THE OPEN PROJECT

Essential Modelling & Bioinformatics to Advance Preclinical Decision Making

Data & Clarity

Tao You & John Prime

Tumour Models, 4 Dec 2019

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Workshop Theme

For any cancer immunotherapy, how to choose an appropriate tumour model to represent a patient population and how to predict clinical efficacy?

- 3 members each team
- Desirable: Each team has at least 1 biologist and 1 pharmacologist
- Brainstorm: Sticky notes, flipchart
- Each team will present the strategy in 3 minutes

It's OK to introduce yourself, say "I don't know", ask for more clarity, say you don't understand, ask what acronyms stand for, ask why and why not, depend on the team, ask for help, not know everything, have quiet times, have loud times, make mistakes, sigh, be excited.

Essential Modelling & Bioinformatics to Advance Preclinical Decision Making

Instructor

Tao You, PhD. PK/PD Modelling & Data Scientist, Beyond Consulting Ltd

- Chemical Engineering, BE, 1998-2002
- Biological and Chemical Engineering, MSc, 2002-2003 — *Singapore–MIT Alliance*
- Cancer Bioinformatics, 2003-2005
- Systems Biology, PhD, 2005-2009
- Systems Biology, Postdoc, 2009-2011
- Physiological Modeller & PK/PD Modeller, 2011-2015
- PK/PD Modelling Lab Head, 2016-2018
- PK/PD Modeller & Data Scientist, Aug 2018 – now

https://www.letsgobeyond.co.uk/testimonials

Disclosure

Tao serves as a PK/PD modelling consultant for

John Prime - Career Overview

PhD Liver cancer (HCC) genetics - Oxford (Prof. J O'D McGee)

2000 - 2003 Amersham Biosciences

- Quantitative proteomics - 2D-DIGE (2D Difference gel electrophoresis)

2003 – 2010 KuDOS Pharmaceuticals / AstraZeneca

- Translational Science/Biomics team leader => Head of Biomics

2010 – 2016 Oncology Research Bioinformatics, MedImmune

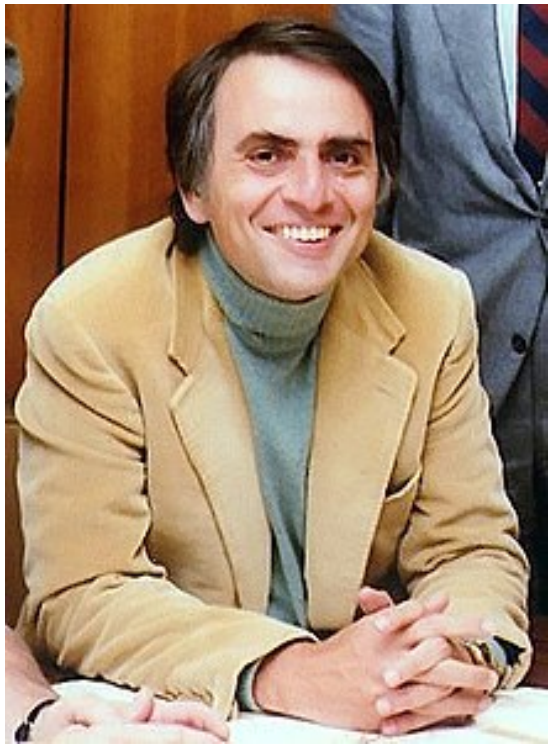
- Led Oncology research (UK & USA) Bioinformatics support for IO projects

2016 – Present Principal Consultant, OncoBioinformatics Consulting

2017 – Present Senior Bioinformatics Scientist, Horizon Discovery

To get the most out of your discussion today

1. Identify the right questions
2. Go deep by asking why (a few times)
3. Sort the fact from fiction

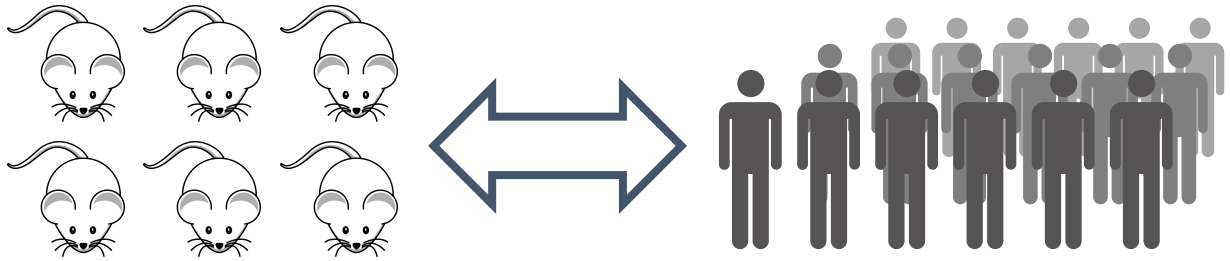


"We can judge our progress by the courage of our questions and the depth of our answers, our willingness to embrace what is true rather than what feels good."

— Carl Sagan (1934-1996)

Objectives

- Recognise the importance of translation
- Xenograft model: Characterisation, translation, evaluation
- Syngeneic model: Characterisation, translation
- Patient population: Characterisation, translation



Agenda

	Duration	Time
• Intro Tao and John	5 mins	
• Participant Intro	10 mins	
• Grouping	5 mins	
• **Lecture 1.** Clinical translation of xenograft models (Tao)	20 mins	9:40am
• **Lecture 2.** Using bioinformatics to aid clinical translation to models (John)	20 mins	
• Group exercise 1: Characterise a patient population for a new CI target and match it to *in vitro*/*in vivo* models	30 mins	10:30am
• Coffee Break / networking / further discussion	10 mins	10:40am
• **Lecture 3.** Clinical translation of syngeneic models (Tao)	20 mins	
• Group exercise 2: Clinical translation of syngeneic models	30 mins	
• Group presentations	20 mins	11:50am
• Discussions & feedbacks	10 mins	12:00pm

Lecture 1. Clinical translation of xenograft models

- The importance of translation
  - Regulatory approval requirements
  - Success rate in oncology drug discovery & development projects
  - 3 pillars & 5Rs

- Clinical translation of xenograft models
  - NCI: Mouse MTD efficacy does not predict clinical efficacy
  - Chemo: Mouse MTD AUC and clinical AUC => clinical fate
  - Chemo: Qualify A2780 (ovarian) xenograft model with PK/PD modelling
  - Chemo & Targeted therapy: Preclinical efficacy => Clinical efficacy (ORR)

Lecture 2. Using bioinformatics to aid clinical translation to models

- **Range of omics data types**
  - Data complexity in clinical/patient samples
  - Value of integrating the right models to the right granularity of patient data

- **Example I:-**
  - Small molecules/targeted therapy – Selecting the right PDX models to match a patient subset
  - Challenges, caveats and pitfalls
- **Tools and omics data sources (i)**

- **Biologics – Cancer Immunotherapy**
  - The challenges of modelling the immune system in cancer
  - Response: Tissue of origin not accurate predictor
  - Cancer immunotherapy – Requires a holistic data paradigm
- **Example II:-**
  - Biologics - Immunotherapy
  - Further challenges, caveats and pitfalls

- **Tools and omics data sources (ii) – Cancer Immunotherapy**

Lecture 3. Clinical translation of syngeneic models

- Clinical translation of syngeneic models
  - Adaptive immunity to tumours
  - Resistance mechanisms to immunotherapy
  - Modelling efficacy: RT/αPD-L1 combination in CT26 syngeneic tumour model

- Group exercise 2

  What are the questions to consider when prospectively translating syngeneic model results into the clinics?

Lecture 1. Clinical translation of xenograft models

- The importance of translation
- Clinical translation of xenograft models

1.1 The importance of translation

- Regulatory approval requirements
- Success rate in oncology drug discovery & development projects
- 3 pillars & 5Rs

Clinical endpoints for cancer trials

Overall Survival

- The time from randomization until death from any cause, and is measured in the intent-to-treat population

Surrogate endpoints based on tumour assessments

- ***Objective Response Rate***: The proportion of patients with tumour size reduction of a predefined amount and for a minimum time period
- ***Disease-Free Survival (Event-Free Survival)***: The time from randomisation until disease recurrence or death from any cause
- ***Complete Response***: No detectable evidence of tumour
- ***Time to Progression***: The time from randomisation until objective tumour progression
- ***Progression-Free Survival***: The time from randomisation until objective tumour progression or death, whichever occurs first

FDA. (2007~2018) Guidance for Industry: Clinical Trial Endpoints for the Approval of Cancer Drugs and Biologics.

FDA regulatory approval requirements

Traditional approval (previously known as “regular approval”)

- Longstanding route of drug approval based on the demonstration of clinical benefit or an effect on a surrogate endpoint known to predict clinical benefits
- Supported by OS
- Supported by ORR in selected settings

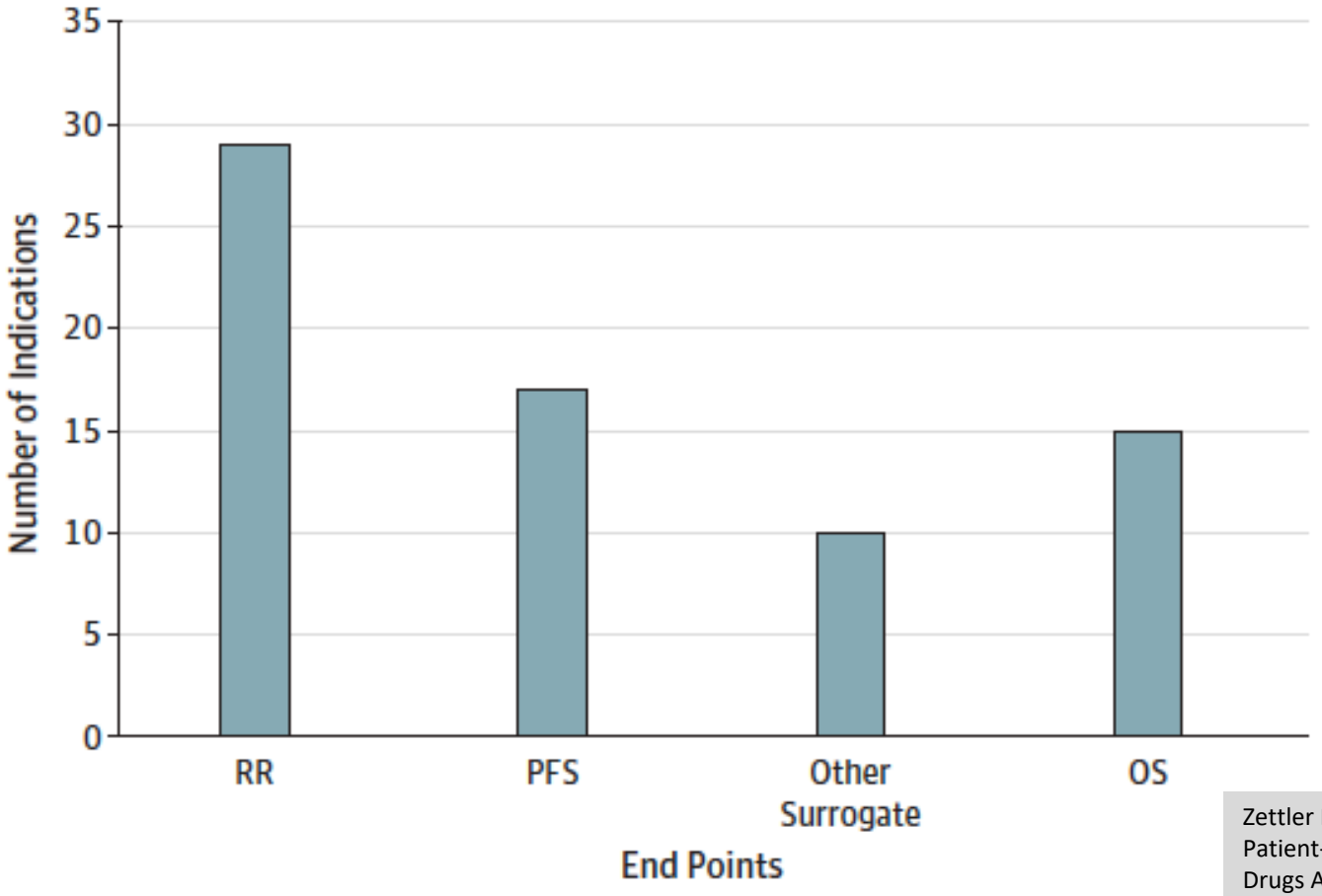
Accelerated approval

- Approval associated with use of a surrogate endpoint or intermediate clinical endpoint that is reasonably likely to predict benefit

FDA. (2007~2018) Guidance for Industry: Clinical Trial Endpoints for the Approval of Cancer Drugs and Biologics.

ORR is extensively used in FDA approvals

Figure 1. Overall Survival and Surrogate End Points Used as the Basis of Approval for 69 Initial Indications of 63 Novel Oncology Drugs Approved by the FDA Between 2011 and 2017[a,b,c]



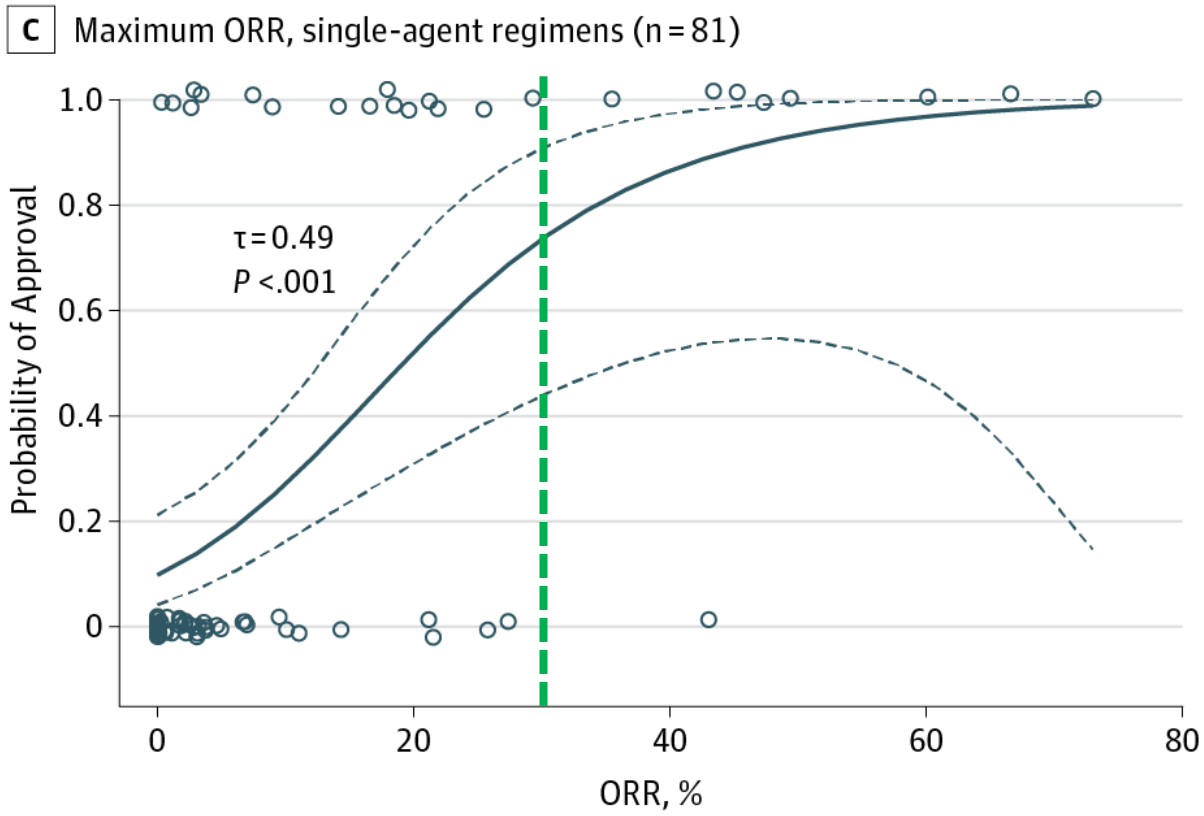
Zettler M *et al*. (2019) Surrogate End Points and Patient-Reported Outcomes for Novel Oncology Drugs Approved Between 2011 and 2017. JAMA Oncol. 3 Jul 2019

ORR is significantly associated with approval decision

Question Is ORR associated with regulatory approval of an anticancer regimen?

Data 1800 trials in advanced solid tumours (1st Oct 2007 ~ 30th Sep 2010): NSCLC, CRC, melanoma, renal cell cancer

Result Association is statistically significant: For single-agents, 89% of regimens with ORR ≥ 30% achieved approval



Oxnard GR *et al*. (2016) Response Rate as a Regulatory End Point in Single-Arm Studies of Advanced Solid Tumors. *JAMA Oncol.* 2(6):772-9.

Appropriate ORR is necessary but insufficient to achieve approval

<u>Can translational modelling predict ORR?</u>

Criticisms of the extensive use of ORR

- ORR is not predictive of OS (many references)
- PFS is not predictive of OS
- DFS is strongly associated with OS only under certain circumstances
- Only ~20% of FDA approved new regimens showed OS improvement (11th Dec 1992~ 31st May 2017)
- The use of ORR and other surrogate endpoints might need restricted to cases where they predict OS

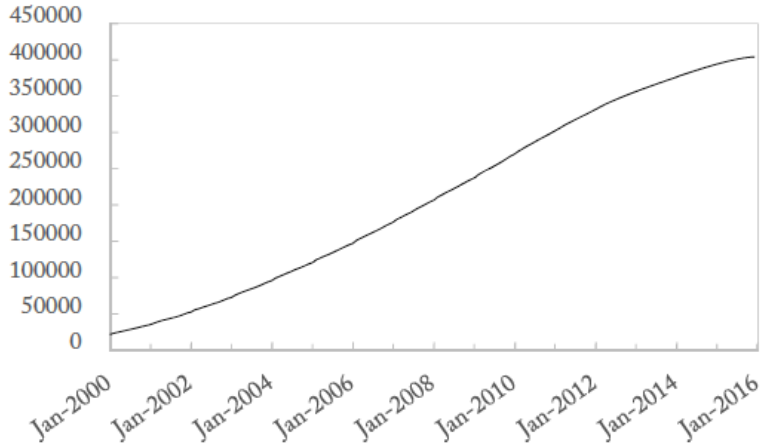
Fischer A *et al*. (2016) Extrapolation from Progression-Free Survival to Overall Survival in Oncology. OHE Research paper 16/07.

Mauguen A et al. (2013) Surrogate endpoints for overall survival in chemotherapy and radiotherapy trials in operable and locally advanced lung cancer: a re-analysis of meta-analyses of individual patients' data. *Lancet Oncol*. 14(7):619-26.

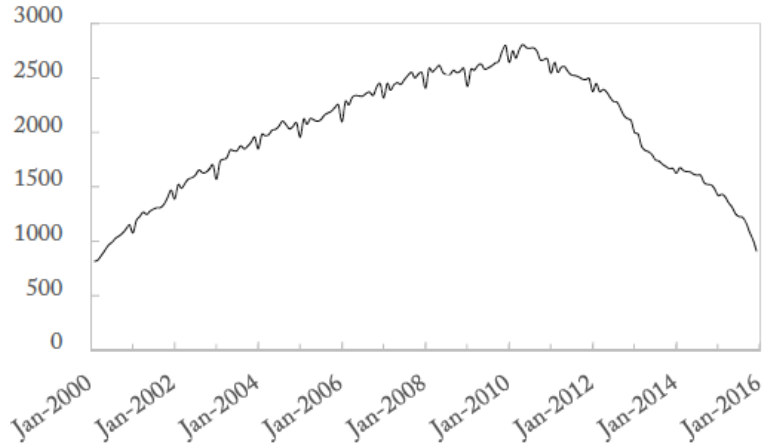
Gyawali B *et al*. (2019) Assessment of the Clinical Benefit of Cancer Drugs Receiving Accelerated Approval. JAMA Intern Med. 179(7):906-913.

Kim C & Prasad V (2016) Strength of Validation for Surrogate End Points Used in the US Food and Drug Administration's Approval of Oncology Drugs Mayo Clin Proc. pii: S0025-6196(16)00125-7.

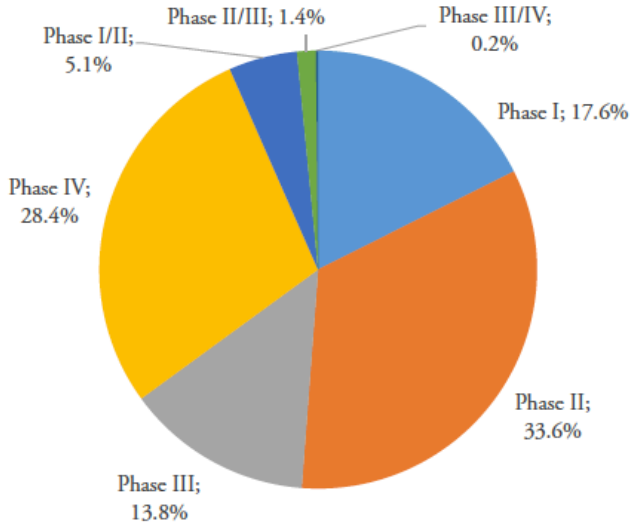
Clinical trials 2000-2015



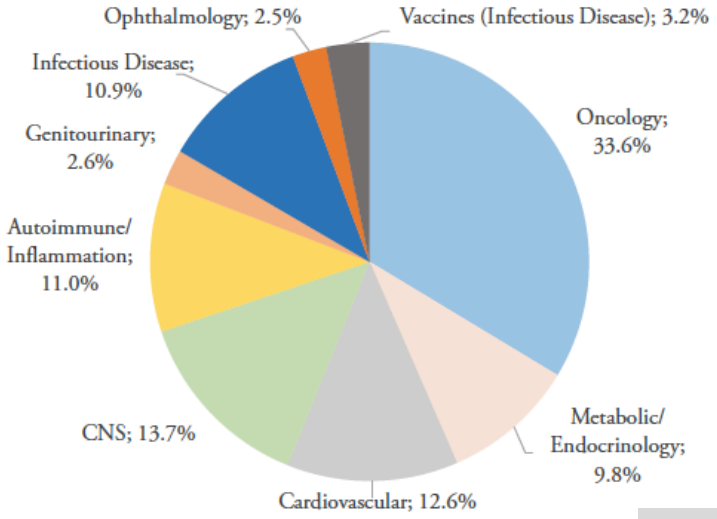
(a) Cumulative number of trials over time



(b) Increase in the number of trials over time



(c) Proportion of trials by phases

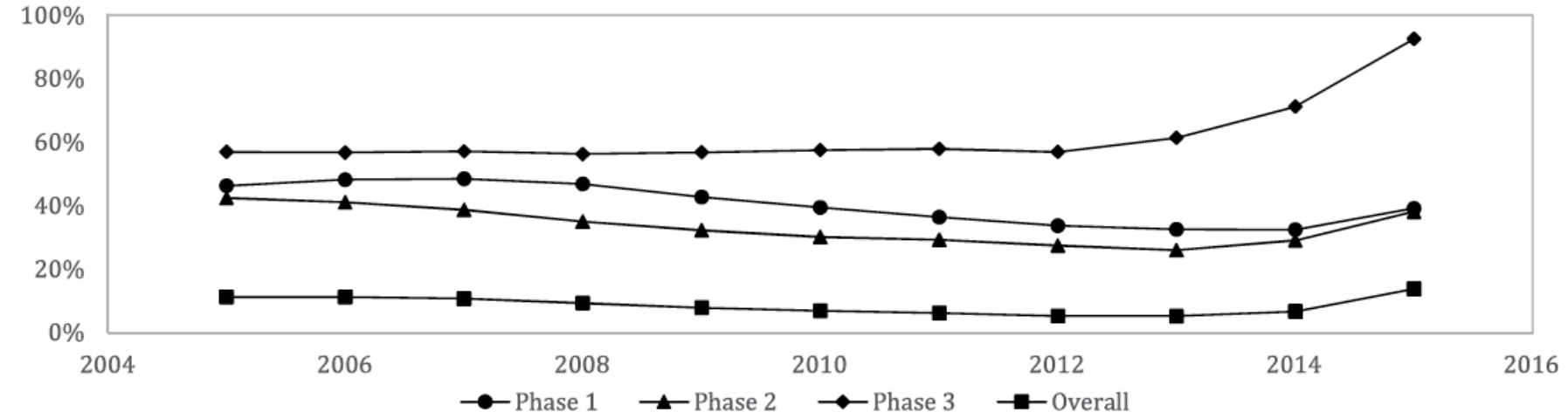


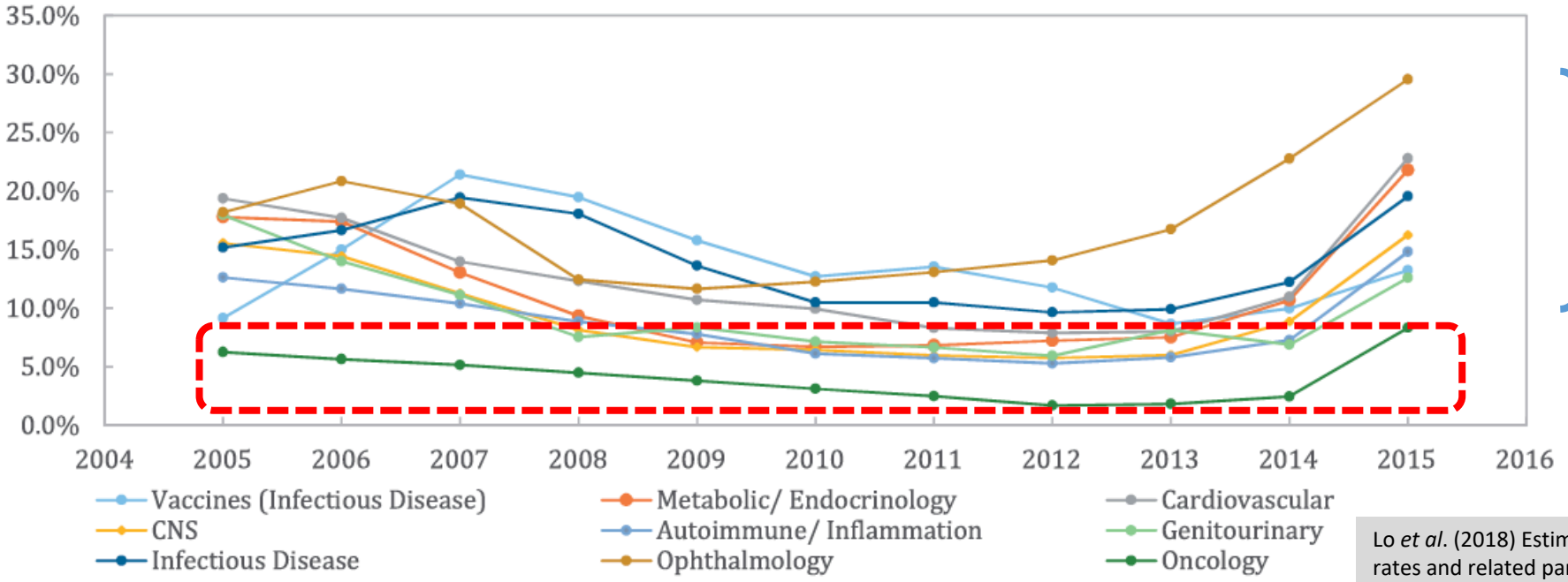
(d) Proportion of trials by therapeutic groups

~186,000 trials in total

Lo *et al*. (2018) Estimation of clinical trial success rates and related parameters. *Biostatistics*. p 1–14

Cancer drug programmes are more risky (on average) than previously thought





$POS_{1,App}$

20.9%

3.4%

Lo *et al*. (2018) Estimation of clinical trial success rates and related parameters. *Biostatistics*. p 1–14

Cancer drug: Phase 2 is key

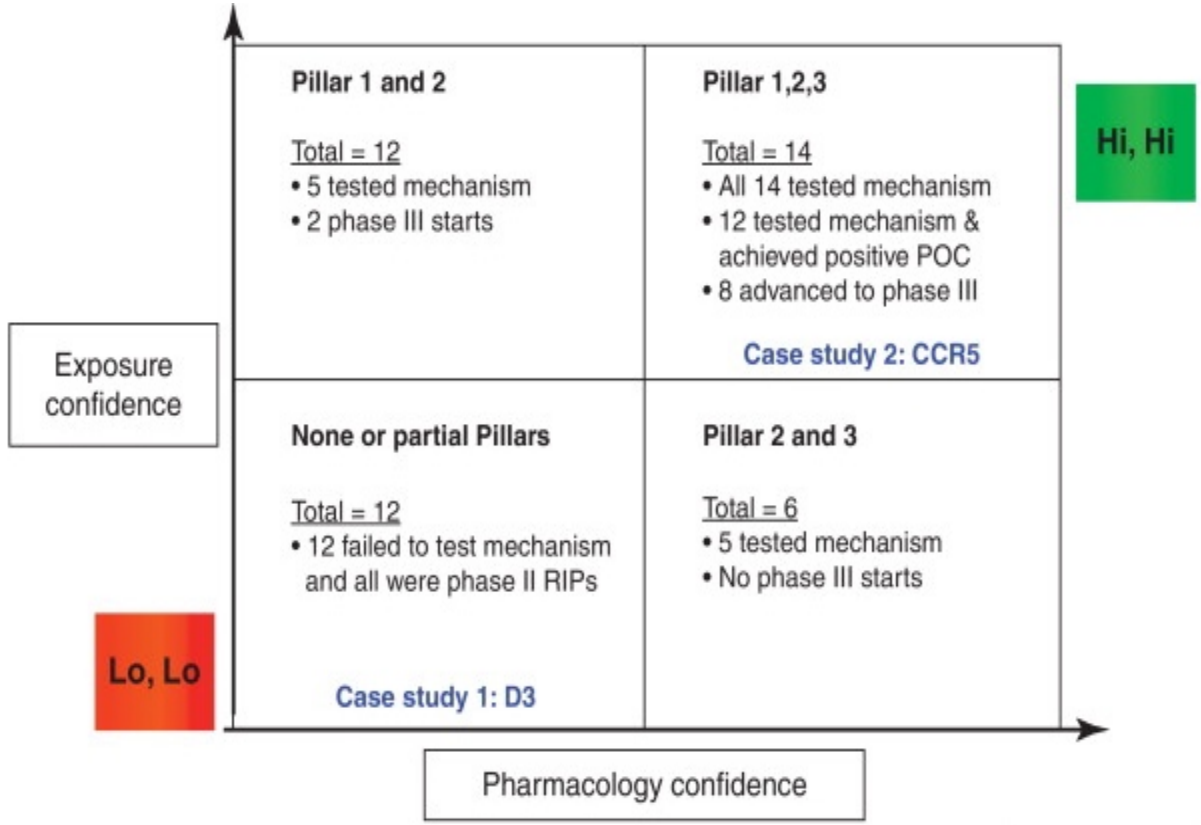
All indications (industry)

	Phase 1 to Phase 2		Phase 2 to Phase 3			Phase 3 to Approval		Overall
Therapeutic group	Total paths	$POS_{1,2}$, % (SE, %)	Total paths	$POS_{2,3}$, % (SE, %)	$POS_{2,APP}$, % (SE, %)	Total paths	$POS_{3,APP}$, % (SE, %)	POS, % (SE, %)
Oncology	17 368	57.6 (0.4)	6533	32.7 (0.6)	6.7 (0.3)	1236	35.5 (1.4)	3.4 (0.2)
Metabolic/ Endocrinology	3589	76.2 (0.7)	2357	59.7 (1.0)	24.1 (0.9)	1101	51.6 (1.5)	19.6 (0.7)
Cardiovascular	2810	73.3 (0.8)	1858	65.7 (1.1)	32.3 (1.1)	964	62.2 (1.6)	25.5 (0.9)
CNS	4924	73.2 (0.6)	3037	51.9 (0.9)	19.5 (0.7)	1156	51.1 (1.5)	15.0 (0.6)
Autoimmune/ Inflammation	5086	69.8 (0.6)	2910	45.7 (0.9)	21.2 (0.8)	969	63.7 (1.5)	15.1 (0.6)
Genitourinary	757	68.7 (1.7)	475	57.1 (2.3)	29.7 (2.1)	212	66.5 (3.2)	21.6 (1.6)
Infectious disease	3963	70.1 (0.7)	2314	58.3 (1.0)	35.1 (1.0)	1078	75.3 (1.3)	25.2 (0.8)
Ophthalmology	674	87.1 (1.3)	461	60.7 (2.3)	33.6 (2.2)	207	74.9 (3.0)	32.6 (2.2)
Vaccines (Infectious Disease)	1869	76.8 (1.0)	1235	58.2 (1.4)	42.1 (1.4)	609	85.4 (1.4)	33.4 (1.2)
Overall	41 040	66.4 (0.2)	21 180	58.3 (2.3)	35.1 (2.2)	7532	59.0 (0.6)	13.8 (0.2)

Lo *et al*. (2018) Estimation of clinical trial success rates and related parameters. *Biostatistics*. p 1–14

Pfizer 3 pillars (2012)

- "Pfizer 3 pillars"
  1. Suitable exposure (site of action, duration)
  2. Sufficient target binding
  3. Adequate pharmacology



Can the flow of medicines be improved? Fundamental pharmacokinetic and pharmacological principles toward improving Phase II survival *Drug Discovery Today.* 17, 419–424 (2012)

Pfizer 3 pillars (2012)

Exposure	Direct Target Engagement	Indirect Target Engagement	Physiological Response	Disease Modulation	Outcome/ Endpoints
	target itself "immediate" target	"downstream" signalling	tumour perfusion blood glucose	cell cycle arrest proliferation apoptosis	cellular growth inhibition tumour growth inhibition ORR PFS OS

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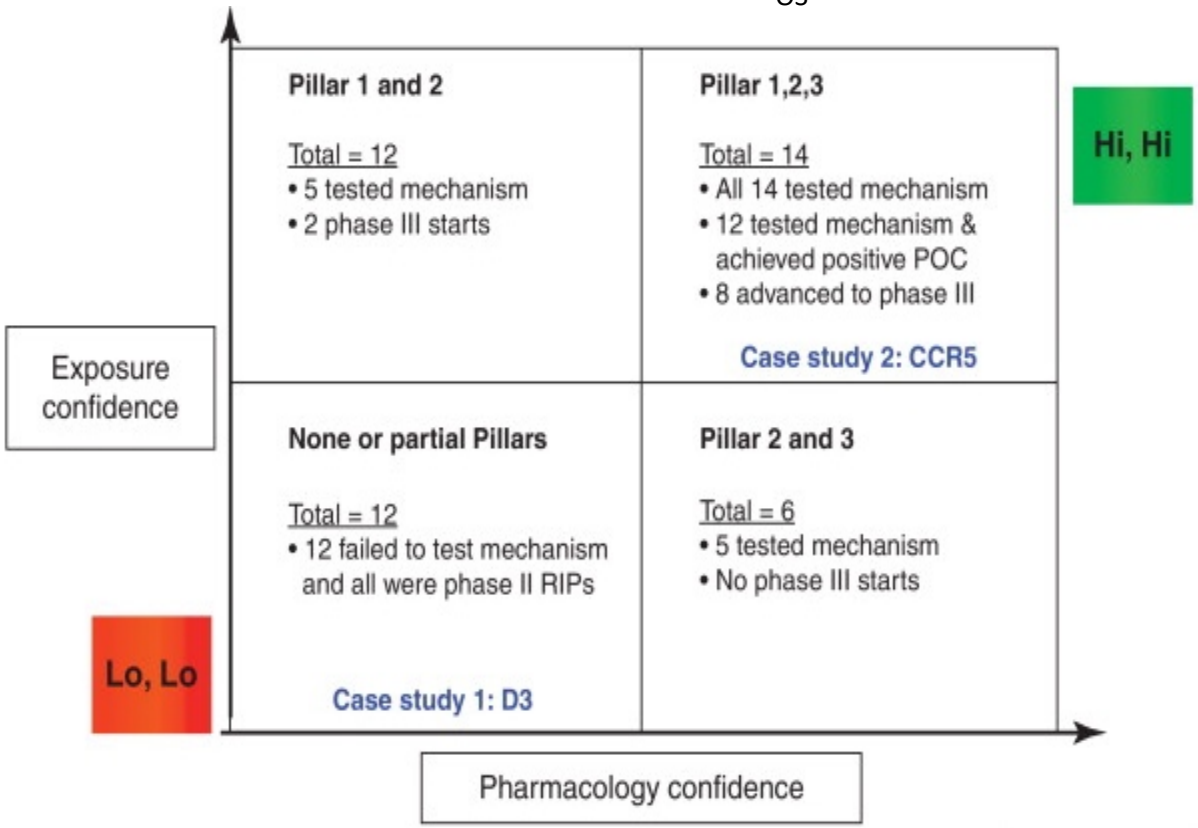

Can the flow of medicines be improved? Fundamental pharmacokinetic and pharmacological principles toward improving Phase II survival *Drug Discovery Today.* 17, 419–424 (2012)

AstraZeneca: Lack of clinical efficacy underpins attrition (2014)

Exposure	Direct Target Engagement	Indirect Target Engagement	Physiological Response	Disease Modulation	Outcome/ Endpoints
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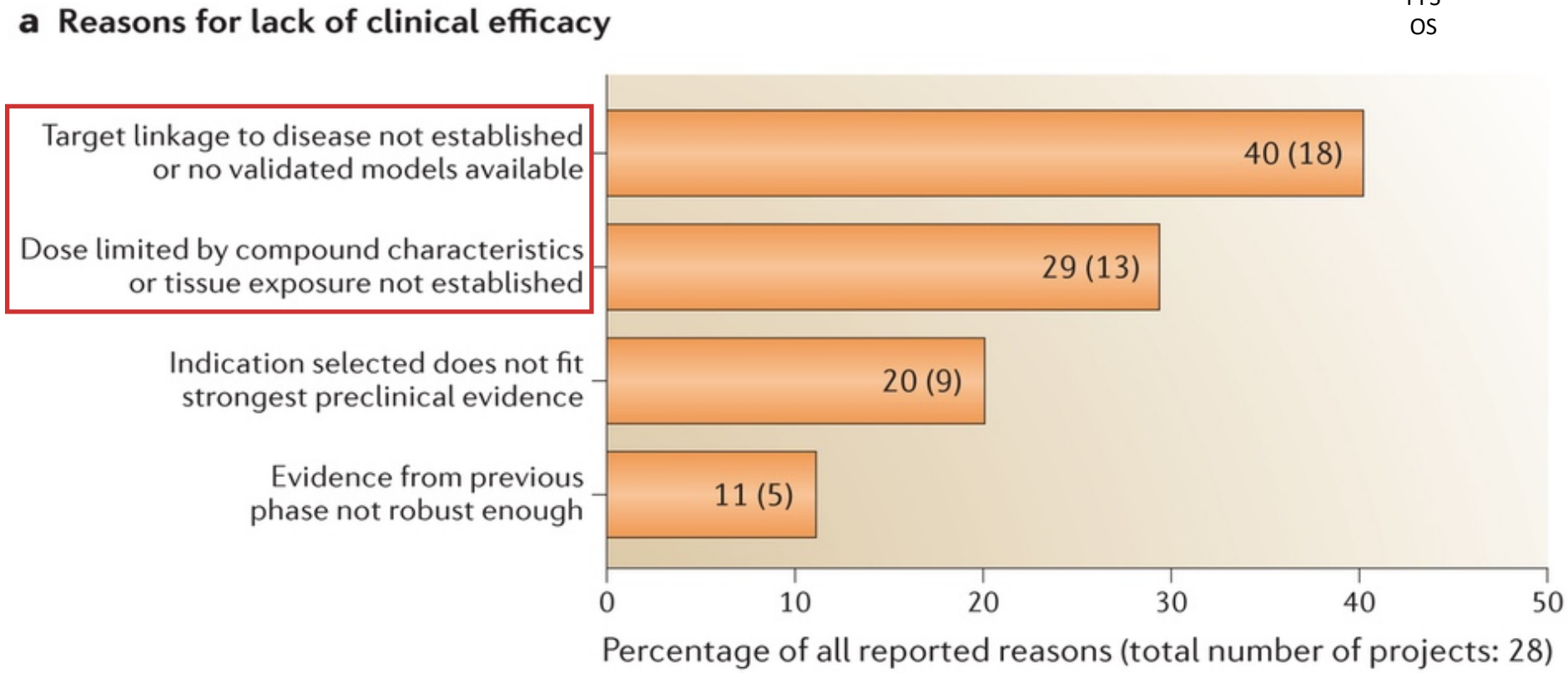
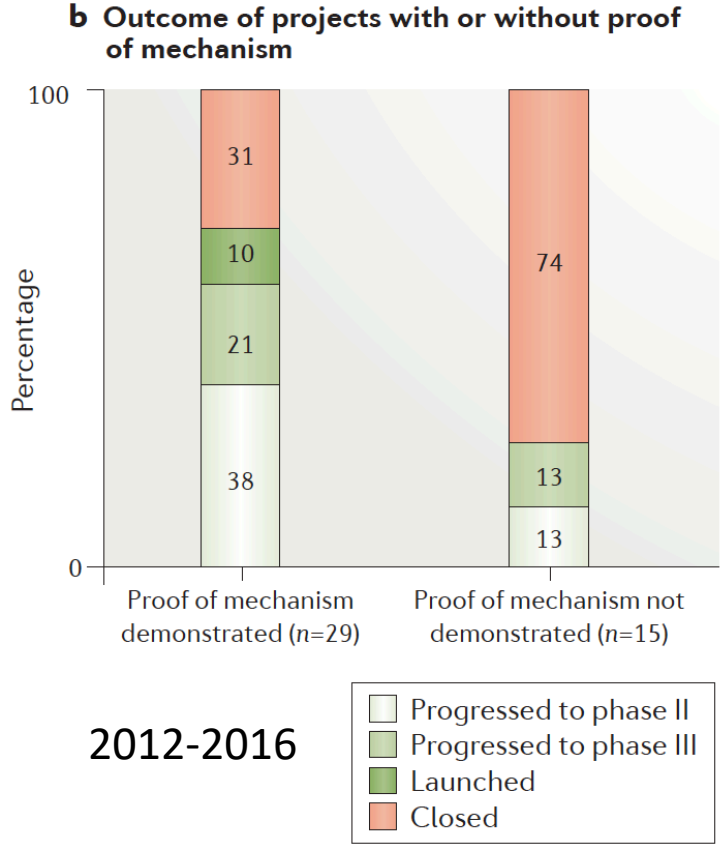


Lessons learned from the fate of AstraZeneca's drug pipeline: a five-dimensional framework
Nature Reviews Drug Discovery **13**, 419–431 (2014) doi:10.1038/nrd4309

Proof of Mechanism is important (AstraZeneca, 2018)

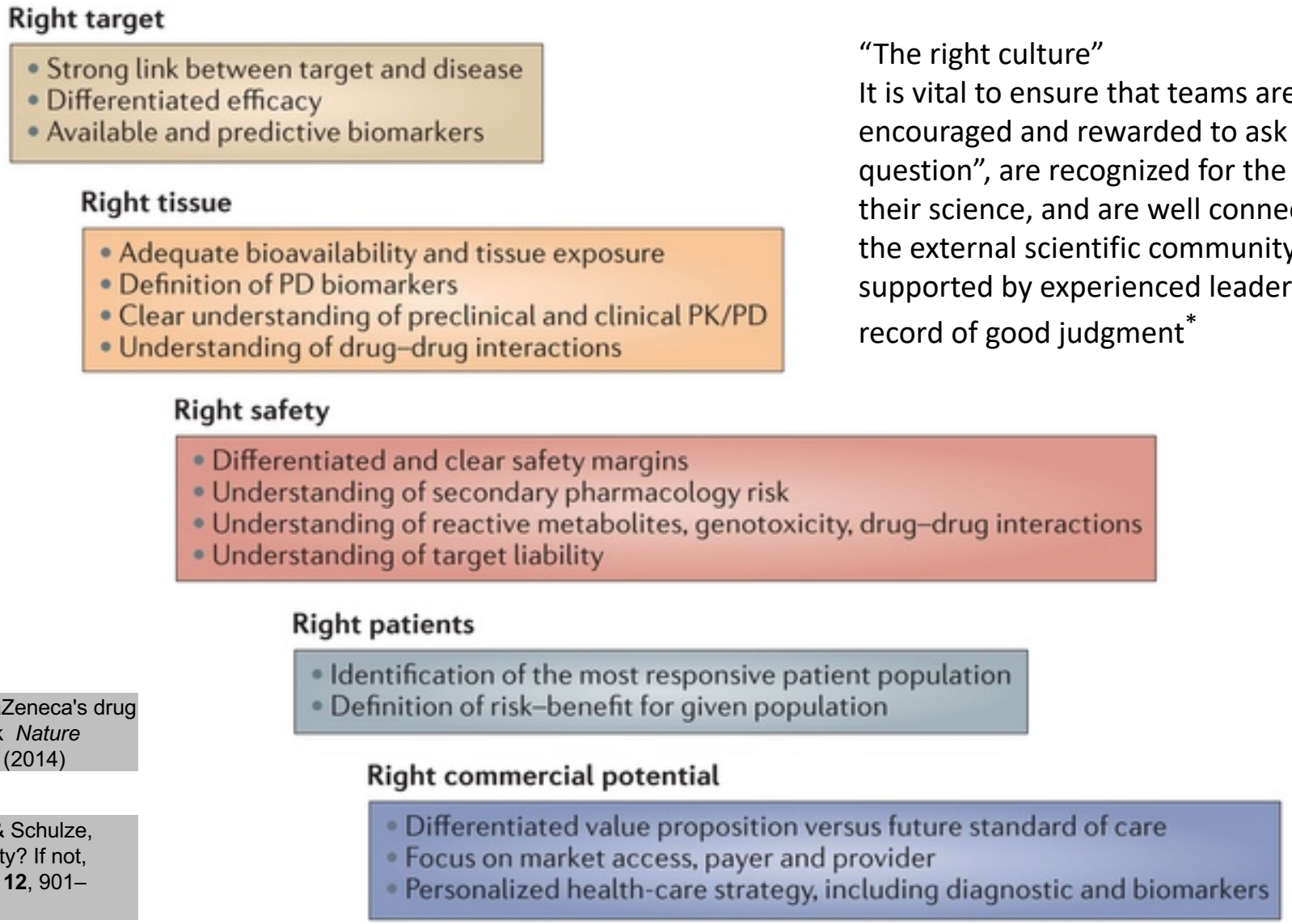
Exposure	Direct Target Engagement	Indirect Target Engagement	Physiological Response	Disease Modulation	Outcome/ Endpoints
	target itself "immediate" target	"downstream" signalling	tumour perfusion blood glucose	cell cycle arrest proliferation apoptosis	cellular growth inhibition tumour growth inhibition ORR PFS OS

- POM demonstrates
  - Target engagement at a predefined and quantifiable level in humans
  - Functional effects
- POM is associated with project fate
  - Consistent with Pfizer 3 pillar results



Morgan P *et al*. Impact of a five-dimensional framework on R&D productivity at AstraZeneca. *Nature Rev. Drug Discov.* **17(3)**, 167-181 (2018).

AstraZeneca 5R framework (2014)



"The right culture"
It is vital to ensure that teams are encouraged and rewarded to ask the "killer question", are recognized for the quality of their science, and are well connected to the external scientific community and supported by experienced leaders with a record of good judgment*

Lessons learned from the fate of AstraZeneca's drug pipeline: a five-dimensional framework *Nature Reviews Drug Discovery* 13, 419–431 (2014)

* Ringel, M., Tollman, P., Hersch, G. & Schulze, U. Does size matter in R&D productivity? If not, what does? *Nature Rev. Drug Discov.* **12**, 901–902 (2013).

Right tissue requires PK/PD understanding

Exposure	Direct Target Engagement	Indirect Target Engagement	Physiological Response	Disease Modulation	Outcome/ Endpoints
	target itself "immediate" target	"downstream" signalling	tumour perfusion blood glucose	cell cycle arrest proliferation apoptosis	cellular growth inhibition tumour growth inhibition ORR PFS OS

Right tissue

- Adequate bioavailability and tissue exposure
- Definition of PD biomarkers
- Clear understanding of preclinical and clinical PK/PD
- Understanding of drug–drug interactions

- Right tissue requires
  - Human PK prediction
  - Clinical POM
  - Reduced failure due to PK/PD issues

Morgan P *et al*. (2018) Impact of a five-dimensional framework on R&D productivity at AstraZeneca. *Nature Rev. Drug Discov.* **17(3)**, 167-181.

Summary: The importance of translation

- Clinical endpoints
  - Regulatory approvals is associated with ORR
  - Obsession with ORR might be unhealthy
  - Predicting ORR is important
- Success rate in oncology drug projects
  - Limited by the lack of efficacy in Ph2 trials
- 3 pillars and 5Rs
  - Key to success:

    Exposure ↔ Target Engagement ↔ Disease Modulation ↔ Outcome
  - PK/PD supports: Target validation, biomarker selection, human PK prediction, safety, qualification of tumour models with clinical evidence
- Can we predict ORR in the clinics?

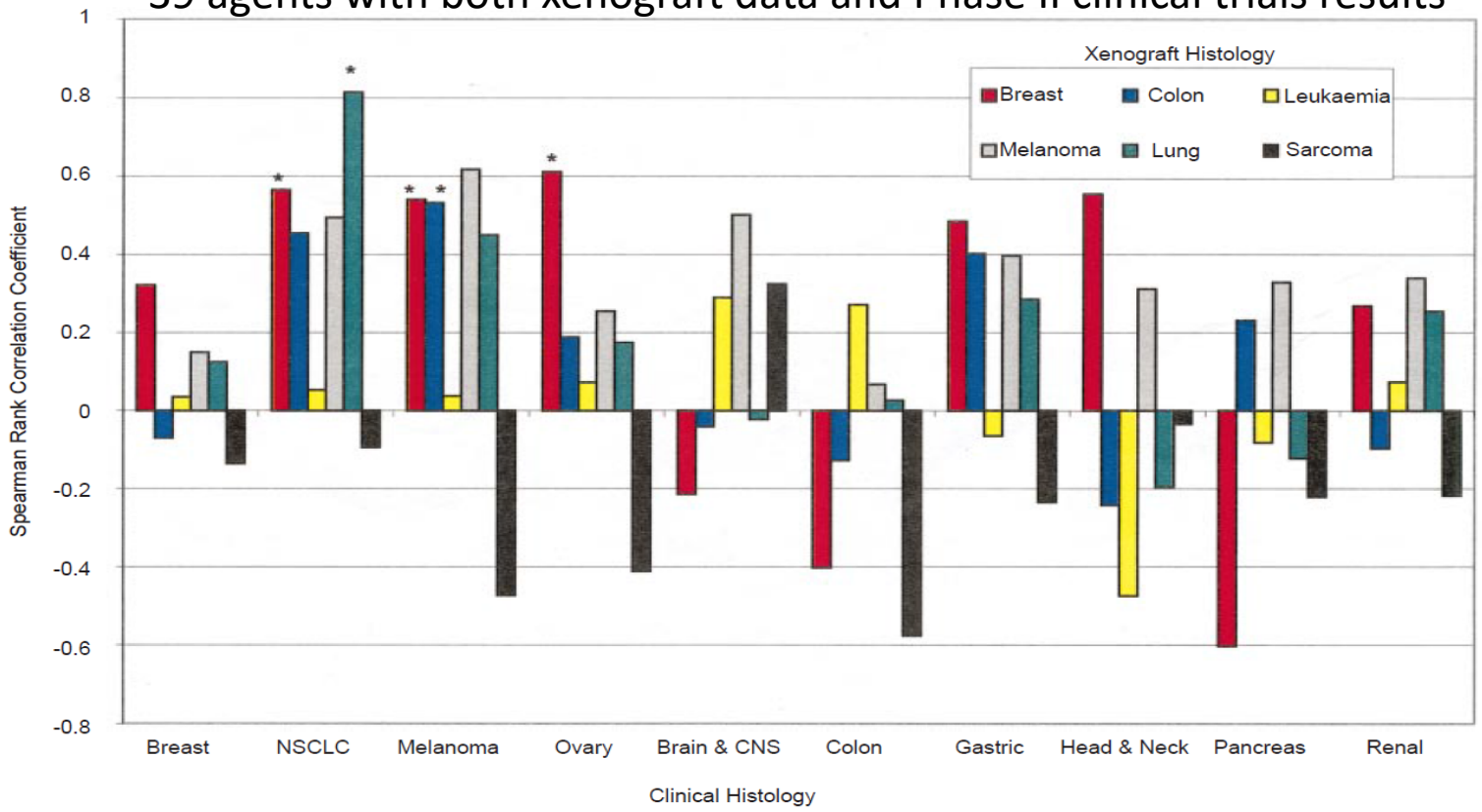
1.2 Clinical translation of xenograft models

- NCI: Mouse MTD efficacy does not predict clinical efficacy
- Chemo: AUC is important
- Chemo: Qualify A2780 (ovarian) xenograft model with PK/PD modelling
- Chemo & Targeted therapy: Preclinical efficacy => Clinical ORR

How reliable are xenograft tumour models?

- **2001** Method: **Mouse MTD efficacy** was compared with clinical response
- **2001** perspectives: For compounds with *in vivo* activity in **>1/3** xenograft models, there was activity in **>1** Phase II trials.

"39 agents with both xenograft data and Phase II clinical trials results"



Johnson et al, British Journal of Cancer (2001) 84(10), 1424–1431

Should mouse xenograft models be abandoned?

Mouse MTD efficacy was only **moderately** predictive of clinical response

Next question: What is the problem?

- Tumour biology difference in growth rates and microenvironment (e.g. surrounding blood vessels, immune cells, fibroblasts, signalling molecules, orthotopic location and extracellular matrix)?
- Exposure differences?

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Is mouse MTD efficacy relevant?

- The ratio R=(AUC-mouse-MTD)/(AUC-humans) was computed for 9 compounds
- Results: R<1 was a necessary, but insufficient condition for success

Drug	Clinical Result	Calculated Ratio: (Mouse MTD AUC) /(Human clin. AUC)	Calculation details
Carzelesin	**Failure**	40	Table 2 (80/2)
DMP840	**Failure**	7	Table 2 (17.5/2.5)
MGI-114	**Failure**	7	p839, col2 text (214/33)
9-AC	**Failure**	4	Kirstein et al., Clin. Canc. Res., 7, 358 (2001)
Sulophenur	**Failure**	3	Table 2 (8/3)
Topotecan	**Success**	0.3	Table 2 (10/3)
Melphalan	**Success**	0.3	Table 2 (1/3.5)
EPO906	**Failure**	0.3	*See Backups*
Irinotecan	**Success**	0.2	Table 2 (16/100)

Peterson and Houghton, Eur. J. Canc., 40, 837 (2004)

Exposure difference is relevant

Looks like exposure difference is relevant

Next question: By correcting the difference, can we find any consistency between preclinical models and clinical data?

- Case study: 10 successful chemotherapy drugs

Can we learn from approved chemotherapies?

Methods

- 10 chemotherapy drugs were tested on mouse A2780 ovarian carcinoma xenografts.
- PK/PD models were constructed to estimate the exposures needed for preclinical tumor shrinkage.

Rocchetti et al., Eur. J. Canc., 43, 1862 (2007)

Simeoni model structure

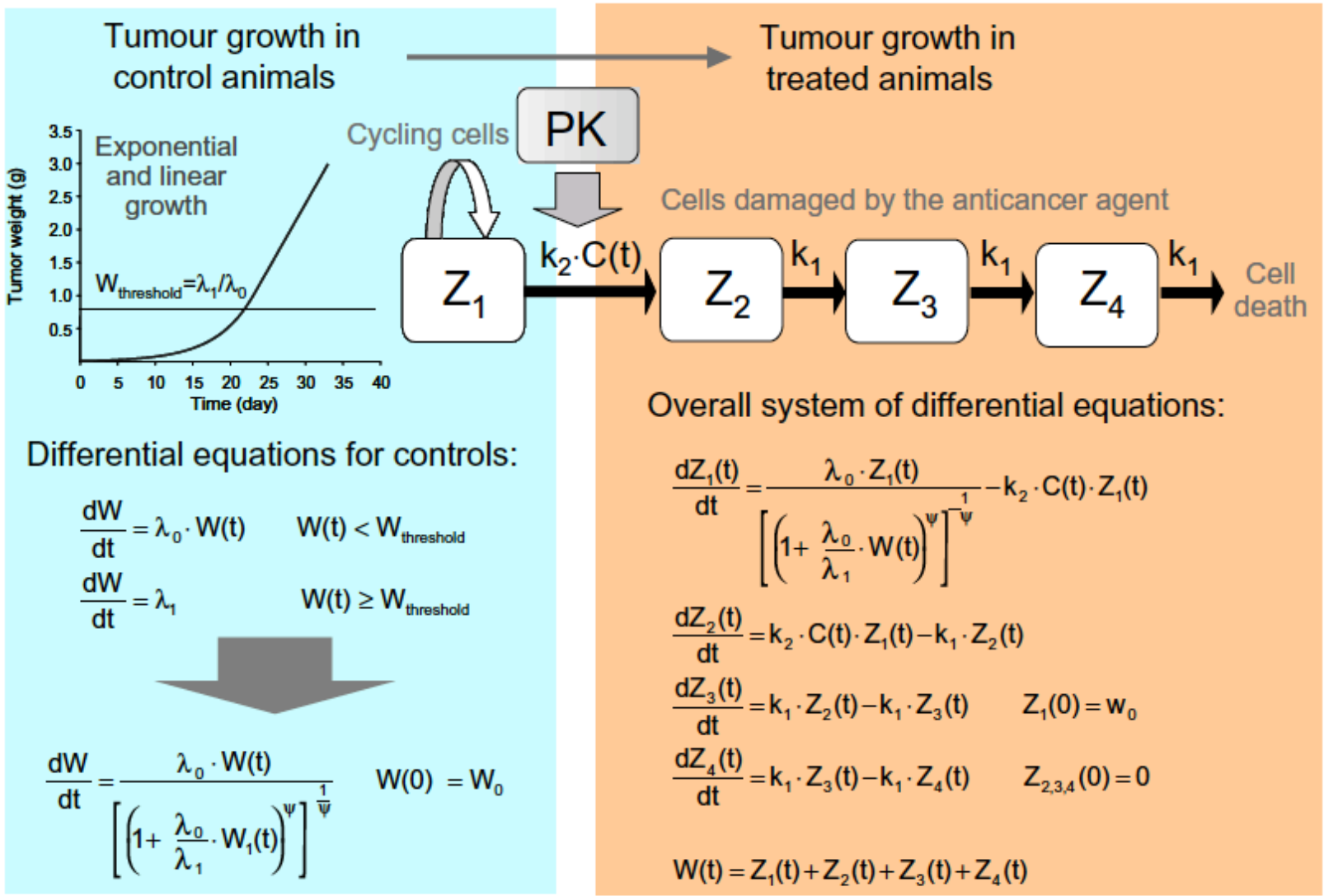


Fig. 1 – Scheme and equations of the PK/PD tumour growth inhibition model.

Rocchetti et al., Eur. J. Canc., 43, 1862 (2007)

PK: All parameters published.

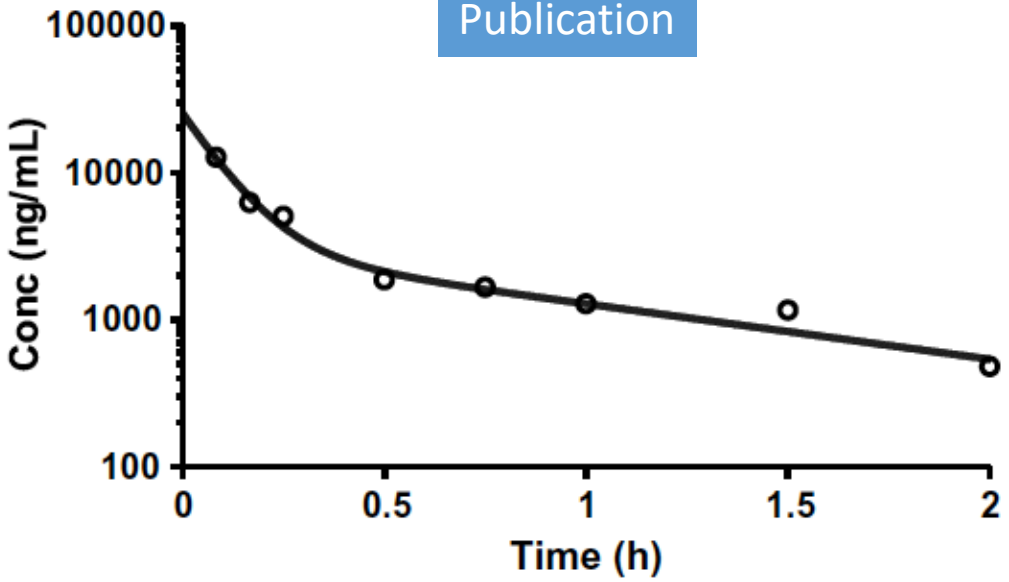
PK verification: Model simulation is plotted to compare with published modelling results.

PD: λ_1 and k_1 were not published in this paper, unfortunately

PD verification: Infer λ_1 and k_1 from tumour growth data and simulate PK/PD model at the expected values to compare with data and published simulation results

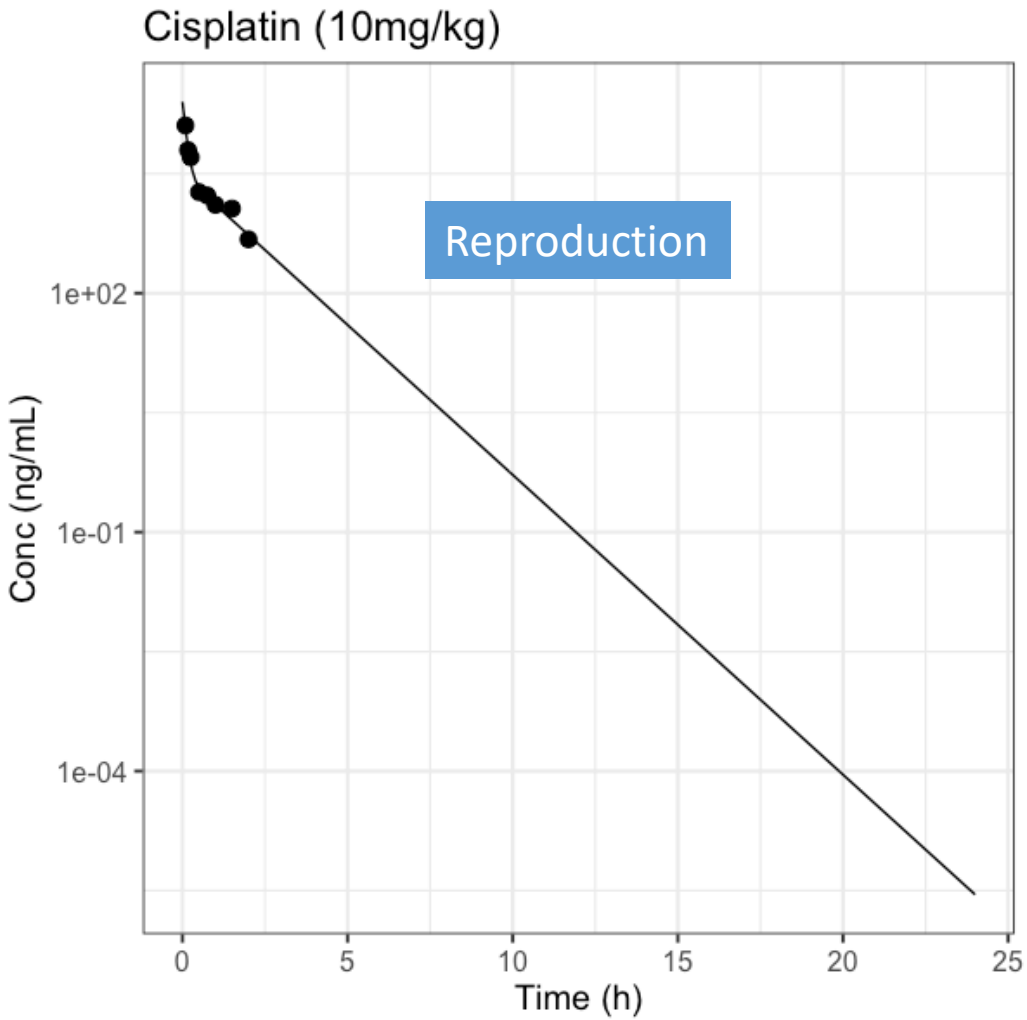
Cisplatin PK

Publication



Rocchetti et al., Eur. J. Canc., 43, 1862 (2007)

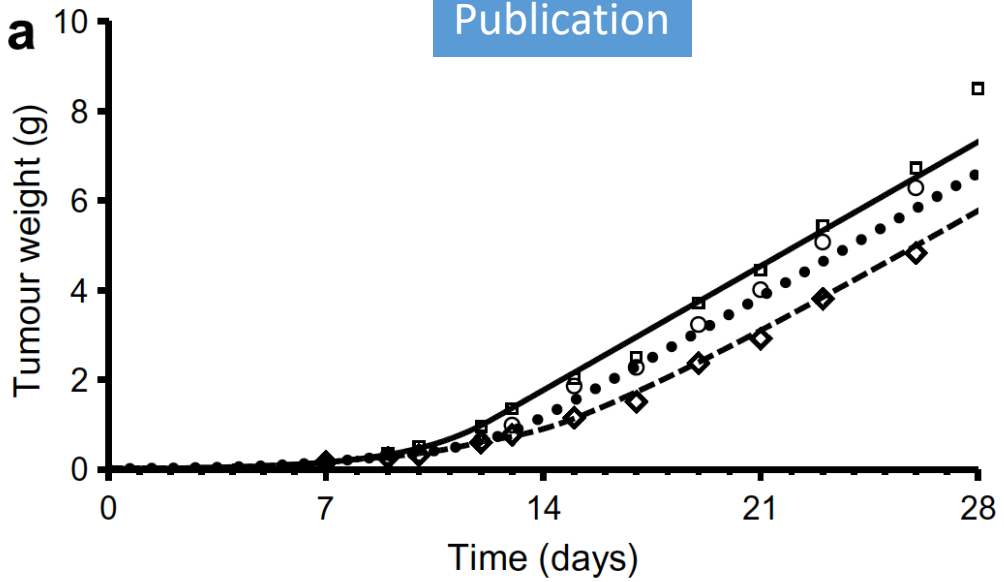
Reproduction



Tao's reproduction of the modelling work (2019)

Cisplatin PD

Publication



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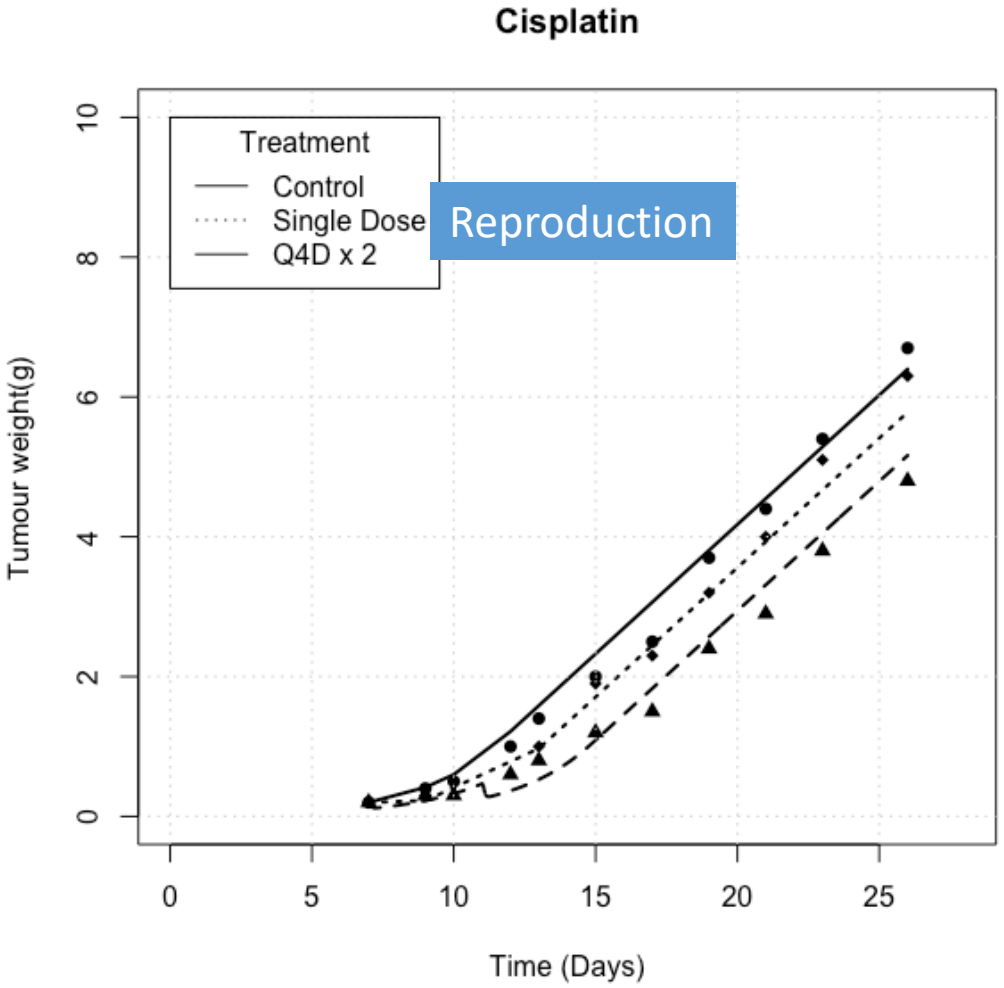


Tao's reproduction of the modelling work (2019)

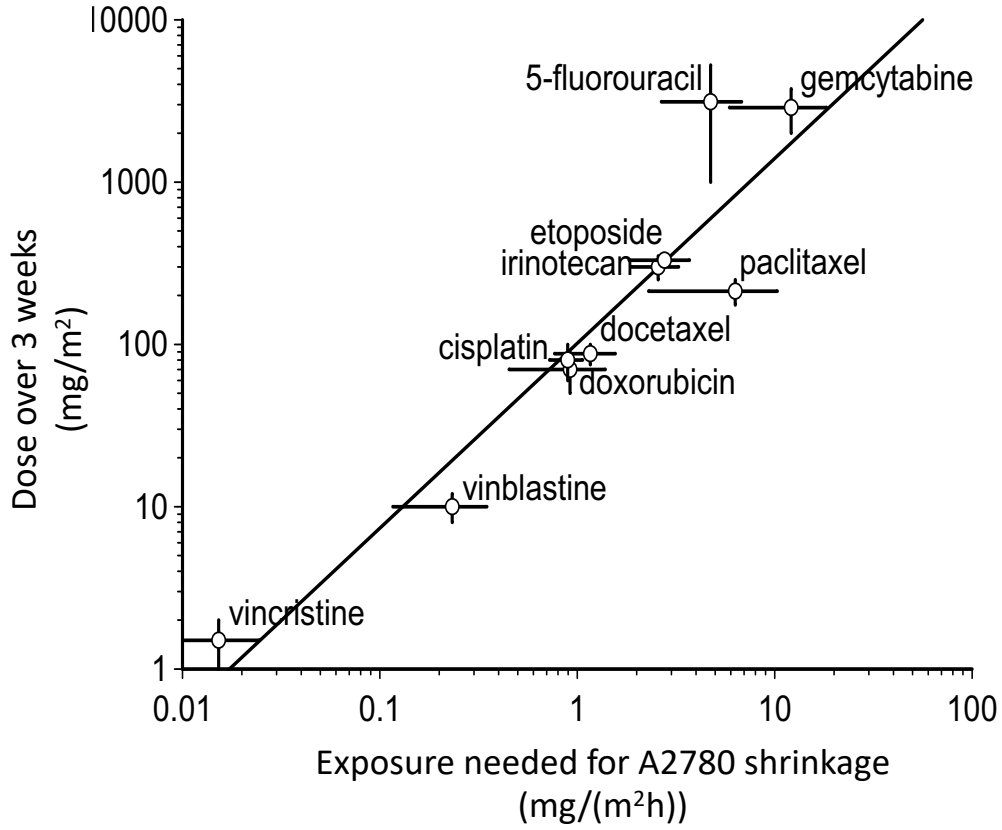
Can we learn from approved chemotherapies?

Methods

- 10 chemotherapy drugs were tested on mouse A2780 ovarian carcinoma xenografts.
- PK/PD models were constructed to estimate the exposures needed for preclinical tumor shrinkage.

Results

- Strong correlations (R = 0.94) was observed between preclinical exposures needed for tumor shrinkage and the exposures achieved in the clinic under standard treatment.



Rocchetti et al., Eur. J. Canc., 43, 1862 (2007)

Exposure difference is relevant

Looks like exposure is relevant to a large extent to clinical success!

Next question: Can we predict clinical failure?

- Case study: 8 chemo/targeted treatments for 10 indications with known clinical outcomes

Compelling preclinical evidence requires…

- What is the minimum preclinical efficacy required for clinical success?
  - Can we establish a robust translational criteria?

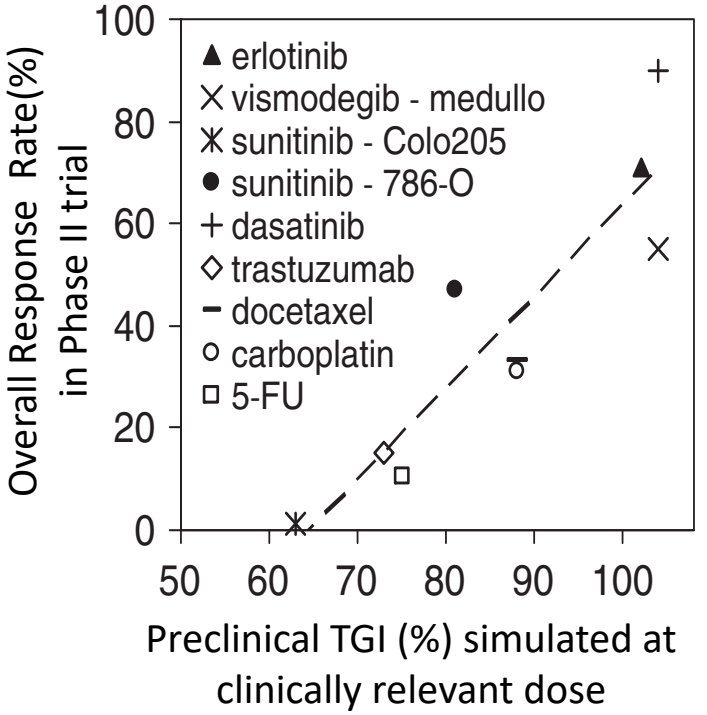
Method

A model-based method to predict *clinical efficacy* based on *preclinical xenograft* studies for both chemotherapies and targeted therapies

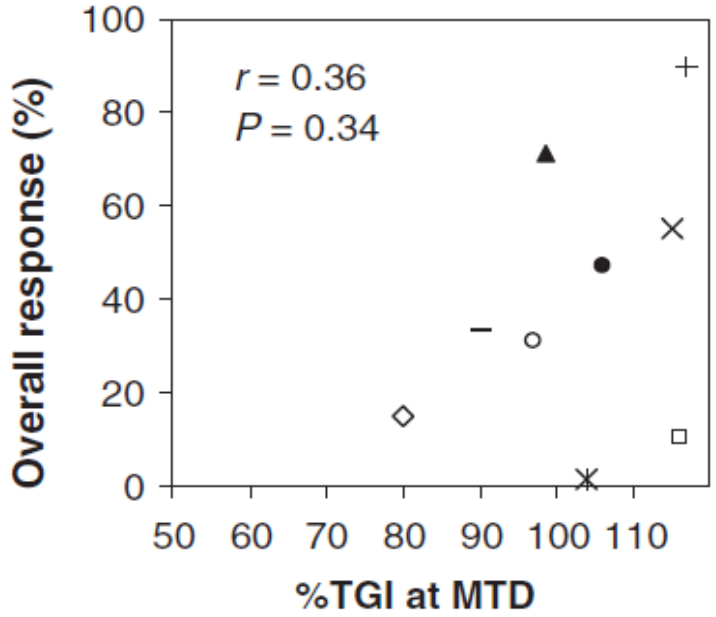
A minimum clinical efficacious exposure can be predicted for *tumor cell-directed therapy*.

Main Limitations

Attaining this minimum clinical exposure is a necessary but not sufficient condition.

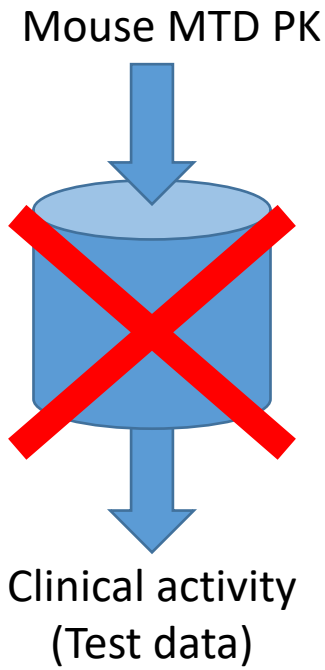


Wong *et al.*, *Clin. Canc. Res.*, 18, 3846, (2012)



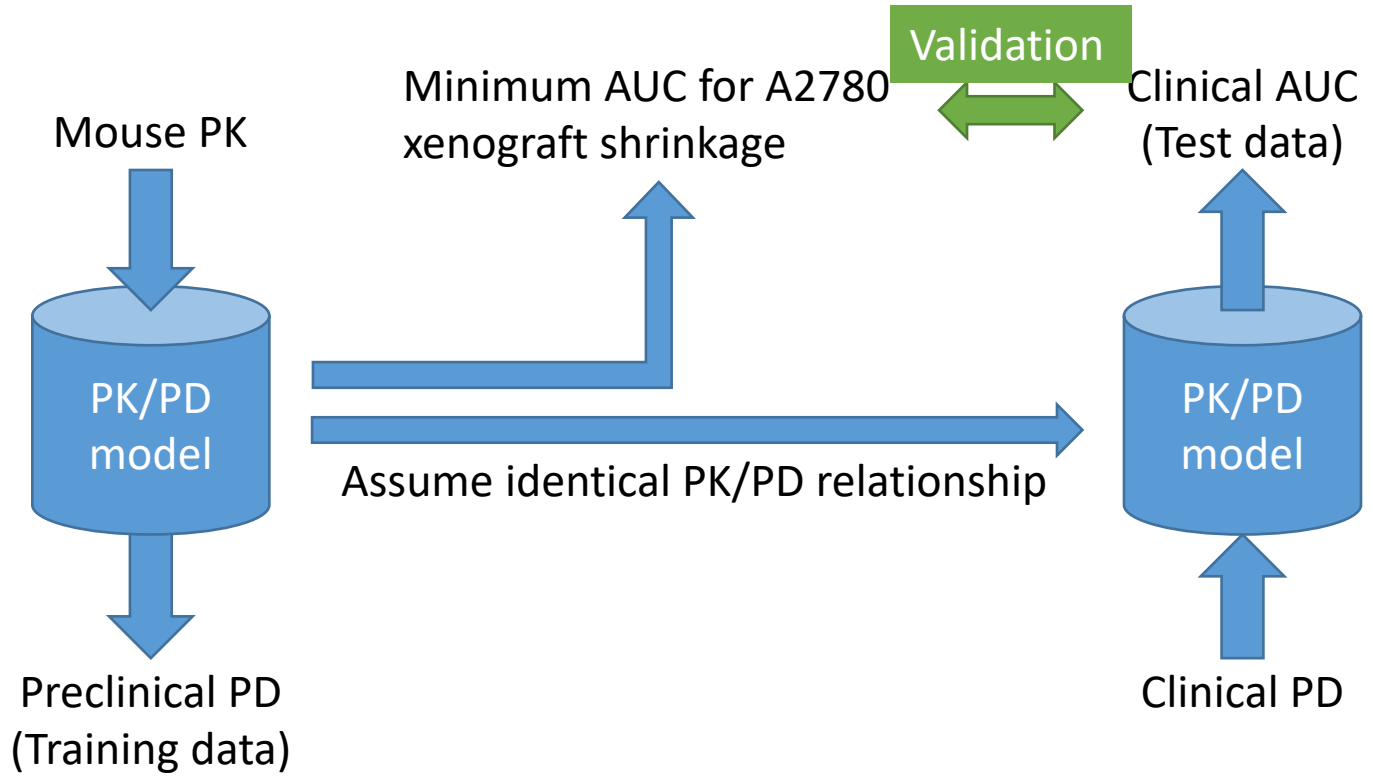
Summary: Clinical translation of xenograft models

- Mouse MTD efficacy was only moderately predictive of clinical response



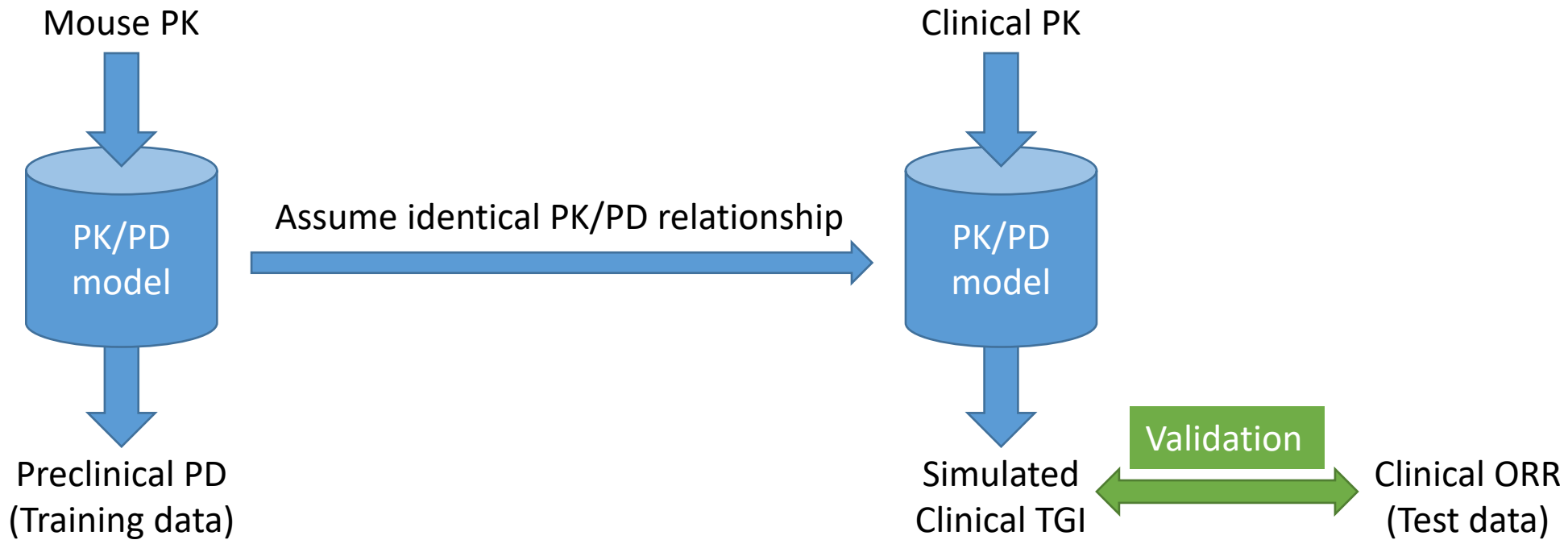
Summary: Clinical translation of xenograft models

- Strong correlations was observed between preclinical exposures needed for tumor shrinkage and the exposures achieved in the clinic under standard treatment



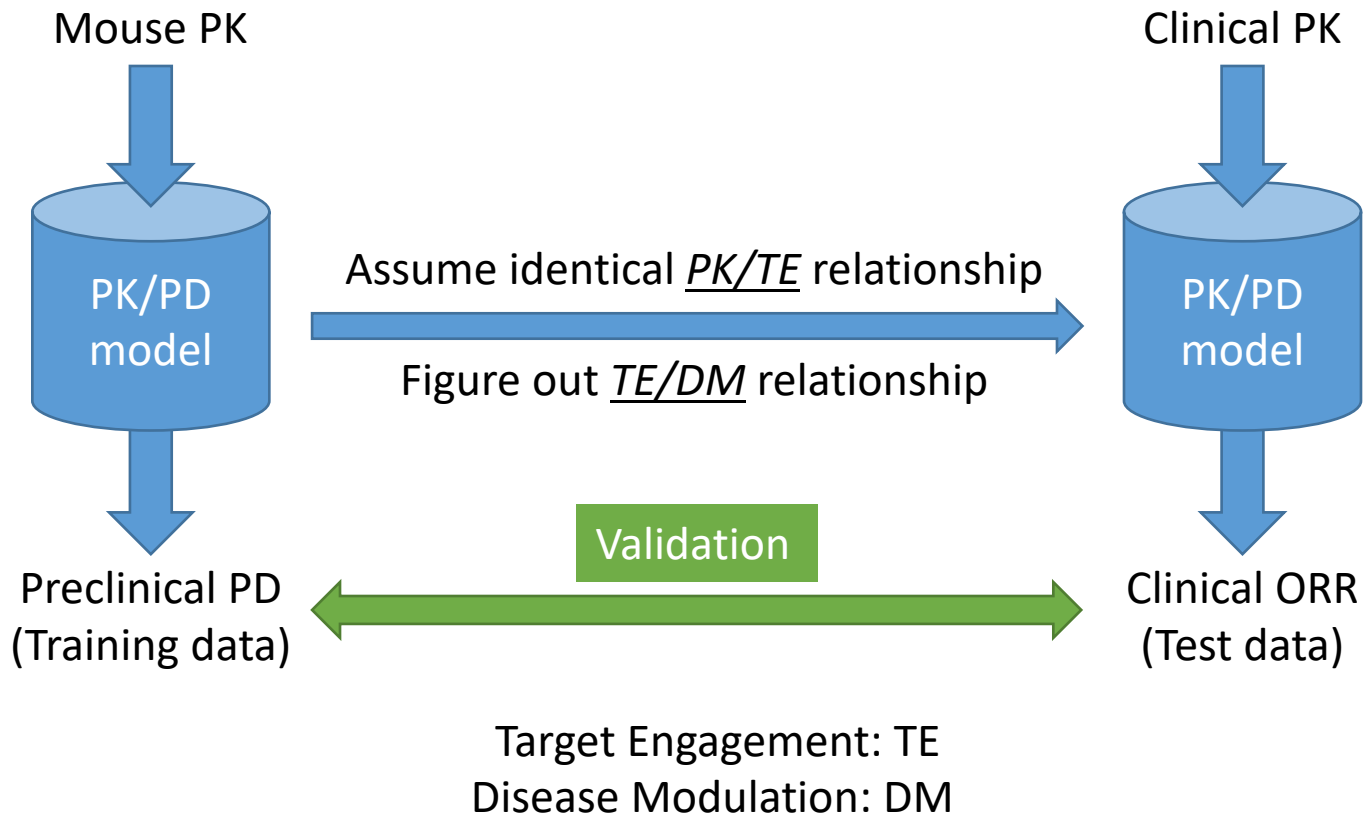
Summary: Clinical translation of xenograft models

- Preclinical efficacy is strongly associated with clinical ORR for some chemotherapies and targeted therapies in certain preclinical models



Summary: Clinical translation of xenograft models

- Translation needs to consider clinical PK
- Translation may assume identical preclinical and clinical PK/PD relationships
- The following scheme makes comparison more straightforward and it requires understanding of growth of preclinical and clinical tumours



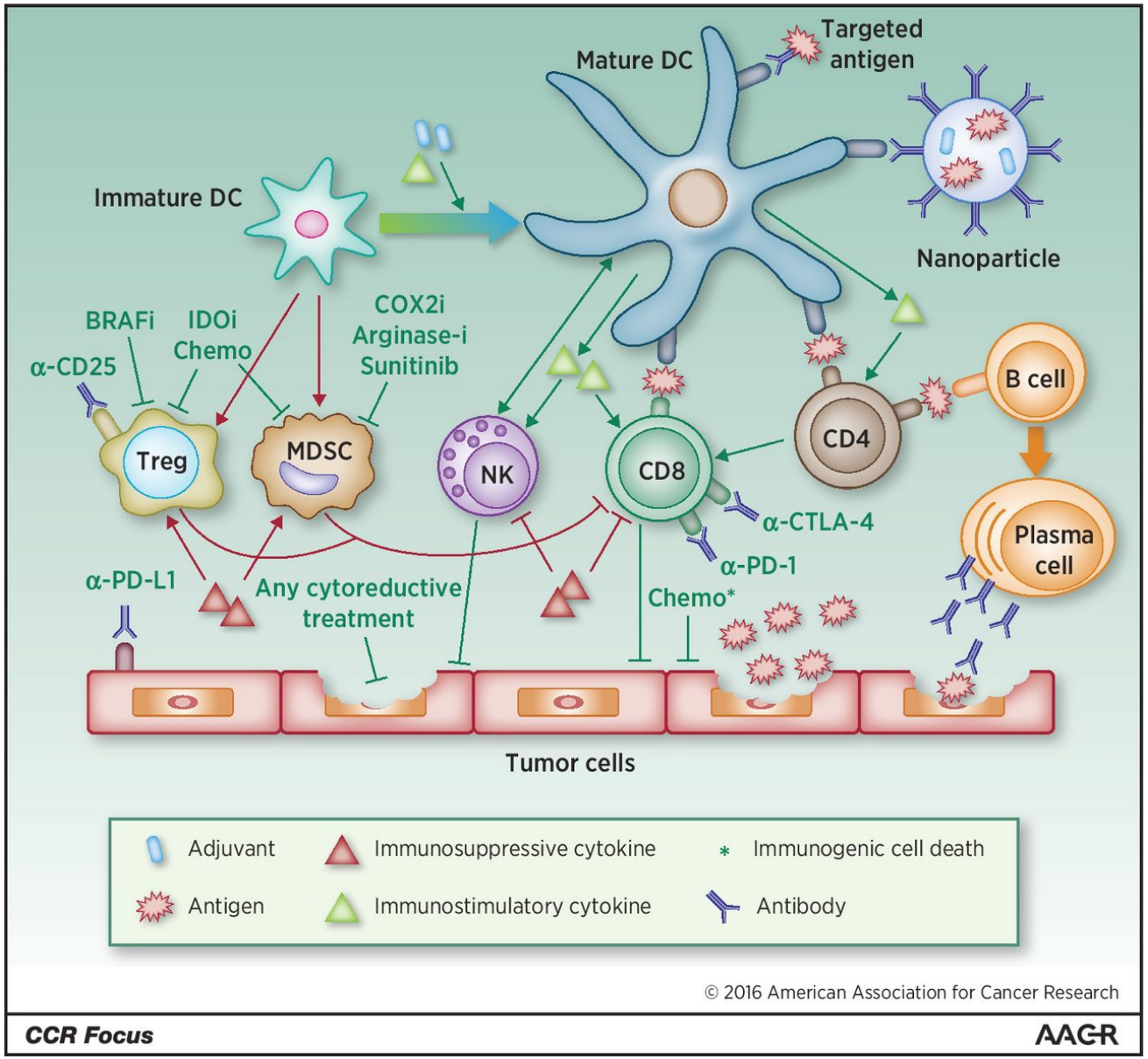
Lecture 3. Clinical translation of syngeneic models

- Adaptive immunity to tumours
- Resistance mechanisms to immunotherapy
- Modelling efficacy: RT/α-PD-L1 combi in CT26 syngeneic tumour model

Adaptive immunity to tumours

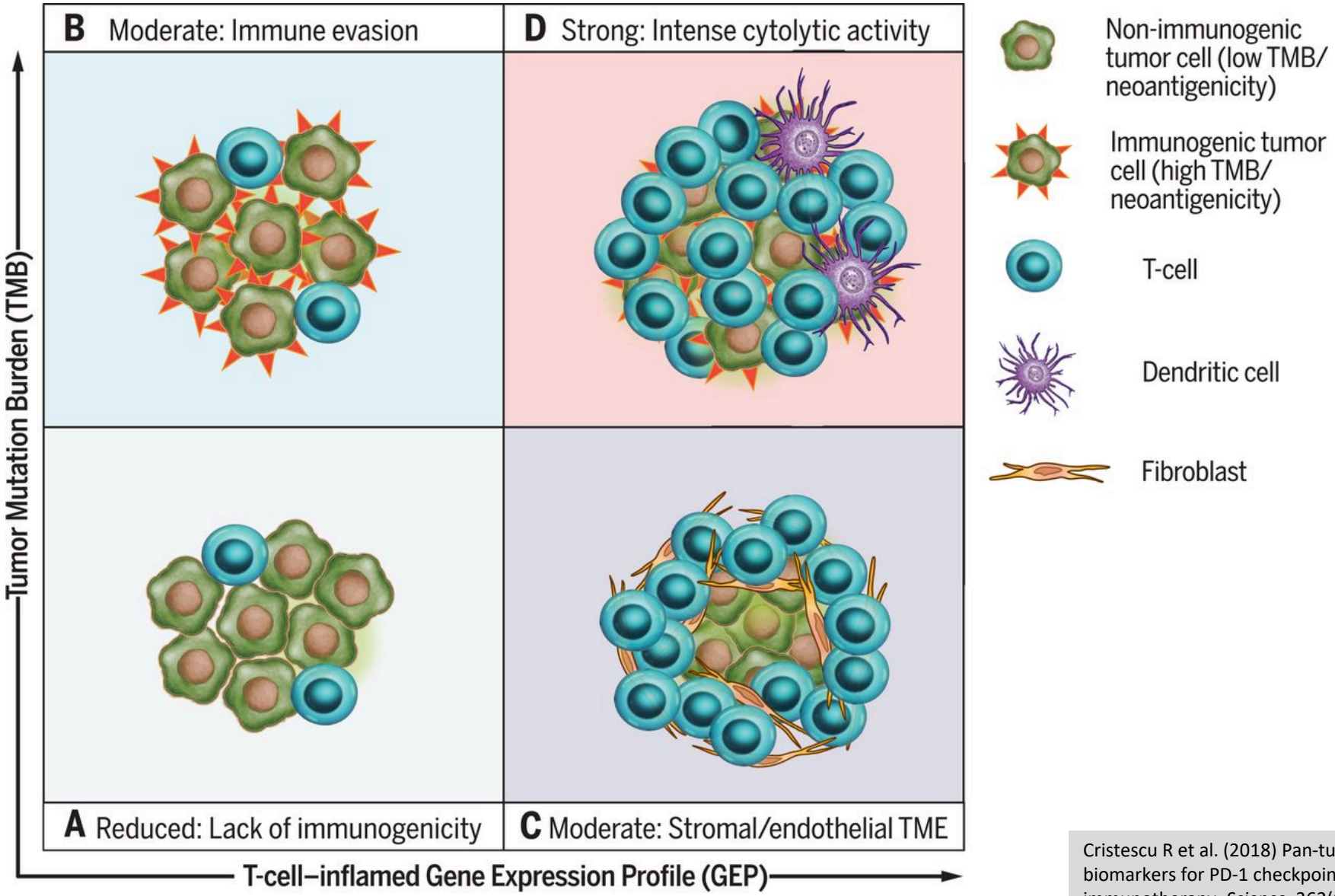
Treatment strategies

- **Induce priming**: Stimulating antigen presenting cells (APC, e.g. dendritic cell)
- **Induce clonal selection and memory response**: Cancer vaccine, *ex vivo* culture of tumour-specific T cells
- **Inhibit regulatory T cells**
- **Block co-inhibitory signals** (e.g. αPD-1, αPD-L1, αCTLA4)



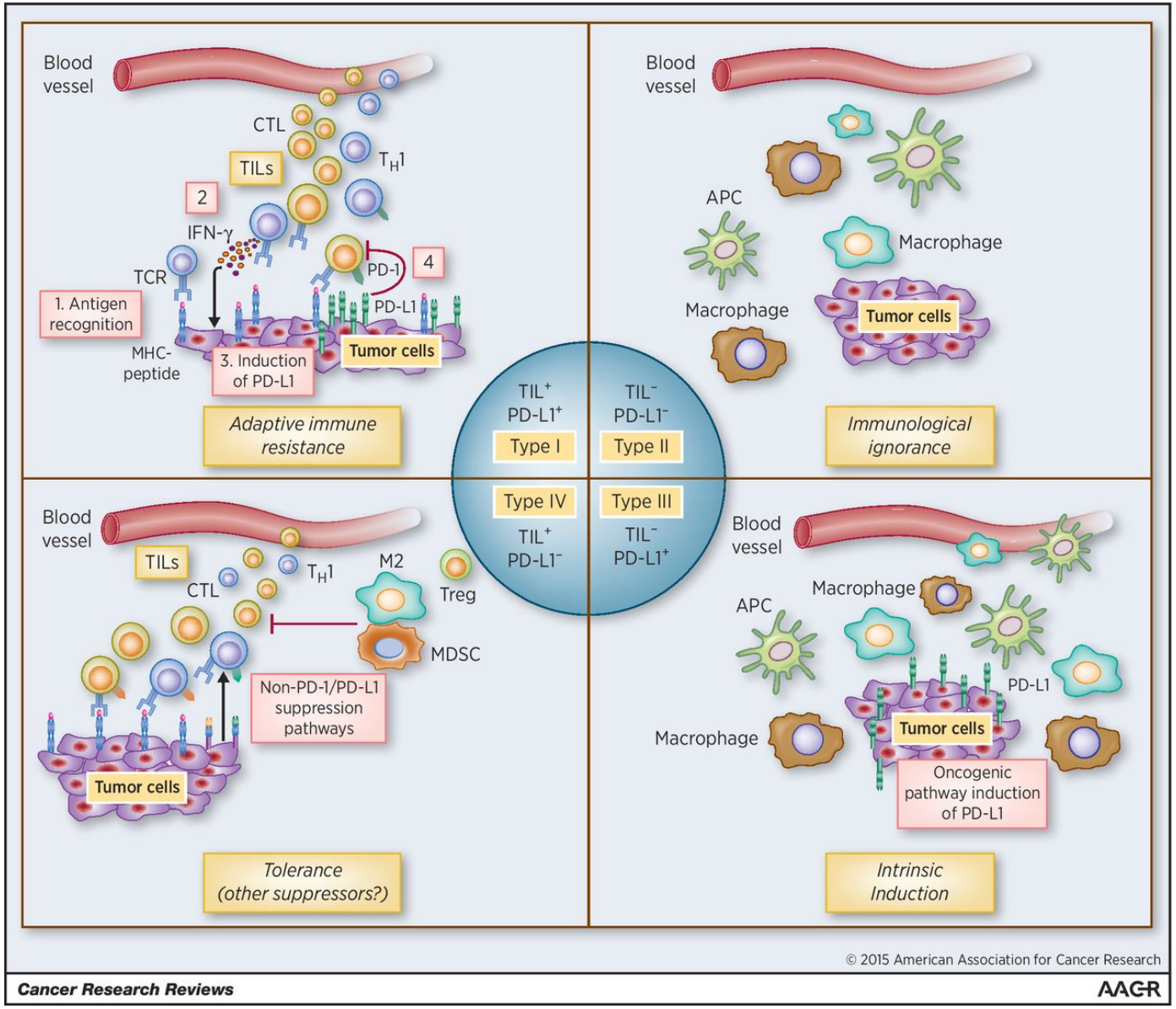
Bol KF et al. (2016) Dendritic Cell–Based Immunotherapy: State of the Art and Beyond. *Clin Cancer Res*. 22(8):1897-906

Most cancers are resistant to αPD-1 monotherapy



Cristescu R et al. (2018) Pan-tumor genomic biomarkers for PD-1 checkpoint blockade-based immunotherapy. *Science*. 362(6411).

αPD-L1 resistance: T-cell Infiltration & PD-L1

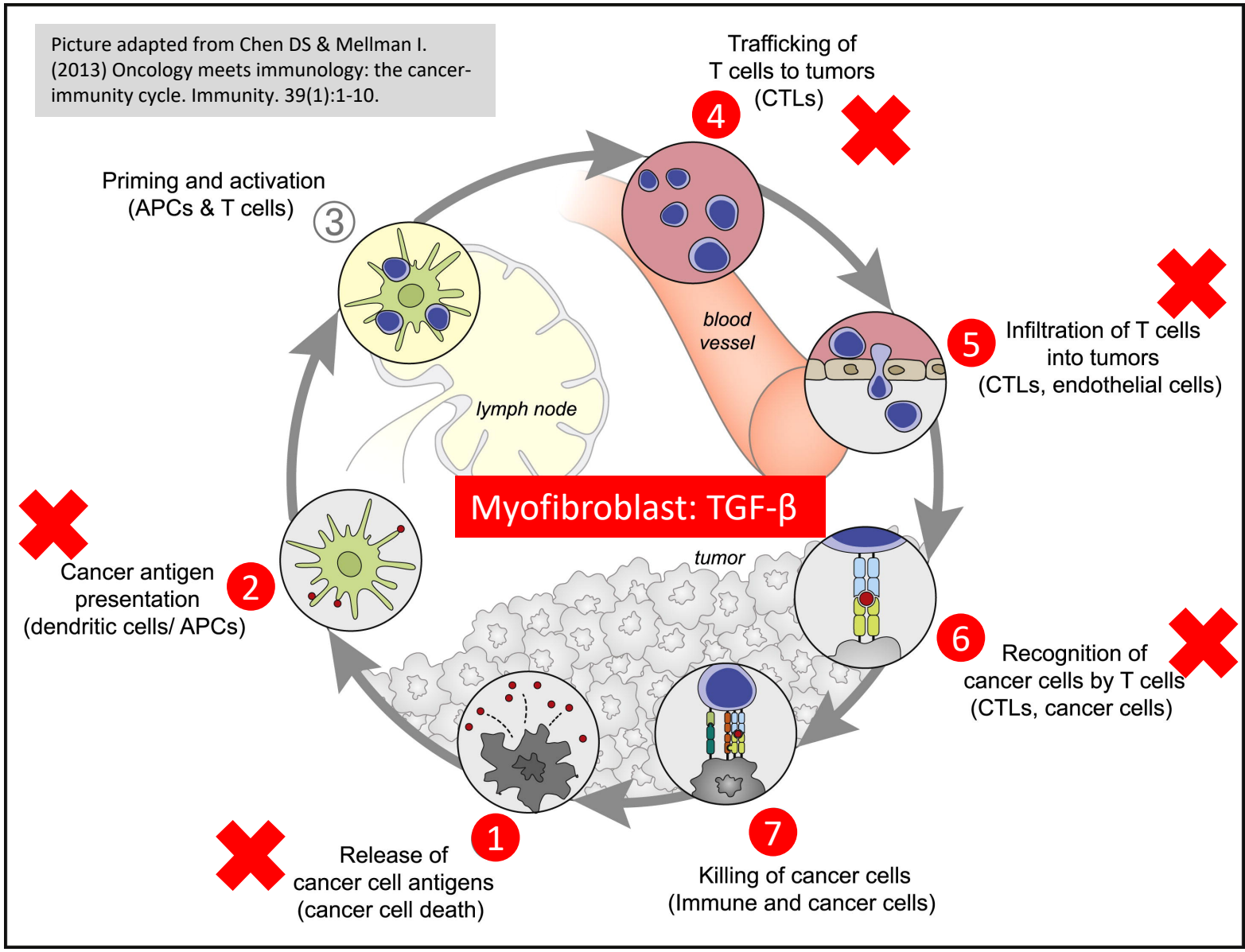


Tang MW *et al*. (2015) Classifying Cancers Based on T-cell Infiltration and PD-L1. *Cancer Res*. 75(11):2139-45.

Extrinsic resistance: Harsh tumour microenvironment

1. **Downregulate MHC / antigen**
2. **Melanoma's intrinsic β-catenin**
   - No CCL4 ⟹ No Ag presentation
3. **PD-1/PD-L1, CTLA4**
4. **Tumour VEGF-A, PGE2, IL-10**
   - Trigger T cell apoptosis
5. **Dysregulated energy metabolism**
   - Lacks glucose
   - Hypoxia ⟹ Adenosine↑
   - Oxidative stress
6. **Myeloid-derived suppressor cell**
   - Depletes essential amino acids
   - IDO promotes T cell tolerance
7. **PD-1/PD-L1, CTLA4**

   Myofibroblasts: TGF-β



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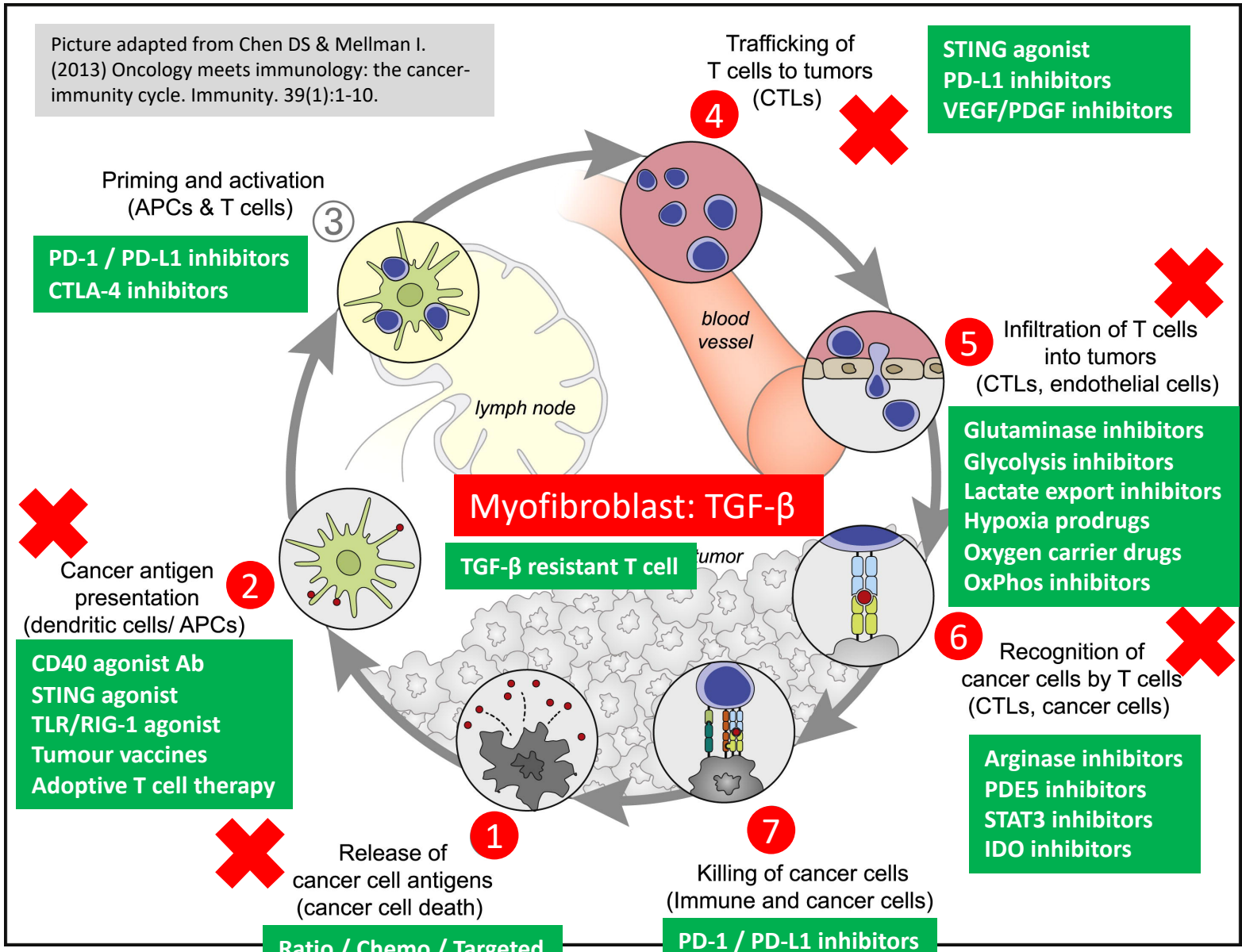


Intrinsic resistance: Primary vs Acquired

- Primary resistance: No response
  - Driven by mutations
  - Melanoma and colon cancer: *JAK1/2* loss-of-function mutations ⇒ PD-L1↓
  - Lung adenocarcinoma: *STK11/LKB1* co-mutation in *KRAS*-mutant cancers ⇒ αPD-L1 resistance
- Acquired resistance: Progression of disease post response
  - Leads to disease progression after 6 months of therapy
  - Lung cancer: Loss of B2M (and hence HLA class I expression) ⇒ αPD-1 / αPD-L1 resistance

Shin DS *et al*. (2017) Primary Resistance to PD-1 Blockade Mediated by JAK1/2 Mutations. Cancer Discovery 7(2):188-201.

Skoulidis F *et al.* (2018) STK11/LKB1 Mutations and PD-1 Inhibitor Resistance in KRAS-Mutant Lung Adenocarcinoma. *Cancer Discov*. 8(7):822-835.

Gettinger S *et al*. (2017) Impaired HLA Class I Antigen Processing and Presentation as a Mechanism of Acquired Resistance to Immune Checkpoint Inhibitors in Lung Cancer . *Cancer Discov*. 7(12):1420-1435.

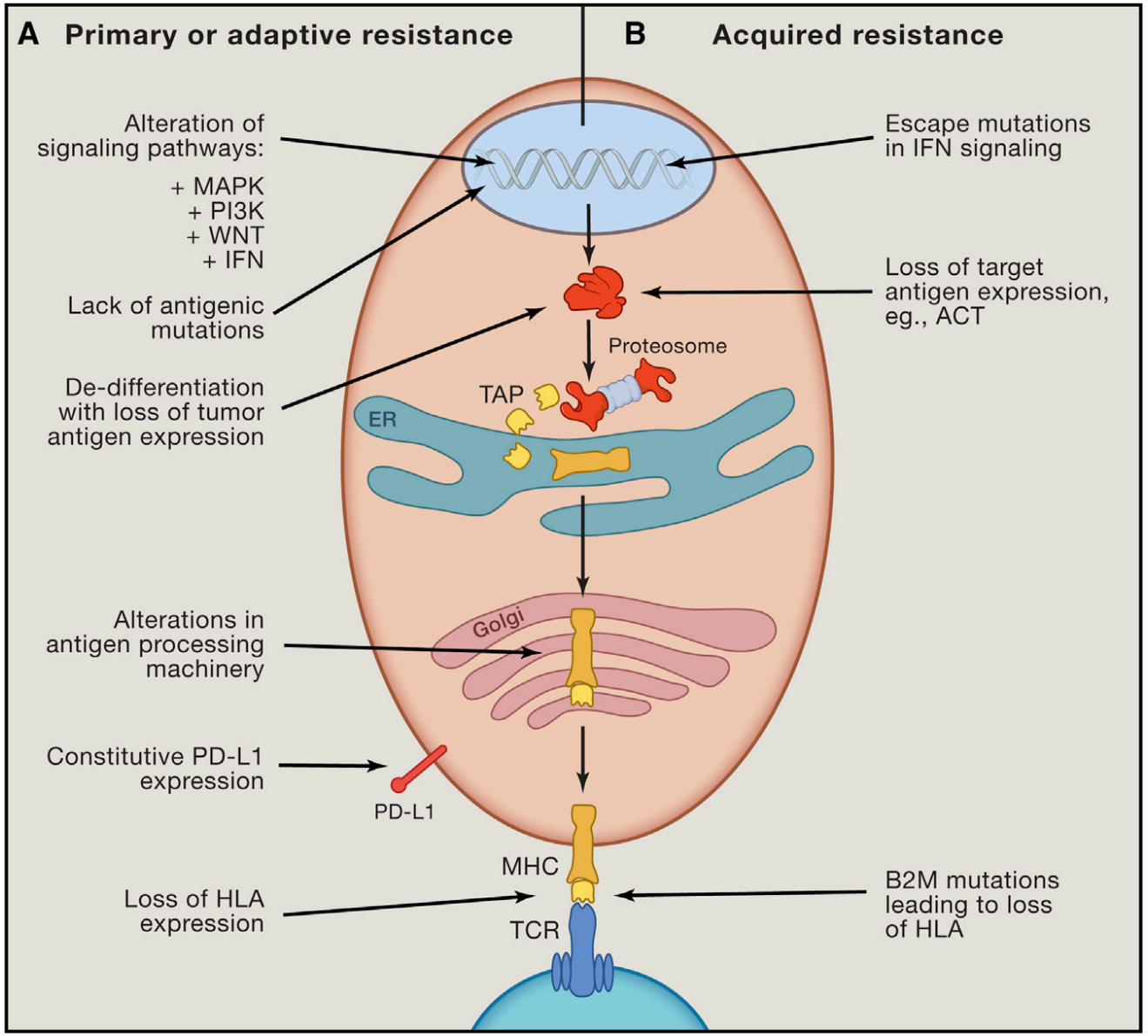
Intrinsic resistance: Primary vs Acquired

Primary resistance: No response

- Driven by mutations

Acquired resistance: Progression of disease post response

- Leads to disease progression after 6 months of therapy



Sharma, P. *et al*. (2017) Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy. CELL, 168(4), 707-723.

Immuno-oncology tumour model challenges

- Characterise baseline
  - How does the tumour model grow under the control condition?

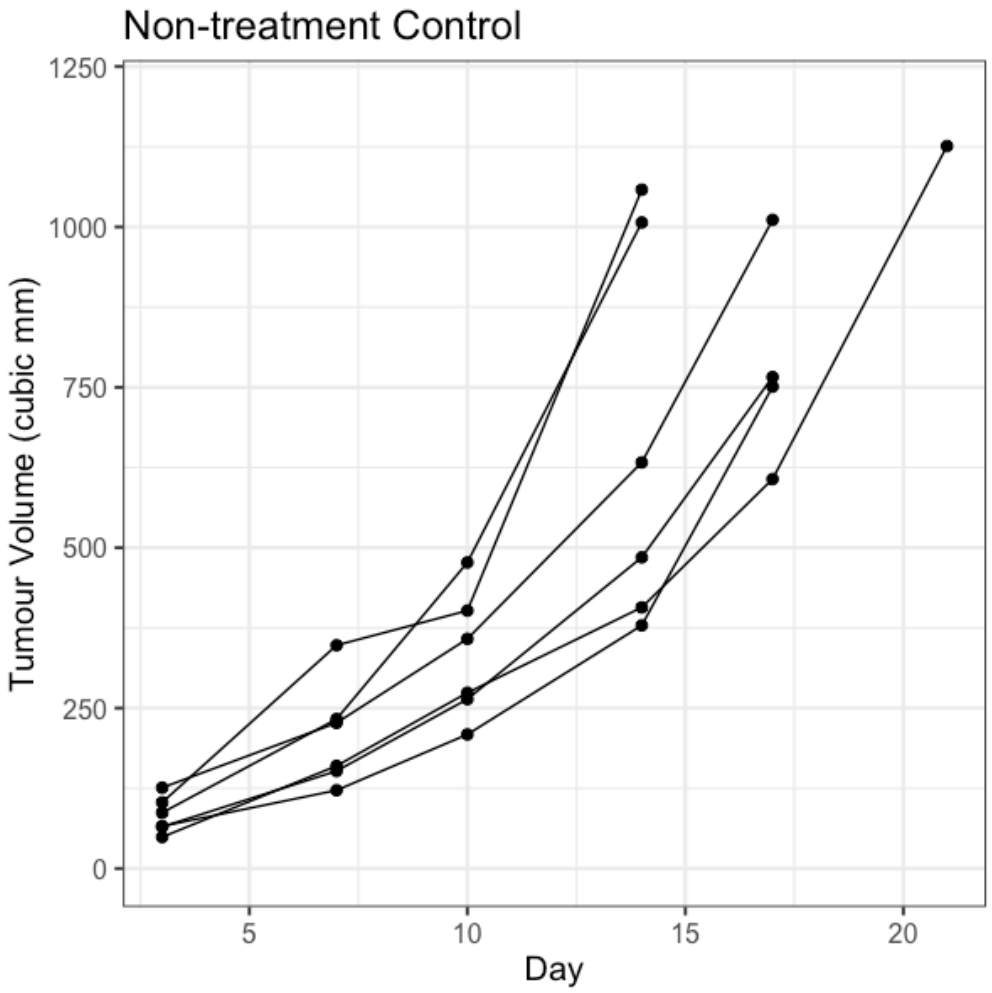
- Confirm mechanism of action of treatment
  - **Exposure ⇔ Target engagement ⇔ Disease Modulation ⇔ Efficacy**
  - Is innate / adaptive immunity appropriately activated?
  - How does the tumour model respond to treatment?

- Translation
  - How to extrapolate the preclinical data to forecast clinical efficacy?

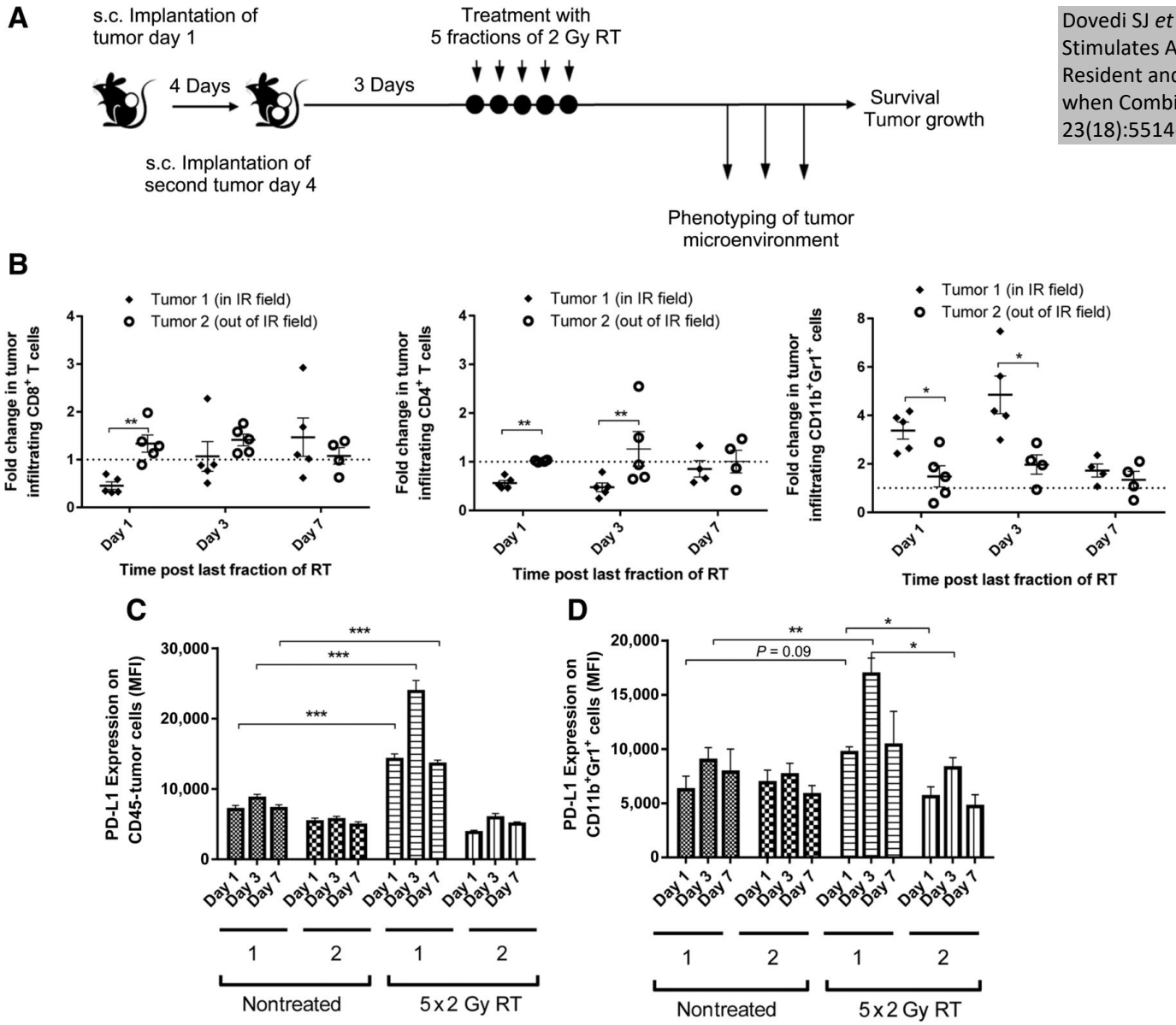
CT26 control experiment overview

- Tumour volume at day 3
  - 49~126mm^3
- Tumour growth
  - No Exceptions: Spontaneous regression, no growth, irregular growth rates
  - Variable speeds and sizes reached
- Possible growth rate law
  - Logistic: Postulates a maximum tumour volume. Used in Kosinsky *et al*. (2018) J Immunother Cancer.
  - Exponential: The most appropriate rate law for these data
  - Cubic (i.e. diameter expands linearly): Nearly as good as exponential rate law
  - Exponential-linear
  - Gompertz

Data: Dovedi SJ *et al*. Acquired Resistance to Fractionated Radiotherapy Can Be Overcome by Concurrent PD-L1 Blockade. *Cancer Res.* 74(19): 5458-68 (2014).

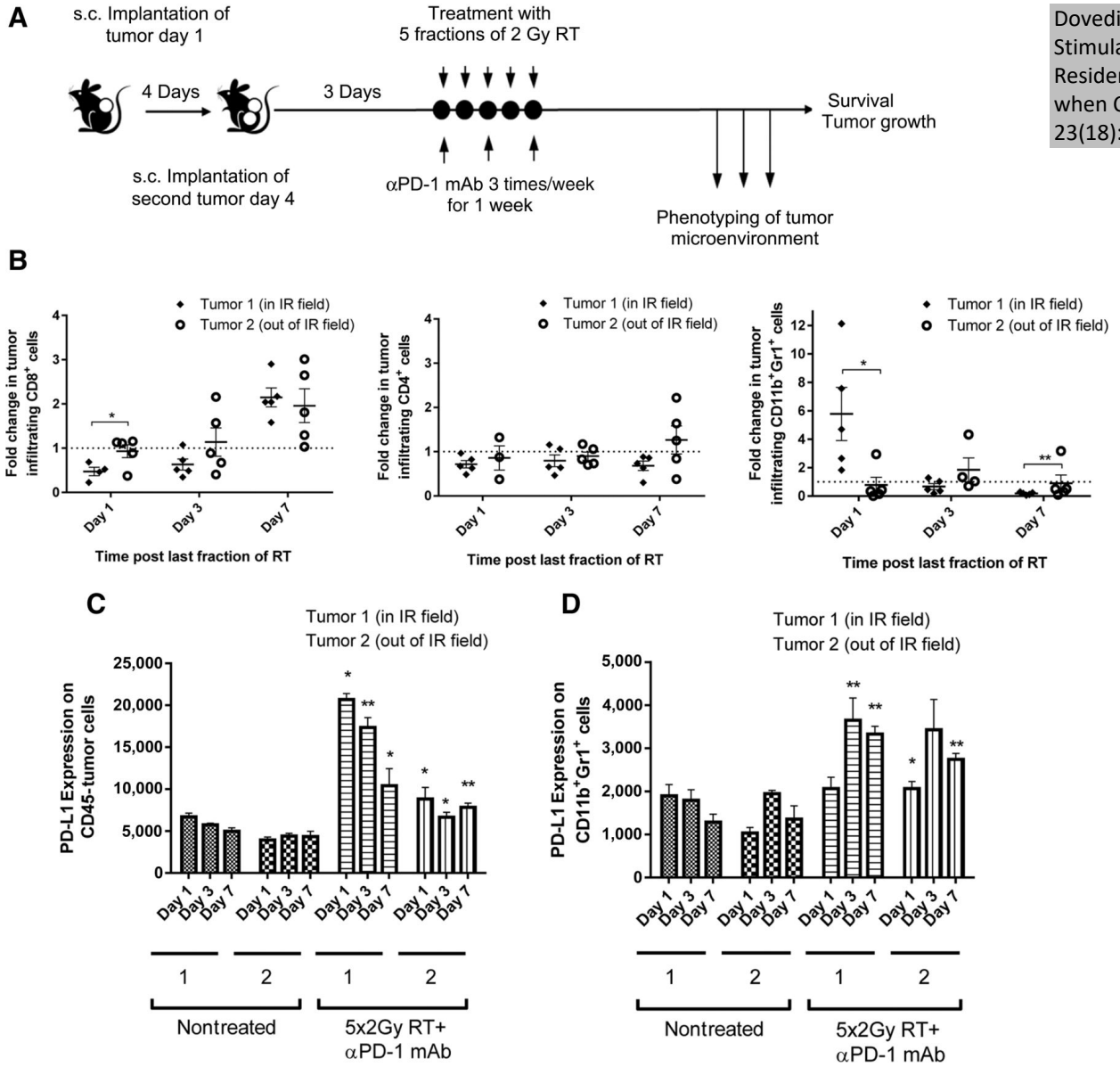


RT in CT26: TE / DM



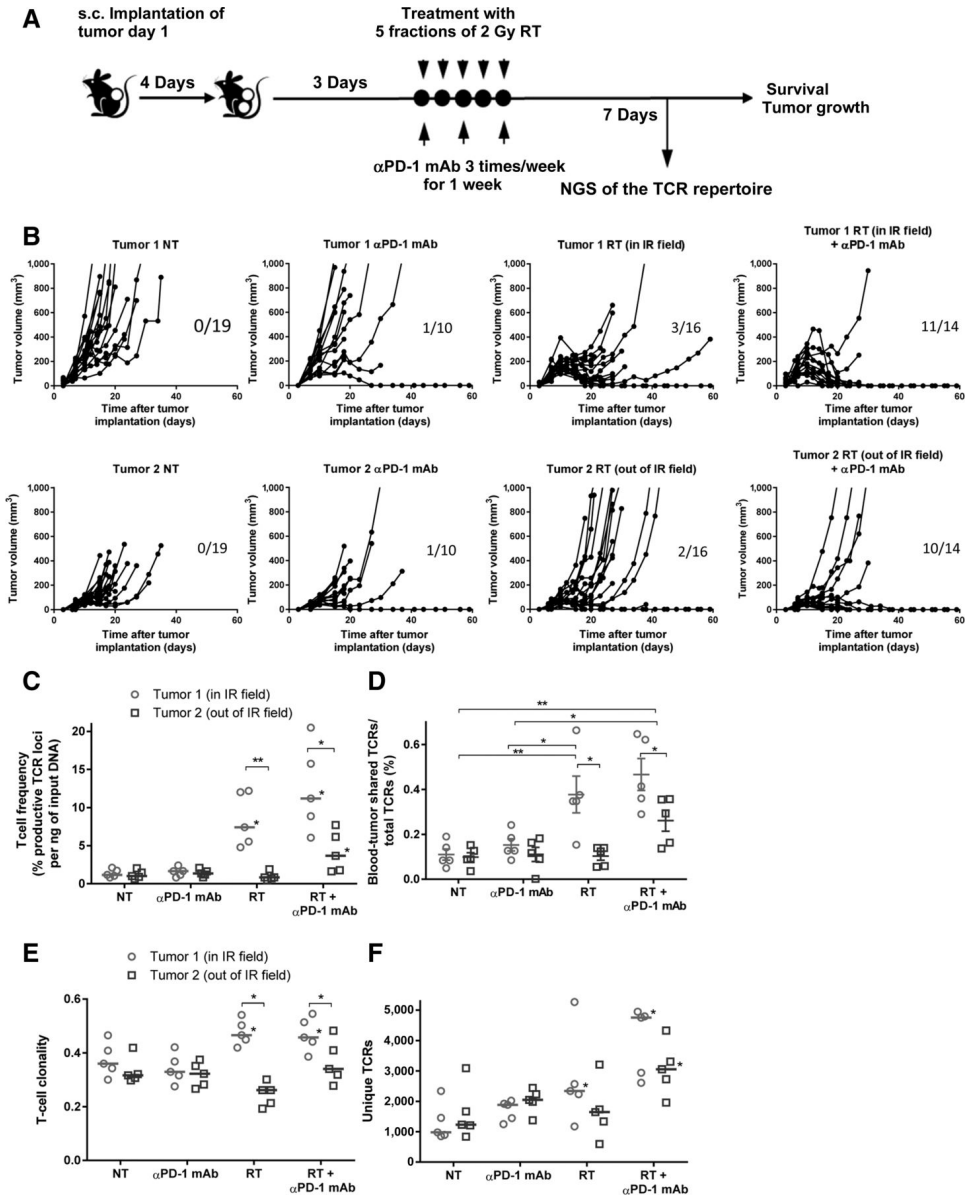
Dovedi SJ *et al*. (2017) Fractionated Radiation Therapy Stimulates Antitumor Immunity Mediated by Both Resident and Infiltrating Polyclonal T-cell Populations when Combined with PD-1 Blockade. *Clin Cancer Res.* 23(18):5514-5526.

RT/αPD-L1 in CT26: TE / DM



Dovedi SJ *et al*. (2017) Fractionated Radiation Therapy Stimulates Antitumor Immunity Mediated by Both Resident and Infiltrating Polyclonal T-cell Populations when Combined with PD-1 Blockade. *Clin Cancer Res.* 23(18):5514-5526.

RT/αPD-L1 in CT26: DM / Efficacy



Dovedi SJ *et al*. (2017) Fractionated Radiation Therapy Stimulates Antitumor Immunity Mediated by Both Resident and Infiltrating Polyclonal T-cell Populations when Combined with PD-1 Blockade. *Clin Cancer Res.* 23(18):5514-5526.

Biological mechanisms

- RT triggers Immunogenic Cell Death
  - Transient increase in tumour antigens
  - Damage-associated molecular patterns (DAMPs) release is RT dose dependent
  - DAMPs ➔ dendritic cells ➔ macrophage phagocytosis / antigen presentation
- PD-L1 responses
  - RT ➔ active immune cells ➔ interferon γ ➔ tumour PD-L1
  - *CD8*$^+$ cell depletion ⊣ RT-dependent PD-L1 induction
  - Tumour PD-L1 ⊣ T cell proliferation, pro-inflammatory cytokine production, antigen-dependent cytotoxicity
  - The PD-L1 induction by RT lasts over 7 days
- *CD8*$^+$*:Treg* ratio
  - Control condition: *CD8*$^+$*:Treg* ratio decreases over time
  - *CD4*$^+$ cell (Th1/2 and *Treg*) depletion ➔ PD-L1
  - Syngeneic CT26 tumours: High baseline *Treg* cell count in tumour

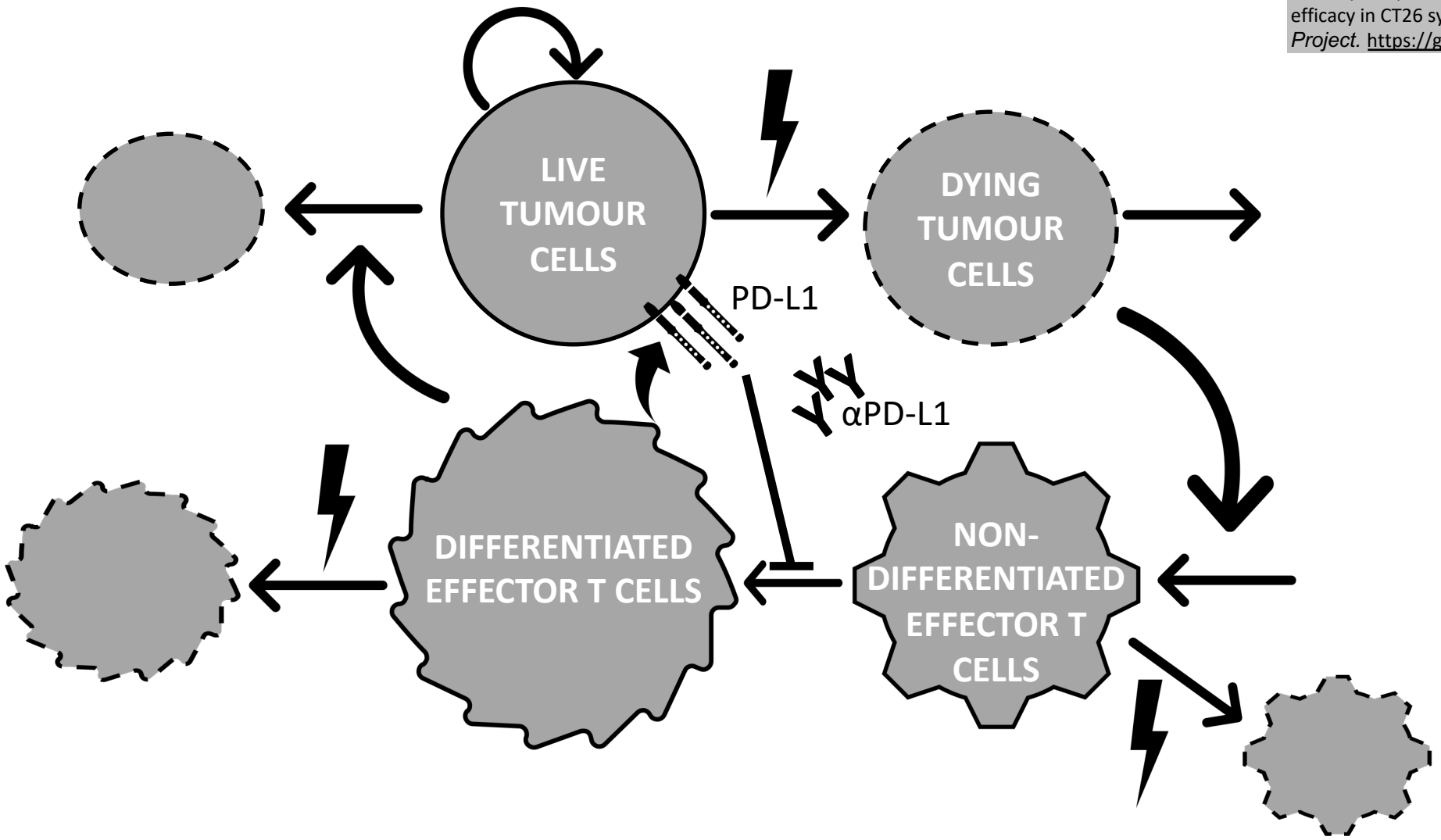
Dovedi SJ *et al*. (2014) Acquired Resistance to Fractionated Radiotherapy Can Be Overcome by Concurrent PD-L1 Blockade. *Cancer Res.* 74(19): 5458-68.

Twyman-Saint VC, *et a*l. (2015) Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. *Nature*. 520:373–7.
Sharabi AB, *et al*. (2015) Stereotactic

Radiation Therapy Augments Antigen-Specific PD-1-Mediated Antitumor Immune Responses via Cross-Presentation of Tumor Antigen. *Cancer Immunol. Res*. 3:345–55.

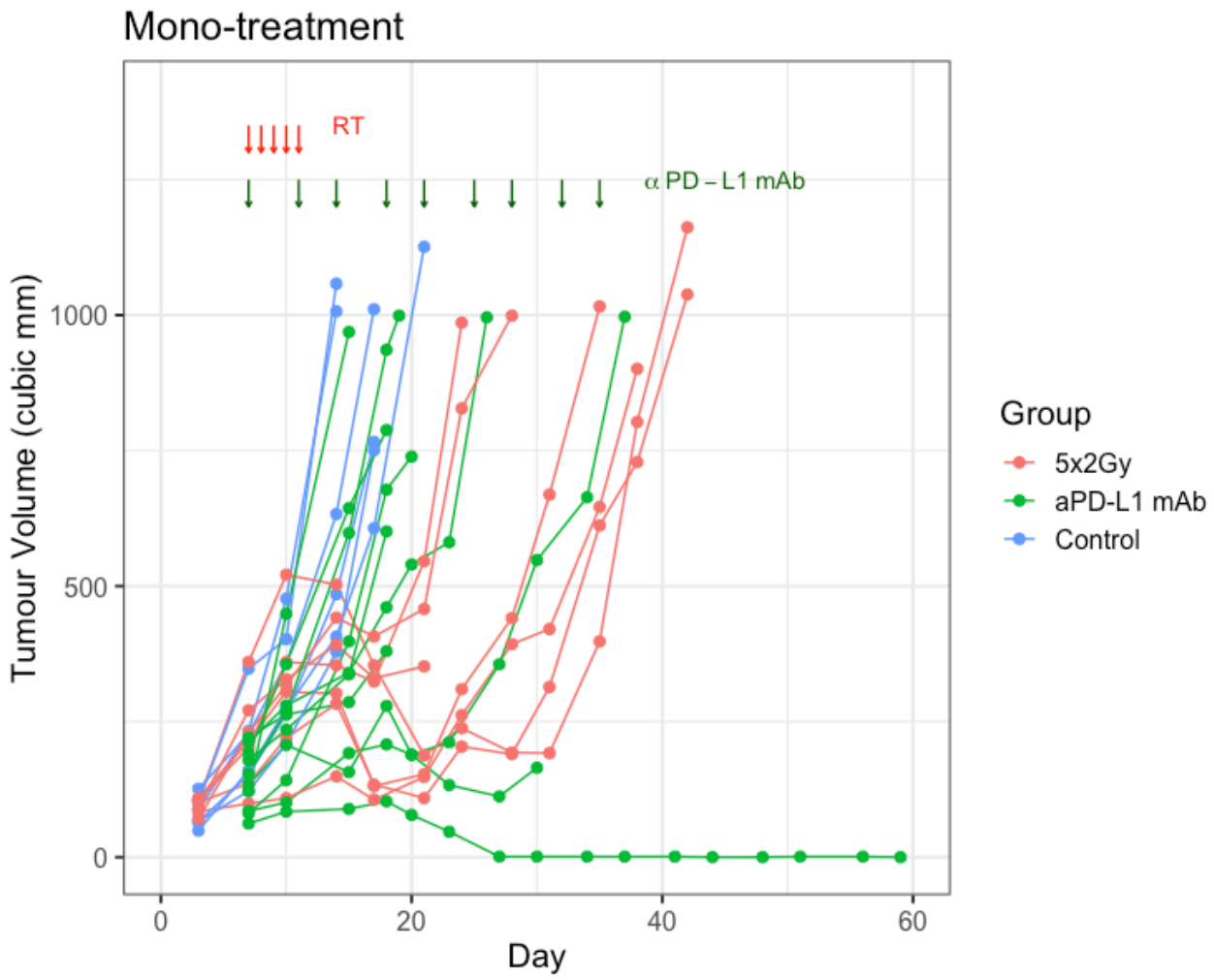
Model diagram

You T. (2019) Modelling of RT/αPD-L1 combination efficacy in CT26 syngeneic mouse model. *The Open Project.* https://github.com/TheOpenProject/



Mono-treatment of CT26

- Radio: 2Gy x 5 (days 7~11)
  - Tumour shrinkage in most mice post day 10
  - Tumour regrowth starts after day 15
  - No complete tumour rejection
- αPD-L1: 3qw for 3 weeks
  - 1/10 complete tumour rejection
  - 9/10 delayed tumour growth



RT: Dovedi SJ *et al*. Acquired Resistance to Fractionated Radiotherapy Can Be Overcome by Concurrent PD-L1 Blockade. *Cancer Res.* 74(19): 5458-68 (2014).

***α*PD-L1**: Dovedi SJ *et al*. Fractionated Radiation Therapy Stimulates Antitumor Immunity Mediated by Both Resident and Infiltrating Polyclonal T-cell Populations when Combined with PD-1 Blockade. *Clin Cancer Res.* 23(18):5514-5526 (2017).

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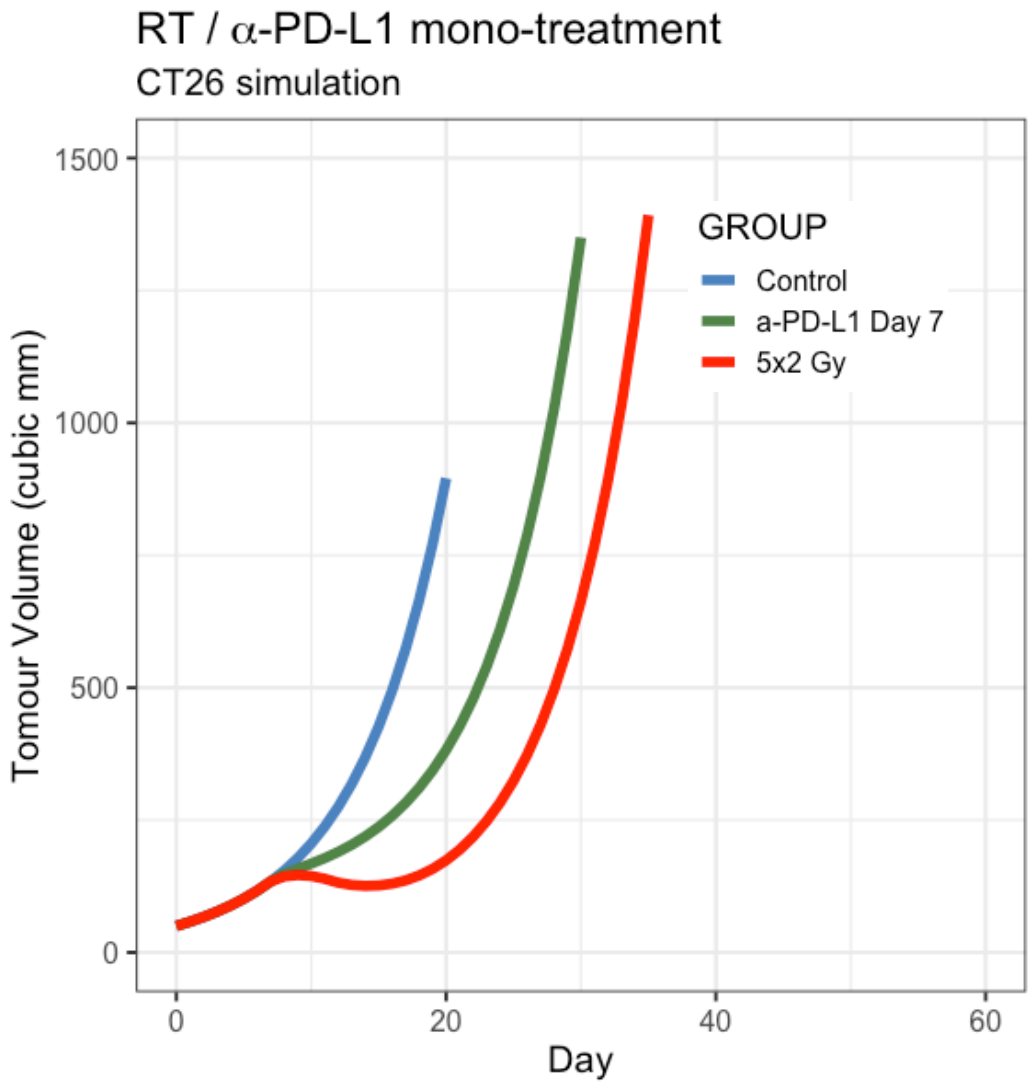
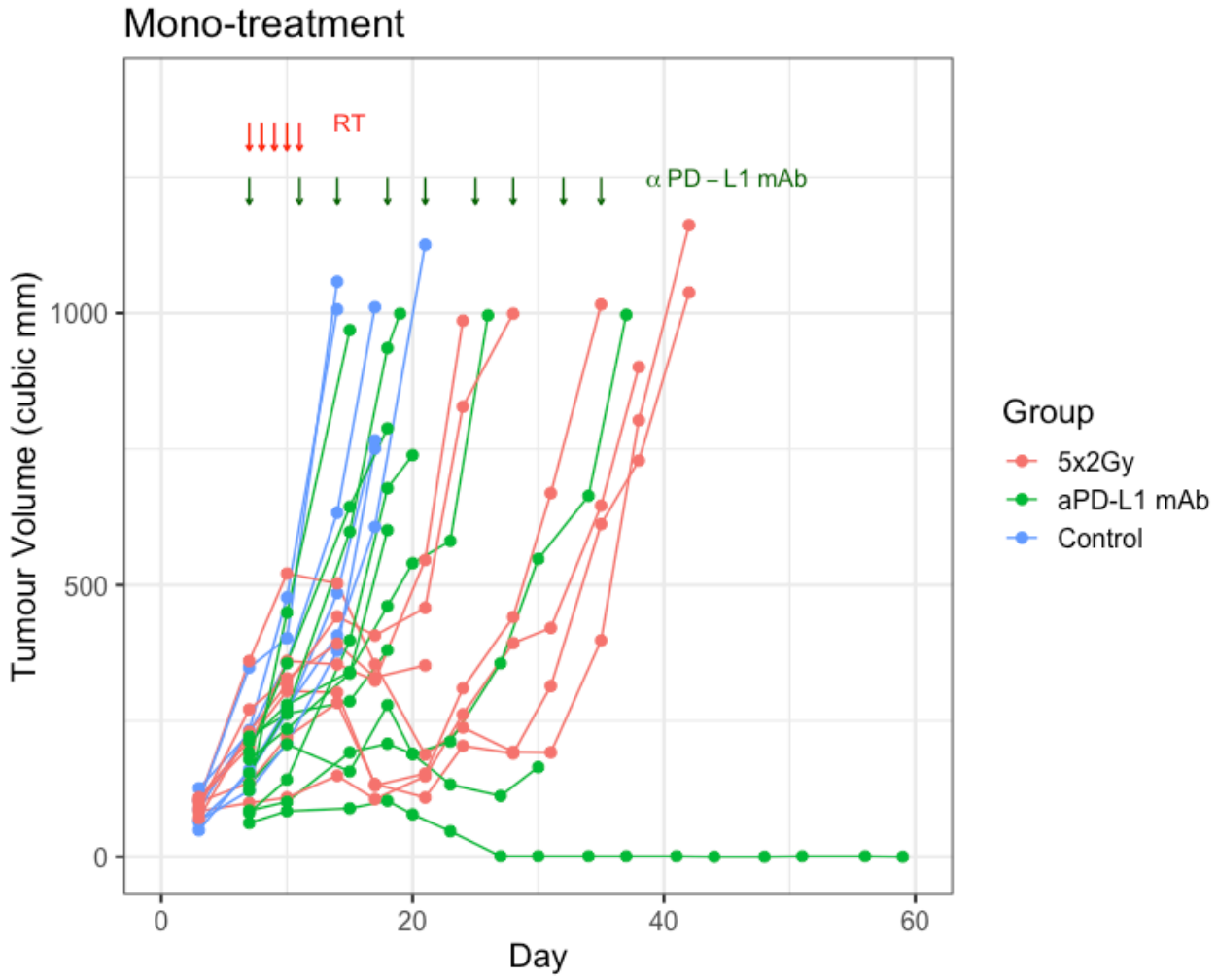


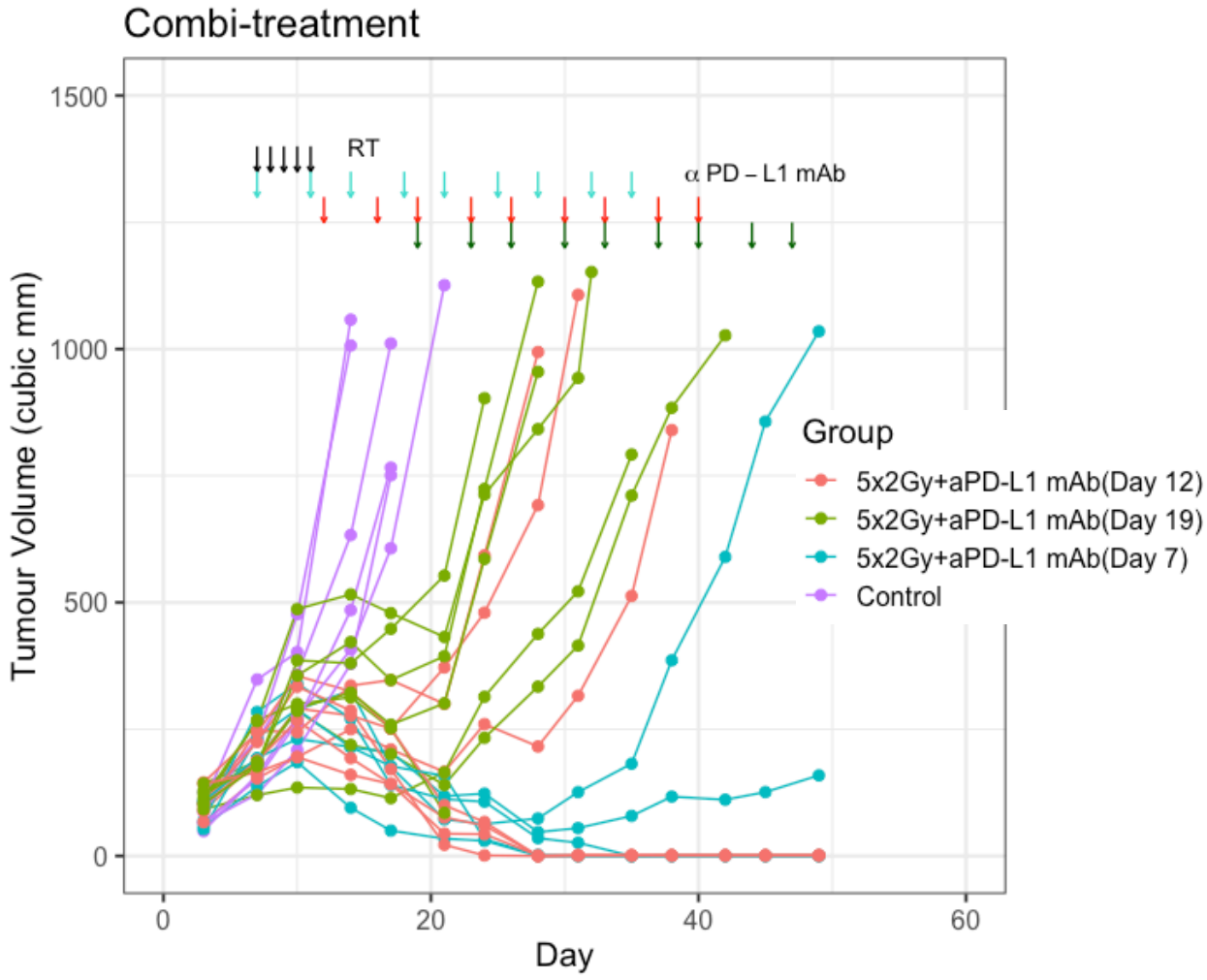


Combi-treatment of CT26

- 2Gy x 5 + αPD-L1 (days 7)
  - 3/5 complete tumour rejection
  - 2/5 delayed tumour growth
- 2Gy x 5 + αPD-L1 (days 12)
  - 4/7 complete tumour rejection
  - 3/7 delayed tumour growth
- 2Gy x 5 + αPD-L1 (days 19)
  - 1/7 overall tumour shrinkage
  - 6/7 delayed tumour growth
- Concurrent dosing has highest efficacy

RT: Dovedi SJ *et al*. (2014) Acquired Resistance to Fractionated Radiotherapy Can Be Overcome by Concurrent PD-L1 Blockade. *Cancer Res.* 74(19): 5458-68.

αPD-L1: Dovedi SJ *et al*. (2017) Fractionated Radiation Therapy Stimulates Antitumor Immunity Mediated by Both Resident and Infiltrating Polyclonal T-cell Populations when Combined with PD-1 Blockade. *Clin Cancer Res.* 23(18):5514-5526.



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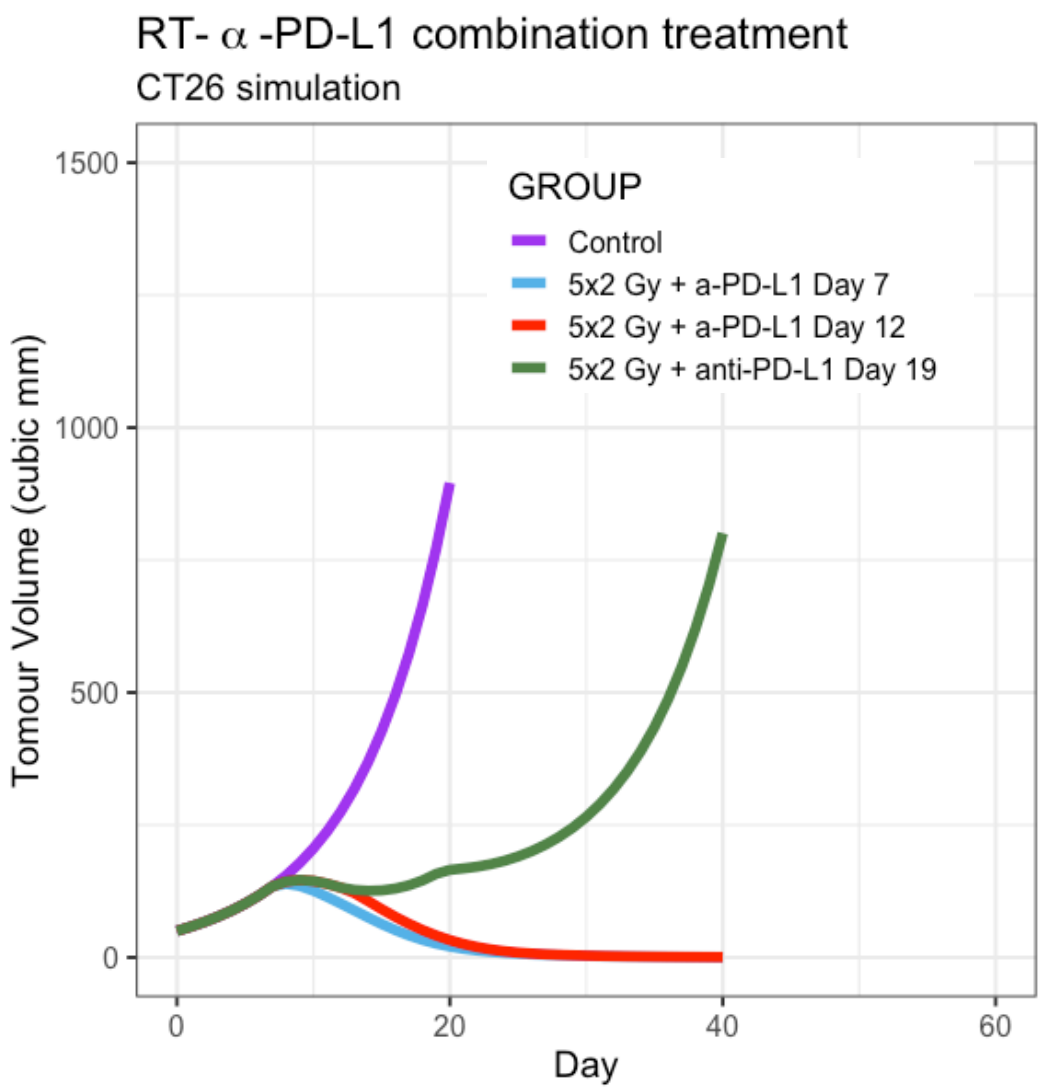
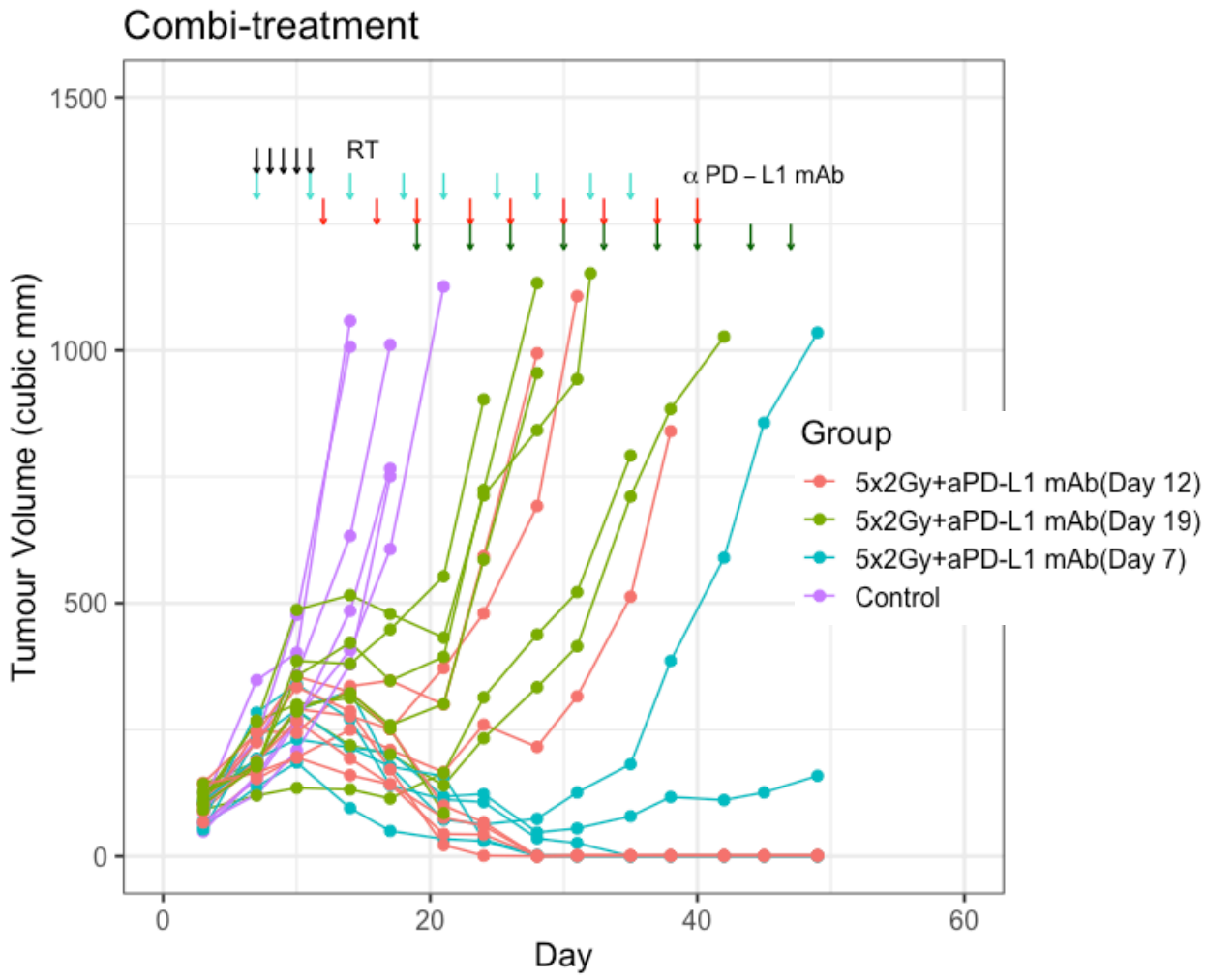




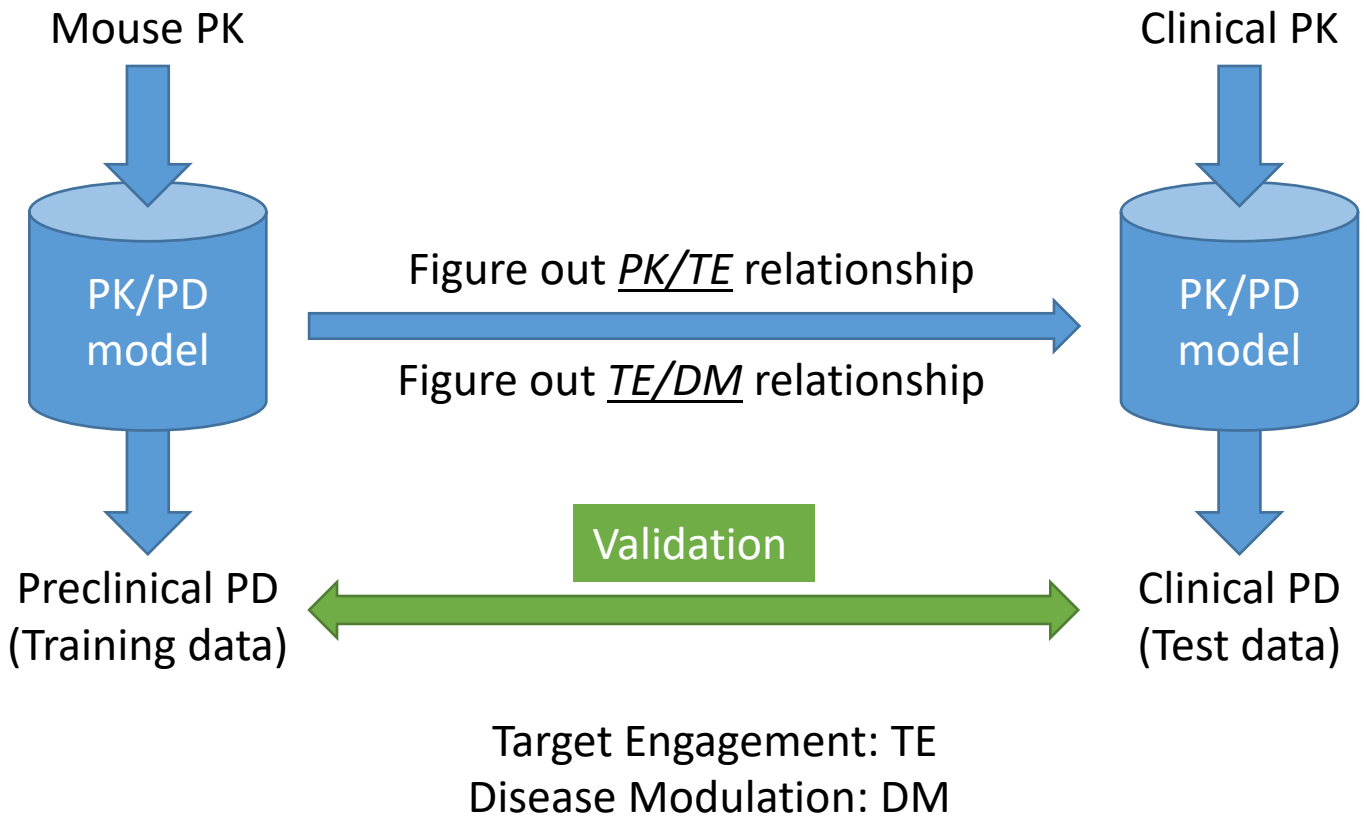
You IR/αPD-L1 CT26 PK/PD model

```
You_IR_PDL1_2019 = function(Time, State, Pars) {
  with(as.list(c(State, Pars)), {
    TV    = State[1] # Tumour cell without DSB (cubic mm)
    TVd   = State[2] # Tumour cell with DSB (cubic mm)
    nTeff = State[3] # Non-differentiated T cells relatively long-lived T cells, which may proliferate and differentiate (a.u.)
    dTeff = State[4] # Terminally differentiated, cytotoxic effector T cells (a.u.)
    PD_L1 = State[5] # Tumoural PD-L1 expression in the TME (a.u.)
    R     = State[6] # Irradiation dose rate (Gray/d)
    D     = State[7] # anti PD-L1 antibody (mAb) dose (nmole)
    mAb   = State[8] # anti PD-L1 antibody (mAb) substance in central compartment (nmole)
    mAb_conc = mAb/Vd
    PD_L1free = PD_L1 / (1+mAb_conc/KD)
    TV_ode = r*TV - alpha*R*TV - e*dTeff*TV
    TVd_ode = alpha*R*TV - mu*TVd
    nTeff_ode = kdTeff - kdTeff*nTeff + kaTeff*nTeff*TVd - km*nTeff*KmPD_L1^2/(KmPD_L1^2+PD_L1free^2) - alpha*R*nTeff
    dTeff_ode = km*nTeff*KmPD_L1^2/(KmPD_L1^2+PD_L1free^2) - ki*dTeff*TV - alpha*R*dTeff
    PD_L1_ode = kdPD_L1 + kp*dTeff - kdPD_L1*PD_L1
    R_ode = 0
    D_ode = -ka*D
    mAb_ode = ka*D - kelmAB*mAb
    return(list(c(TV_ode,TVd_ode,nTeff_ode,dTeff_ode,PD_L1_ode,R_ode,D_ode,mAb_ode)))
  })
}
# Parameters used to generate "Sim - mono.png" and "Sim - combi.png"
ini  <- c(TV=50, TVd=0, nTeff=1, dTeff=0, PD_L1=1, R=0, D=0, mAb=0)
param = c(r = 0.15, alpha = 0.16, e = 0.15, mu = 0.1725, kdTeff = 1,
          kaTeff = 0.01, km = 1, KmPD_L1 = 0.2, ki = 0.005, kp = 0.1,
          kdPD_L1 = 0.1, Vd = 0.003, ka = 8.0, kelmAB = 0.15, KD = 30)
```

You T. (2019) Modelling of RT/αPD-L1 combination efficacy in CT26 syngeneic mouse model. *The Open Project.* https://github.com/TheOpenProject/

Summary: Clinical translation of syngeneic models

- Translation needs to consider clinical PK
- Translation may *not* assume identical preclinical and clinical PK/PD relationships
- Translation requires understanding of PK/TE/DM relationships of preclinical and clinical tumours



What are the questions to consider when prospectively translating syngeneic model results into the clinics?

1. **Downregulate MHC / antigen**
2. **Melanoma's intrinsic β-catenin**
   - No CCL4 ⟹ No Ag presentation
3. **PD-1/PD-L1, CTLA4**
4. **Tumour VEGF-A, PGE2, IL-10**
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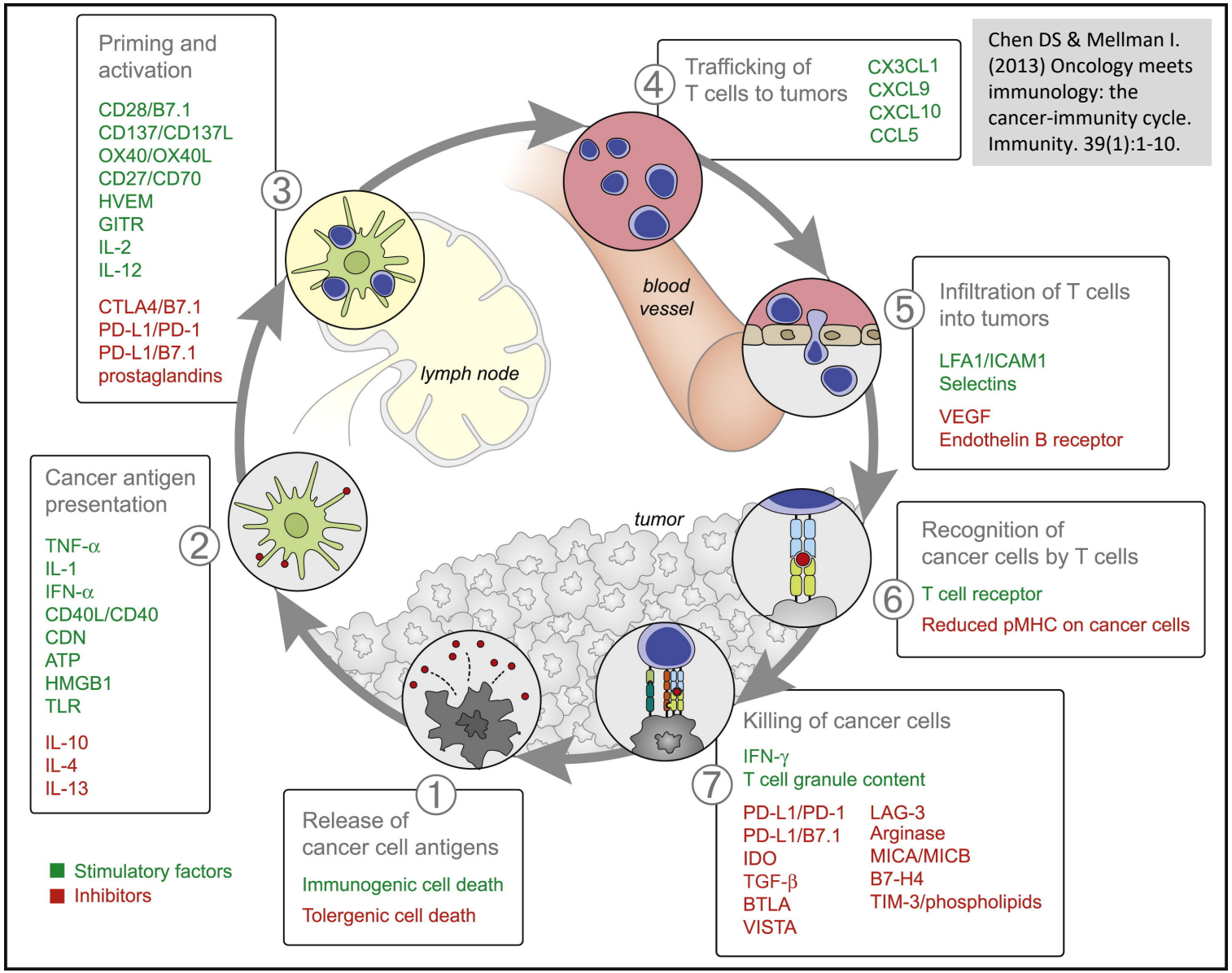
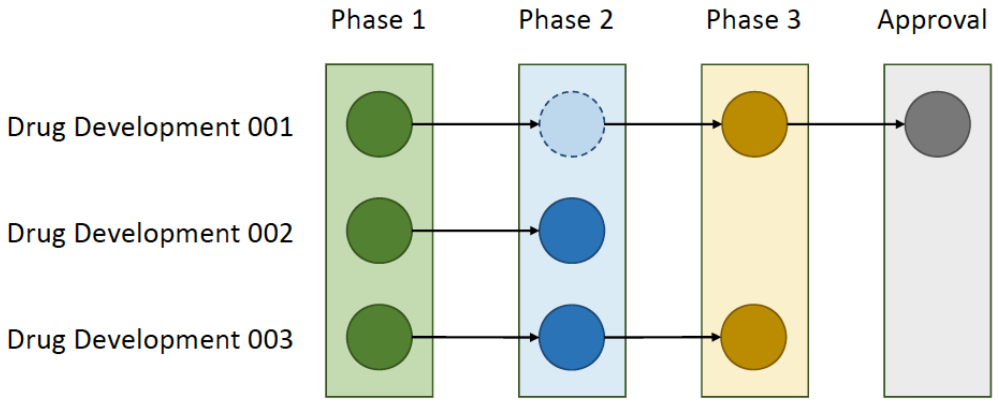


Chen DS & Mellman I. (2013) Oncology meets immunology: the cancer-immunity cycle. Immunity. 39(1):1-10.

What is the one action I will take after the workshop and why?

Supporting slides

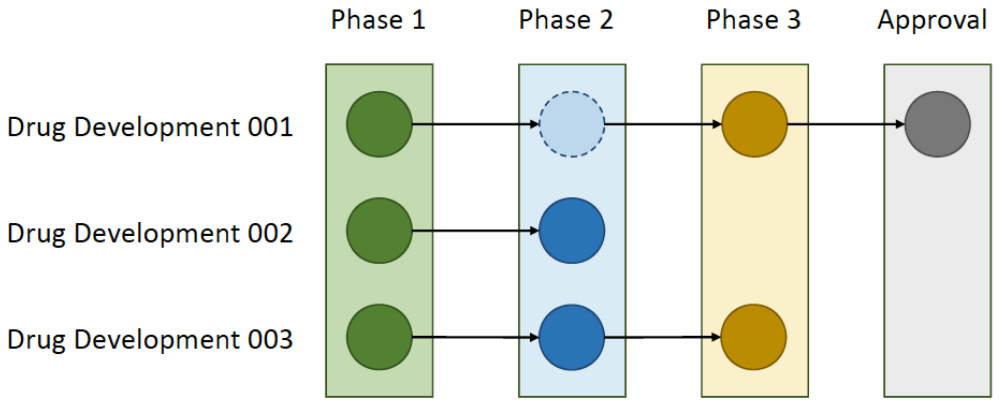
Probability of Success (POS)



- Phase-by-phase counting
  - $POS_{1,2} = 1$
  - $POS_{2,3} = \frac{1}{2}$
  - $POS_{3,App} = \frac{1}{2}$
  - $POS_{1,App} = 1x \frac{1}{2} x \frac{1}{2} = \frac{1}{4}$
  - Widely used in the past
  - Ignore missing trials

Lo *et al*. (2018) Estimation of clinical trial success rates and related parameters. *Biostatistics*. p 1–14

Probability of Success (POS)



- Path-by-path counting
  - Missing Phase 2 is inferred
  - $POS_{1,2} = 1$
  - $POS_{2,3} = \frac{2}{3}$
  - $POS_{3,App} = \frac{1}{2}$
  - $POS_{1,App} = 1x \frac{2}{3} x \frac{1}{2} = \frac{1}{3}$
  - Used by Lo *et al*
  - Considers missing, in progress and terminated trials
  - More accurate description than phase-by-phase

Lo *et al*. (2018) Estimation of clinical trial success rates and related parameters. *Biostatistics*. p 1–14

Path-by-path formal definition

- Trial status

$$s = \begin{cases} ip, & \text{if all the trials are in progress} \\ t, & \text{if the program failed to proceed to phase } i+1 \text{ (i.e., terminated)} \\ m, & \text{if the phase transition can be inferred to be missing} \end{cases}$$

- Conservation law

Total trials in phase j

Observable trials in phase j

$$n^{j+1} = n^j + n^j_m - n^j_{ip} - n^j_t \qquad \forall j = 1, 2, 3$$

Total active trials in phase j

Probability of success (Ph j to j+1)

$$\text{POS}_{j,j+1}(\text{Path-by-Path}) = \frac{n^{j+1}}{n^j + n^j_m - n^j_{ip}}$$

Observable trials in phase j+1

Total active trials in phase j

Probability of success (Ph 1 to registration)

$$\text{POS}_{1,\text{APP}}(\text{Path-by-Path}) = \frac{n^{\text{Approval}}}{n^1 + n^1_m - n^1_{ip} - n^2_{ip} - n^3_{ip}}$$

Phase-by-phase formal definition

- Trial status

$$s = \begin{cases} ip, & \text{if all the trials are in progress} \\ t, & \text{if the program failed to proceed to phase } i+1 \text{ (i.e., terminated)} \\ m, & \text{if the phase transition can be inferred to be missing} \end{cases}$$

- Conservation law

Total trials in phase j

Observable trials in phase j

$$n^{j+1} = n^j + n_m^j - n_{ip}^j - n_t^j \qquad \forall j = 1, 2, 3$$

Total active trials in phase j

$$\mathrm{POS}_{j,j+1}^p = \frac{n^{j+1} - n_m^j}{n^j - n_{ip}^j}$$

Observed transitions to phase j

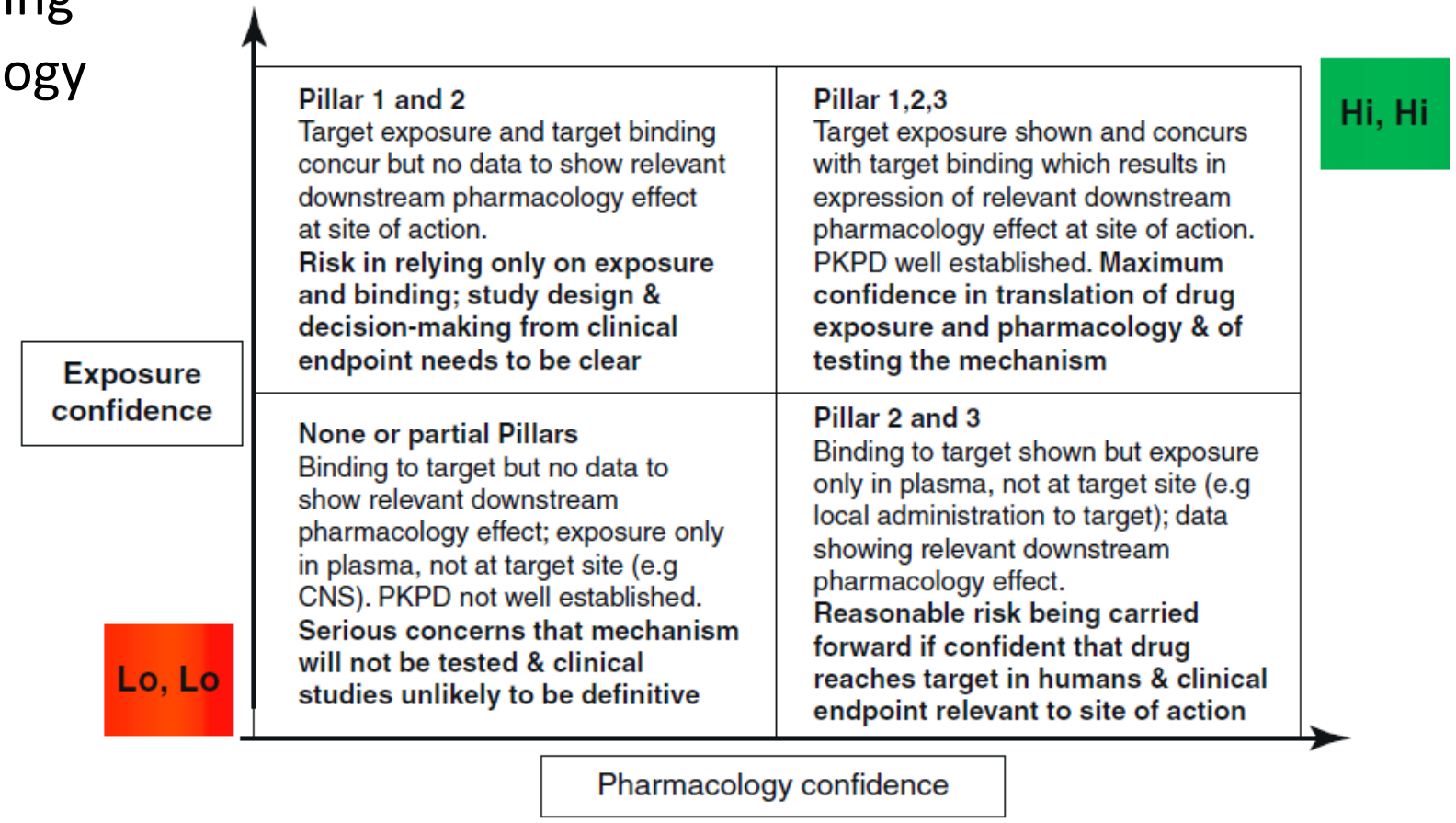
Observed active trials in phase j

$$\mathrm{POS}_{1,\mathrm{APP}}^p = \prod_{j \in \{1,2,3\}} \mathrm{POS}_{j,j+1}^p$$

- If no missing trials, the two models are identical

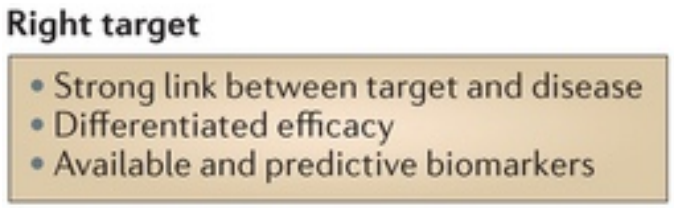
Pfizer 3 pillars

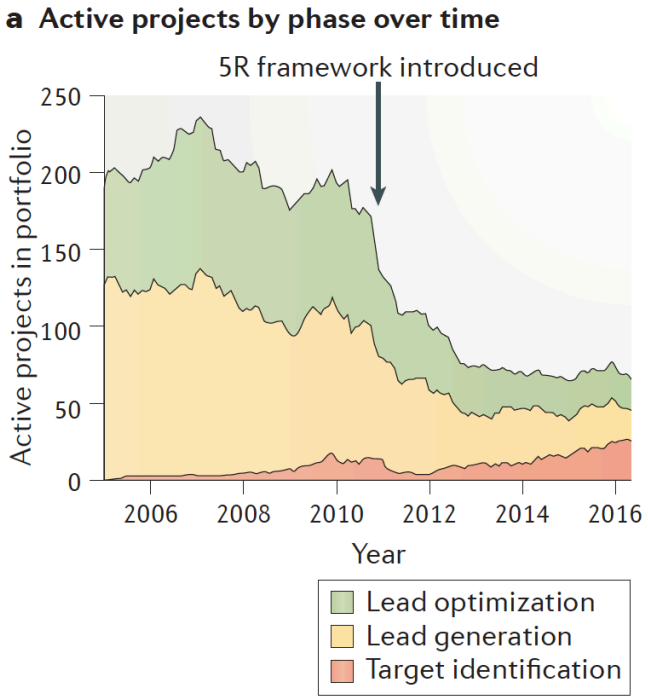
- "Pfizer 3 pillars"
  1. Suitable exposure (site of action, duration)
  2. Sufficient target binding
  3. Adequate pharmacology



Can the flow of medicines be improved? Fundamental pharmacokinetic and pharmacological principles toward improving Phase II survival *Drug Discovery Today.* 17, 419–424 (2012)

Right target



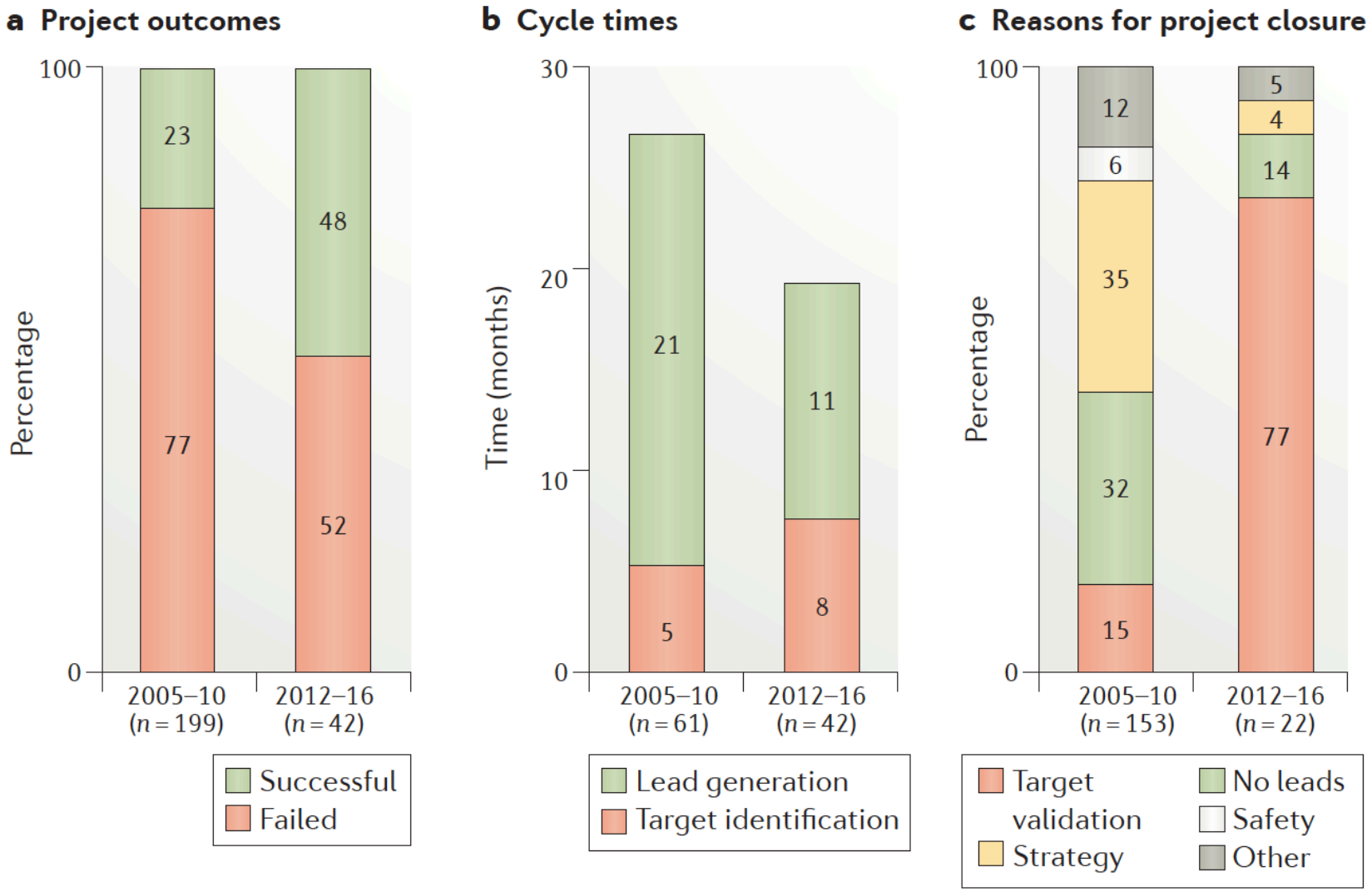


Morgan P *et al*. Impact of a five-dimensional framework on R&D productivity at AstraZeneca. *Nature Rev. Drug Discov.* **17(3)**, 167-181 (2015).

- **"The most important of the 5Rs"**
  - Verify target validity using genome editing
    - Invalidate a target earlier than previously possible
    - CRISPR
      - Invalidate MTH1 as an oncology target
      - Identify isotypes for SIK- mediated inflammatory responses
    - TALEN: Transcription activator-like effector nuclease

AstraZeneca 5R framework

- **Improve target validation to reduce attrition in early-stage discovery**
  - Genome editing
  - Genomics
  - Humanised preclinical models



Morgan P *et al*. Impact of a five-dimensional framework on R&D productivity at AstraZeneca. *Nature Rev. Drug Discov.* **17(3)**, 167-181 (2015).

How to apply “right commercial potential”

- **Adapting “right commercial potential”**
  - Projects can be mistakenly driven by an overemphasis on commercial potential
  - Candidate selection
    - It is years from launch: commercial valuation cannot be accurate
    - This decision point focuses primarily on efficacy, safety and differentiation
  - Ph III investment decision needs a thorough commercial assessment
    - Patient population size
    - Unmet medical need
    - Required differentiation (vs the standard of care)
    - Payer criteria for reimbursement
    - Competitive environment
    - Sales projections

Morgan P *et al*. Impact of a five-dimensional framework on R&D productivity at AstraZeneca. *Nature Rev. Drug Discov.* **17(3)**, 167-181 (2015).

Translational modelling framework

- Limitations of the approach/conclusions
- How to apply translational modelling framework to best support drug projects?
- Where does it add value?
- How define and support immuno-oncology projects?

	Exposure	Target Engagement	Disease Modulation	Outcome
In vitro	Medium	Direct / Indirect	DM	Efficacy
In vivo	Plasma Tissue	Direct / Indirect	DM	Efficacy
Clinical	Plasma	Direct / Indirect	DM	Efficacy

Translational modelling tools

- Human PK prediction: Small molecules, Large molecules (PBPK)
- Safety prediction: Cardiac safety, bone marrow toxicity
- Decision making with PK/PD modelling

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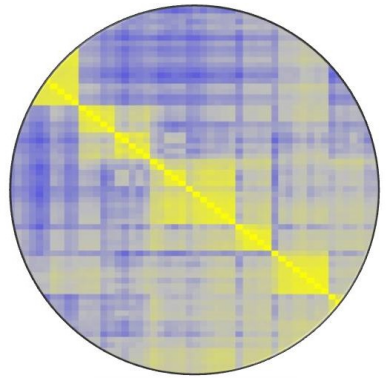

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