For which patients shall the drug work, and how?

Mathematical and Computational Biology in Drug Discovery (MCBDD)
Module V

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May-June 2023

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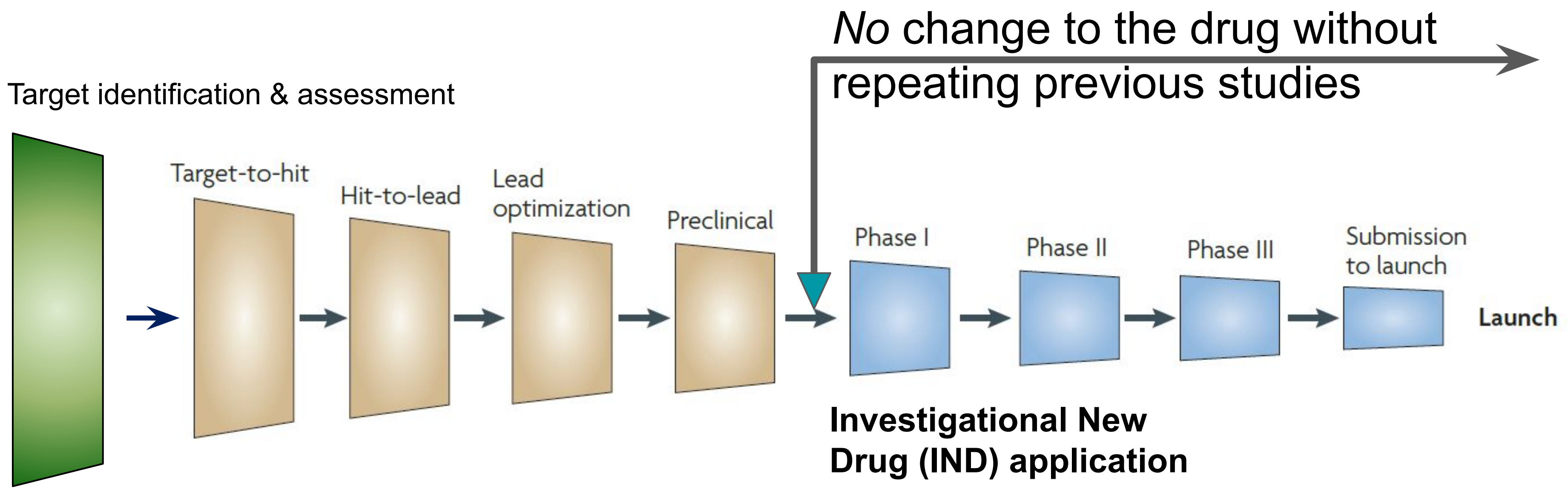
Outline of Module V

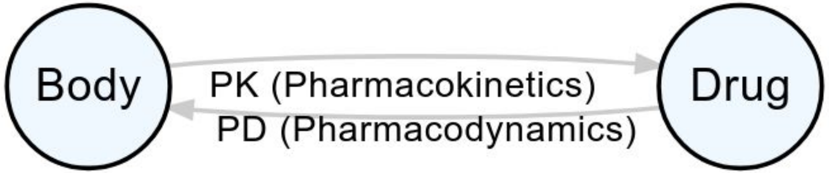
- Lecture 11
    - Biomarker for dose prediction
    - Biomarker for patient-stratification and biology understanding: Merck/Genentech
    - Challenges and caveats
- Lecture 12
    - Integrating statistical and mechanistic modelling: Griffiths *et al.*
    - Mechanistic modelling of biological systems: from Boolean network to Agent-based modelling
    - Causal inference

From drug discovery to drug development

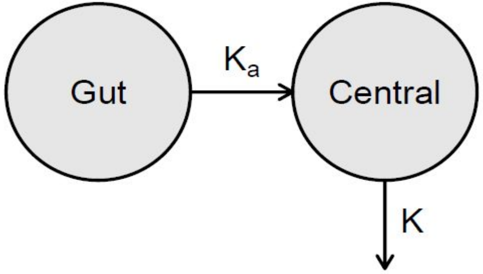


A refresher of PK/PD Modelling

(A)



(B)

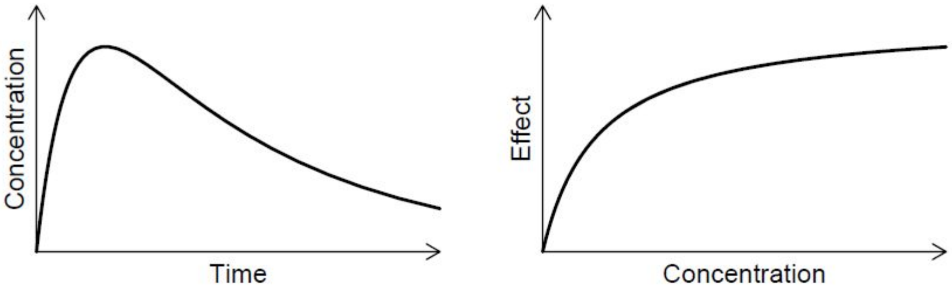


$$\frac{dA_{gut}}{dt} = -K_a \cdot A_{gut}$$

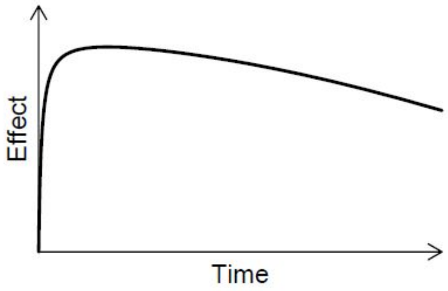
$$\frac{dA}{dt} = \overbrace{F \cdot K_a \cdot A_{gut}}^{\text{from gut}} - \overbrace{K \cdot A}^{\text{elimination}}$$

$$A_{oral}(t) = \frac{K_a F A_0}{K_a - K} \left(\exp(-K \cdot t) - \exp(-K_a \cdot t)\right)$$

(C)

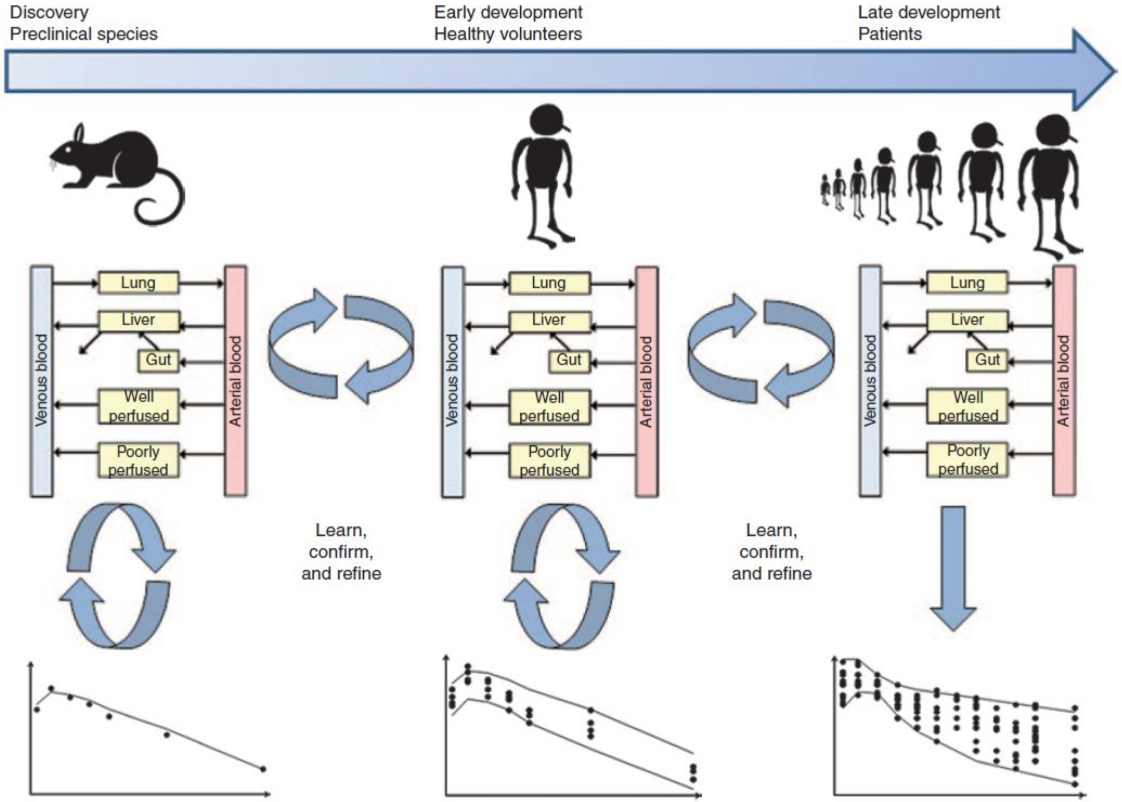


(a) PK model (b) PD model



(c) Combined PK/PD model

Physiologically-based pharmacokinetic modelling (PBPK) is a natural extension of PK modelling



Phases of clinical trials

Investigational New Drug (IND) application (before Phase I)

New Drug Application (NDA) (after Phase III)

Phase 0*	Phase I ~70%	Phase II ~50%	Phase III ~60%
• **Aim:** Getting PK/PD data to verify the drug behaves as expected. • **Dose:** *Microdosing, e.g. 1% of predicted dose.* • **Subjects:** <15 healthy subjects • **Time:** A few weeks	• Finding safe dose ranges and optimal dosing regimens with further PK/PD data. • Sub-therapeutic single and multiple ascending doses • 20-100 healthy subjects (patients) • A few months	• Assessing efficacy and safety profiles of the drug, and determining the dosing regimen. • Therapeutic dose • Usually 100-300 patients with a specific disease • A year or longer	• Comparing efficacy, effectiveness, and safety profiles with the standard-of-care treatment option. • Therapeutic dose • Usually 300-3000 patients • Usually several years

Empirical, stratified, and individualized medicine

Empirical medicine

- Vaccines
- Non-steroid anti-inflammatory drugs (NSAIDs)

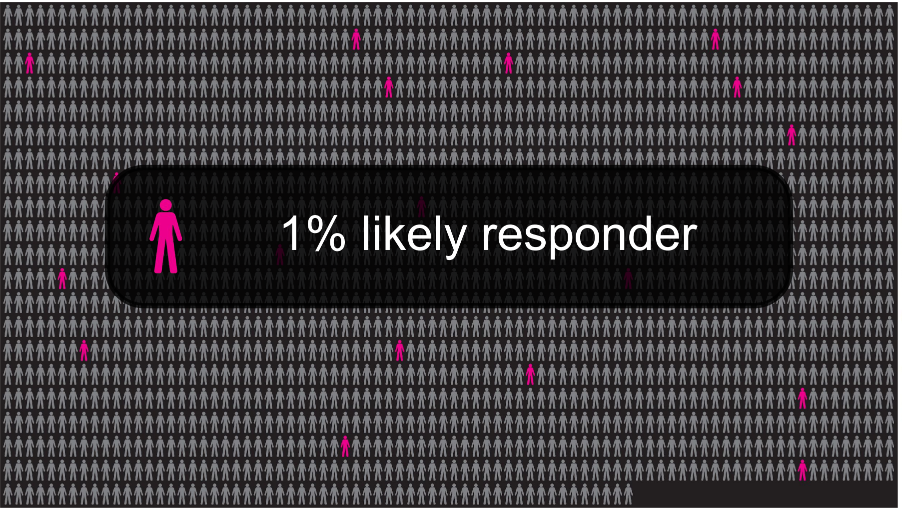
Stratified Medicine

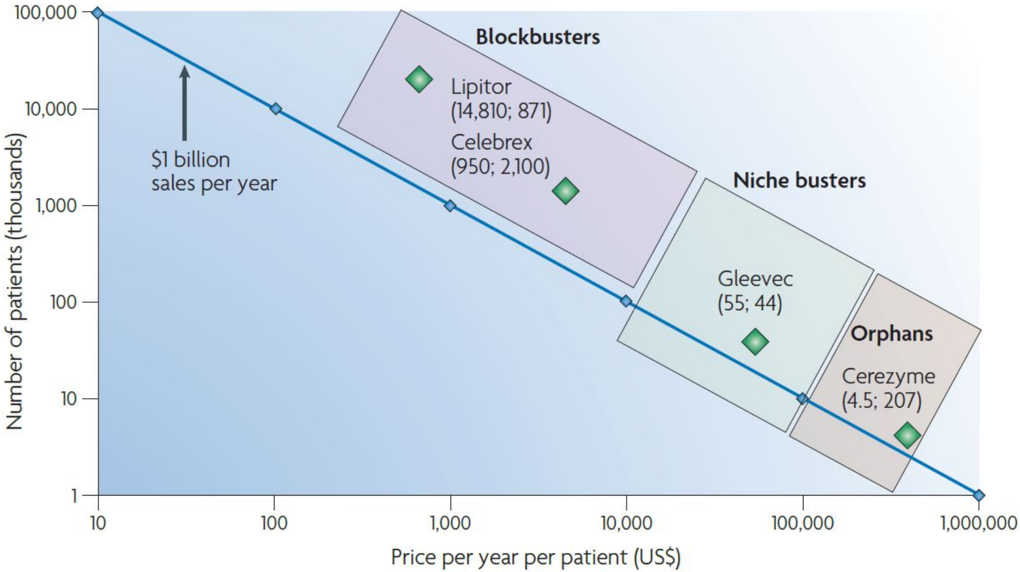
- Vemurafenib (Zelboraf)
- Trastuzumab (Herceptin)

Individualized medicine

- CAR-T therapy

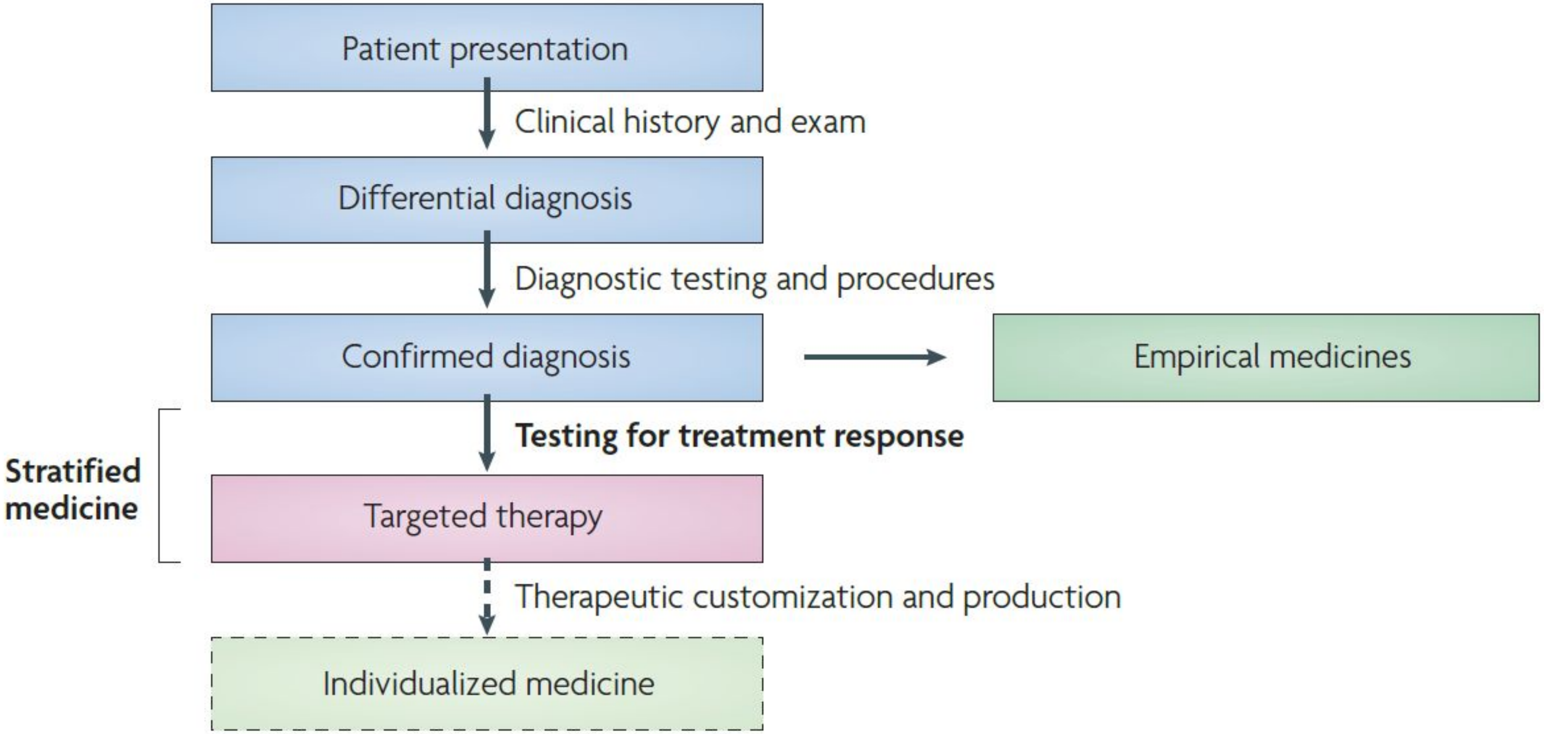
Why stratified medicines are becoming popular?



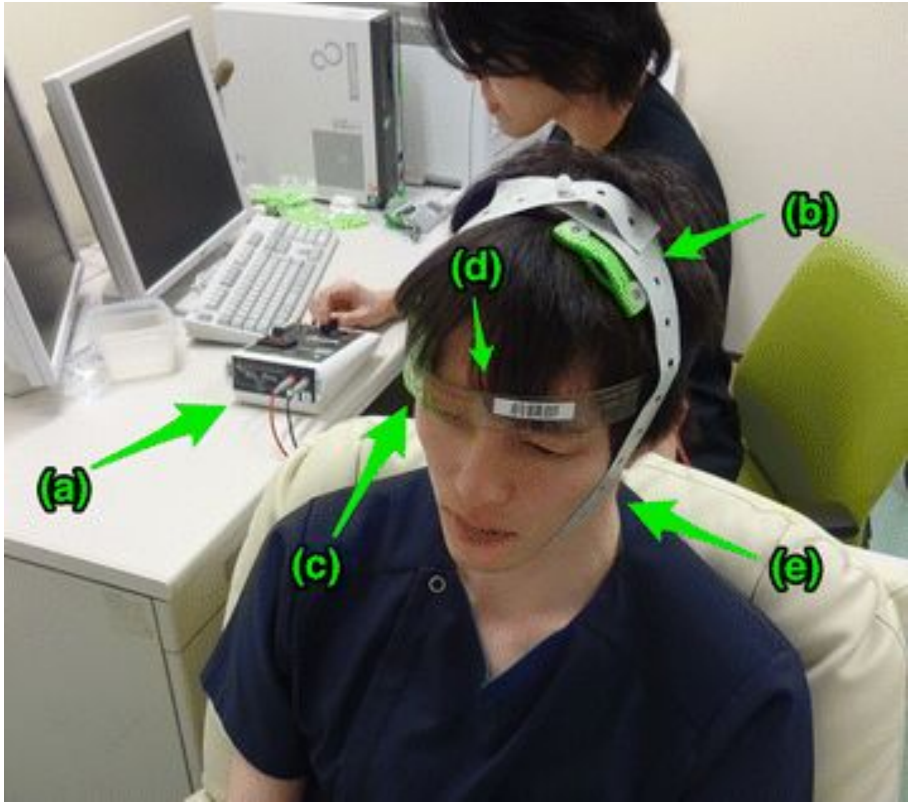


Medical reasons | **Commercial reasons**

Empirical, stratified, and individualized medicine in the clinical context



Transcranial Direct Current Stimulation (tDCS)



Transcranial Direct Current Stimulation (tDCS)

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LIFTiD tDCS Gerät zur Verbesserung von Fokus, Aufmerksamkeit, Gedächtnis und Produktivität

Marke: LIFTiD

Preis: 169,00 €

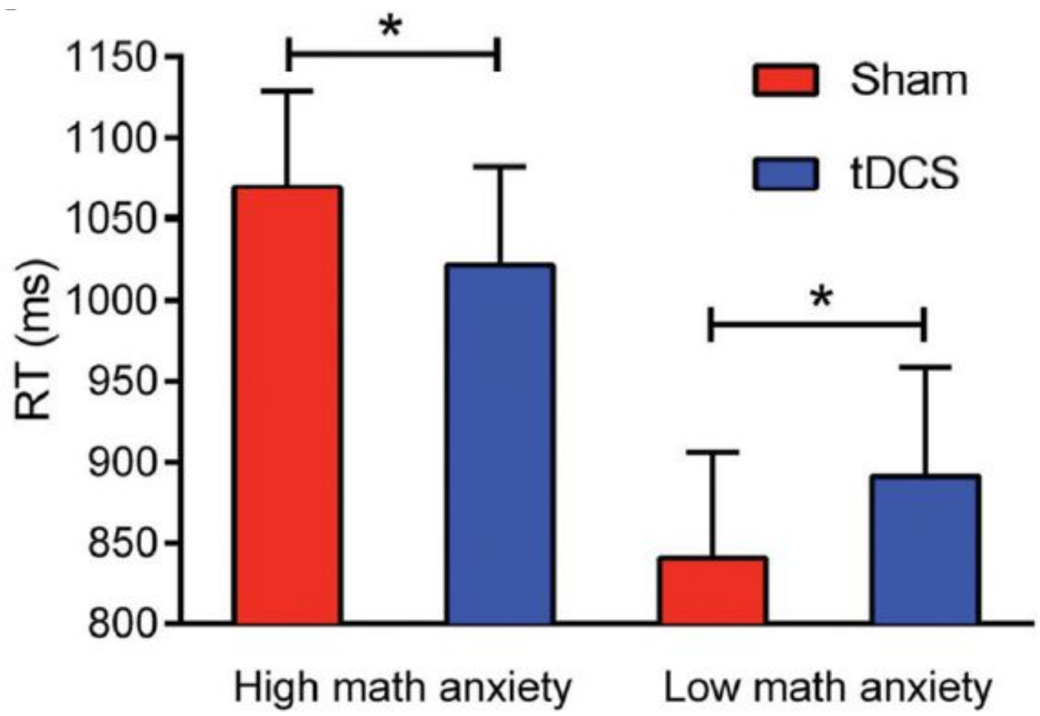
Preisangaben inkl. USt. Abhängig von der Lieferadresse kann die USt. an der Kasse variieren. Weitere Informationen.

Ausgaben im Blick behalten und **8€ Aktionsgutschein** sichern: Jetzt Amazon-Konto aufladen Mehr erfahren

- Verbessern Sie Ihre Leistung: Entwickelt für Gamer, Studenten, vielbeschäftigte Profis, Musiker und Sportler. Jeder kann LIFTiD verwenden, um seine Leistung zu erhöhen, es dauert nur 20 Minuten, um LIFTID während Ihrer Lieblingsaufgabe zu verwenden.
- tDCS leicht gemacht: leicht (nur 70 Gramm), keine Kabel und einfach zu bedienen (Plug 'n Play). Einfach die Pads anfeuchten, aufsetzen und losdrücken. Gerät läuft automatisch für 20 Minuten bei 1,2 mA.
- Reisesicher: Wiederaufladbarer, langlebiger Lithium-Ionen-Akku. Vergessen Sie die Suche nach einem 9V Akku, der LIFTID Gerät Akku ist langlebig und schnell zu laden. Liftid ist eine Freisprecheinrichtung und benötigt keine Basiseinheit. Absolut tragbar. Verwenden Sie es beim Sitzen, Stehen, Gehen oder Dehnen.

Not tested in randomized clinical trials (https://clinicaltrials.gov)

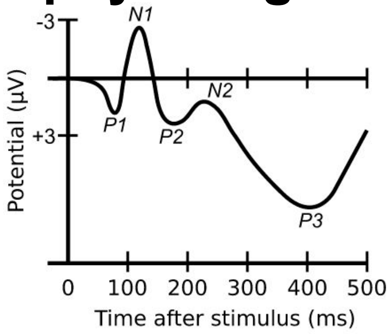
Cognitive Enhancement or Cognitive Cost? It depends!



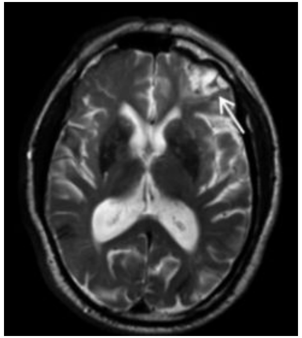
Biomarkers

A objectively measured and evaluated characteristic as an indicator of (1) normal biological process, (2) pathogenic processes, or (3) pharmacological responses to a therapeutic intervention.

Electro-physiological

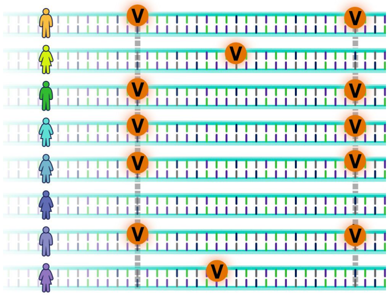


Imaging



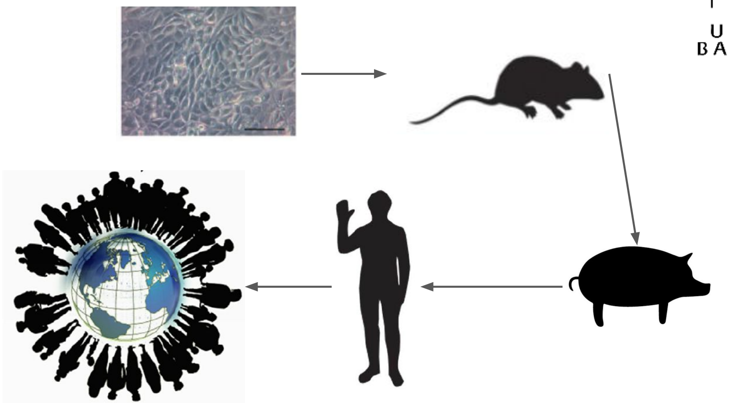
Functional

Molecular



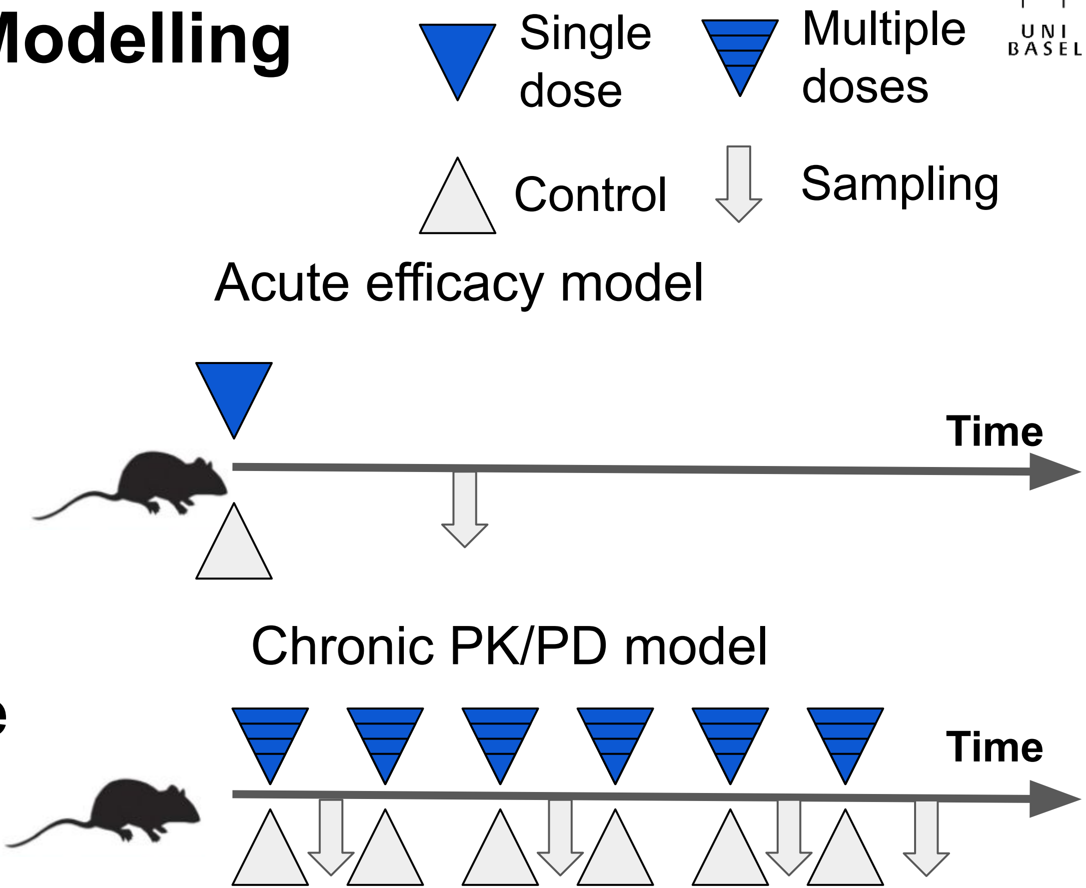
Applications of biomarkers

1. Compound optimization and differentiation from competitors in preclinical study
2. **Human-dose prediction in translational PK/PD modelling**
3. **Patient stratification in clinical studies**



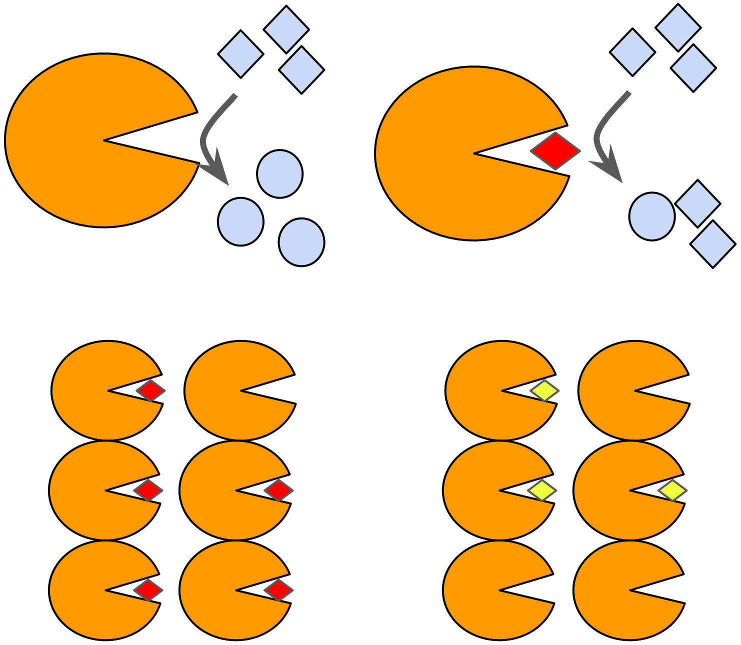
Translational PK/PD Modelling

Samples from blood and the target organ can be analysed for pharmacokinetics, pharmacodynamics, and dose-exposure-response relationships.



Target Occupancy as Biomarkers

Target occupancy, percentage of the protein target occupied by drugs, affects **target engagement,** which describes the process a drug interacts with its intended protein target in a living system to induce downstream effects (Mechanism of Action).

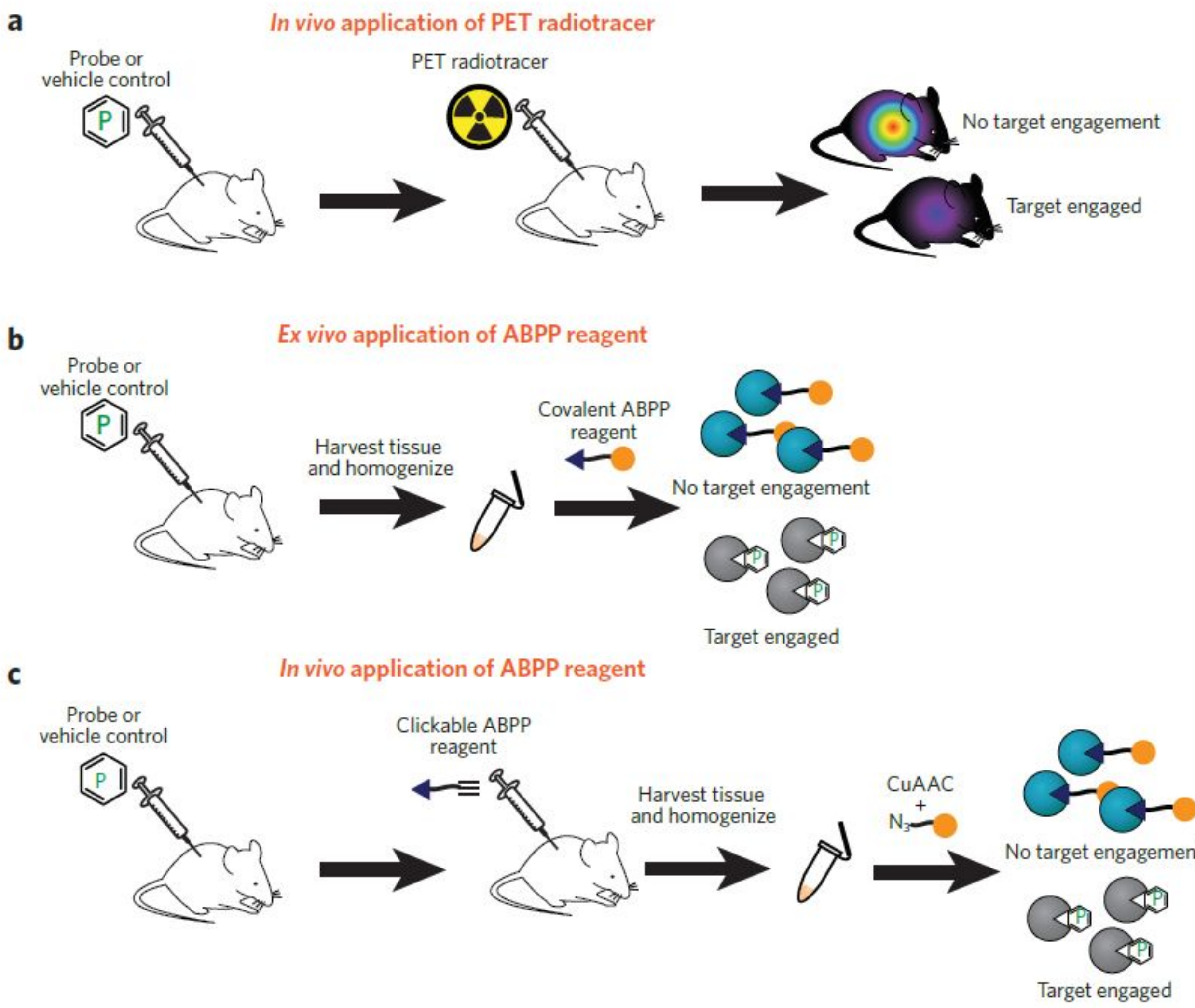


Target occupancy of 83% and 50%, respectively

Target occupancy and engagement profiling in vivo

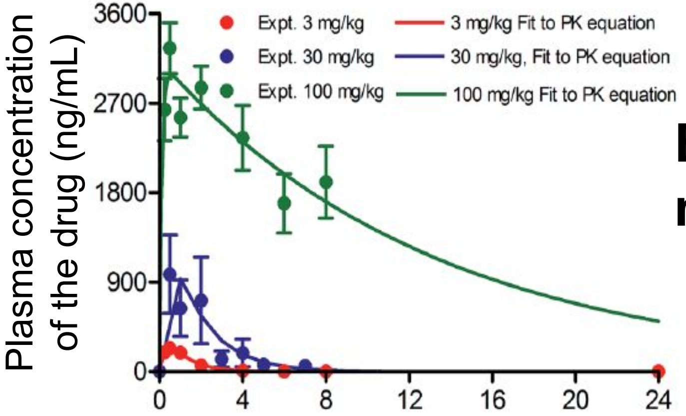
ABPP: Activity-based protein profiling; PET: positron-emission tomography.

Both ABPP reagent and radiotracer binds to the same protein target.

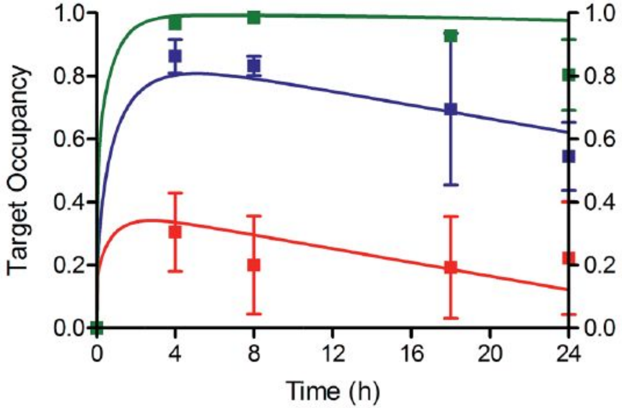


Target occupancy as a biomarker links pharmacokinetics and pharmacodynamics

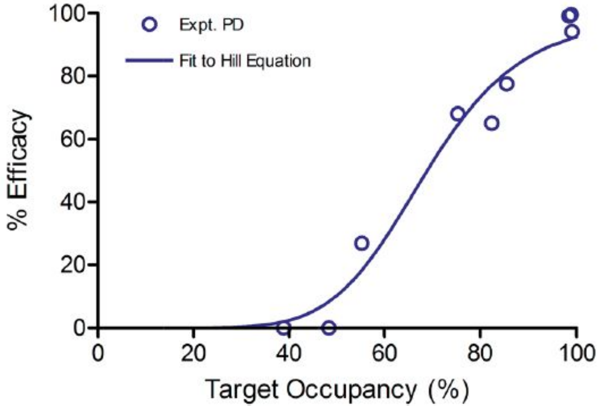
PK modelling



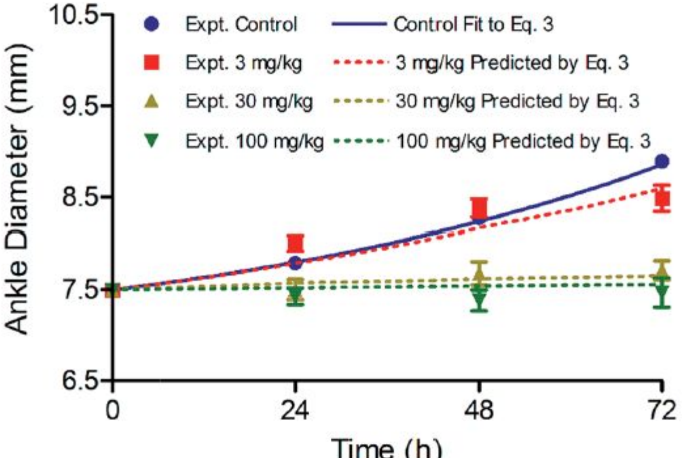
Biomarker modelling



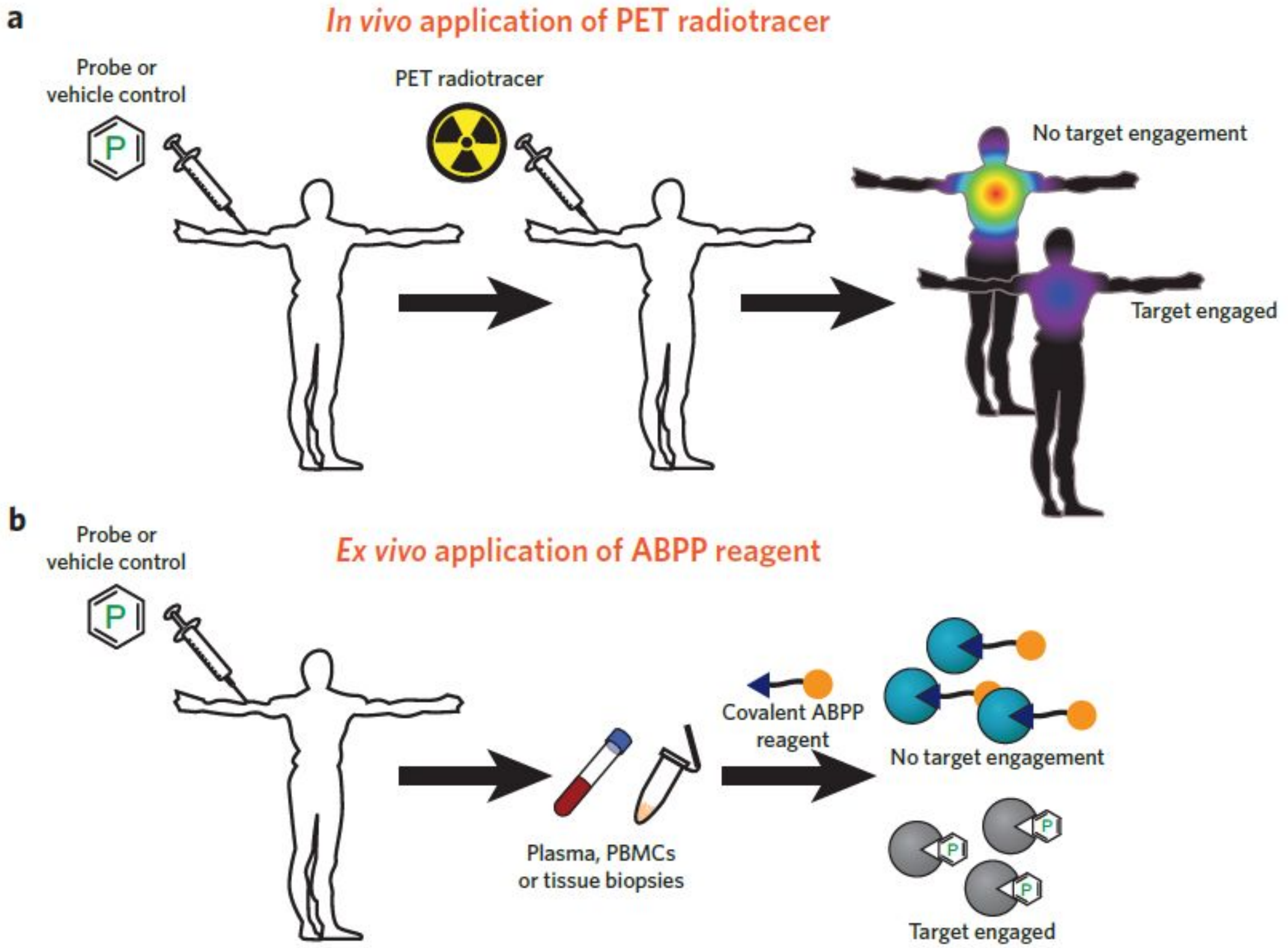
PD modelling



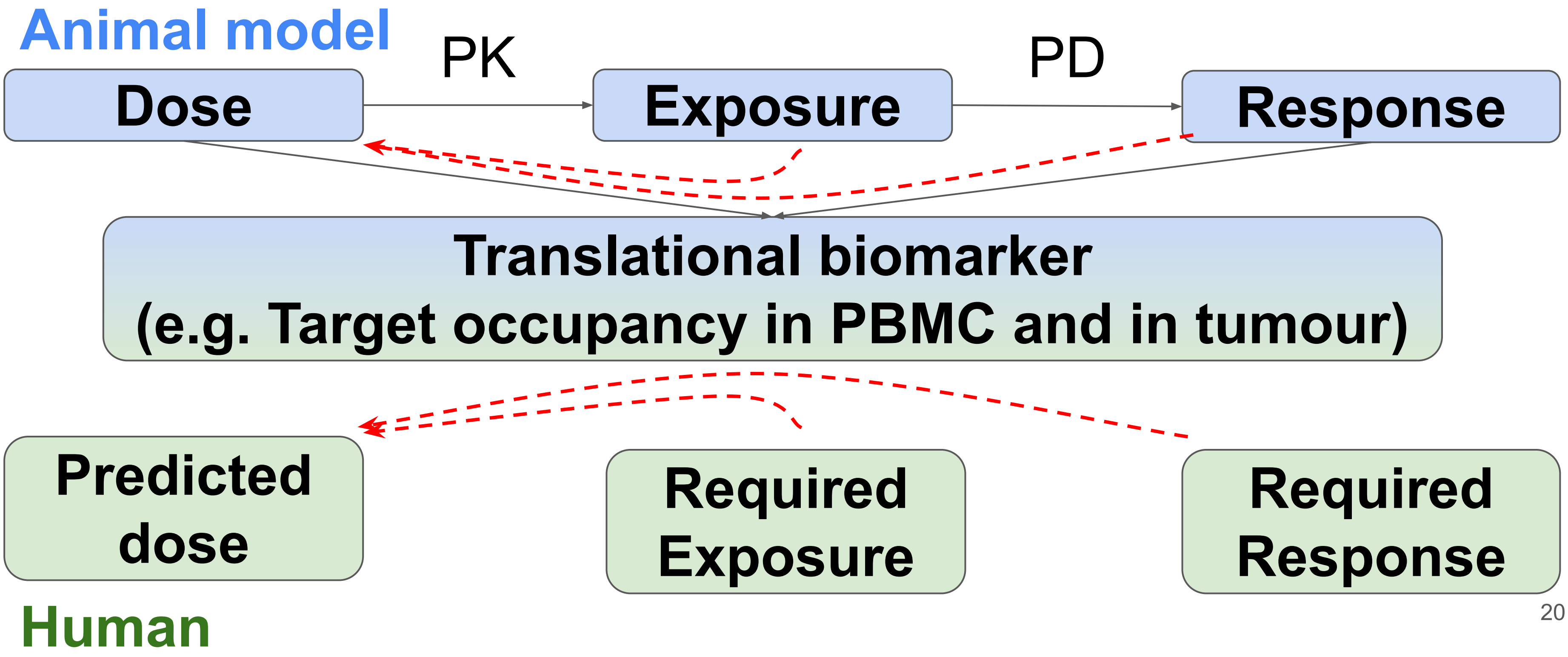
PK/PD modelling



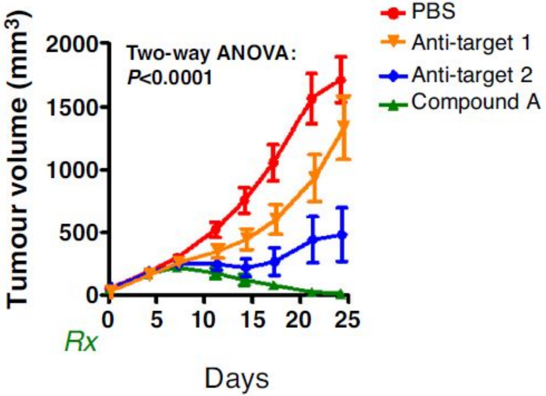
Target occupancy and engagement profiling in human



A mental model of biomarker for human-dose prediction

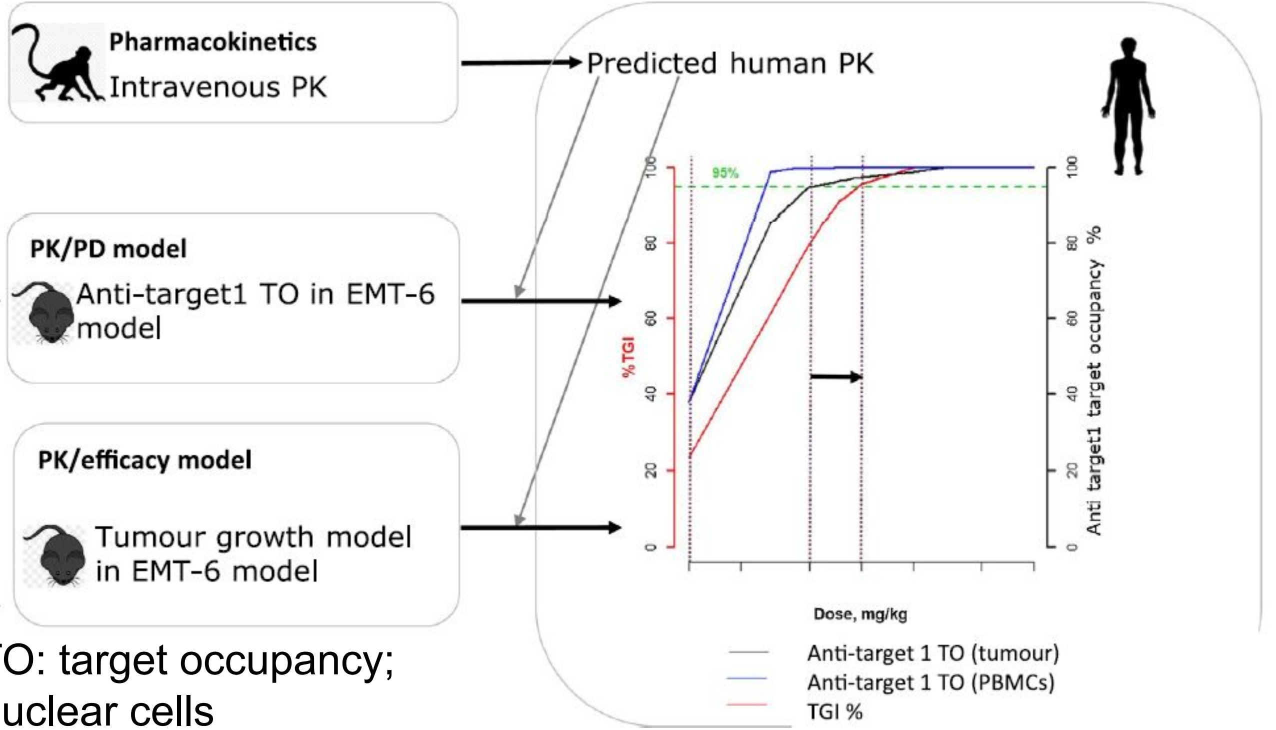


A real-word example with a bispecific antibody



Anti-target 1 TO required for efficacy in tumour and PBMCs

Efficacious concentration



TGI: tumour growth inhibition; TO: target occupancy; PBMC, peripheral blood mononuclear cells

Exposure-response in animal model and translatable biomarkers are essential for dose prediction

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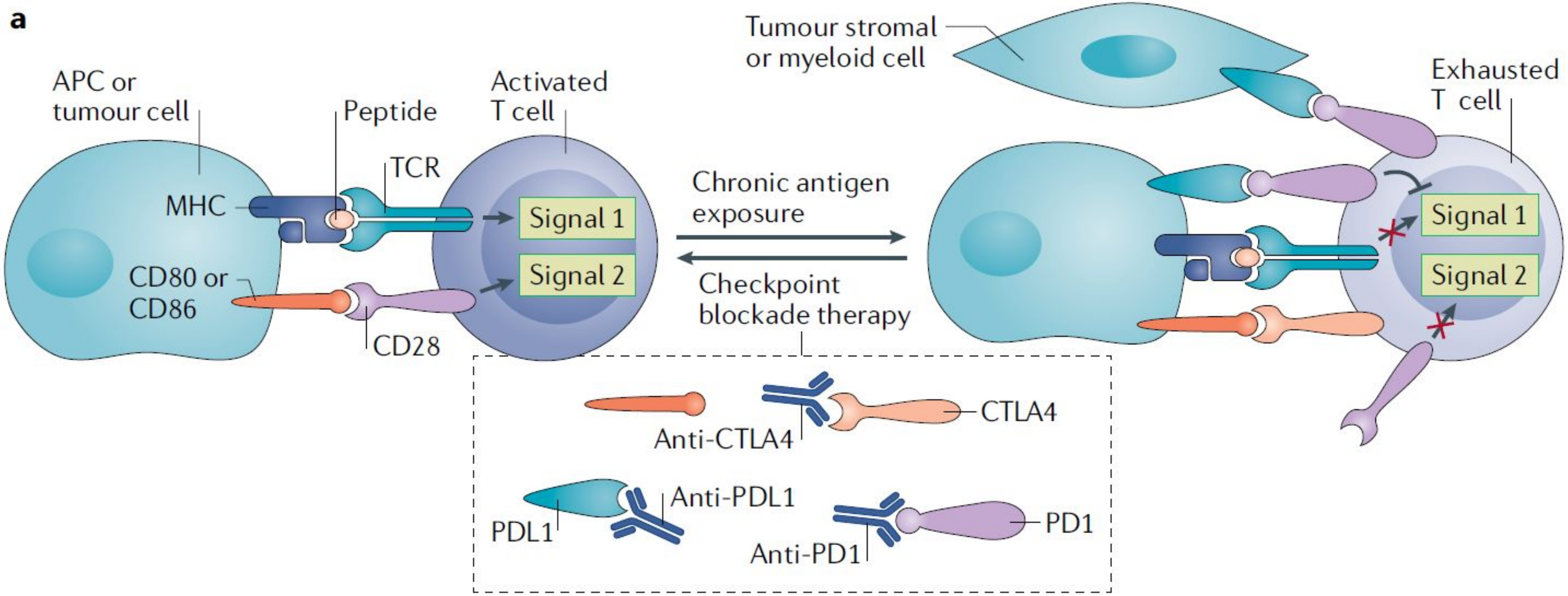
TABLE 2

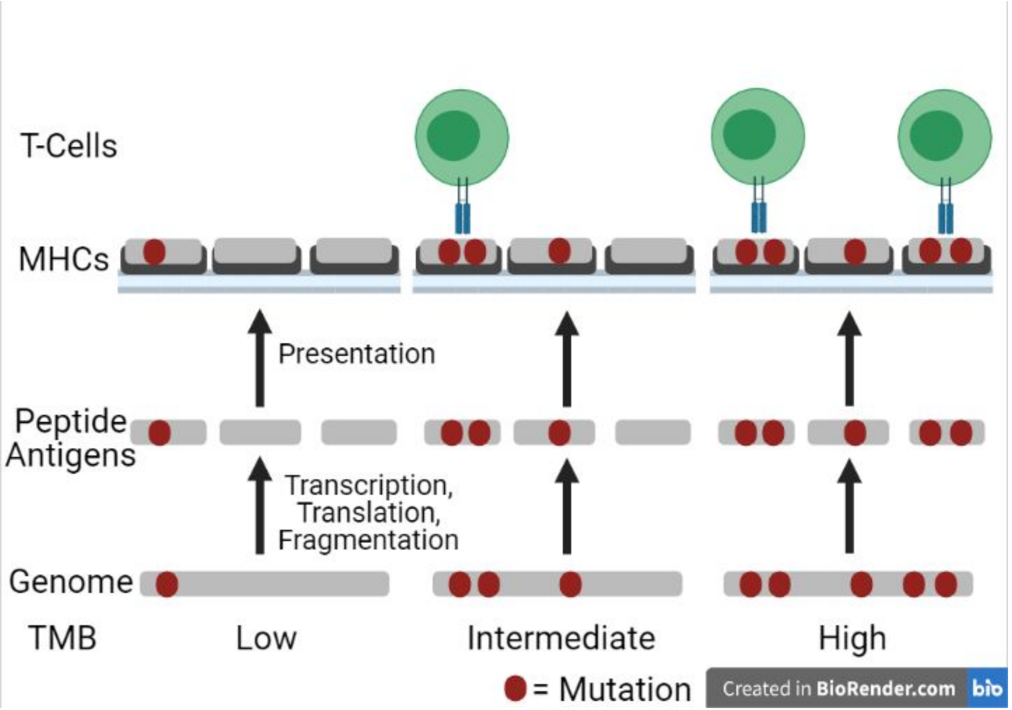
Correlation of responses to dose-related questions (Q) of TmX Guide to dose prediction successes or observation of efficacy in the clinic

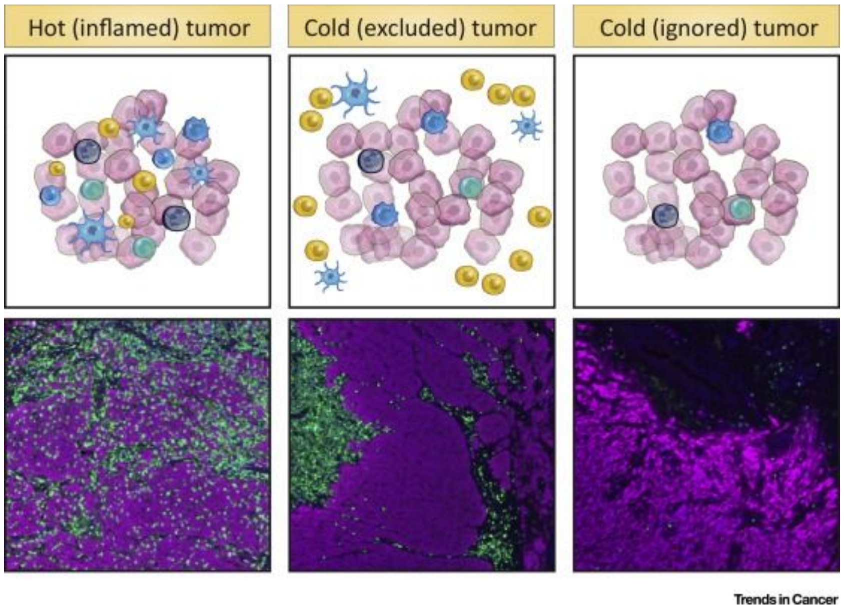
Category	Q1: desired exposure–response in appropriate animal model?	Q2: Translatable biomarkers?	Number of drugs for which model-based active dose prediction is within twofold or clinical efficacy is observed within predicted dose range out of total number in category
1	Yes	Yes	5/6
2[a]	No	No	1/6
3	No	Yes	2/2
4[b]	Yes	No	0/1

Molecular basis of cancer immunotherapy



Tumour mutation burden and immune phenotype may affect the effect of immunotherapy

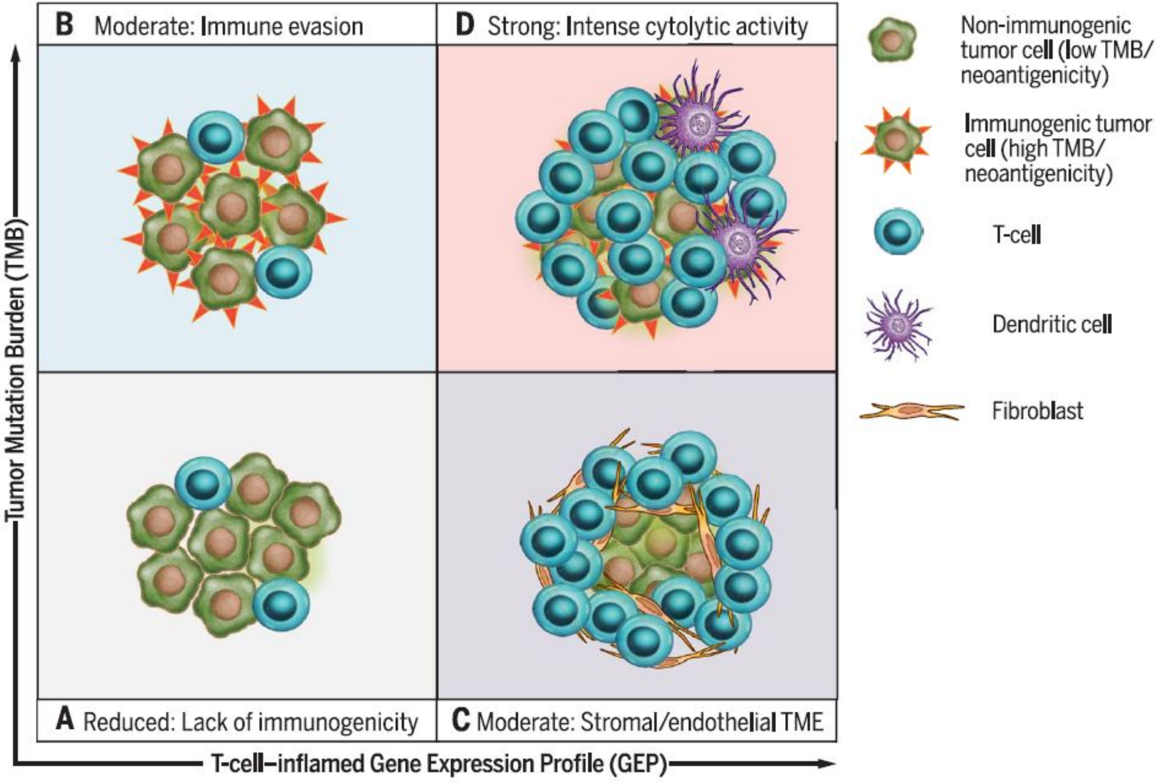




MHC: Major Histocompatibility Complex; TMB:Tumour Mutation Burden.

Cristescu *et al*. established TMB and T-cell-inflamed Gene Expression Profile (GEP) as biomarkers

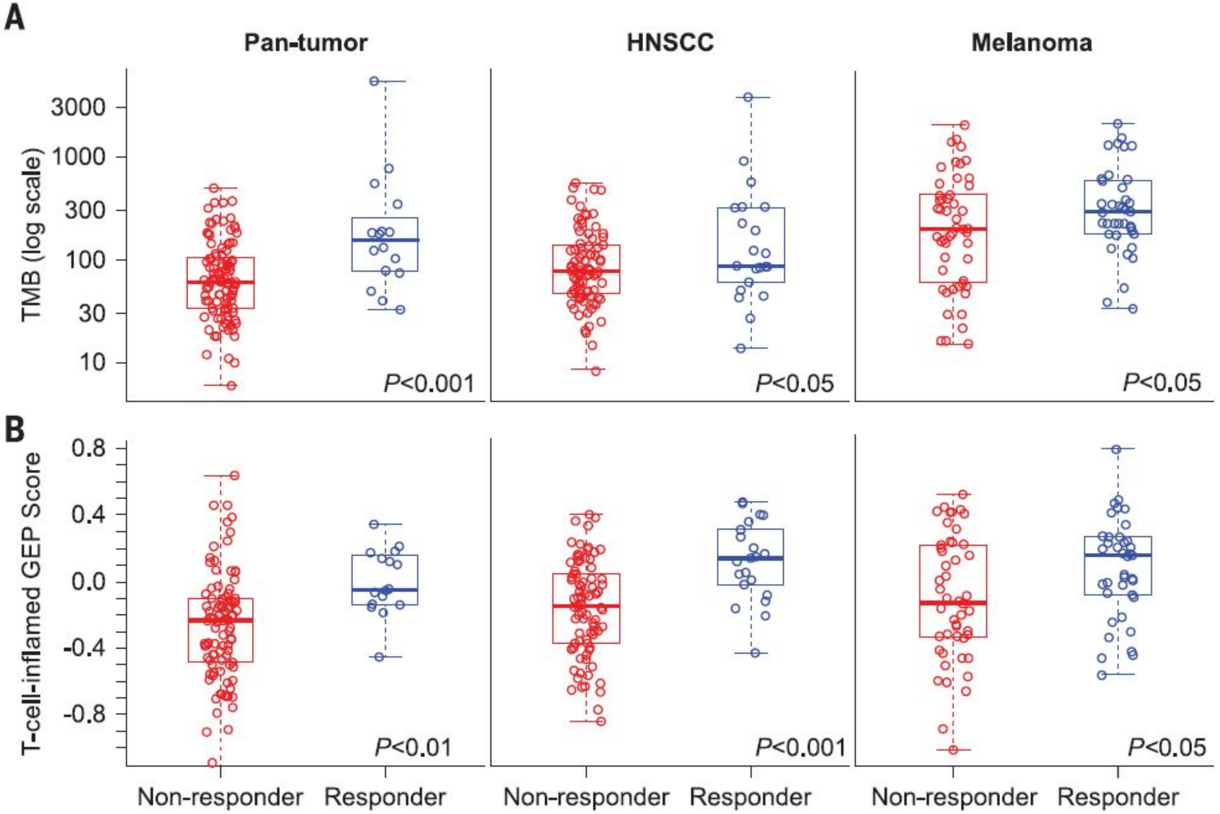
Patients with high tumor mutation burden **AND** a T-cell-inflamed gene expression profile (TME) are more likely to respond to cancer immunotherapy.



Univariate analysis establishes correlation between TMB/GEP and responsiveness

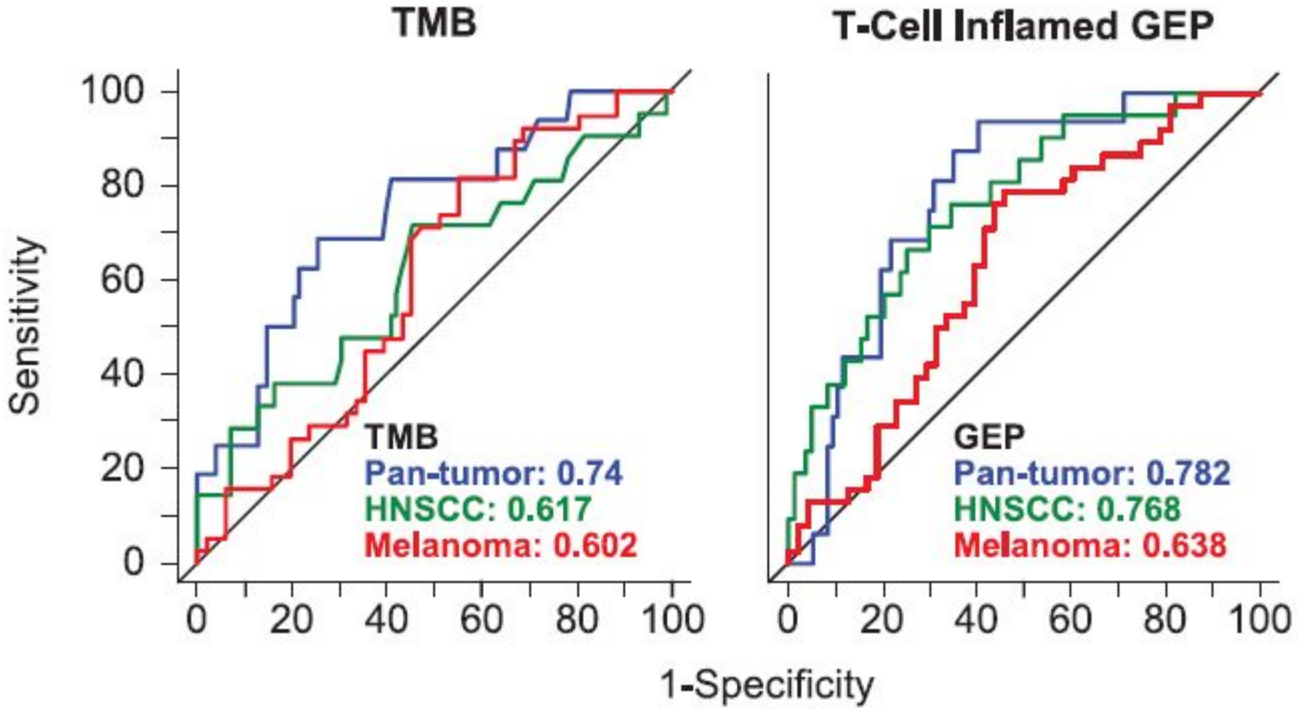
GEP: weighted sum of normalized expression of 18 genes related with immune response (CCL5, CD27, CD274 (PD-L1), CD276 (B7-H3), CD8A, CMKLR1, CXCL9, CXCR6, HLA-DQA1, HLA-DRB1, HLA-E, IDO1, LAG3, NKG7, PDCD1LG2 (PDL2), PSMB10, STAT1, and TIGIT).

HNSCC: head and neck cancer



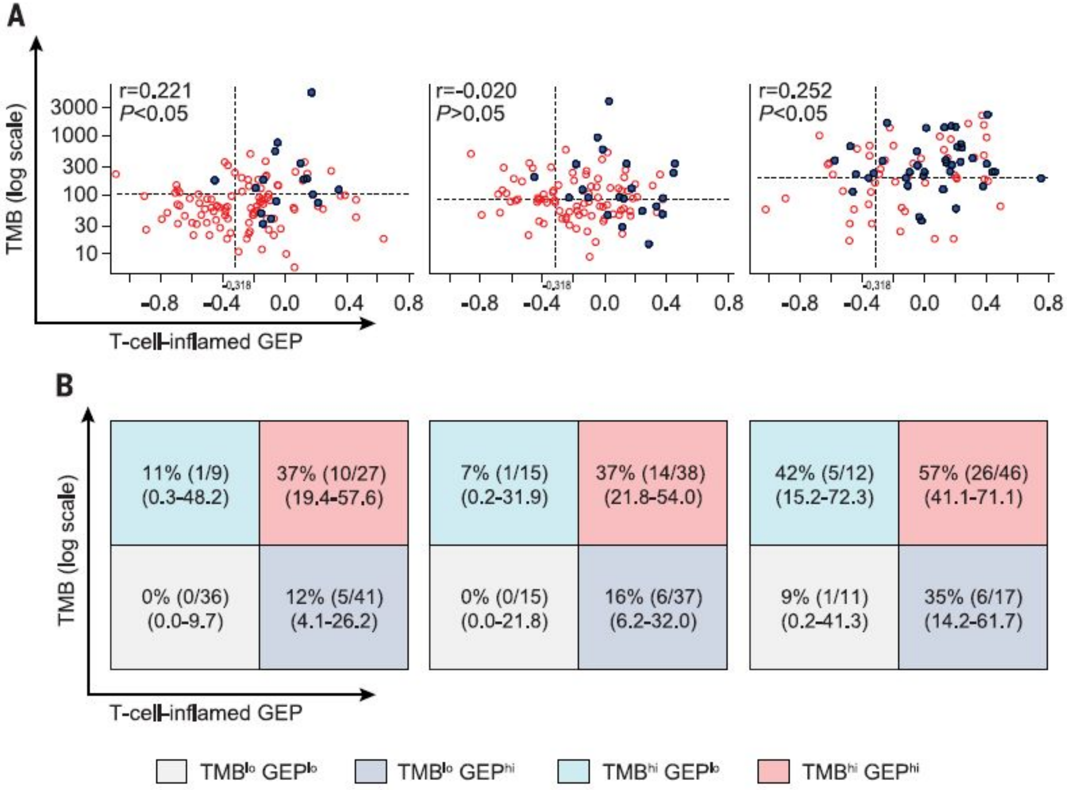
Both TMB and GEP can partially predict responsiveness

Receiver Operating Characteristic (ROC) curves of using either TMB or GEP for binary classification. Metrics: Area Under ROC (AUROC)

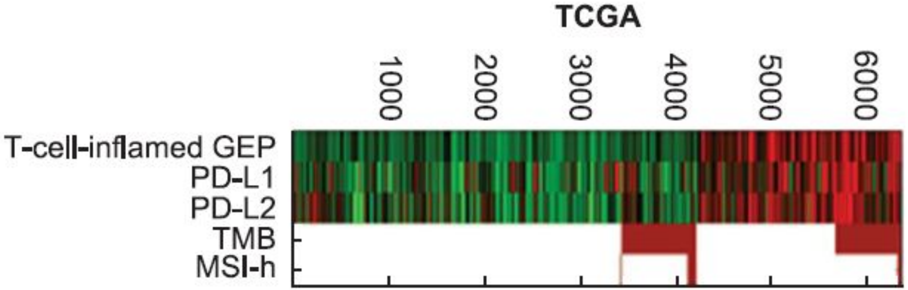


High TMB and high GEP are associated with higher responsiveness to anti-PD1 antibody treatment

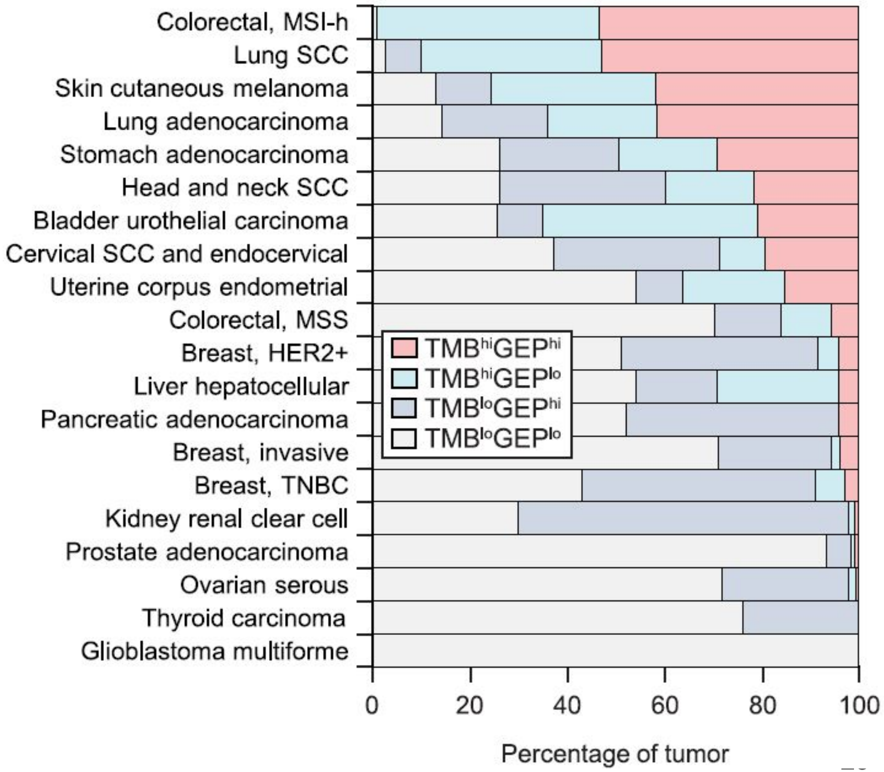
- From left to right: three patient cohorts (pan-cancer; head-and-neck cancer; melanoma)
- Open red circles: non responders; Black dots: responders.



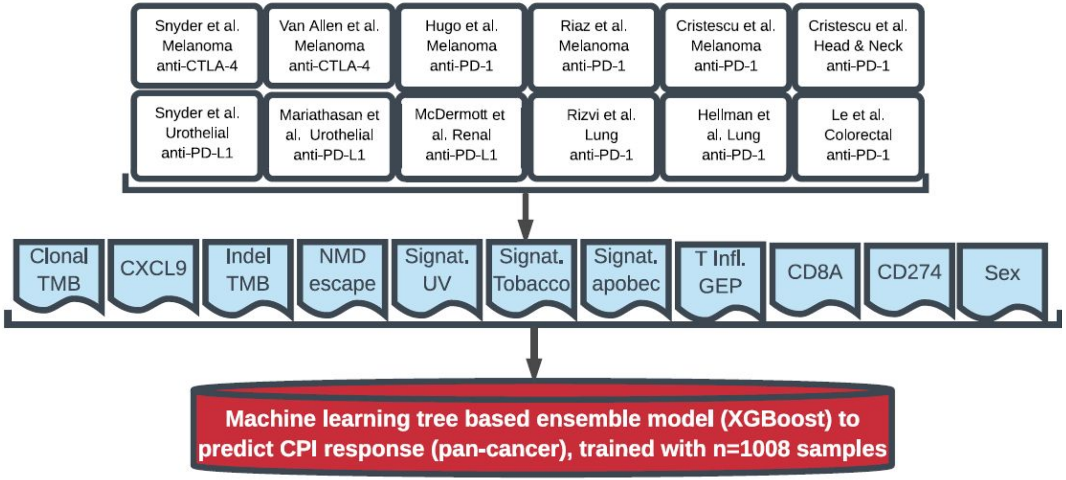
Data mining in public cancer database TCGA suggests potential indications

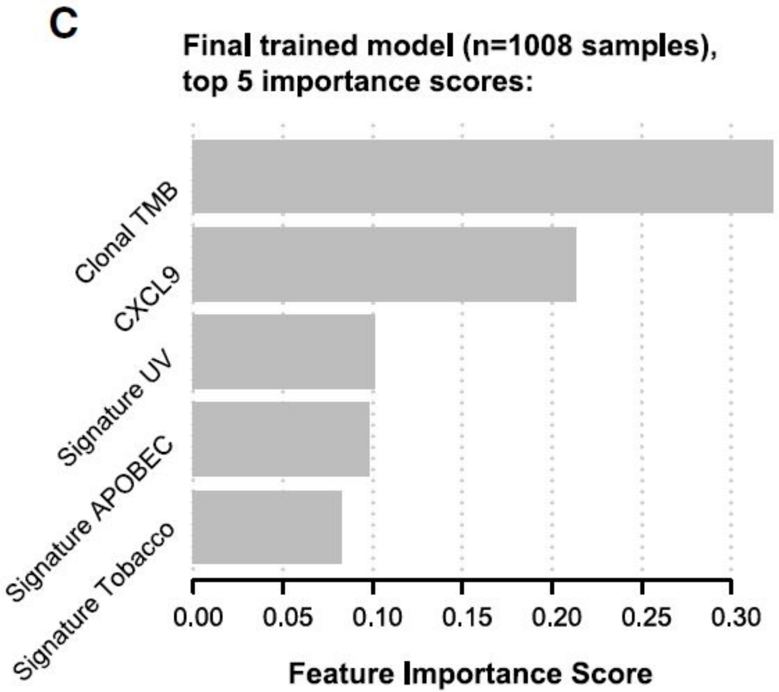


TMB Cut-off (WES)	GEP ≤top tertile (%)	GEP >top tertile (%)
>100	11	12
≤100	55	22



Meta-analysis (Litchfield *et al.* 2021) confirms TMB and T-cell infiltration as predictors of responsiveness

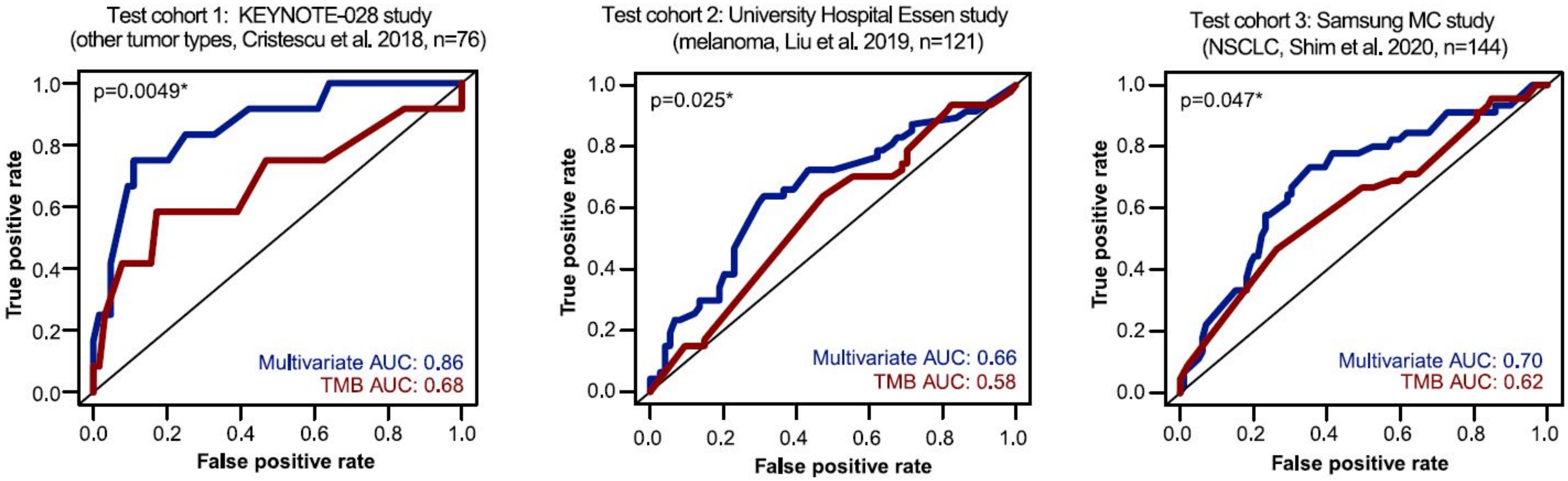




CXCL9 is a chemokine that enhances recruitment of cytotoxic CD8^{+} T cells into the tumor.

The multivariate classifier improves performance, but to predict responsiveness is an open question

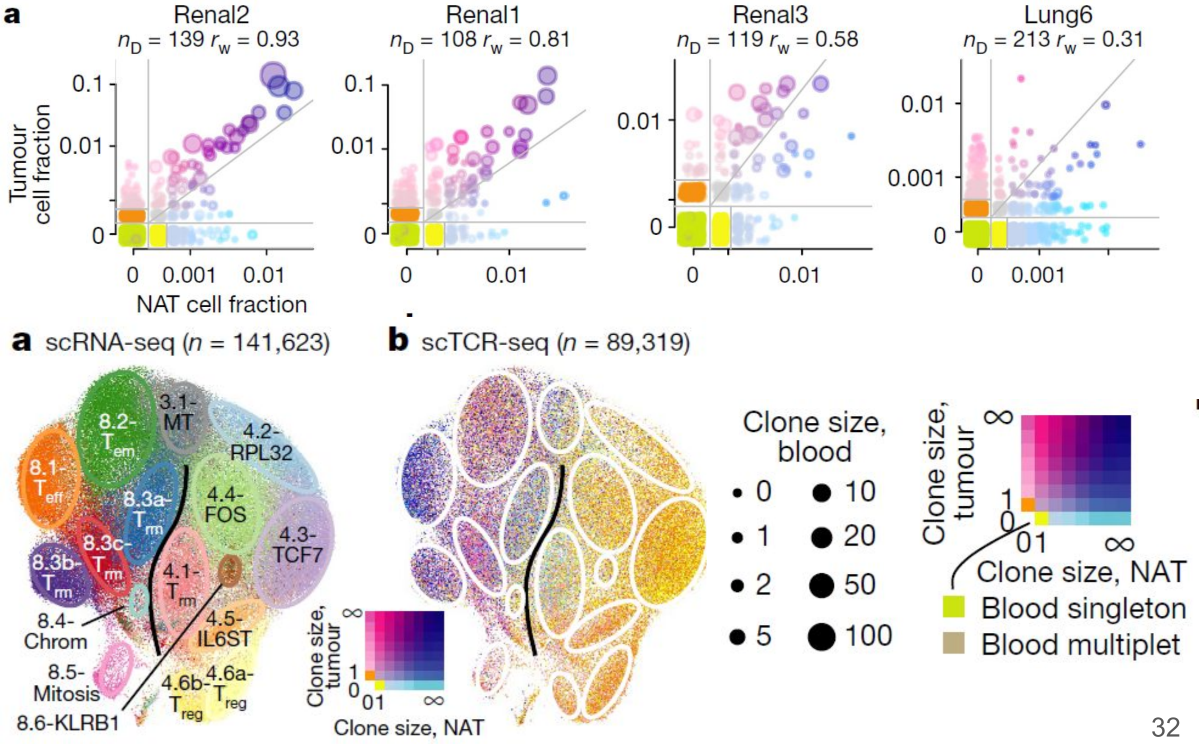
Testing of TMB versus multivariable CPI stratifier performance in three independent test cohorts (total n=341):



Wu *et al.* characterized T cells in tumour, normal adjacent tissue (NAT), and blood using single-cell RNA and TCR sequencing

- Expanded clonotypes (T cells) found in the tumour and normal adjacent tissue can also typically be detected in peripheral blood.

- Intra-tumoural T cells, especially in responsive patients, are replenished with fresh, non-exhausted replacement cells from sites outside the tumour.



Caveats and challenge

- The curse of dimensionality
- Separation of mechanistic modelling and of statistical modelling
- Lack of causal models

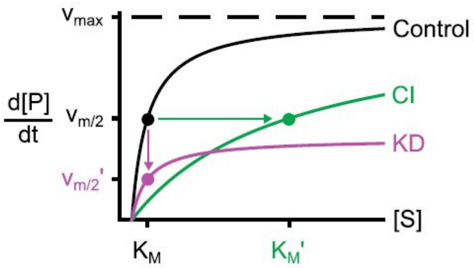
Conclusions

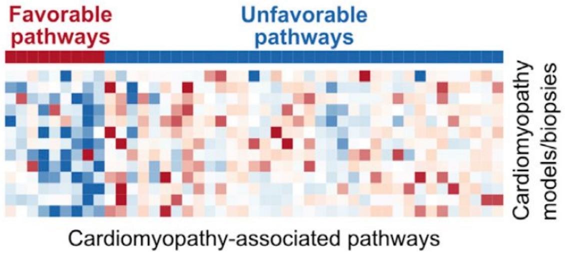
- Biomarkers (1) guide compound optimization and differentiation in preclinical studies, (2) support human dose prediction in translational PK/PD studies, and (3) allow patient stratification in clinical trials;
- Mathematical and computational biology is indispensable for biomarker identification;
- Caveats in biomarker identification calls for integrated mechanistic and statistical modelling to establish causal relations.

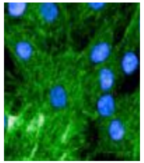
Outline of lecture 12

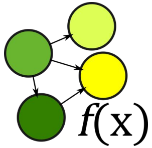
- An example of integrating statistical and mechanistic modelling: Griffiths *et al.*
- Mechanistic modelling of biological systems: from Boolean network to Agent-based modelling
- Causal inference
- Where can we go from here

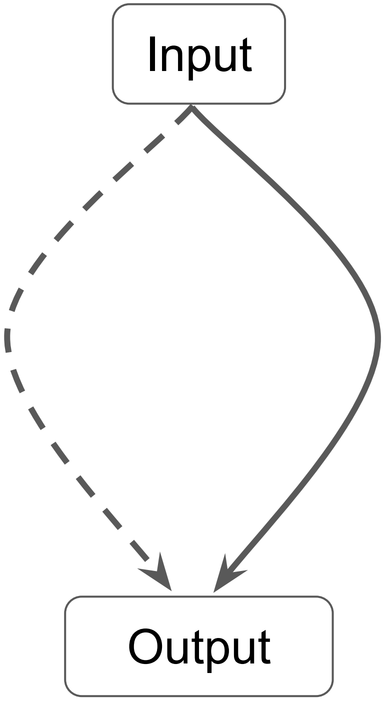
Drug discovery relies on *in vitro*, *in vivo*, and computational models across scales

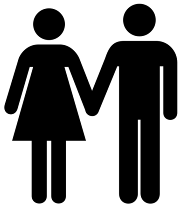


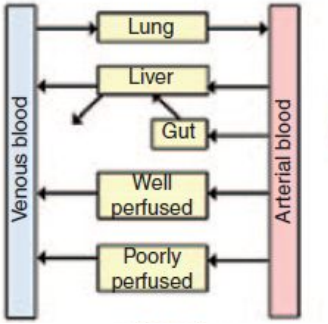


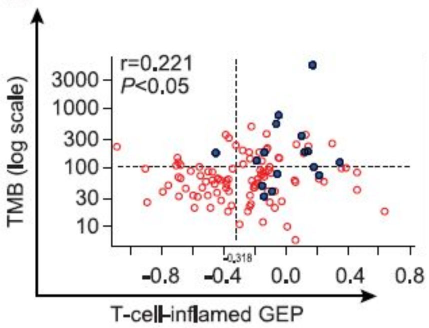








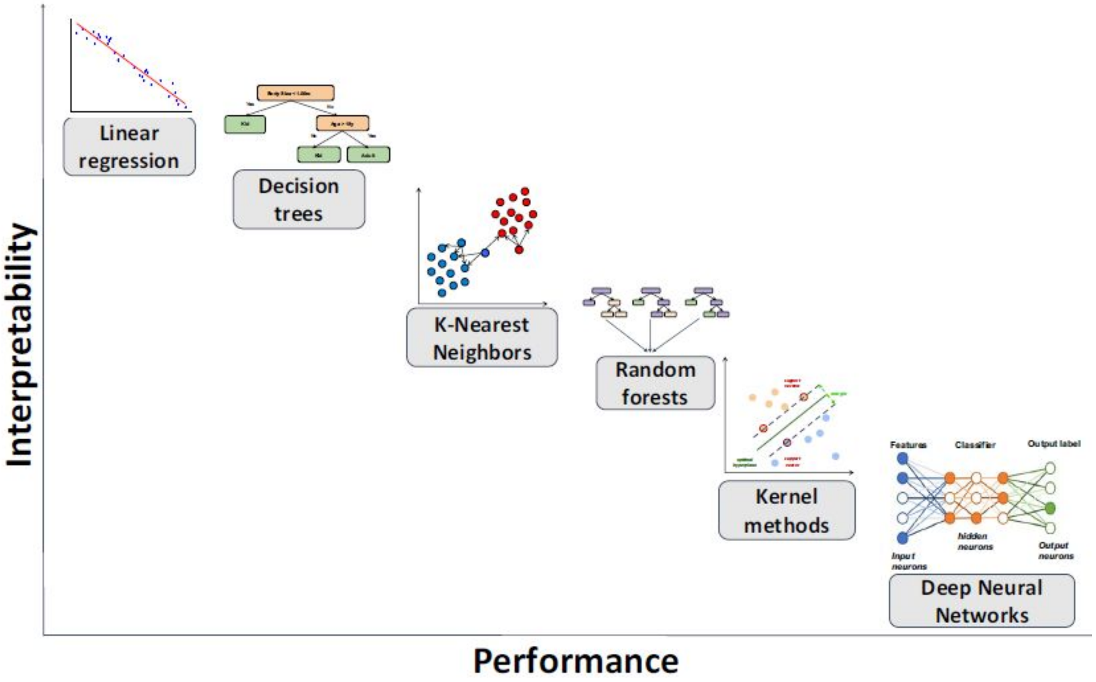




Examples of molecular, omics and cellular, organ and system, and population modelling

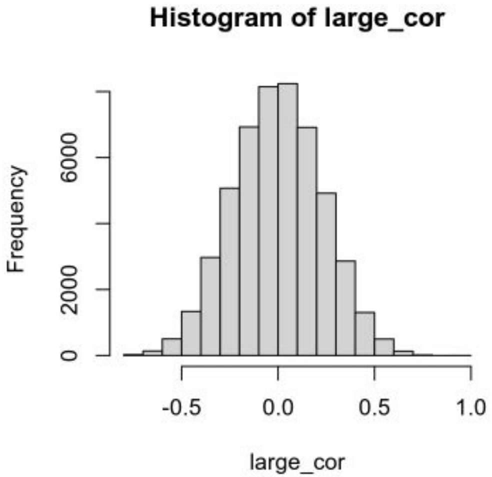
Most statistical models predict output without knowing underlying mechanisms

Useful for hypothesis generation and exploratory analysis, dangerous for extrapolation and ‘black-box’ prediction.

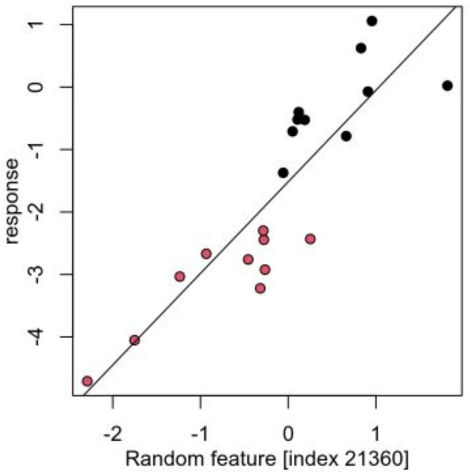


Simulation help us mind the curse of dimensionality

```
set.seed(1887)
patient_group <- gl(2,10)
response <- c(rnorm(10, 0), rnorm(10, -3))
random_features_large <- matrix(rnorm(20*50000), nrow=20)
large_cor <- cor(response, random_features_large, method="spearman")
hist(large_cor)
```




```
largest_cor_ind <- which.max(large_cor)
{
  compactPar()
  plot(random_features_large[, largest_cor_ind],
       response,
       bg=patient_group,pch=21,
       xlab=sprintf("Random feature [index %d]", largest_cor_ind))
  abline(lm(response ~ random_features_large[, largest_cor_ind]))
}
```

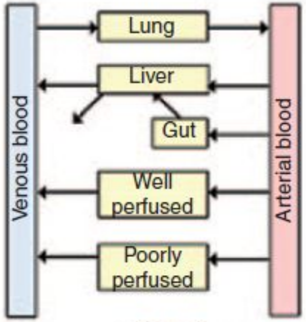


Mechanistic and computational models *explain*

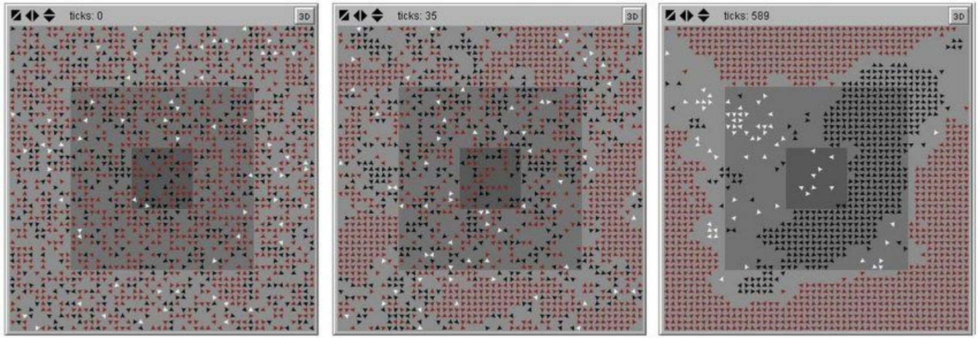
Compartment models

$$\frac{d[LR]}{dt} = k_1[L][R] - k_2[LR]$$

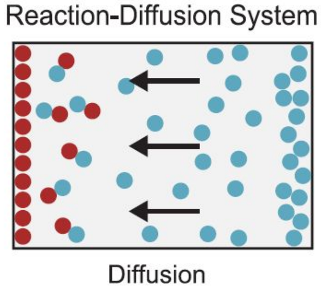
Kinetics of ligand-target interaction



Particle models

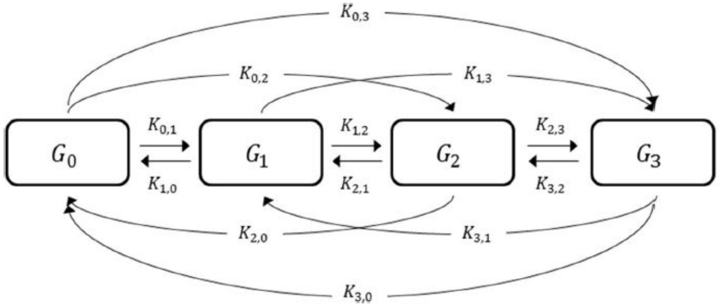


Transport models

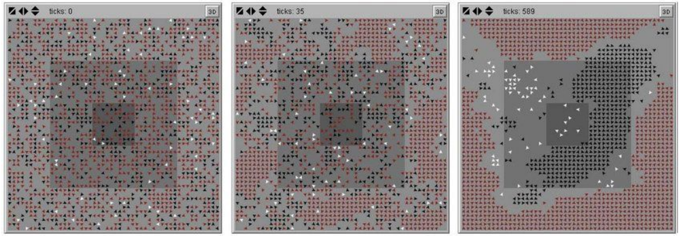


$$\frac{\partial u}{\partial t} = D \underbrace{\frac{\partial^2 u}{\partial x^2}}_{\text{Diffusion}} + \underbrace{ku}_{\text{Binding}}$$

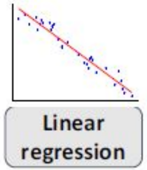
Finite state models

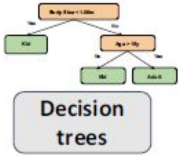


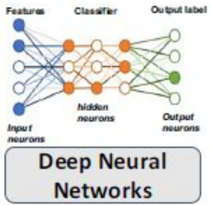
Integrating models across scales is a grand challenge in drug discovery

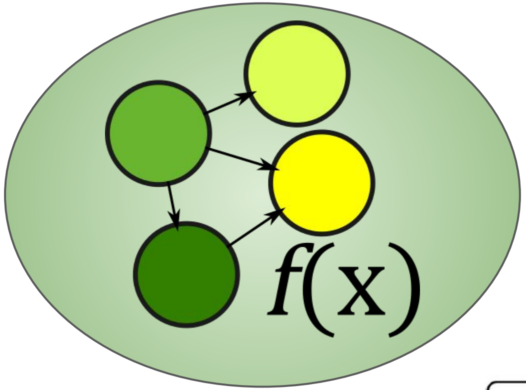


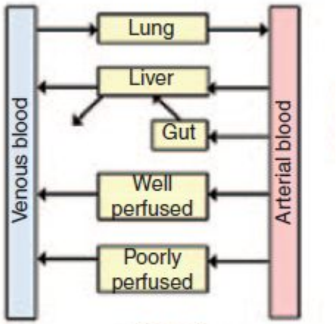
$$\frac{d[LR]}{dt} = k_1[L][R] - k_2[LR]$$

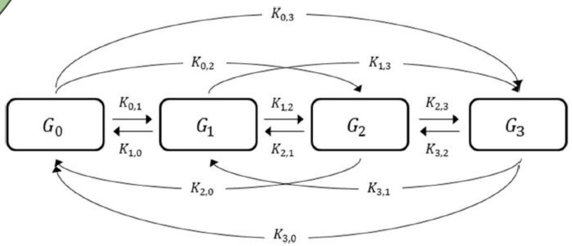








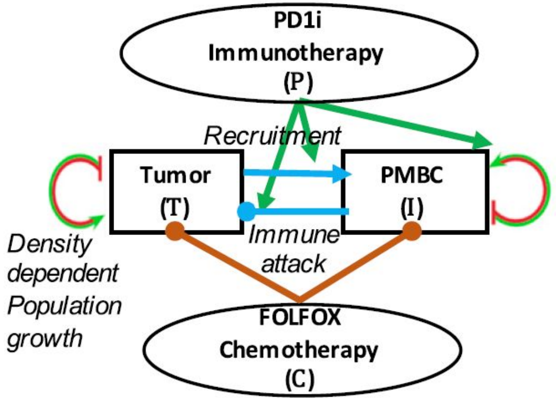


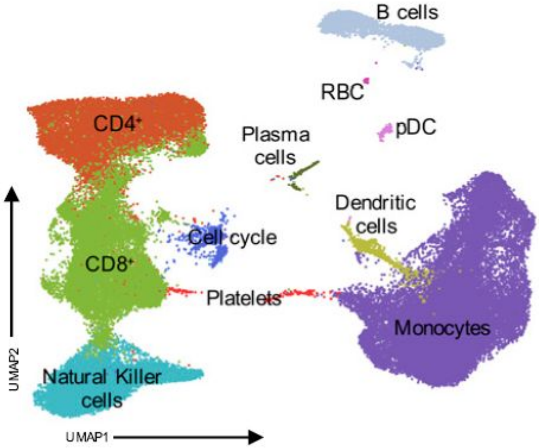


$$\frac{\partial u}{\partial t} = \underbrace{D\frac{\partial^2 u}{\partial x^2}}_{\text{Diffusion}} + \underbrace{ku}_{\text{Binding}}$$

An example of integrated modelling

- Griffiths et al. (PNAS 2020) profiled peripheral immune cell abundance in time series following treatment of Gastrointestinal (GI) tumours with immunotherapy in a small clinical trial.
- The authors used **compartment models** to characterize cell-cell interactions and analysed **single-cell omics data** to reveal immune cell abundance, pathway activity patterns, and differentiation status.



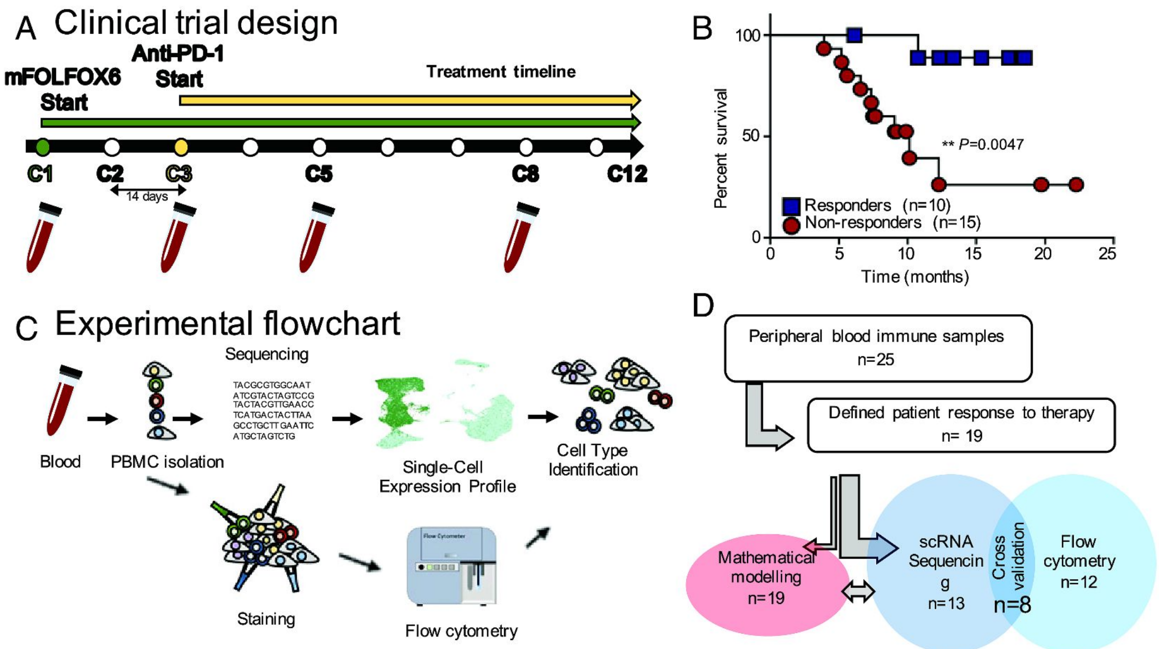


How this study enriches our knowledge

- **Facts**
  - Clinical response to immune checkpoint inhibitors varies substantially.
  - Possible contributing factors correlate only weakly with patient response, including (1) tumor cell mutational load and antigen production, (2) immune-cell infiltration and signalling status, (3) Cross-talk between tumour and immune cells.
  - It is challenging to obtain tumour tissue samples.
- **Questions**: Can circulating immune cells serve as a surrogate measurement of a tumour's interaction with the host immune cells and reflect response to therapy early in the course of treatment?
- **Conclusions**: It is possible to predict patient response with the evolution of peripheral immune cell abundance and signalling over time, as well as how immune cell interact with the tumor.

Design of the clinical trial

- mFOLFOX6 (modified FOLFOX6): a chemotherapy regiment.
- Patient response was assessed by RECIST (Response Evaluation Criteria in Solid Tumors) 1.1 guidelines, using computer tomography (CT).

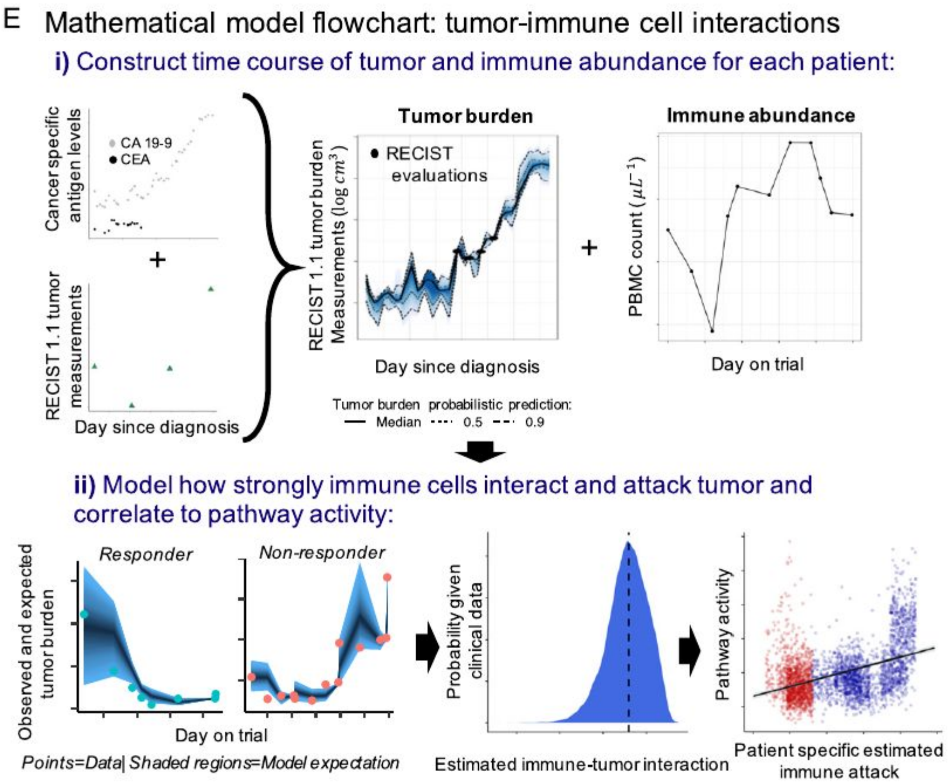


Mathematical modelling of tumour-immune cell interactions

Model inputs (all in time series):

- Tumour burden, inferred by combining antigen values and RECIST evaluation with a *Gaussian process* latent variable model.
- Abundance of PBMCs

Model output: estimated ability of immune cells to kill tumour cells



Gaussian Process

The Lotka-Volterra model of predator-prey relationships

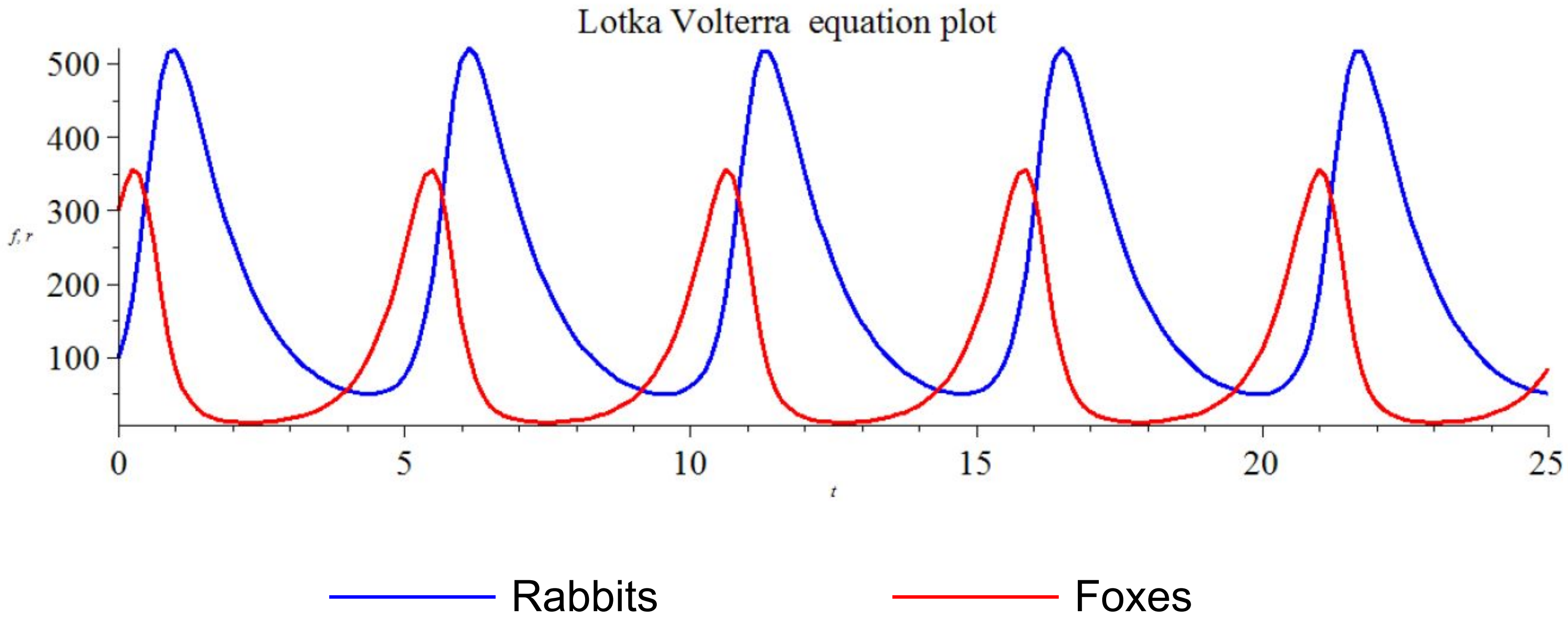
- The Lotka-Volterra equations modelling predator-prey relationships.

$$\frac{dx}{dt} = \alpha x - \beta xy, \tag{1}$$
$$\frac{dy}{dt} = -\gamma y + \delta xy, \tag{2}$$

where

- x is the number of prey (*e.g.* rabbits),
- y is the number of predator (*e.g.* foxes),
- $\frac{dx}{dt}$ and $\frac{dy}{dt}$ represent growth rates of the two populations,
- t represents time,
- α, β, γ, and δ are real parameters specifying the interaction of the two species.

The Lotka-Volterra equations, visualized

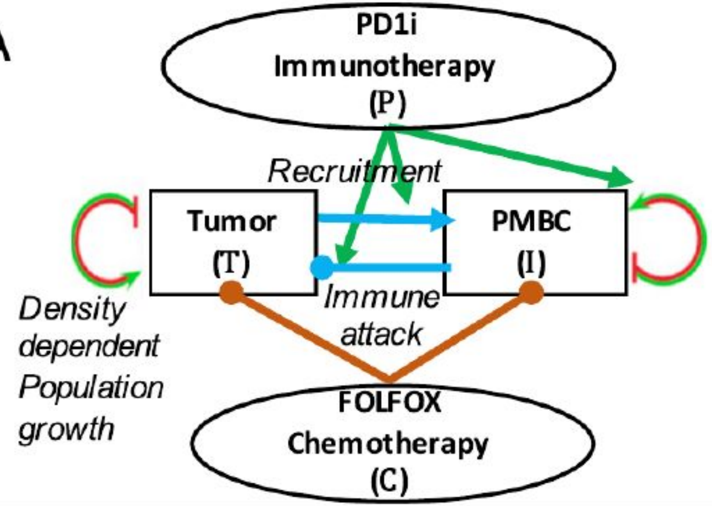


Modelling of interactions between tumour and immune cells

Modelling assumptions:

- Tumor cells are attacked by immune cells
- Tumor cells recruit immune cells
- Chemotherapy kills both tumour and immune cells
- Anti-PD1 inhibitor immunotherapy impacts immune proliferation, recruitment, and cytotoxic tumor activity.

A

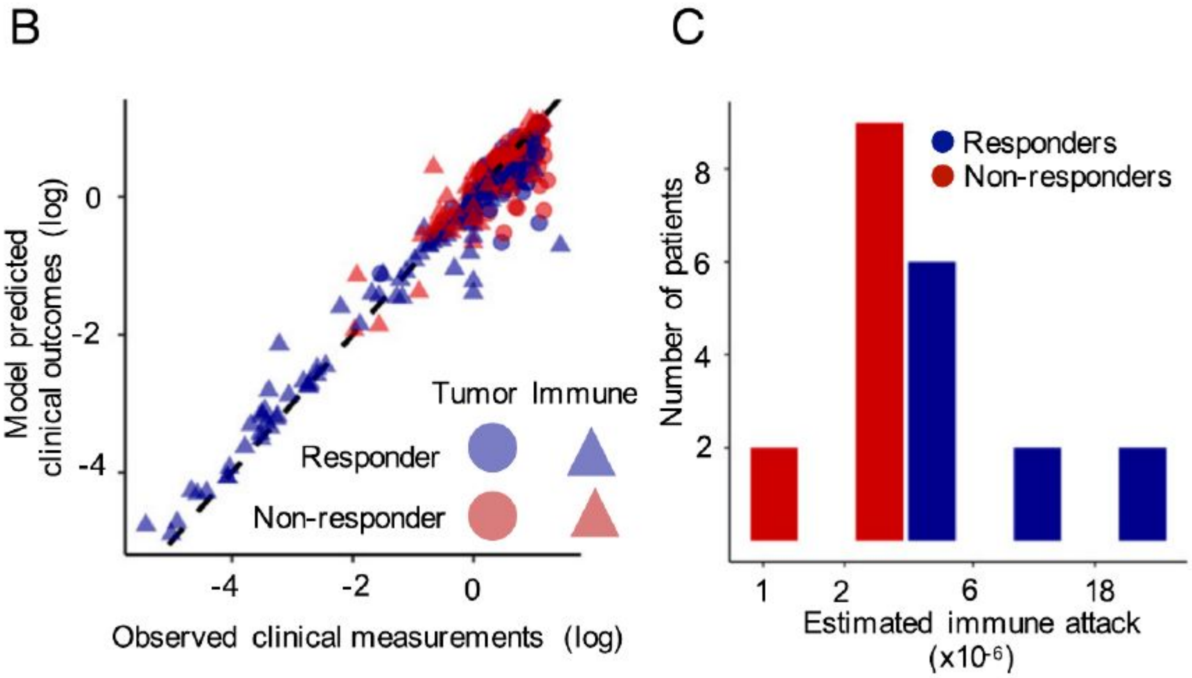


$$RGR_T = \frac{1}{T}\frac{dT}{dt} = \overbrace{r_T(1-\gamma_T T)}^{\text{Tumor growth}} - \overbrace{(\alpha + \beta_\varphi P)I}^{\text{Immune attack}} - \overbrace{\sum_i \vec{\mu_T}[i]\, C_i}^{\text{Chemotherapy}}$$

$$RGR_I = \frac{1}{T}\frac{dI}{dt} = \underbrace{(r_I + \beta_r P)(1-\gamma_I I)}_{\text{Immune growth}} + \underbrace{(\lambda + \beta_\lambda P)T}_{\text{Recruitment}} - \underbrace{\sum_i \vec{\mu_I}[i]\, C_i}_{\text{Chemotherapy}}$$

Model prediction and performance

- The strength of immune-tumor interaction is estimated by statistically fitting the growth rate of immune cells and tumor size to model predictions.
- Changes in tumor burden and immune cell abundance are described by data fitting, using a Bayesian hierarchical model.



Multilevel/hierarchical models

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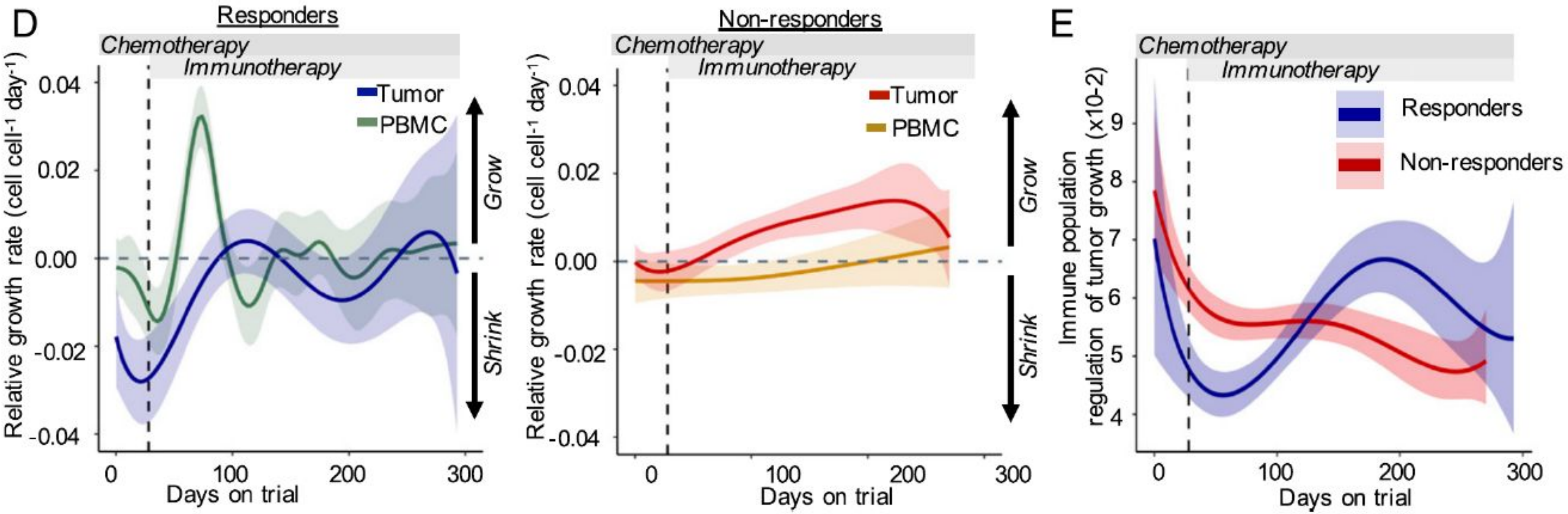
Profiles of relative growth rates differ between responders and non-responders

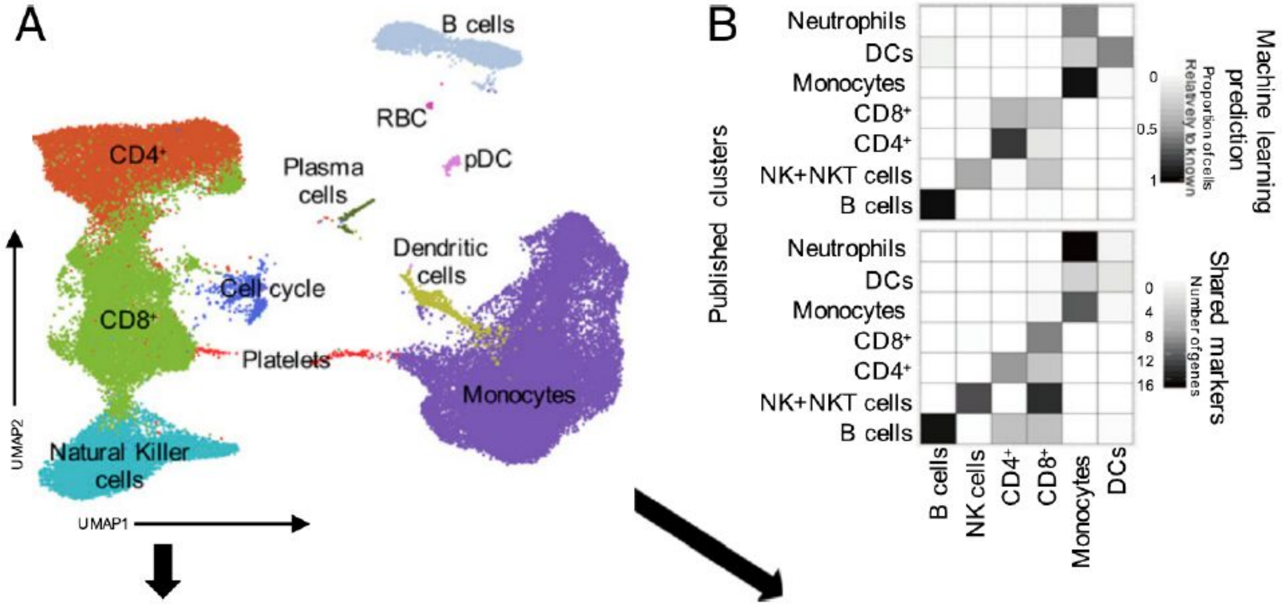


- Neither tumor nor PBMC responds to chemotherapy in non-responders.
- Responders have lower PBMC abundance in general at baseline.

Immune cell population identified by scrnaseq

PBMCs were analysed at three time points:

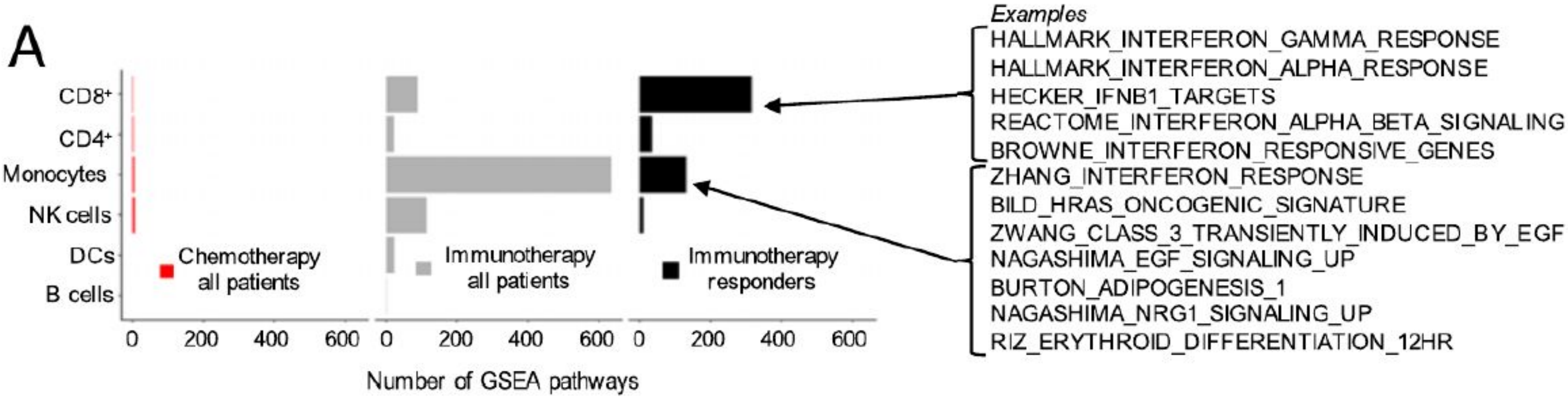
1. Cycle 1 (C1): baseline before treatment;
2. Cycle 3 (C3): chemotherapy alone;
3. Cycle 5 (C5): chemotherapy + anti-PD-1.



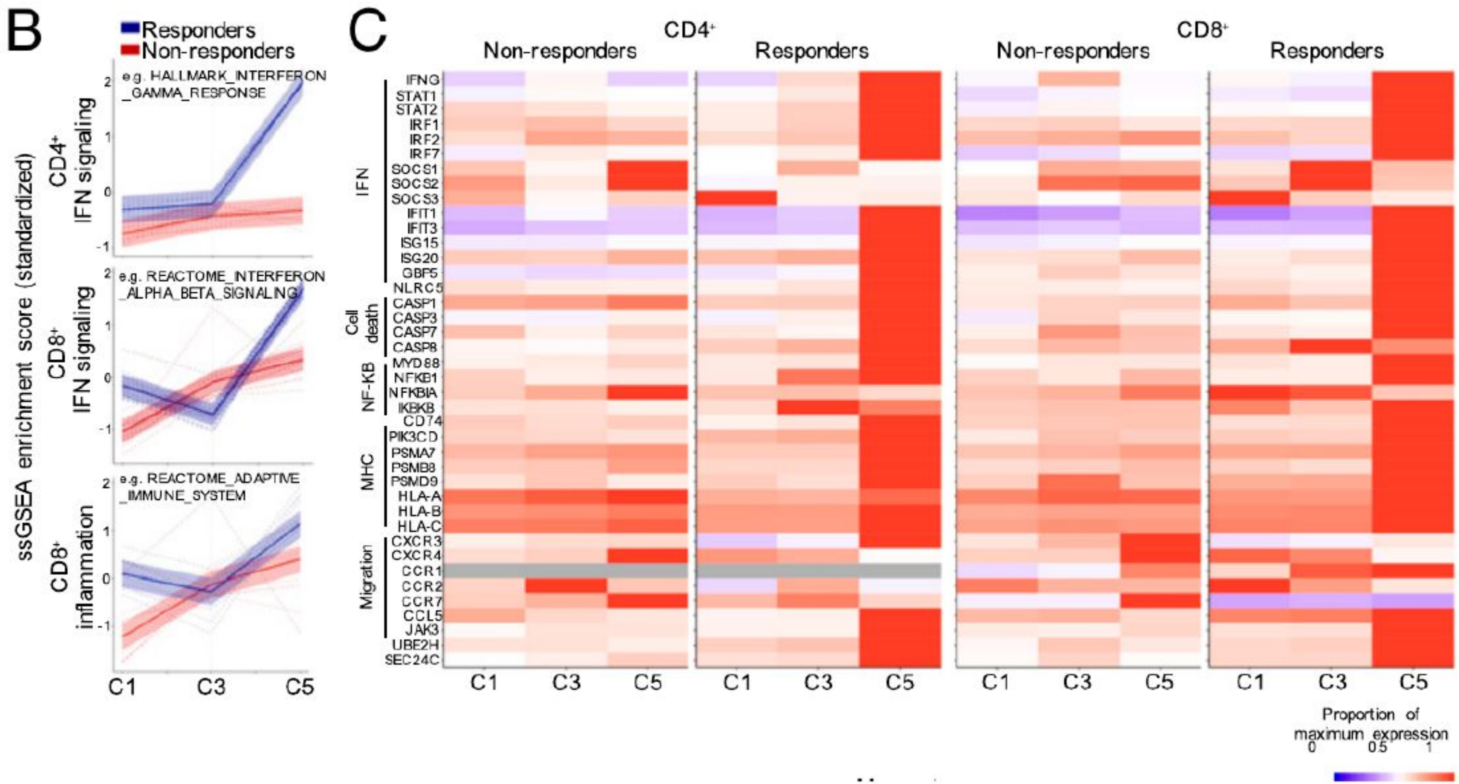
A total number of 70781 cells from 13 patients (7 responders and 6 non-responders) were profiled.

Pathway analysis

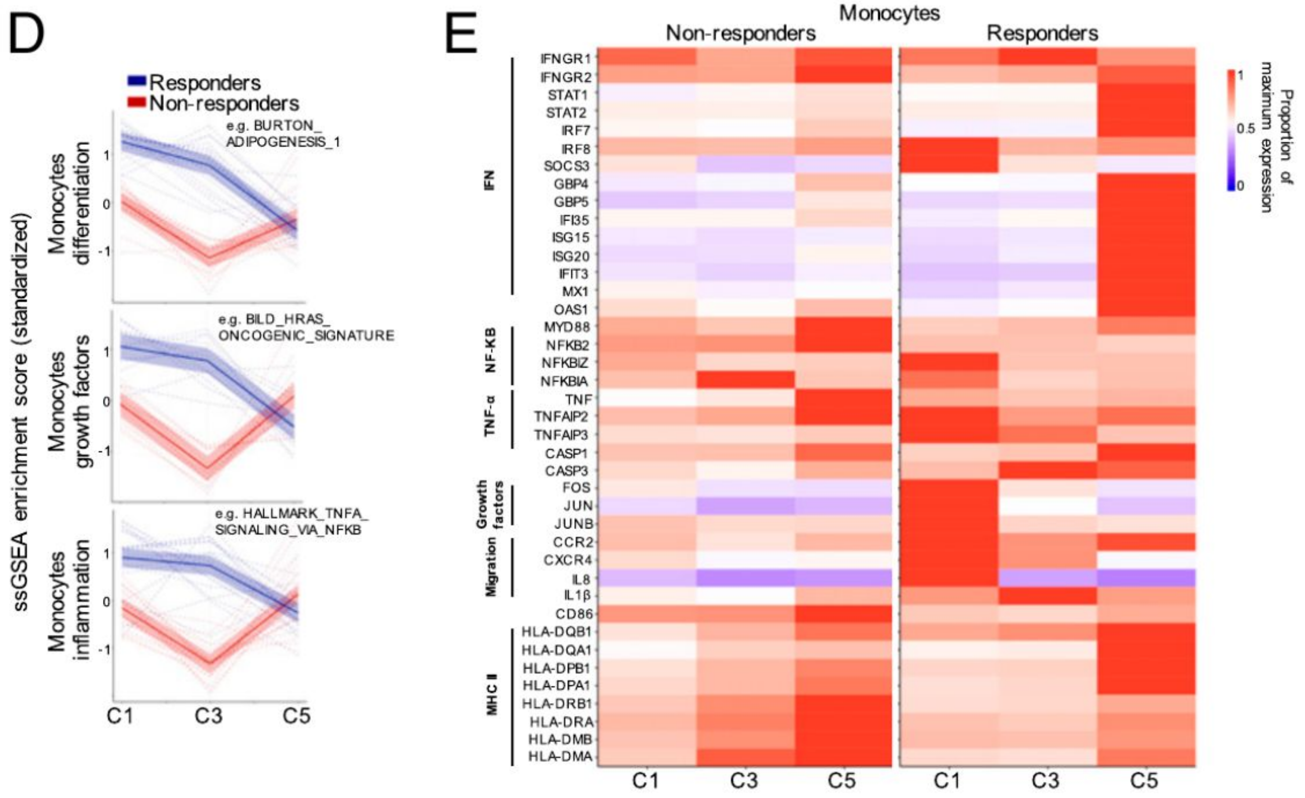
Single-sample gene-set enrichment analysis was performed to identify pathway differences before therapy, during chemotherapy, and during the early combo of chemotherapy and immunotherapy using a *random effects linear model*.



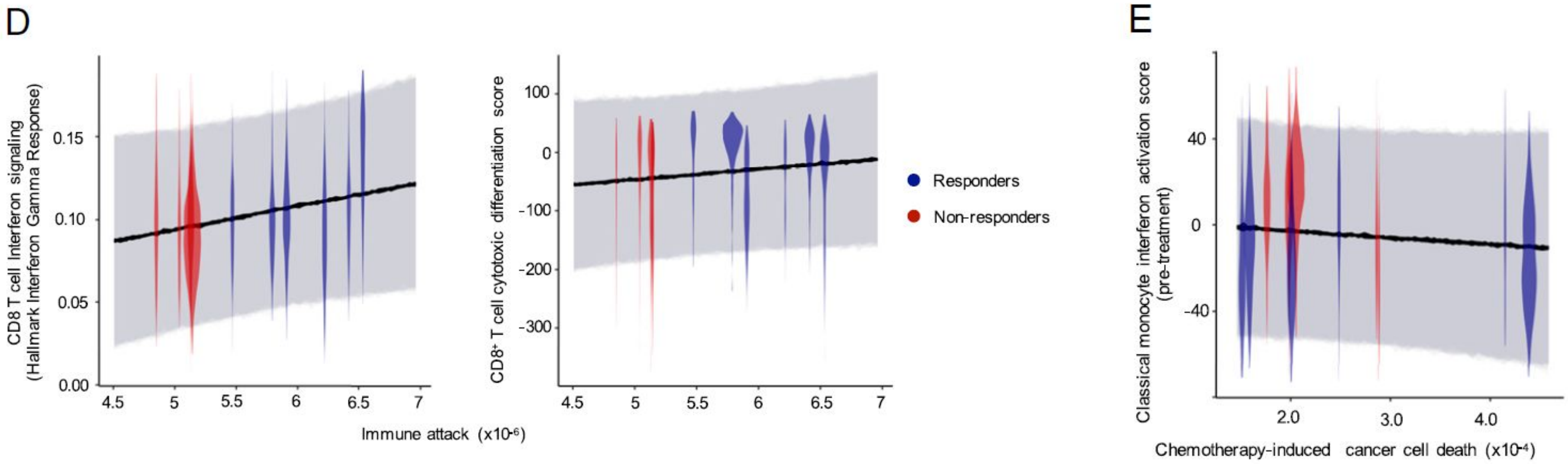
Responders show changes in T-cell signalling during treatment



Responders show changes in monocyte signalling during treatment



Associations between omics data and inferred model parameter



Impact and limitation of the study

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- *On the biological side,* the results suggest that peripheral blood phenotypes can be used as biomarker of patient responsiveness to therapy. The idea seems to be confirmed the findings by Wu *et al.*, *Peripheral T cell expansion predicts tumour infiltration and clinical response*, Nature 2020.
- *On the modelling side*, the study integrates machine learning, omics data analysis, mathematical modelling techniques to link macroscopic findings, for instance antigen and RECIST scores, with cellular findings, including scRNAseq and flow cytometry. This study exemplifies what we call *multiscale modelling of drug mechanism and safety*.
- We do not know why some patients respond to anti-PD-1 or anti-PDL1 therapies better than other patients based on findings reported in both papers. Nevertheless, both studies suggest that immune cells in peripheral blood may be used as biomarkers in certain settings.

Bonus: Mathematical modelling of epidemiology

The SIR (S=susceptible, I=infectious, R=removed) model modelling epidemiology (without viral dynamics, $N = S + I + R$).

$$\frac{dS}{dt} = -\frac{\beta IS}{N}, \tag{3}$$

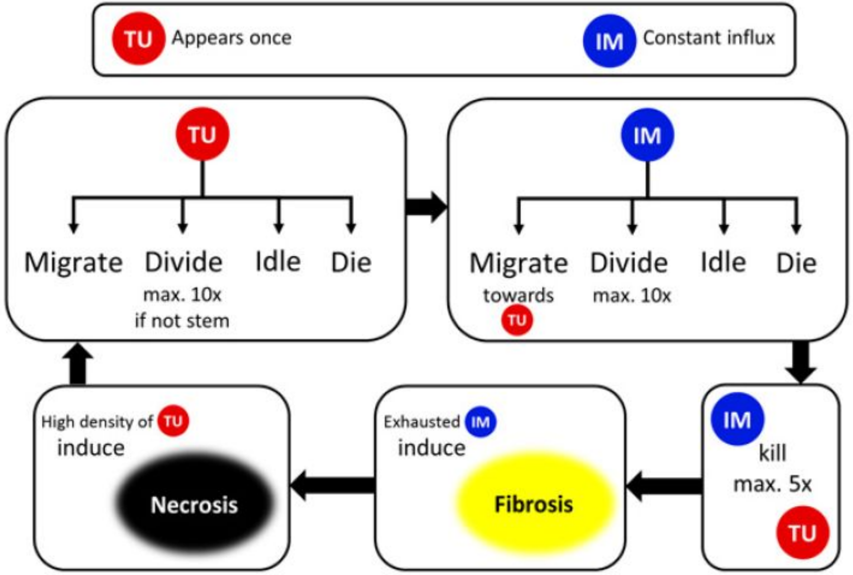
$$\frac{dI}{dt} = \frac{\beta IS}{N} - \gamma I, \tag{4}$$

$$\frac{dR}{dt} = \gamma I \tag{5}$$

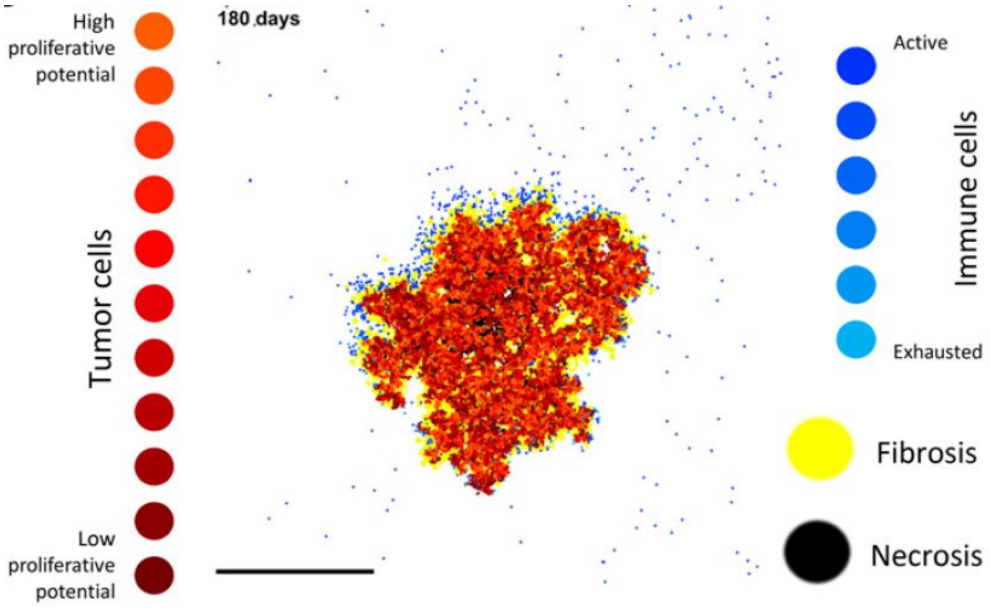
Agent-based modelling of cancer immunotherapy for colorectal cancer without high TMB

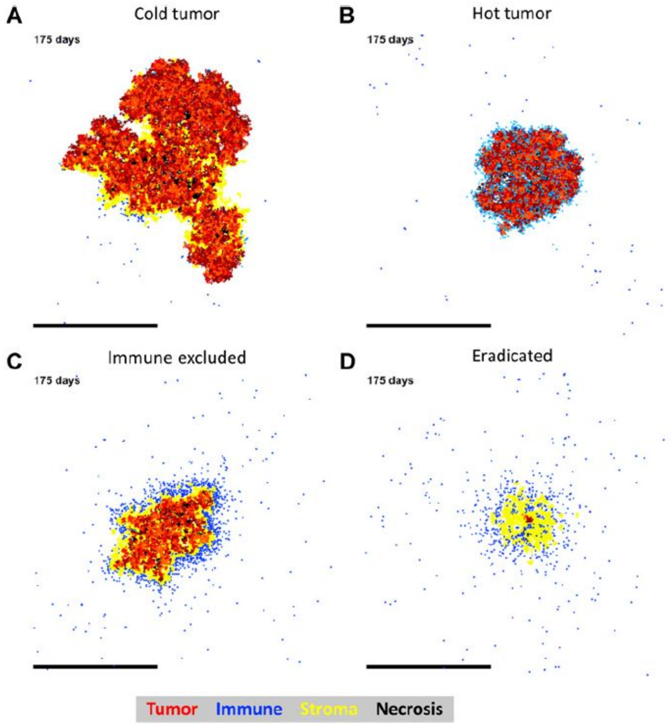
Table 1. Assumptions for the model and references for each assumption

Assumption	Ref.
All cells can migrate, proliferate, and die.	Trivial
Tumor cells are composed of stem cells and non-stem cells. Stem cells can divide symmetrically with a fixed probability.	(14)
Stem cells can proliferate indefinitely, all other cells die after a fixed number of proliferation cycles.	(14)
All cells can spontaneously enter apoptosis.	Own data
Tumor cells can spontaneously enter necrosis.	Own data
Tumor cells that are far from the outer margin have a higher probability of entering necrosis than those cells closer to the margin.	Own data
Immune cells are generated through a steady influx into the domain and proliferation within the domain.	(32), own data
Immune cells move by a "random walk" but have a tendency to migrate toward tumor cells.	(31–33), own data
Immune cells can kill adjacent tumor cells whenever they are close enough. Killing, like other events in the model, occurs stochastically with a fixed probability and is not regulated by other factors.	(23)
Immune cells can kill five times before they become exhausted, which means that they cannot kill anymore but can still proliferate.	(23, 34)
Activated immune cells give rise to stroma through a desmoplastic reaction (stroma reaction). For simplicity, this behavior is restricted to immune cells that have successfully killed five times in the model.	(35, 36)
By default, cells cannot migrate through stroma, but stromal permeability can be increased optionally.	(37)

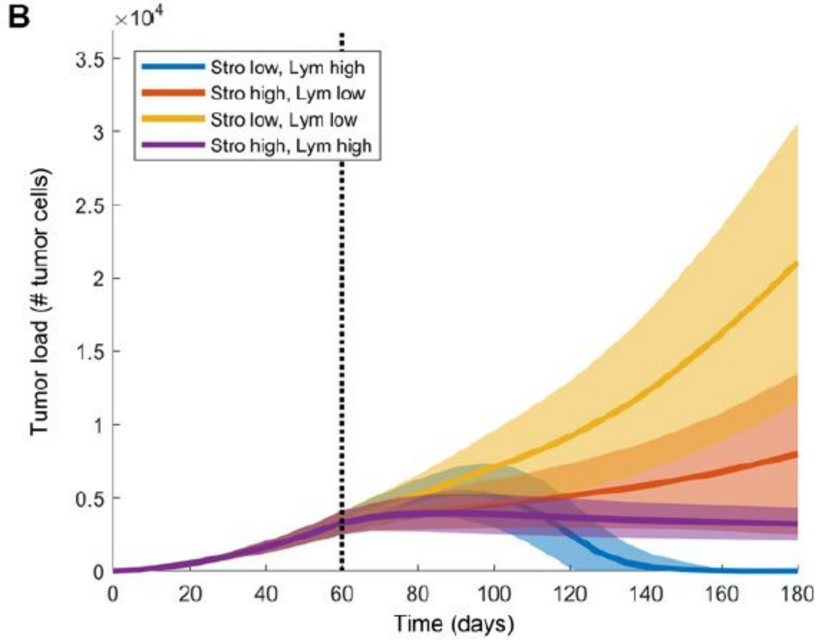


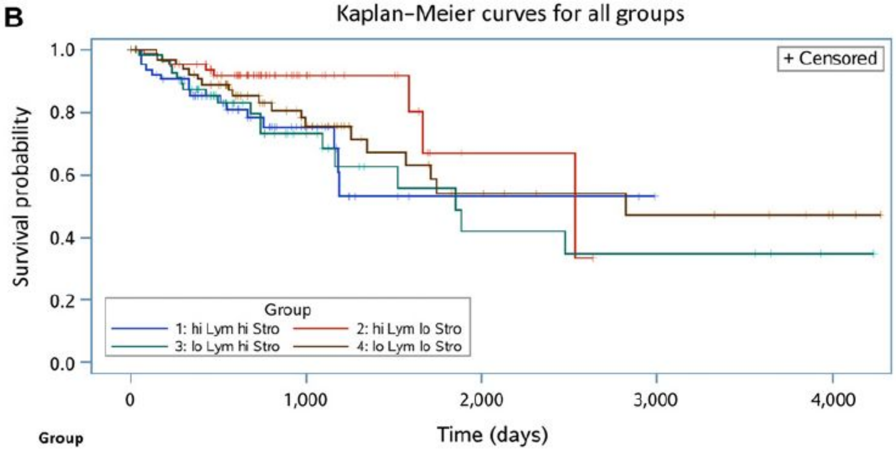
Agent-based modelling of cancer immunotherapy for colorectal cancer without high TMB





Counterfactual and statistical analysis allow us *learn* from the models *confirm* the learnings



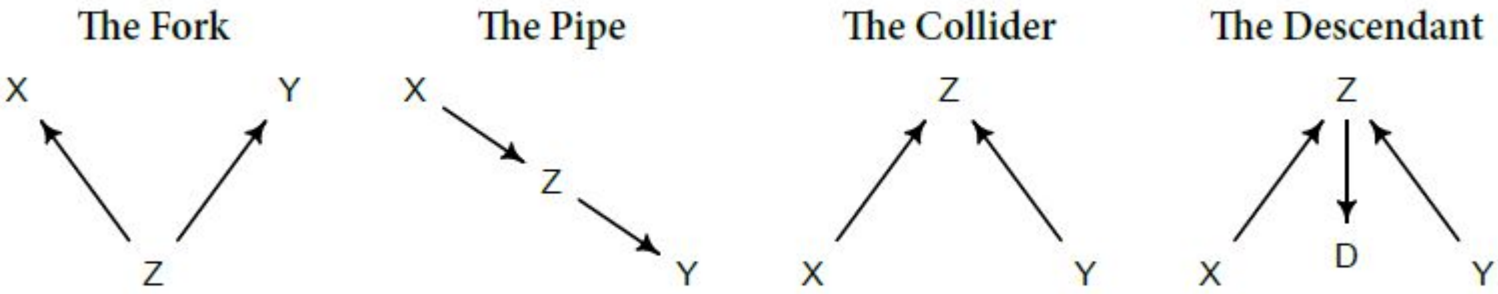


Group	0	1,000	2,000	3,000	4,000
1	66	16	2	0	
2	65	17	2	0	
3	59	17	6	5	1
4	70	28	11	7	3

Bradford Hill Criteria for causation

1. **Strength** (effect size)
2. **Consistency** (reproducibility)
3. ***Specificity***
4. **Temporality**
5. **Biological gradient** (dose-response relationship)
6. ***Plausibility***
7. **Coherence**
8. **Experiment**
9. **Analogy** (similarity)
10. **Reversibility (proposed by others)**

Statistical causal inference with Directed Acyclic Graphs (DAGs)



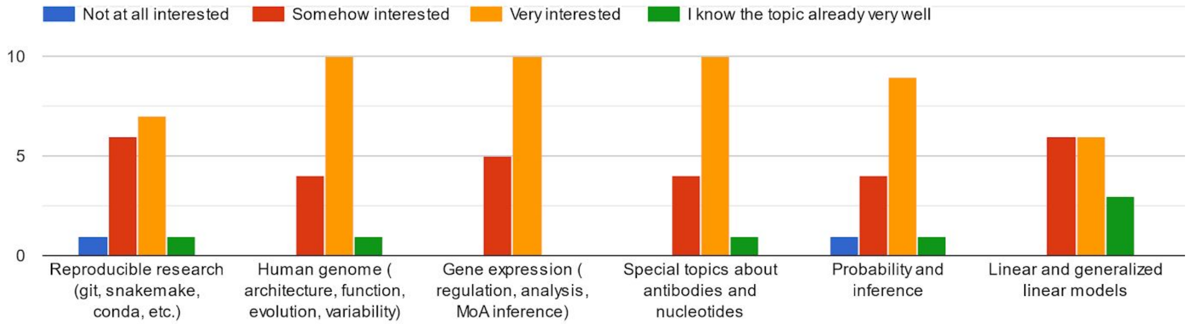
Reading: chapter 1-6 of *Statistical Rethinking* (2nd Edition)

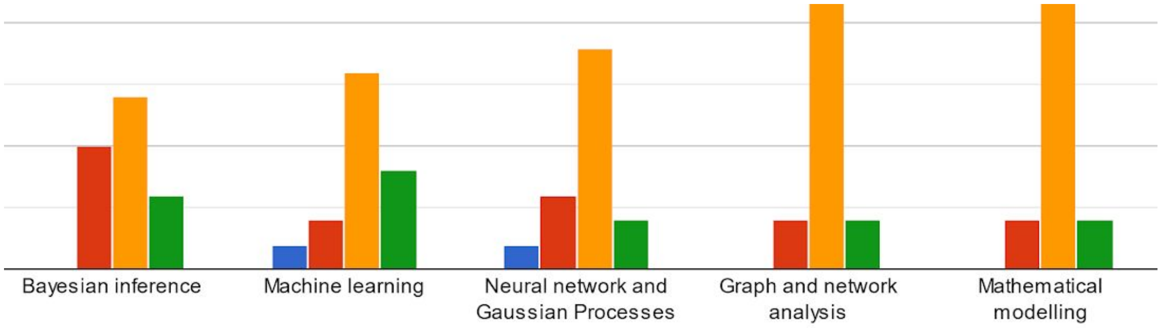
Conclusions

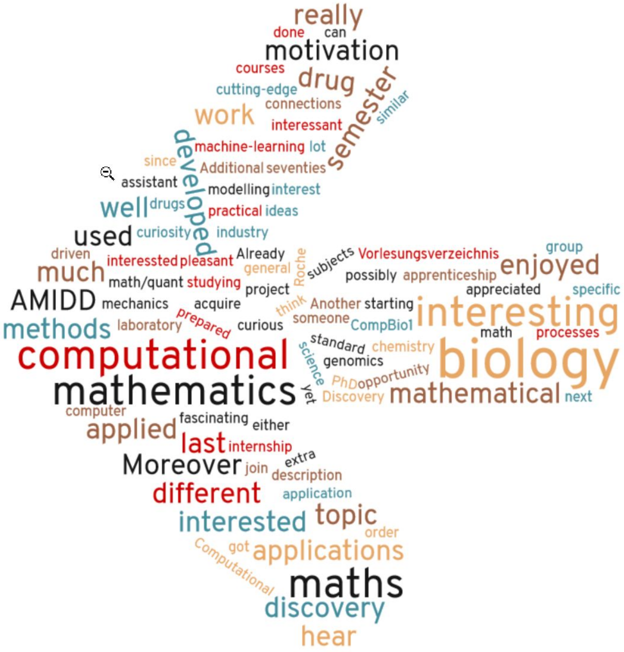
- Understanding how drugs work and how to develop better drugs requires *causal reasoning*, for which there are no scientific consensus yet.
- I personally argue for integrated mechanistic, computational, and statistical modelling across scales as a approach towards causal reasoning.
- Mathematical and computational biology is indispensable to address this grand challenge.

Ways to learn more about mathematical & computational biology in drug discovery

- **People** around you, both with the same and different backgrounds;
- **Reading**, including Journal *Nature Reviews Drug Discovery*, blogs *In the Pipeline*, *CureFFI*, and newsletter *This Week in Mathematical Oncology;*
- **Online courses**: *Statistical Rethinking* by Richard McElreath, with freely available lecture videos on YouTube*, and Information Theory, Inference, and Learning Algorithms* by David MacKay, with freely available lecture videos.







Your interest and motivation *motivated* me. THANK YOU!

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