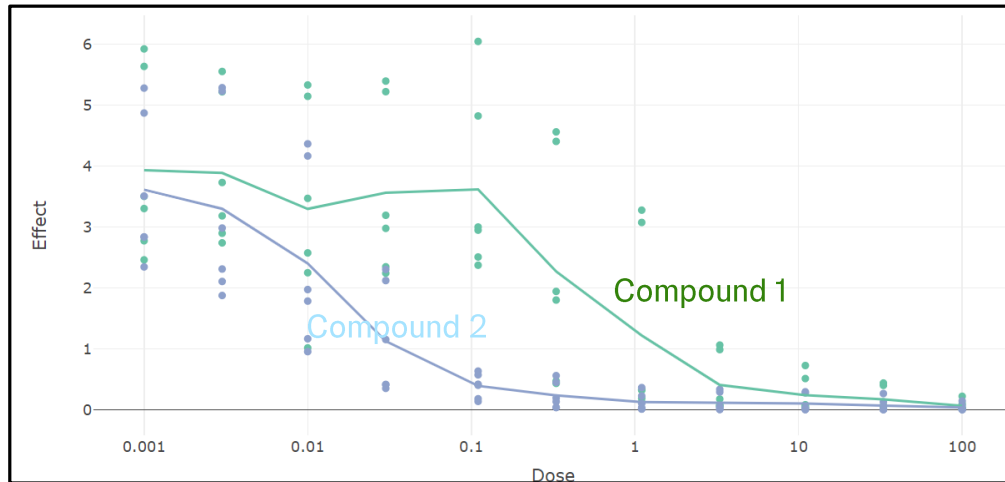
M0 time-concentration profile:



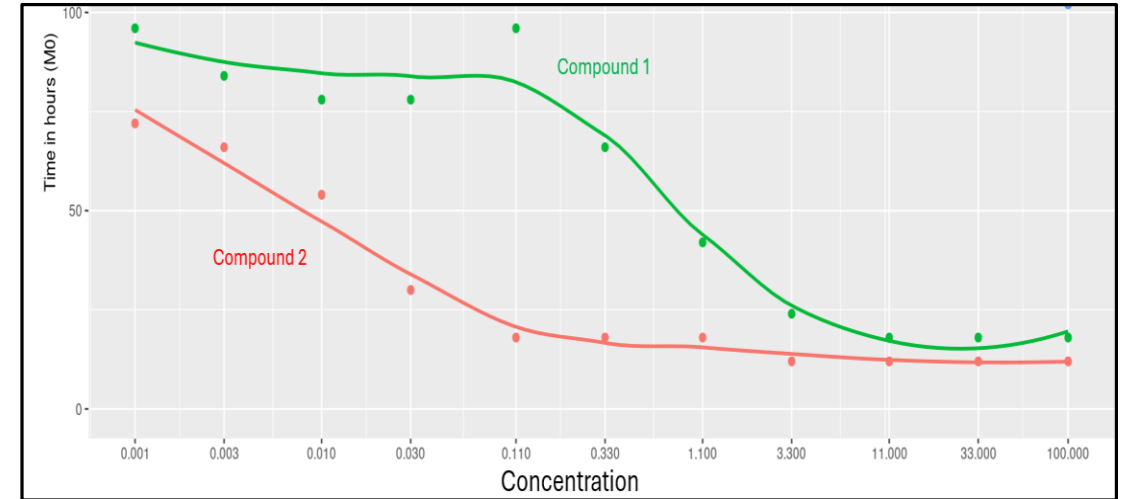
- Both compounds showed significant effect much earlier than the last time point,
- Compound 2 showed earlier significant killing in time and concentration
- Compound 1 shows a steeper decline in time-concentration profile

Comparing compounds: Dose response & killing kinetic

Dose response analysis:



M0 kinetic time-concentration profile



	EC50 estimate (95% CI)
Compound 1	0.47 (0.18, 1.21)
Compound 2	0.014 (0.0068, 0.028)

- Both compounds are able to kill all cancer cells (same lower asymptote)
- Compound 2 more potent (start killing at lower concentration)
- Compound 2 start killing not only at lower concentrations, but also at earlier timepoints

Complement each other

Conclusions

- MO metric provides additional information on the time-concentration dynamic of compounds by providing the specific time points at which clear effect started to show-up.
- Instead of significant effect, also a specific desired (pre-defined) minimal amount of killing can be used to extract a time profile.
- Creating time concentration profiles are pragmatic approach to get a view on killing kinetic without extensive kinetic modelling.

Acknowledgement



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Thank you

