What's in our translational science toolbox?

Preclinical oncology model selection in the genomic era – a biotech's perspective

- Current problems and opportunities; what have we learnt from past experience?
- First-time right? Delivering POC studies to inform the path to the clinic
- From EMT to TME: exploiting data-driven model selection (exemplified by case studies)

Current problems and opportunities - what have we learnt from past experience?

Evaluating efficacy – 10 years on

BIOBUSINESS BRIEFS

TRIAL WATCH

NATURE REVIEWS | DRUG DISCOVERY
VOLUME 10 | MAY 2011 | 1

Phase II failures: 2008–2010

Well-conducted Phase II clinical trials provide the data required to determine whether there is a case to be made, both scientifically and commercially, for progressing a drug candidate into Phase III trials. At present, however, Phase II success rates are lower than at any other phase of development. Analysis by the Centre for Medicines Research (CMR) of projects from a group of 16 companies (representing approximately 60% of global R&D spending) in the CMR International Global R&D database reveals that the Phase II success rates for new development projects have fallen from 28% (2006–2007) to 18% (2008–2009), although these success rates do vary between therapeutic areas and between small molecules and biologics. As the current likelihood of a drug successfully progressing through Phase III to launch is 50% (*Nature Rev. Drug Discov.* **10**, 87; 2011), the overall attrition of late-stage drug development seems to be unsustainably high.

To help understand these trends, Thomson Reuters Life Science Consulting analysed the 108 reported Phase II failures from 2008 to 2010 for new drugs and major new indications of existing drugs (*Drug News Perspect.* **22**, 39–51; 2009; *Drug News Perspect.* **23**, 48–63; 2010; *Drugs Today*, **47**, 27–51; 2011). Out of these, 87 reported the reasons for failure (FIG. 1a): 51% (44 out of 87) were due to insufficient efficacy, 29% (25 out of 87) were due to strategic reasons and 19% (17 out of 87) were due to clinical or preclinical safety reasons. Out of the 25 failures that were terminated for strategic reasons, 16 involved validated targets such as peroxisome proliferator activated receptor-γ (PPARγ) and factor Xa, therefore suggesting that some of these failures were due to inadequate differentiation from more advanced drugs in the same class or from drugs with similar indications in another mechanistic class. Out of the 21 failures for which reasons were not reported, 17 involved validated targets, although not always in an approved indication for drugs affecting that target. Again, it would seem reasonable to conclude that some of these failures were due to insufficient evidence of an efficacy advantage over a more advanced drug; however, it is important not to rule out that failure could be due to the change in the benefit–risk balance of a known target in a new patient population. These data also show that 68% (73 out of 108) of failures fell into four therapeutic areas (FIG. 1b): alimentary/metabolism, cancer, cardiovascular, and neuroscience. Notably, 61% (14 out of 23) of failures in alimentary/metabolism are for diabetes.

Although it is difficult to draw conclusions from these data, the finding that a substantial proportion of Phase II failures were due to strategic reasons suggests that one important underlying factor could be overlapping R&D activity between companies with drugs in Phase II trials. This raises the question of whether an increase in collaborative efforts between companies up to the point of proof-of-concept for novel targets or mechanisms might be more cost- and time-effective.

John Arrowsmith is a Scientific Director at Thomson Reuters, 77 Hatton Garden, London EC1N 8JS, UK.

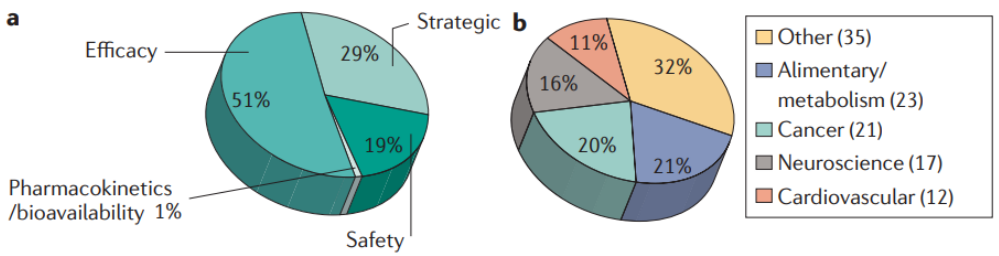


Figure 1 | **Phase II failures: 2008–2010.** The 108 failures are divided according to reason for failure when reported (87 drugs) (**a**) and therapeutic area (**b**).

Biostatistics (2019) **20**, 2, *pp.* 273–286
doi:10.1093/biostatistics/kxx069
Advance Access publication on January 31, 2018

Estimation of clinical trial success rates and related parameters

CHI HEEM WONG, KIEN WEI SIAH

MIT Computer Science and Artificial Intelligence Laboratory & Department of Electrical Engineering and Computer Science, Cambridge, MA 02139, USA and MIT Sloan School of Management and Laboratory for Financial Engineering, Cambridge, MA 02142, USA

ANDREW W. LO*

MIT Computer Science and Artificial Intelligence Laboratory & Department of Electrical Engineering and Computer Science, Cambridge, MA 02139, USA, MIT Sloan School of Management and Laboratory for Financial Engineering, Cambridge, MA 02142, USA, and AlphaSimplex Group, LLC, Cambridge, MA 02142, USA

alo-admin@mit.edu

SUMMARY

Previous estimates of drug development success rates rely on relatively small samples from databases curated by the pharmaceutical industry and are subject to potential selection biases. Using a sample of 406 038 entries of clinical trial data for over 21 143 compounds from January 1, 2000 to October 31, 2015, we estimate aggregate clinical trial success rates and durations. We also compute disaggregated estimates across several trial features including disease type, clinical phase, industry or academic sponsor, biomarker presence, lead indication status, and time. In several cases, our results differ significantly in detail from widely cited statistics. For example, oncology has a 3.4% success rate in our sample vs. 5.1% in prior studies. However, after declining to 1.7% in 2012, this rate has improved to 2.5% and 8.3% in 2014 and 2015, respectively. In addition, trials that use biomarkers in patient-selection have higher overall success probabilities than trials without biomarkers.

Keywords: Clinical phase transition probabilities; Clinical trial statistics; Probabilities of success.

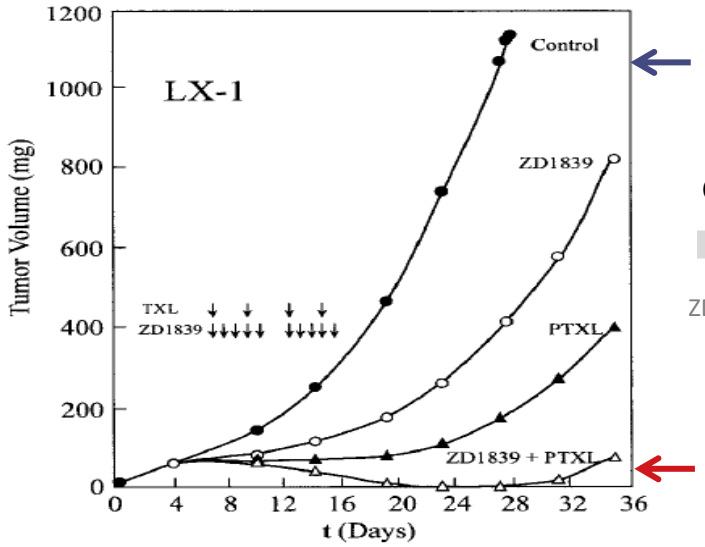
Probability of Success[2] by Clinical Trial Phase and Therapeutic Area

	P1 to P2	P2 to P3	P3 to Approval	Overall
Oncology	57.6	32.7	35.5	3.4
Metabolic/Endocrinology	76.2	59.7	51.6	19.6
Cardiovascular	73.3	65.7	62.2	25.5
Central Nervous System	73.2	51.9	51.1	15.0
Autoimmune/Inflammation	69.8	45.7	63.7	15.1
Genitourinary	68.7	57.1	66.5	21.6
Infectious Disease	70.1	58.3	75.3	25.2
Ophthalmology	87.1	60.7	74.9	32.6
Vaccines (Infectious Disease)	76.8	58.2	85.4	33.4
Overall	66.4	48.6	59.0	13.8
Overall (Excluding Oncology)	73.0	55.7	63.6	20.9

Source: Chi Heem Wong, Kien Wei Siah, Andrew W Lo. "Estimation of clinical trial success rates and related parameters." *Biostatistics* 20(2): April 2019, Pages 273-286. Published online: 31 January 2018. DOI: 10.1093/biostatistics/kxx069

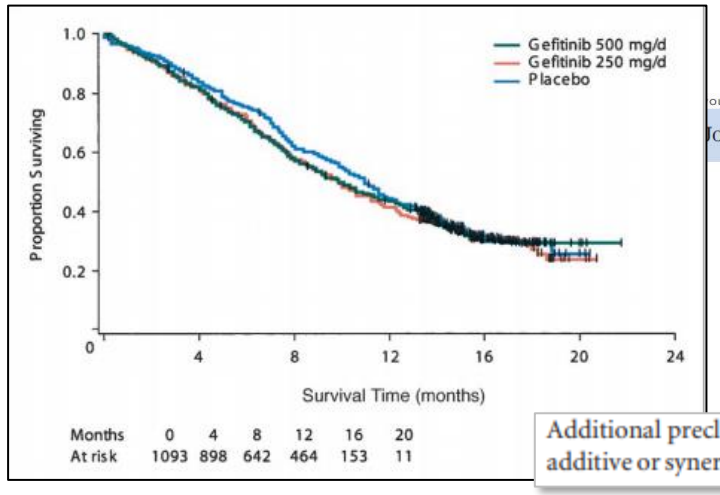
Problem: Lack of historical correlation?

PRECLINICAL ***vs.*** **CLINICAL**



NSCLC:
Gefitinib + chemo

ZD1839 = gefitinib = Iressa®



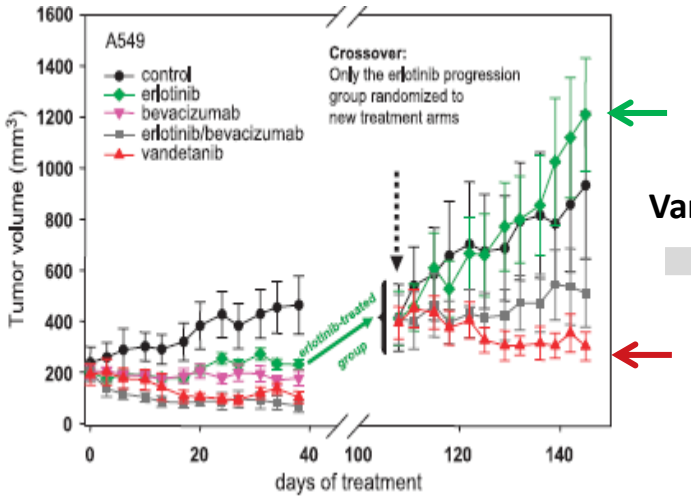
INTACT 1 PhIII
Gem-Cis +/- Gefitinib

VOLUME 22 · NUMBER 5 · MARCH 1 2004
JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

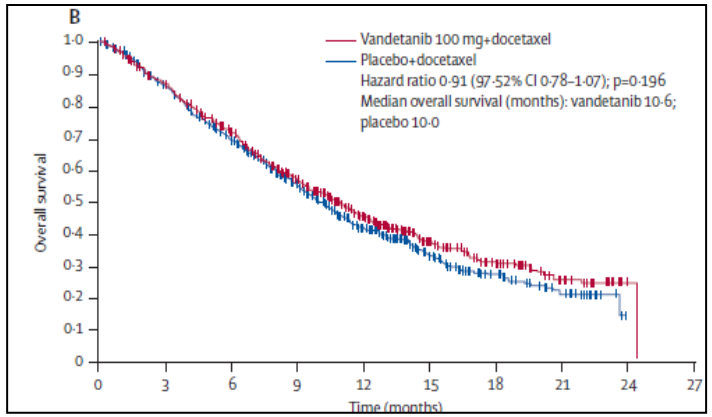
Gefitinib in Combination With Gemcitabine and Cisplatin in Advanced Non–Small-Cell Lung Cancer: A Phase III Trial—INTACT 1

Giuseppe Giaccone, Roy S. Herbst, Christian Manegold, Giorgio Scagliotti, Rafael Rosell, Vincent Miller, Ronald B. Natale, Joan H. Schiller, Joachim von Pawel, Anna Pluzanska, Ulrich Gatzemeier, John Grous, Judith S. Ochs, Steven D. Averbuch, Michael K. Wolf, Pamela Rennie, Abderrahim Fandi, and David H. Johnson

Additional preclinical studies may shed light on the lack of additive or synergistic activity in unselected human tumors.



NSCLC:
Vandetanib + chemo



ZODIC PhIII
Docetaxel +/- Vandetanib

Modest advantages in PFS & ORR but not OS

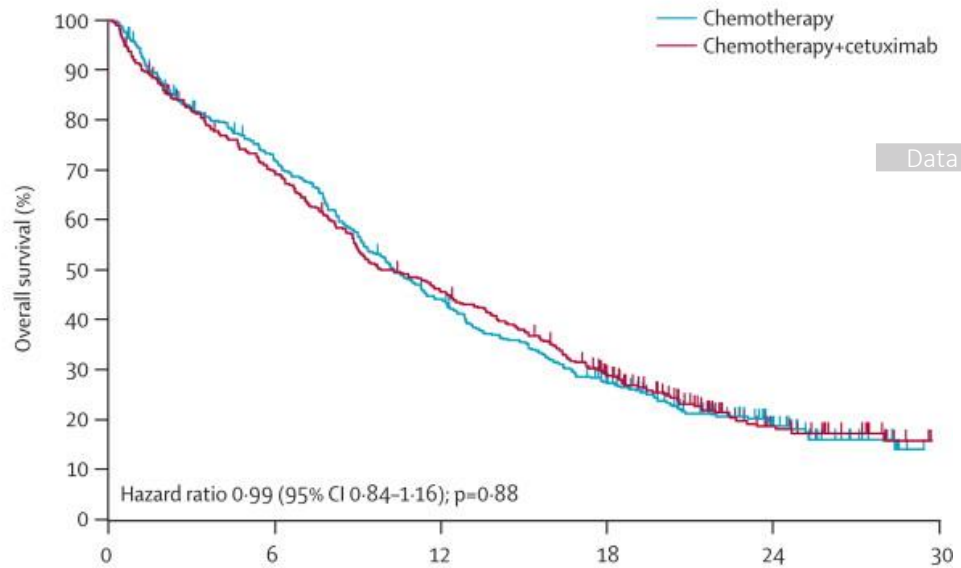
ClinicalTrials.gov: NCT00312377

BETA PhIII
Erlotinib +/- bevacizumab

Important to translate back: Lessons from FLEX Trial

ClinicalTrials.gov number NCT00148798

FLEX CLINICAL TRIAL



Data re-analysed

FLEX CLINICAL TRIAL (retrospective analysis)

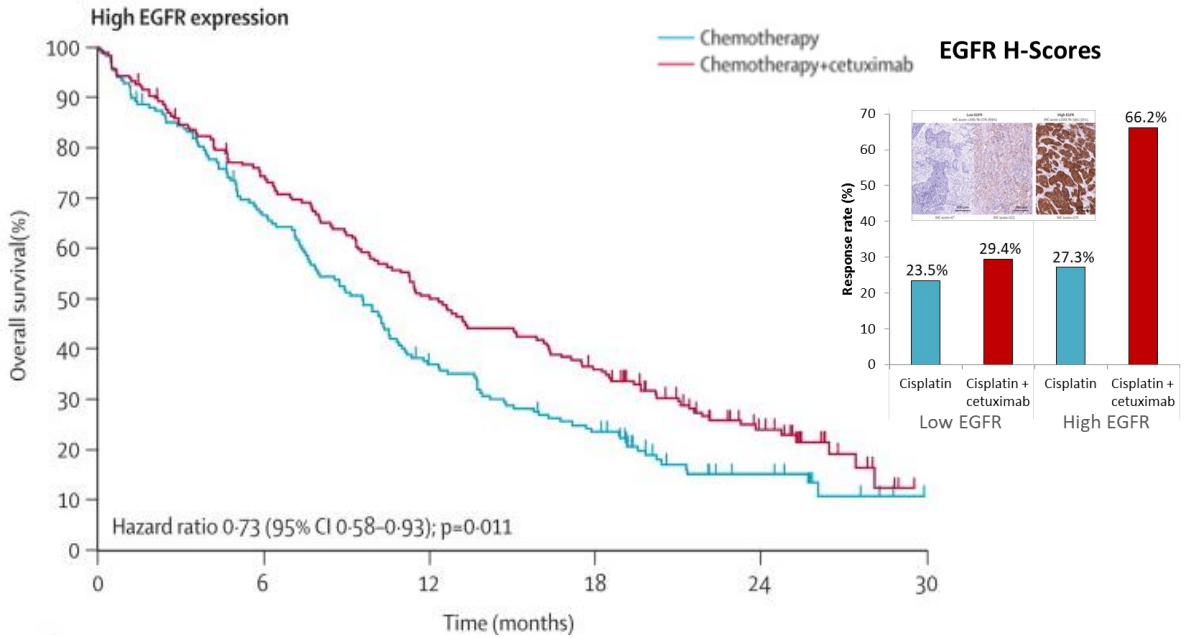
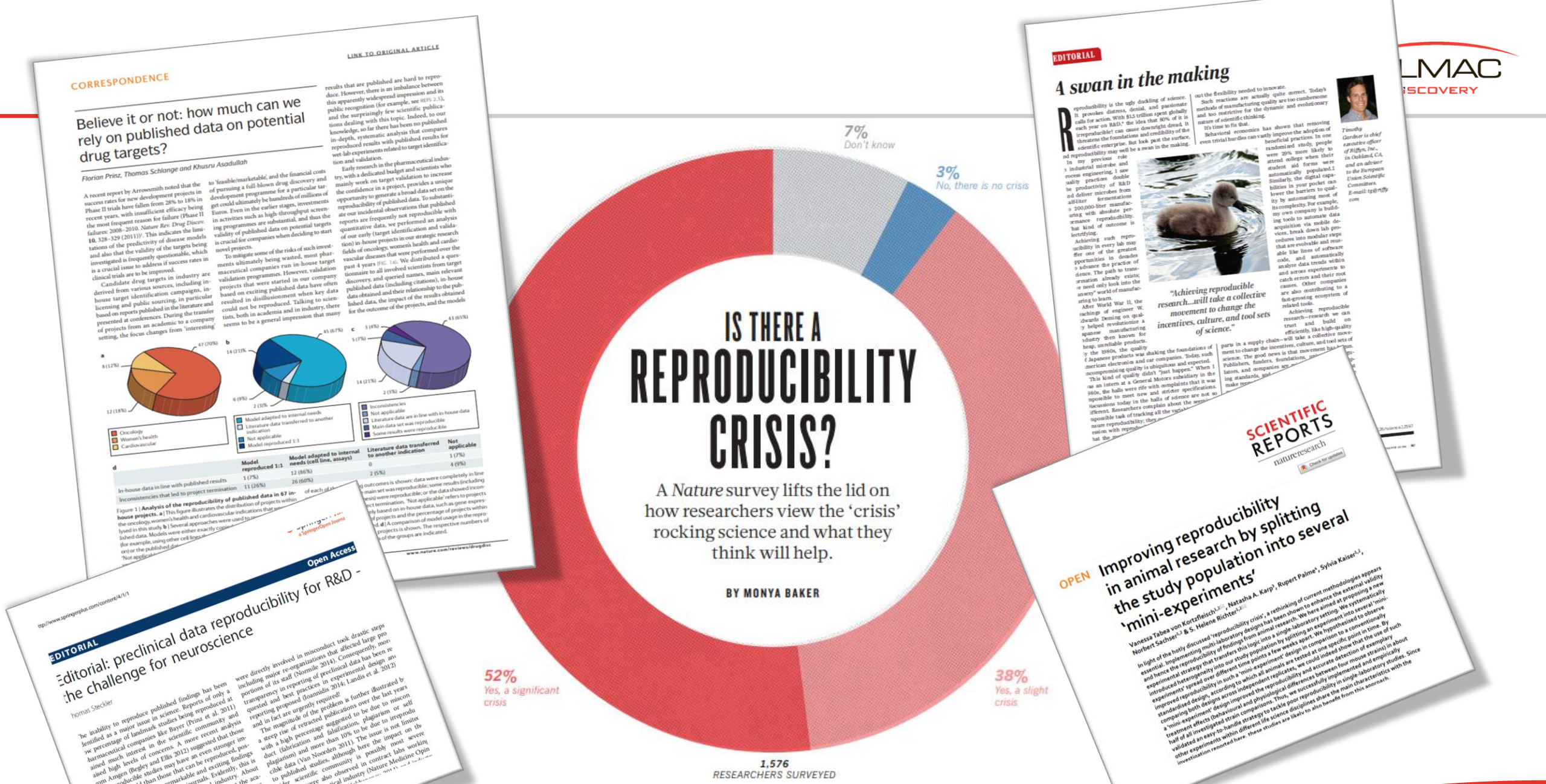


Figure taken from Pirker *et al.*, The Lancet Oncology (2012) 13, 33-42

ClinicalTrials.gov number NCT00148798

- No survival benefit to cisplatin + cetuximab treatment observed
- Response to cisplatin + cetuximab treatment significantly associated with high EGFR expression (retrospectively)
- NSCLC PDX data confirmed benefit of combination but only when EGFR H-score was re-assessed (cut-off $\geq$ 200)… A case of 'informed predictability'?


CORRESPONDENCE
Believe it or not: how much can we rely on published data on potential drug targets?
Florian Prinz, Thomas Schlange and Khusru Asadullah
LINK TO ORIGINAL ARTICLE
EDITORIAL
A swan in the making
Open Access
Editorial: preclinical data reproducibility for R&D - the challenge for neuroscience
Thomas Steckler
7%
Don't know
3%
No, there is no crisis
IS THERE A REPRODUCIBILITY CRISIS?
A Nature survey lifts the lid on how researchers view the 'crisis' rocking science and what they think will help.
BY MONYA BAKER
52%
Yes, a significant crisis
38%
Yes, a slight crisis
1,576
RESEARCHERS SURVEYED
SCIENTIFIC REPORTS
nature research
OPEN
Improving reproducibility in animal research by splitting the study population into several 'mini-experiments'

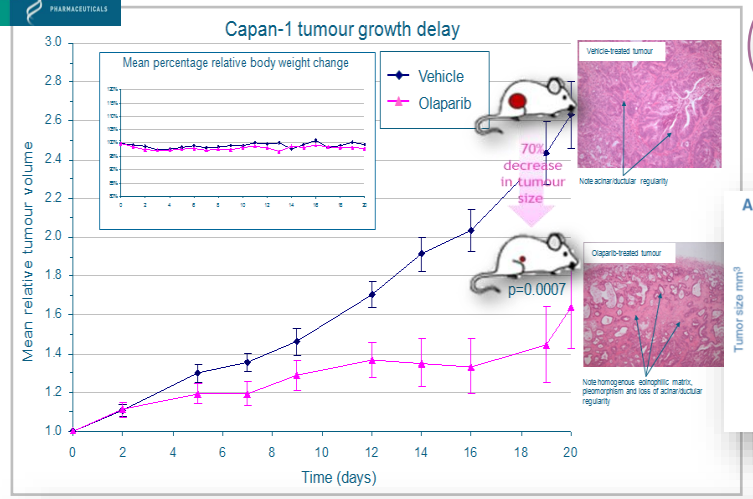
First-time right? Delivering POC studies to inform the path to the clinic

History: Olaparib (Lynparza®)

From discovery to first-in-class clinical practice and the models in between

1

BRCA2 mutant cell-line derived xenograft model

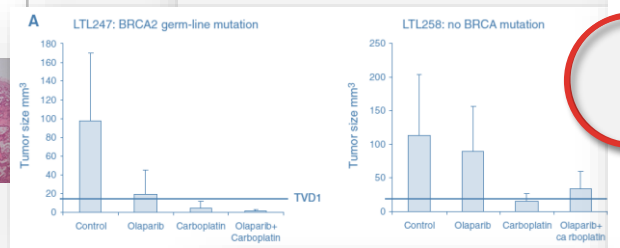


4

Cancer Therapy: Preclinical

Tumor Growth Inhibition by Olaparib in *BRCA2* Germline-Mu… Patient-Derived Ovarian Cancer Tissue Xenografts

Ursula Kortmann[1,2], Jessica N. McAlpine[3], Hui Xue[2], Jun Guan[2], Gavin Ha[4], Sophie … Sharaz Shafait[5], Alan Lau[6], Aaron N. Cranston[5], Mark J. O'Connor[6], David G. Hunts… Yuzhuo Wang[2,8], and C. Blake Gilks[7]



LYNPARZA™ approved by the US food and drug administration for the treatment of advanced ovarian cancer in patients with germline BRCA-mutations

AstraZeneca

PUBLISHED
19 December 2014

5

The NEW ENGLAND JOURNAL of MEDICI…

ESTABLISHED IN 1812 JULY 9, 2009

Inhibition of Poly(ADP-Ribose) Polymerase in … from *BRCA* Mutation Carriers

Peter C. Fong, M.D., David S. Boss, M.Sc., Timothy A. Yap, M.D., Andrew Tutt, M.D., Ph… Marja Mergui-Roelvink, M.D., Peter Mortimer, Ph.D., Helen Swaisland, B.Sc., Ala… Mark J. O'Connor, Ph.D., Alan Ashworth, Ph.D., James Carmichael, M.D., Stan B… Jan H.M. Schellens, M.D., Ph.D., and Johann S. de Bono, M.D., Ph.D

ABSTRACT

BACKGROUND

The inhibition of poly(adenosine diphosphate [ADP]–ribose) polymerase (PARP) is a potential synthetic lethal therapeutic strategy for the treatment of cancers with specific DNA-repair defects, including those arising in carriers of a *BRCA1* or *BRCA2* mutation. We conducted a clinical evaluation in humans of olaparib (AZD2281), a novel, potent, orally active PARP inhibitor.

METHODS

This was a phase 1 trial that included the analysis of pharmacokinetic and pharmacodynamic characteristics of olaparib. Selection was aimed at having a study population enriched in carriers of a *BRCA1* or *BRCA2* mutation.

RESULTS

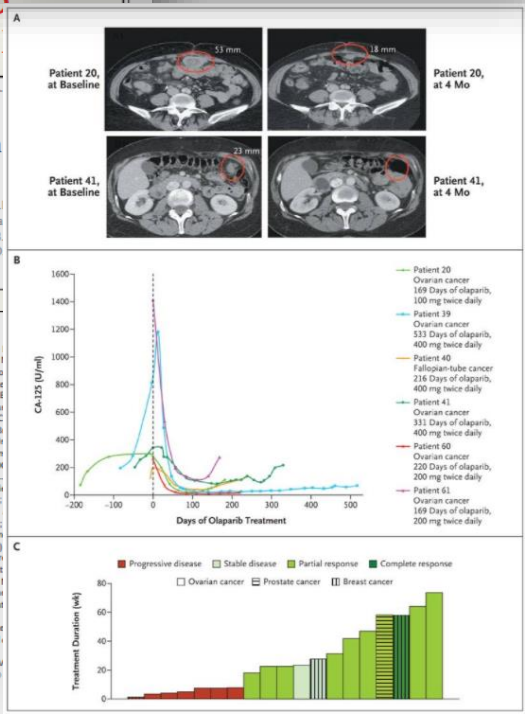
We enrolled and treated 60 patients; 22 were carriers of a *BRCA1* or *BRCA2* mutation and 1 had a strong family history of *BRCA*-associated cancer but declined to undergo mutational testing. The olaparib dose and schedule were increased from 10 mg daily for 2 of every 3 weeks to 600 mg twice daily continuously. Reversible dose-limiting toxicity was seen in one of eight patients receiving 400 mg twice daily (grade 3 mood alteration and fatigue) and two of five patients receiving 600 mg twice daily (grade 4 thrombocytopenia and grade 3 somnolence). This led us to enroll another cohort, consisting only of carriers of a *BRCA1* or *BRCA2* mutation, to receive olaparib at a dose of 200 mg twice daily. Other adverse effects included mild gastrointestinal symptoms. There was no obvious increase in adverse effects seen in the mutation carriers. Pharmacokinetic data indicated rapid absorption and elimination; pharmacodynamic studies confirmed PARP inhibition in surrogate samples (of peripheral-blood mononuclear cells and plucked eyebrow-hair follicles) and tumor tissue. Objective antitumor activity was reported only in mutation carriers, all of whom had ovarian, breast, or prostate cancer and had received multiple treatment regimens.

CONCLUSIONS

Olaparib has few of the adverse effects of conventional chemotherapy, inhibits PARP, and has antitumor activity in cancer associated with the *BRCA1* or *BRCA2* mutation. (ClinicalTrials.gov number, NCT00516373.)

N ENGL J MED 361;2 NEJM.ORG JULY 9, 2009

123



2

Research Article

Poly(ADP-Ribose) Polymerase-1 Inhibitor Treatment Regresses Autochthonous *Brca2/p53*-Mutant Mammary Tumors *In vivo* and Delays Tumor Relapse in Combination with Carboplatin

Trevor Hay,[1] James R. Matthews,[1] Lucie Pietzka,[1] Alan Lau,[3] Aaron Cranston,[3] Anders O.H. Nygren,[4] Anthony Douglas-Jones,[2] Graeme C.M. Smith,[3] Niall M.B. Martin,[3] Mark O'Connor,[3] and Alan R. Clarke[1]

[1]Department of Genetics, School of Biosciences, and [2]Department of Pathology, Wales College of Medicine, School of Medicine, Cardiff University, Cardiff, United Kingdom; [3]KuDOS Pharmaceuticals Ltd., Cambridge, United Kingdom; and [4]MRC-Holland, Amsterdam, The Netherlands

3

PNAS

High sensitivity of BRCA1-deficient mammary tumors to the PARP inhibitor AZD2281 alone and in combination with platinum drugs

Sven Rottenberg[a], Janneke E. Jaspers[a], Ariena Kersbergen[a], Eline van der Burg[a], Anders O. H. Nygren[b], Serge A. L. Zander[a], Patrick W. B. Derksen[a,1], Michiel de Bruin[a], John Zevenhoven[c], Alan Lau[d], Robert Boulter[d], Aaron Cranston[d], Mark J. O'Connor[d], Niall M. B. Martin[d], Piet Borst[a], and Jos Jonkers[a,2]

[a]Division of Molecular Biology and Centre for Biomedical Genetics and [c]Division of Molecular Genetics, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands; [b]MRC–Holland, Willem Schoutenstraat 6, 1057 DN Amsterdam, The Netherlands; and [d]KuDOS Pharmaceuticals, Ltd., 410 Cambridge Science Park, Milton Road, Cambridge, CB4 0PE, United Kingdom

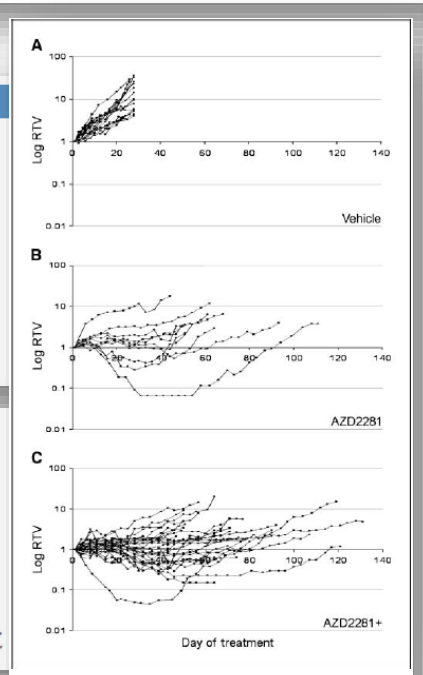
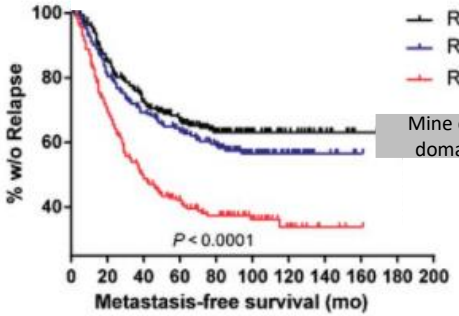


Figure 2. Long-term responses of *Brca2/p53*-deficient tumors from mice treated daily with 50 mg/kg AZD2281. *A*, 28 d of daily vehicle treatment. *B*, 28 d of daily 50 mg/kg AZD2281 treatment. *C*, continuous daily 50 mg/kg AZD2281 treatment. *X axis*, day of treatment; *Y axis*, log RTV; each line represents the response of an individual tumor.

POC Studies to inform the Path to Clinic

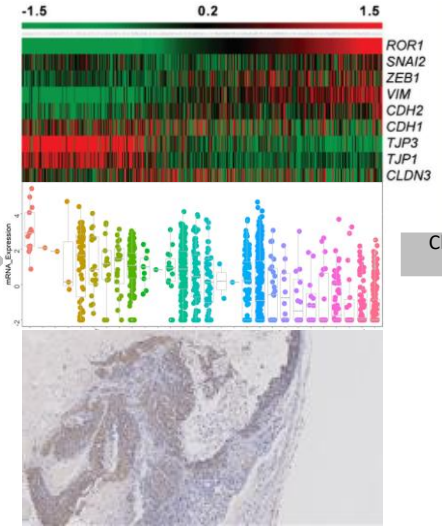
From model short-listing to patient selection

Clinical indication

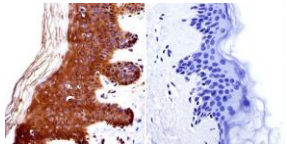


Mine datasets from public domain & multiple CROs

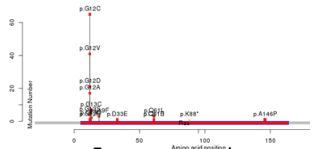
Choose models with range of target expression



Characterise available biobank tissues



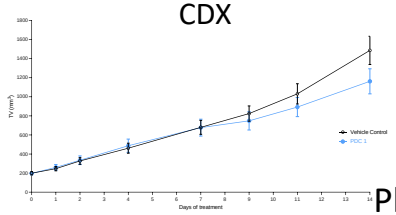
Characterise and perform efficacy studies in shortlisted models



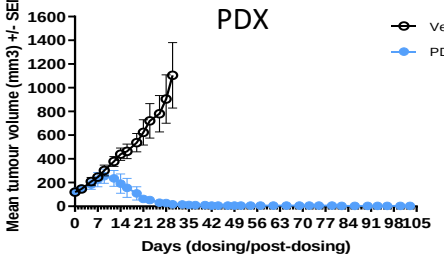
Exome sequencing

Efficacy studies

CDX

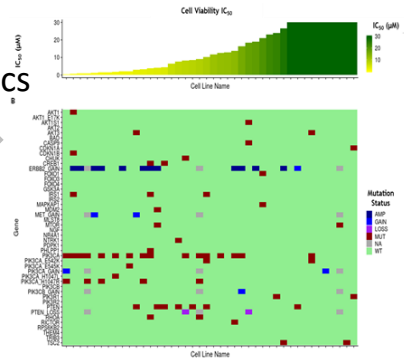


PDX

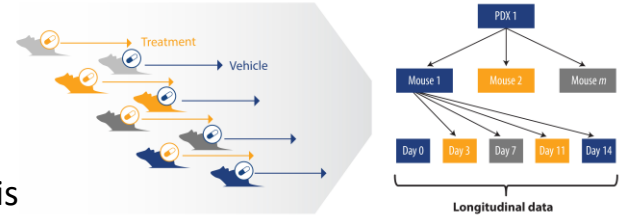


Pharmagenomics

Determinants of sensitivity Biomarker validation



Evaluation in more PDX models

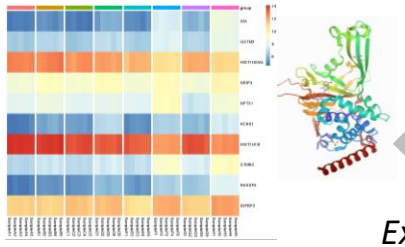


Linear Mixed Models Approach to Mouse Clinical Trial Analysis

CrownBio

Bioinformatic-designed multi-site mouse PDX mini-trial to confirm additional indications and refine patient selection approach

Ex vivo analysis

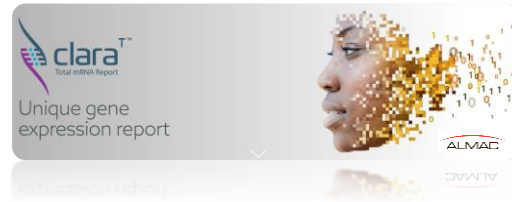


In-depth analysis of PD biomarkers, responder (and non-responder) samples

Confirms target indication

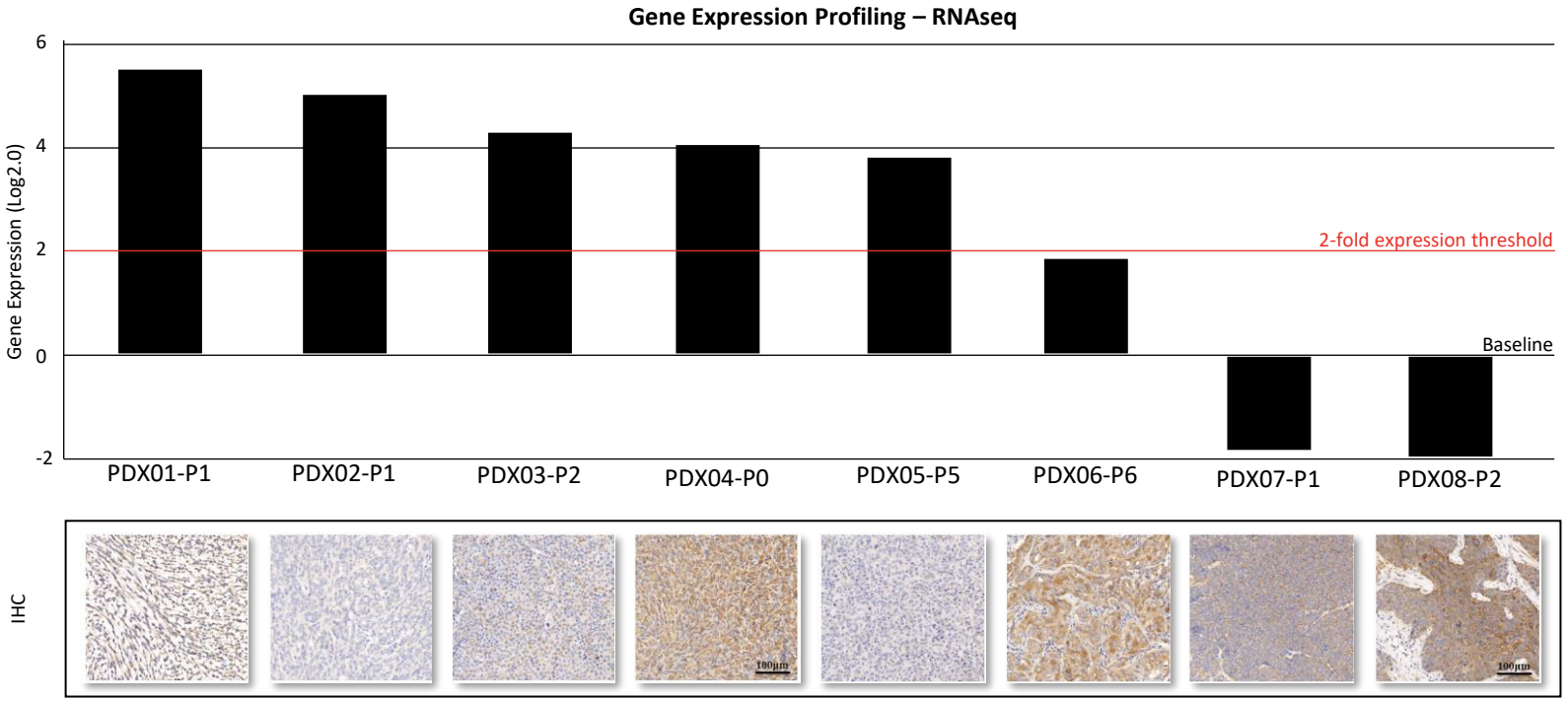
Identifies new indications

Patient selection



RNAseq screening as a gate to select models – a cautionary tale

- Models screened for target expression by RNAseq and ranked according to gene expression score (shown opposite); *data kindly supplied gratis by CRO*
- Confirmatory IHC screen conducted to validate model choice (shown opposite)
- Concordance of model selection approaches by RNASeq and IHC is less than 40%. Raises questions about validity of model selection on a single method



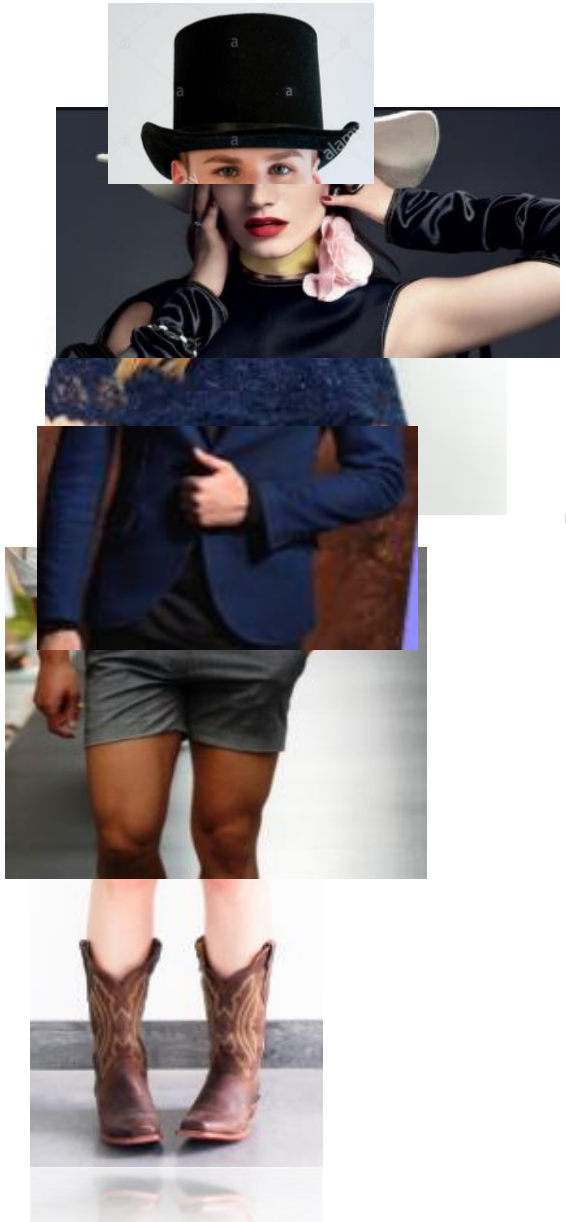
PDX model	RNAseq	IHC
PDX01	✓	✓✓
PDX02	✓	✗
PDX03	✓	✓
PDX04	✓	✓✓
PDX05	✓	✗
PDX06	✗	✓✓✓
PDX07	✗	✓
PDX08	✗	✓✓✓

Data shown is real-world data but PDX model IDs are redacted to protect confidential company intellectual property

RNA-IHC concordance = 37.5%
(*insufficient confidence*)

Could be explained by heterogeneity of PDX/sampling, cross-reactivity of IHC Ab for human and mouse target expression, cross-reactivity with another [unknown] protein or simply that RNA levels do not translate to protein expression

Building a better picture: the right model for the right question



GEMMs

orthotopics

humanised models

Syngeneics

Co-cultures

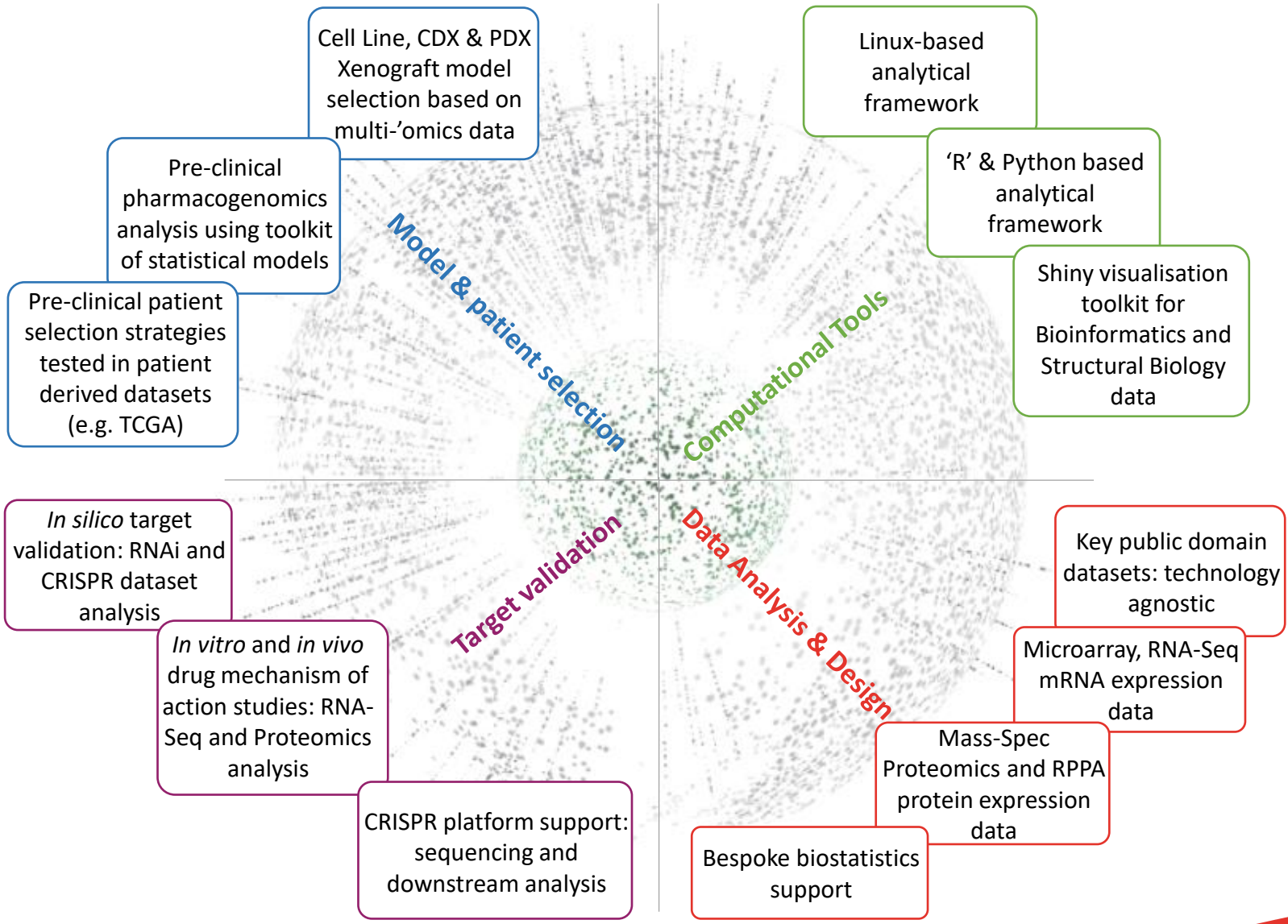
PDX

Organoids/PDXOs

CRISPR

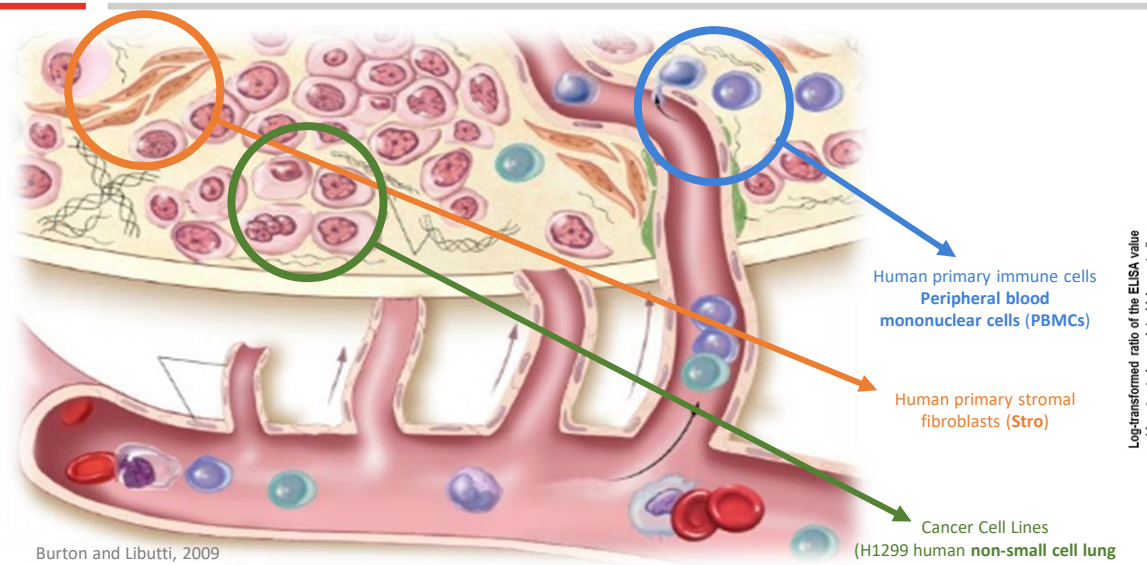
cdx

Systems Biology Toolbox: connecting the dots to translate discoveries to cures

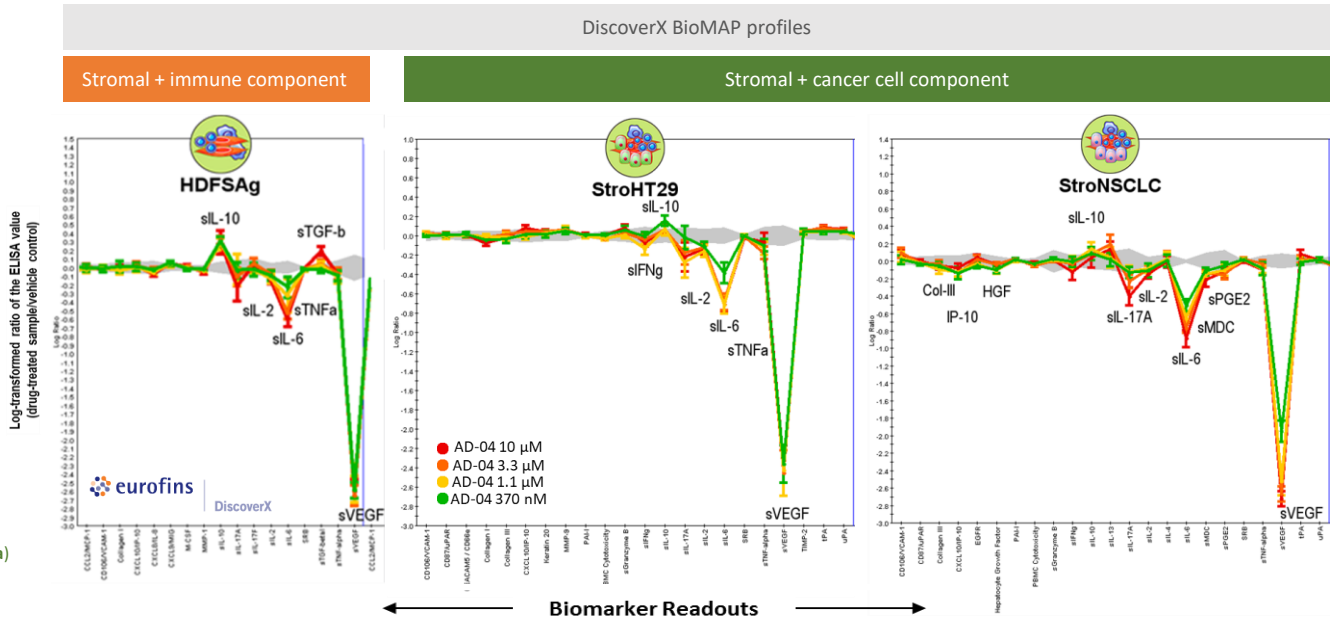


From EMT to TME: exploiting data-driven model selection

Deconstructing the Tumour Microenvironment:

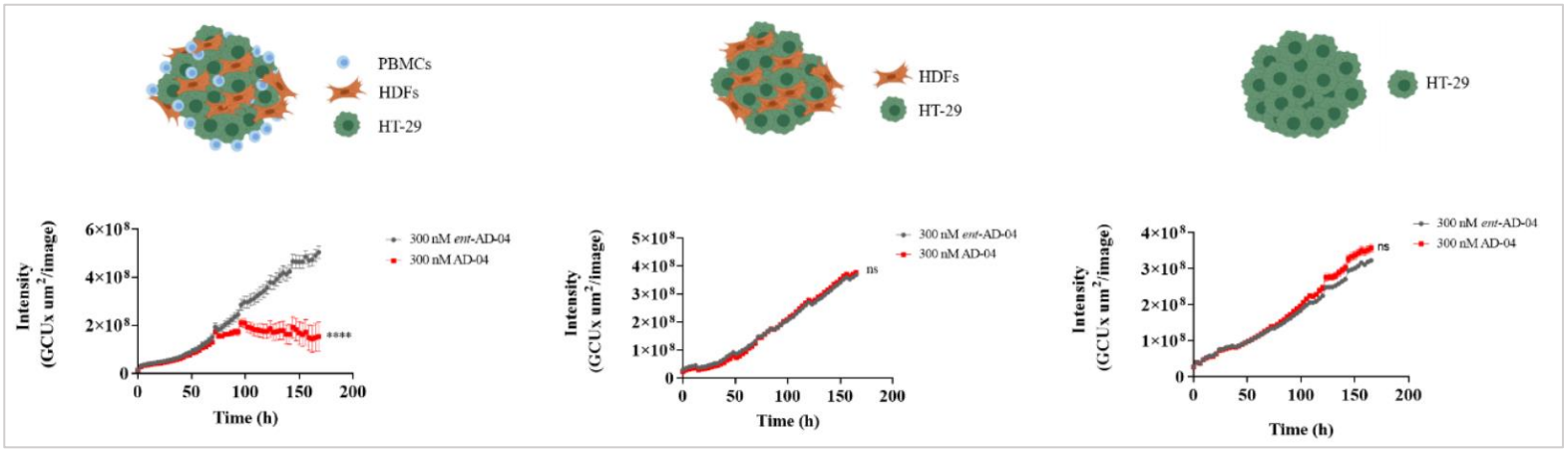


Burton and Libutti, 2009



- Early lead molecule profiled in Eurofins DiscoverX BioMAP co-culture biomarker discovery system
- Inhibitor generated very striking biological marker profiles when evaluated in complex co-culture cellular systems
- Strong modulation of sVEGF observed across dose range in HDF + PBMC and HDF + cancer cell panels
- Suggests biological mechanism is mediated via the stromal cell component

- Validation of the biological MOA was achieved in-house in spheroid [triple] co-culture systems → → →



AD-04 = USP7 inhibitor; *ent*-AD-04 = inactive enantiomer

Reconstructing the Tumour Microenvironment:

TME models:

2D culture options:

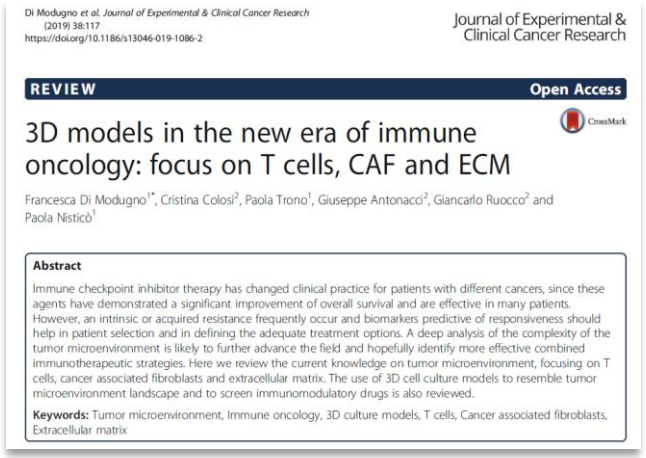
- The use of conditioned medium
- The use of transwell techniques
- Seeding cancer cells on fibroblasts

3D culture options:

- Colony assay in soft agar or Matrigel
- Spheroids
- Organoids, PDXO

In vivo models:

- Syngeneics (limitations: mouse immune system, limited tumours)
- CDX (limitations: homogeneous, drift/selection on plastic, immunocompromised hosts)
- PDX (limitations: immunocompromised hosts, [stromal] tissue drift)
- Humanised PDX (fewer limitations? but expensive)
- GEMMs (limitations: mouse immune system, slow to grow)


Di Modugno et al. Journal of Experimental & Clinical Cancer Research (2019) 38:117
https://doi.org/10.1186/s13046-019-1086-2

Journal of Experimental & Clinical Cancer Research

REVIEW Open Access

3D models in the new era of immune oncology: focus on T cells, CAF and ECM

Francesca Di Modugno[1*], Cristina Colosi[2], Paola Trono[1], Giuseppe Antonacci[2], Giancarlo Ruocco[2] and Paola Nisticò[1]

Abstract

Immune checkpoint inhibitor therapy has changed clinical practice for patients with different cancers, since these agents have demonstrated a significant improvement of overall survival and are effective in many patients. However, an intrinsic or acquired resistance frequently occur and biomarkers predictive of responsiveness should help in patient selection and in defining the adequate treatment options. A deep analysis of the complexity of the tumor microenvironment is likely to further advance the field and hopefully identify more effective combined immunotherapeutic strategies. Here we review the current knowledge on tumor microenvironment, focusing on T cells, cancer associated fibroblasts and extracellular matrix. The use of 3D cell culture models to resemble tumor microenvironment landscape and to screen immunomodulatory drugs is also reviewed.

Keywords: Tumor microenvironment, Immune oncology, 3D culture models, T cells, Cancer associated fibroblasts, Extracellular matrix

3D TME remodelling schemes

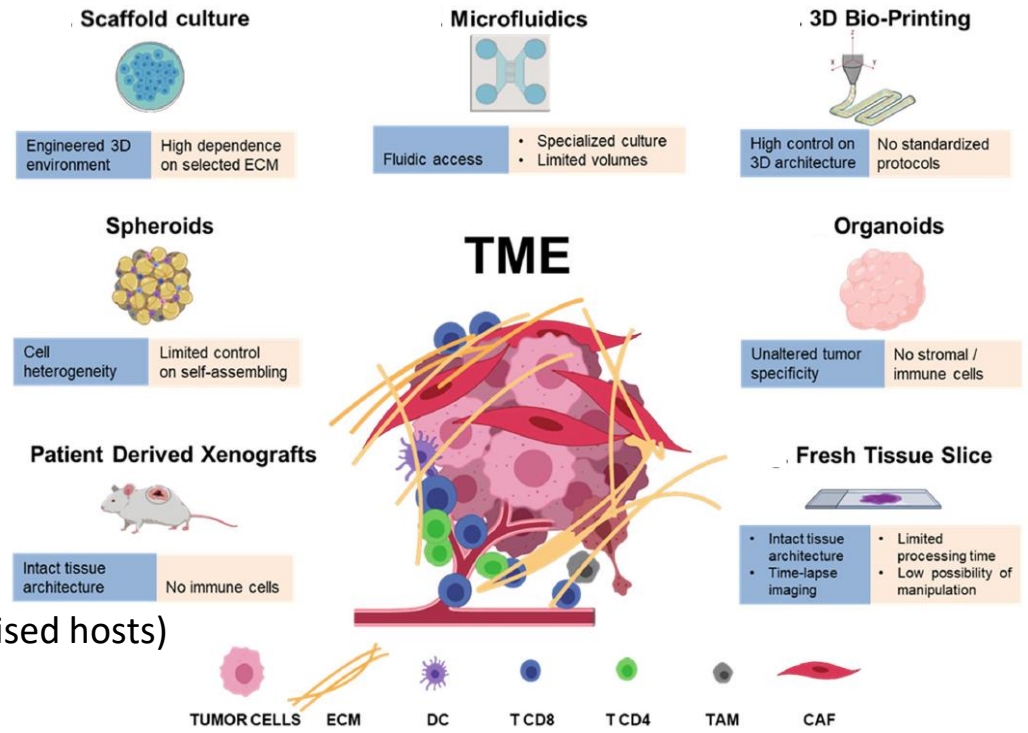


Figure taken from: Di Modugno *et al.*, 2019. J Exp & Clin Can Res 38:117

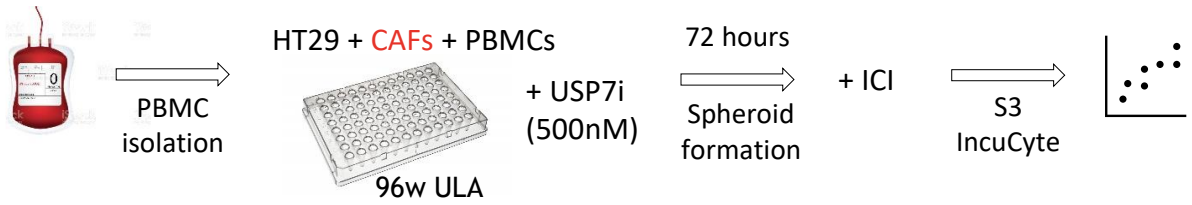
Goals

To develop a 3D high-throughput assay that allows us to investigate the infiltration, activation and function of immune cells, and tumour cell cytotoxicity upon different immune checkpoint inhibitors/compound treatments

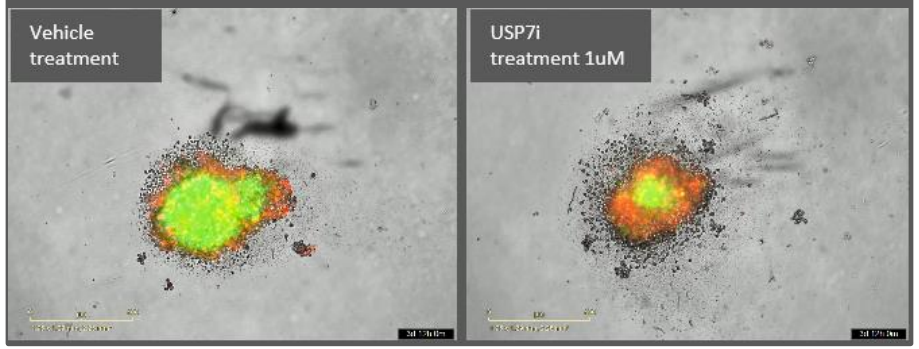
In-house development of tumour immune microenvironment (TIME), using tumour cell lines with or without primary dermal fibroblasts (HDFs) or cancer-associated fibroblasts (CAFs) co-cultured with human immune cells in non-adherent growth conditions

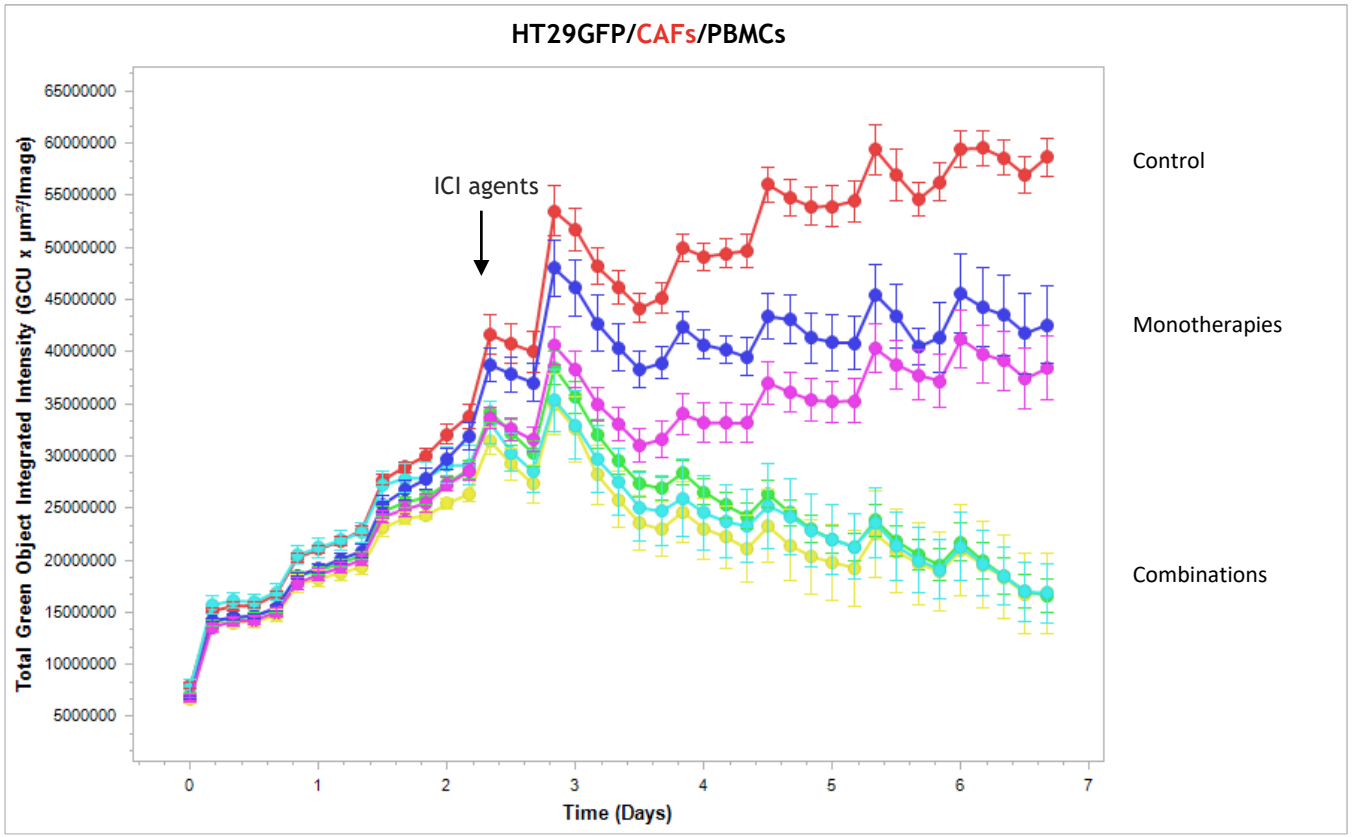
Reconstructing the Tumour Microenvironment (*ex vivo*):

3D Spheroid Tri-culture Assays for Compound Profiling:



- 3D spheroid tri-culture of cancer cells, CAFs and PBMCs
- 96-well plate format; 1 spheroid per well
- Treat with test agents (mono or combo)
- Measure in real time on IncuCyte

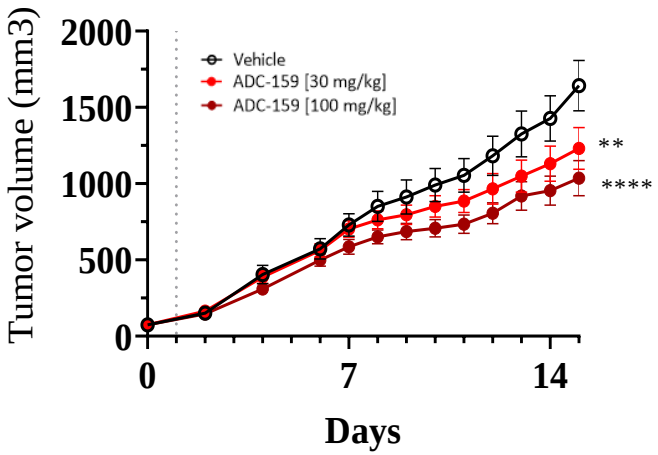


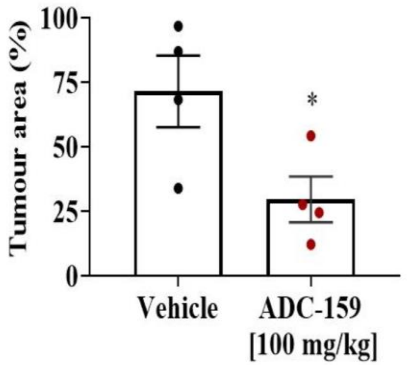


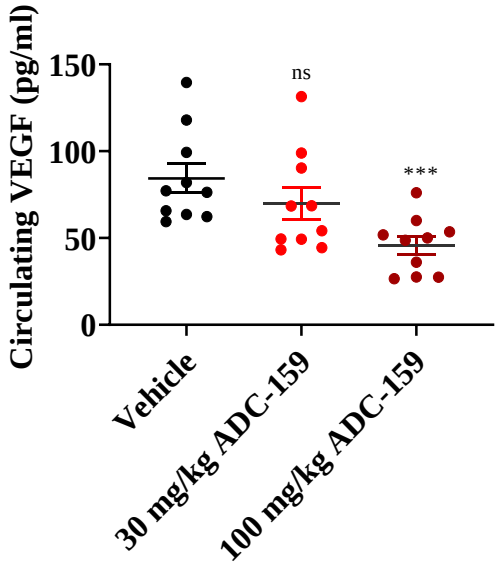
HT29GFP + CAF + PBMCs (freshly isolated PBMCs) + ICI agent(s)

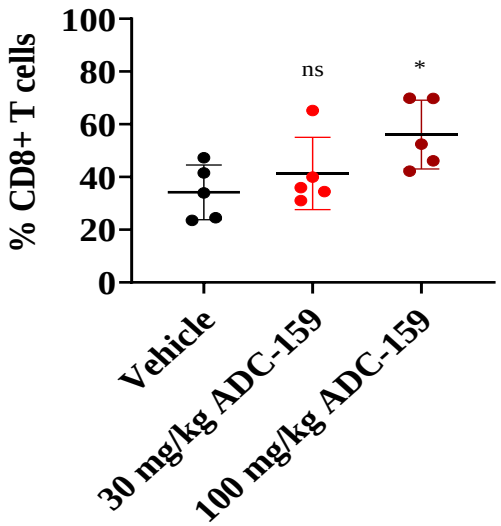
Syngeneics, [serendipity] and systems biology

Helping to connect the dots to translate from discoveries to cures



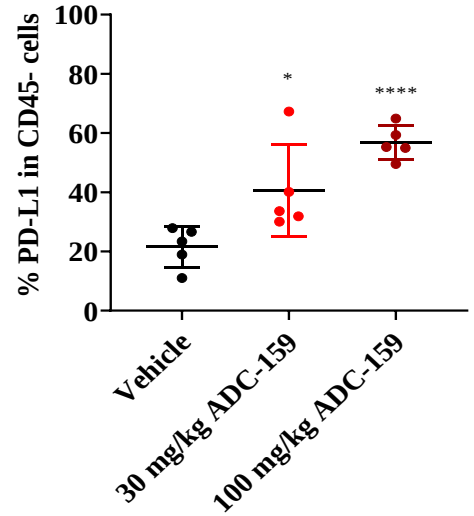


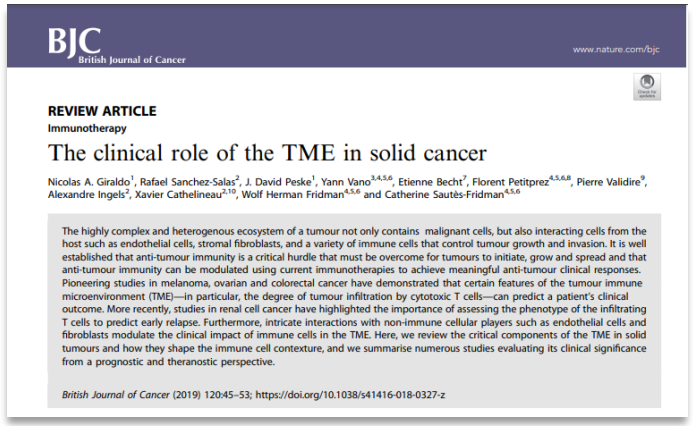




- Treatment of CT26 syngeneic tumours with ADC-159 as a monotherapy in an initial POC study produced statistically, but still only modest effects on tumour growth (by calliper measurement)
- However, analysis of the tumours revealed remodelling of the TME on multiple levels
- Analysis of circulating VEGF revealed a dose-dependent decrease with ADC-159 treatment (as predicted by the DiscoverX co-culture assay)
- FACS analysis of the treated CT26 tumours demonstrated an increase in $CD8^+$ T cells suggesting a modulation of the tumour microenvironment

- Also of note, we observed a dose-dependent increase in the level of PD-L1 on $CD45^-$ cells (endothelial, fibroblasts and cancer cells)




BJC
British Journal of Cancer
www.nature.com/bjc

REVIEW ARTICLE
Immunotherapy

The clinical role of the TME in solid cancer

Nicolas A. Giraldo[1], Rafael Sanchez-Salas[2], J. David Peske[1], Yann Vano[3,4,5,6], Etienne Becht[7], Florent Petitprez[4,5,6,8], Pierre Validire[9], Alexandre Ingels[2], Xavier Cathelineau[2,10], Wolf Herman Fridman[4,5,6] and Catherine Sautès-Fridman[4,5,6]

The highly complex and heterogenous ecosystem of a tumour not only contains malignant cells, but also interacting cells from the host such as endothelial cells, stromal fibroblasts, and a variety of immune cells that control tumour growth and invasion. It is well established that anti-tumour immunity is a critical hurdle that must be overcome for tumours to initiate, grow and spread and that anti-tumour immunity can be modulated using current immunotherapies to achieve meaningful anti-tumour clinical responses. Pioneering studies in melanoma, ovarian and colorectal cancer have demonstrated that certain features of the tumour immune microenvironment (TME)—in particular, the degree of tumour infiltration by cytotoxic T cells—can predict a patient's clinical outcome. More recently, studies in renal cell cancer have highlighted the importance of assessing the phenotype of the infiltrating T cells to predict early relapse. Furthermore, intricate interactions with non-immune cellular players such as endothelial cells and fibroblasts modulate the clinical impact of immune cells in the TME. Here, we review the critical components of the TME in solid tumours and how they shape the immune cell contexture, and we summarise numerous studies evaluating its clinical significance from a prognostic and theranostic perspective.

British Journal of Cancer (2019) 120:45–53; https://doi.org/10.1038/s41416-018-0327-z

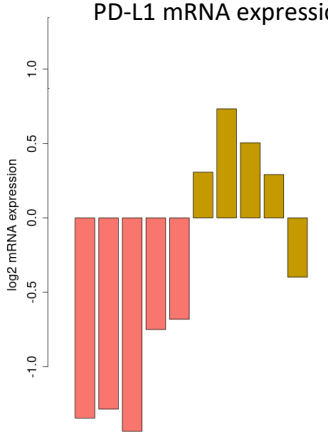
ADC-159 = oral USP7 inhibitor

Syngeneics, [serendipity] and systems biology

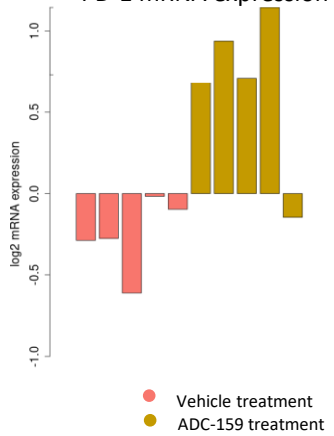
Helping to connect the dots to translate from discoveries to cures

- Analysis of CT26 syngeneic tumours treated with ADC-159 (an orally bioavailable version of AD-04) at the transcriptomics level confirmed the FACS findings
- Immune checkpoint molecules, PD-1 and PD-L1 are transcriptionally upregulated following treatment with ADC-159
- These finding suggest that a immunologically cold tumour could be sensitised to an agent targeting the PD-(L)1 axis
- CT26 tumours were treated with ADC-159, an anti-PD-L1 agent or the two agents in combination and tumour growth followed over time

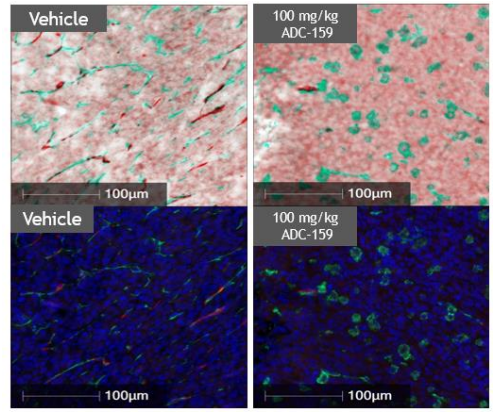
PD-L1 mRNA expression



PD-1 mRNA expression



Tumour vasculature abrogated with ADC-159

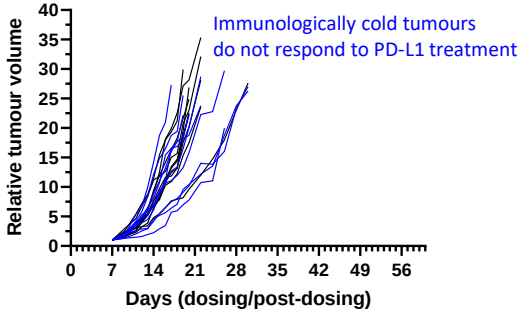


CD31 endothelial vessel marker; NG2 pericyte marker

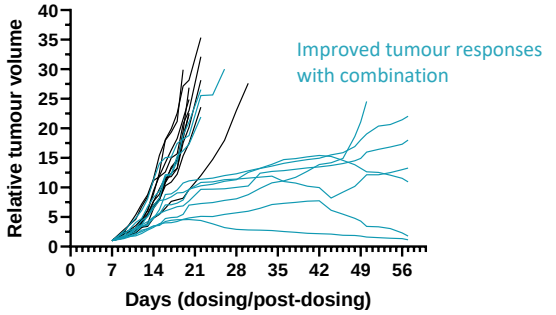
As hypothesised, an increase in PDL-1 expression, infiltrating CD8+ T cells, and reduced VEGF levels resulted in improved responses to an anti PD-L1 agent. Provides *in vivo* POC that treatment with ADC-159 can modulate the tumour microenvironment to turn immunologically cold tumours, warm ↘

→ Confirmation of ↑ in tumour infiltrating cells by *Ultivue* IO multiplex panel *(planned)*

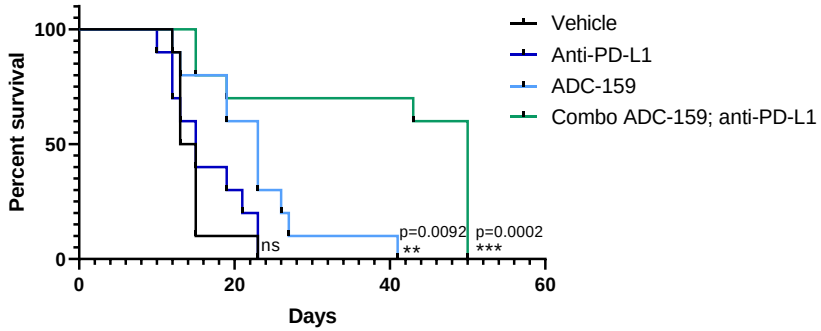
Veh vs Anti-PD-L1 10mg/kg ip BIW



Veh vs ADC-159 75mg/kg po QD; anti-PD-L1 10mg/kg ip BIW

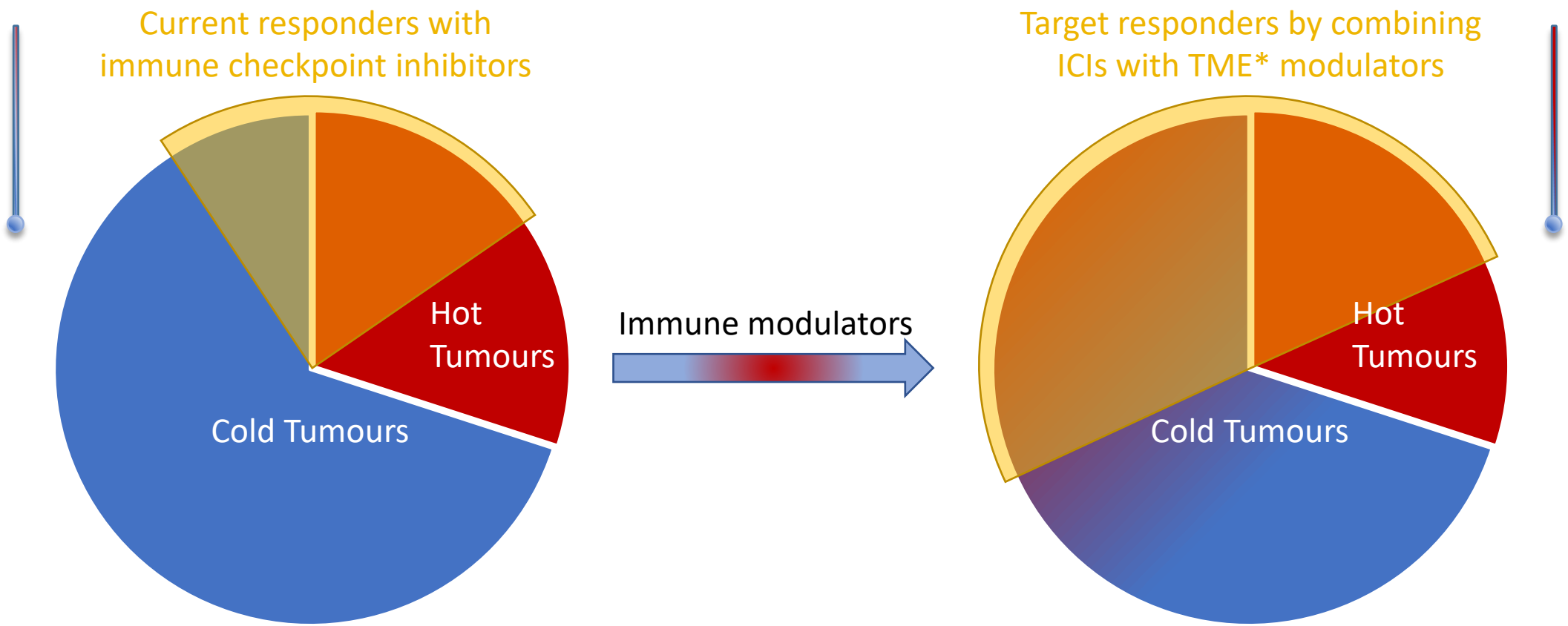


Kaplan-Meier (surrogate) survival



In addition, the combination treatment was very well tolerated with no body weight loss or adverse clinical signs observed over a 7-week dosing period

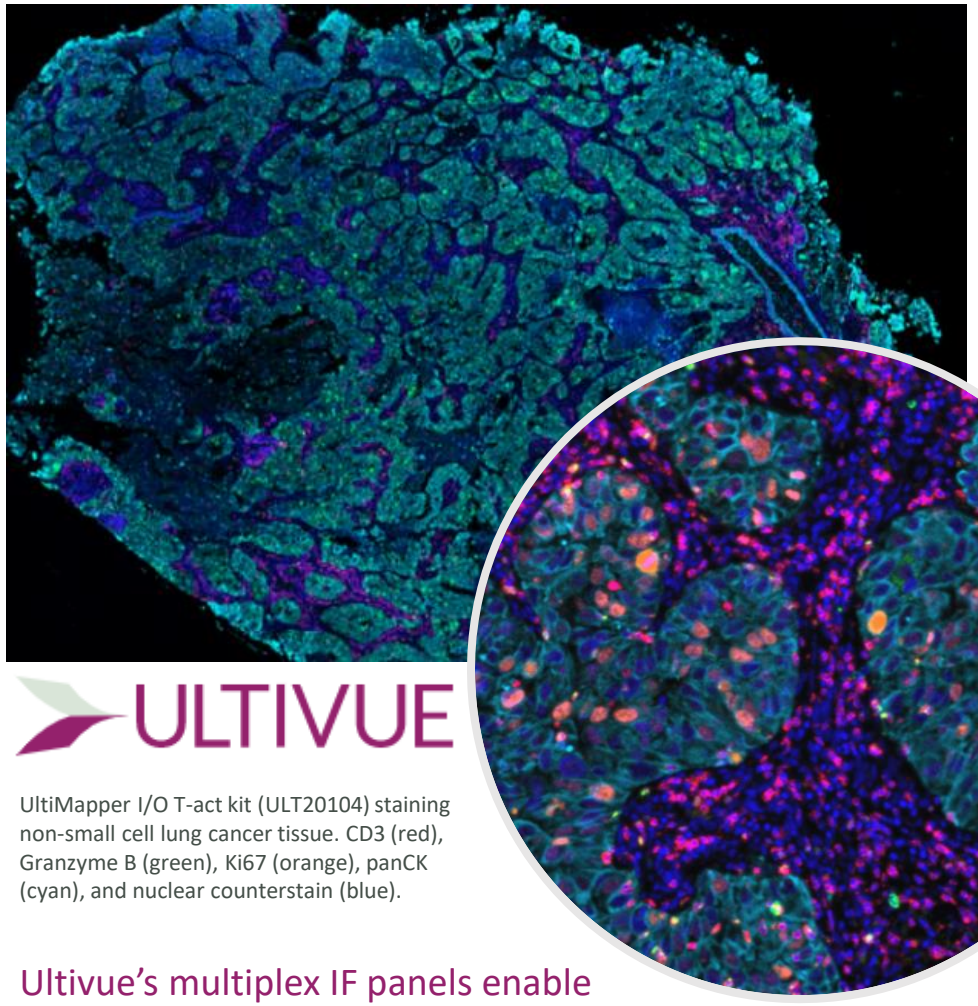
Turning up the Temperature in the Tumour Microenvironment



Combination therapies to improve patient responses

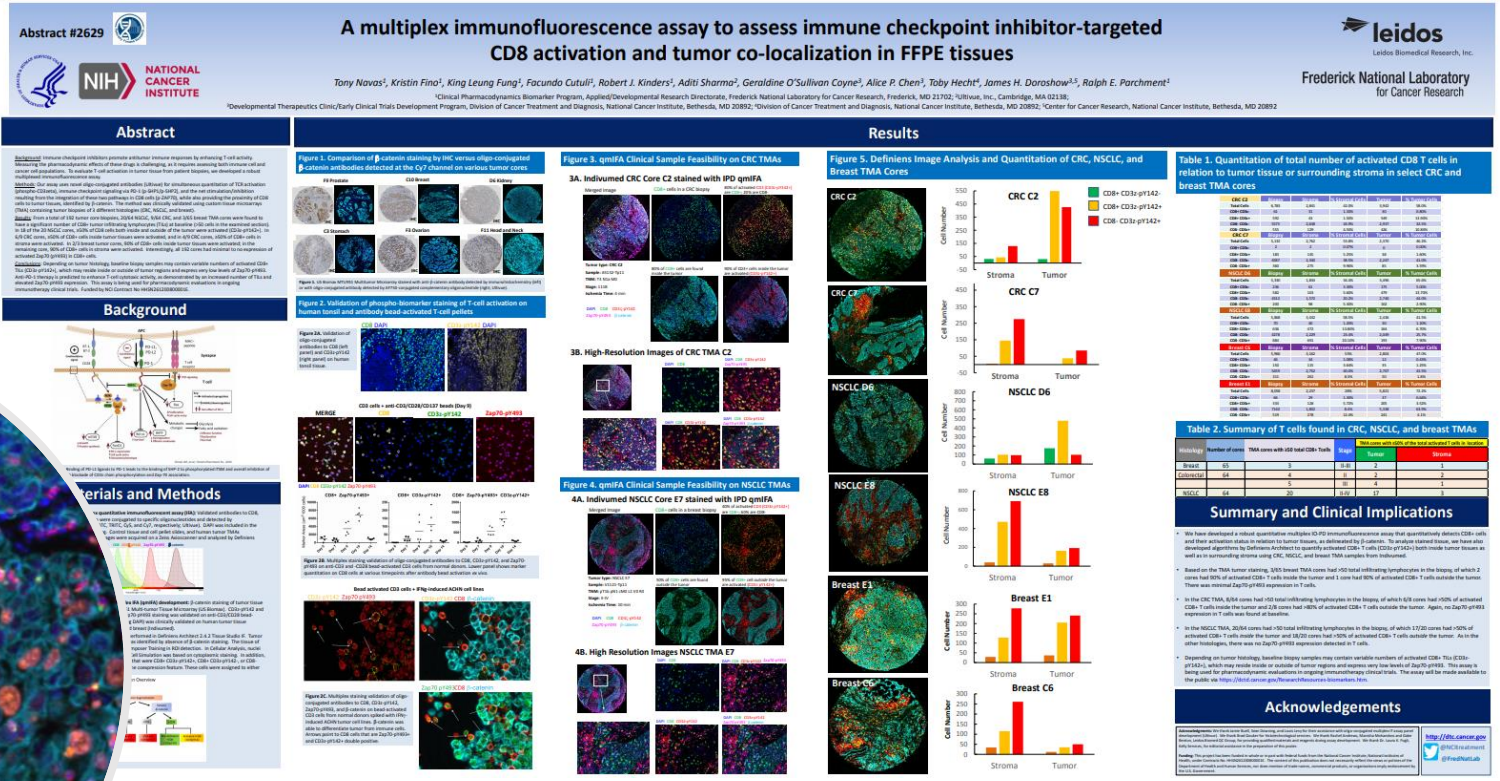
- Unmet need in 2nd line NSCLC: <20% response rate in a PD-L1 unselected population
  (Borghaei *et al.*, 2015 NEJM 373:1627-1639; Nishio *et al.*, 2015, 33:15, 8027)

Turning up the Temperature in the Tumour Immune Microenvironment



ULTIVUE

UltiMapper I/O T-act kit (ULT20104) staining non-small cell lung cancer tissue. CD3 (red), Granzyme B (green), Ki67 (orange), panCK (cyan), and nuclear counterstain (blue).



Abstract #2629

A multiplex immunofluorescence assay to assess immune checkpoint inhibitor-targeted CD8 activation and tumor co-localization in FFPE tissues

Tony Navas[1], Kristin Fino[1], King Leung Fung[1], Facundo Cutuli[2], Robert J. Kinders[2], Aditi Sharma[2], Geraldine O'Sullivan Coyne[3], Alice P. Chen[3], Toby Hecht[4], James H. Doroshow[3,5], Ralph E. Parchment[1]

[1]Clinical Pharmacodynamics Biomarker Program, Applied/Developmental Research Directorate, Frederick National Laboratory for Cancer Research, Frederick, MD 21702; [2]Ultivue, Inc., Cambridge, MA 02138; [3]Developmental Therapeutics Clinic/Early Clinical Trials Development Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Bethesda, MD 20892; [4]Division of Cancer Treatment and Diagnosis, National Cancer Institute, Bethesda, MD 20892; [5]Center for Cancer Research, National Cancer Institute, Bethesda, MD 20892

NIH NATIONAL CANCER INSTITUTE

leidos Leidos Biomedical Research, Inc.

Frederick National Laboratory for Cancer Research

Abstract

Background

Materials and Methods

Results

Figure 1. Comparison of β-catenin staining by IHC versus oligo-conjugated β-catenin antibodies detected at the Cy7 channel on various tumor cores

Figure 2. Validation of phospho-biomarker staining of T-cell activation on human tonsil and antibody bead-activated T-cell pellets

Figure 3. qmIFA Clinical Sample Feasibility on CRC TMAs

3A. Indivumed CRC Core C2 stained with IPD qmIFA

3B. High-Resolution Images of CRC TMA C2

Figure 4. qmIFA Clinical Sample Feasibility on NSCLC TMAs

4A. Indivumed NSCLC Core E7 stained with IPD qmIFA

4B. High Resolution Images NSCLC TMA E7

Figure 5. Definiens Image Analysis and Quantitation of CRC, NSCLC, and Breast TMA Cores

CRC C2

CRC C7

NSCLC D6

NSCLC E8

Breast E1

Breast C6

CD8+ CD3ε-pY142-

CD8+ CD3ε-pY142+

CD8- CD3ε-pY142+

Cell Number

Stroma

Tumor

Table 1. Quantitation of total number of activated CD8 T cells in relation to tumor tissue or surrounding stroma in select CRC and breast TMA cores

Table 2. Summary of T cells found in CRC, NSCLC, and breast TMAs

Histology	Number of cores	TMA cores with ≥50 total CD8+ T cells	Breast	TMA cores with ≥50% of the total activated T cells in location	
				Tumor	Stroma
Breast	65	3	8-9	2	1
Colorectal	64	4	11	2	2
		5	9	4	1
NSCLC	64	20	8-9	17	3

Summary and Clinical Implications

- We have developed a robust quantitative multiplex IO PD immunofluorescence assay that quantitatively detects CD8+ cells and their activation status in relation to tumor tissues, as delineated by β-catenin. To analyze stained tissue, we have also developed algorithms by Definiens Architect to quantify activated CD8+ T cells (CD3ε-pY142+) both inside tumor tissues as well as in surrounding stroma using CRC, NSCLC, and breast TMA samples from Indivumed.
- Based on the TMA tumor staining, 3/65 breast TMA cores had >50 total infiltrating lymphocytes in the biopsy, of which 2 cores had 90% of activated CD8+ T cells inside the tumor and 1 core had 90% of activated CD8+ T cells outside the tumor. There was minimal Zap70-pY493 expression in T cells.
- In the CRC TMA, 8/64 cores had >50 total infiltrating lymphocytes in the biopsy, of which 6/8 cores had >50% of activated CD8+ T cells inside the tumor and 2/8 cores had >80% of activated CD8+ T cells outside the tumor. Again, no Zap70-pY493 expression in T cells was found at baseline.
- In the NSCLC TMA, 20/64 cores had >50 total infiltrating lymphocytes in the biopsy, of which 17/20 cores had >50% of activated CD8+ T cells inside the tumor and 18/20 cores had >50% of activated CD8+ T cells outside the tumor. As in the other histologies, there was no Zap70-pY493 expression detected in T cells.
- Depending on tumor histology, baseline biopsy samples may contain variable numbers of activated CD8+ TILs (CD3ε-pY142+), which may reside inside or outside of tumor regions and express very low levels of Zap70-pY493. This assay is being used for pharmacodynamic evaluations in ongoing immunotherapy clinical trials. The assay will be made available to the public via https://dctd.cancer.gov/ResearchResources/biomarkers.htm.

Acknowledgements

This project has been funded in whole or in part with Federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. HHSN261200800001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

http://dtc.cancer.gov

@NCItreatment

@FredNatLab

The antibody panel in the UltiMapper I/O T-act kit enables users to identify activated T cells. CD3 is a marker of T cells. Granzyme B is produced, stored, and released by cytotoxic T cells to mediate cellular death in combination with perforin. Ki67 is a nuclear protein that is a marker of proliferation. Co-expression of CD3, Granzyme B, and Ki67 indicates proliferating cytotoxic T cells. SOX10 is a tumour marker for melanomas while panCK detects carcinomas (provided in a cocktail).

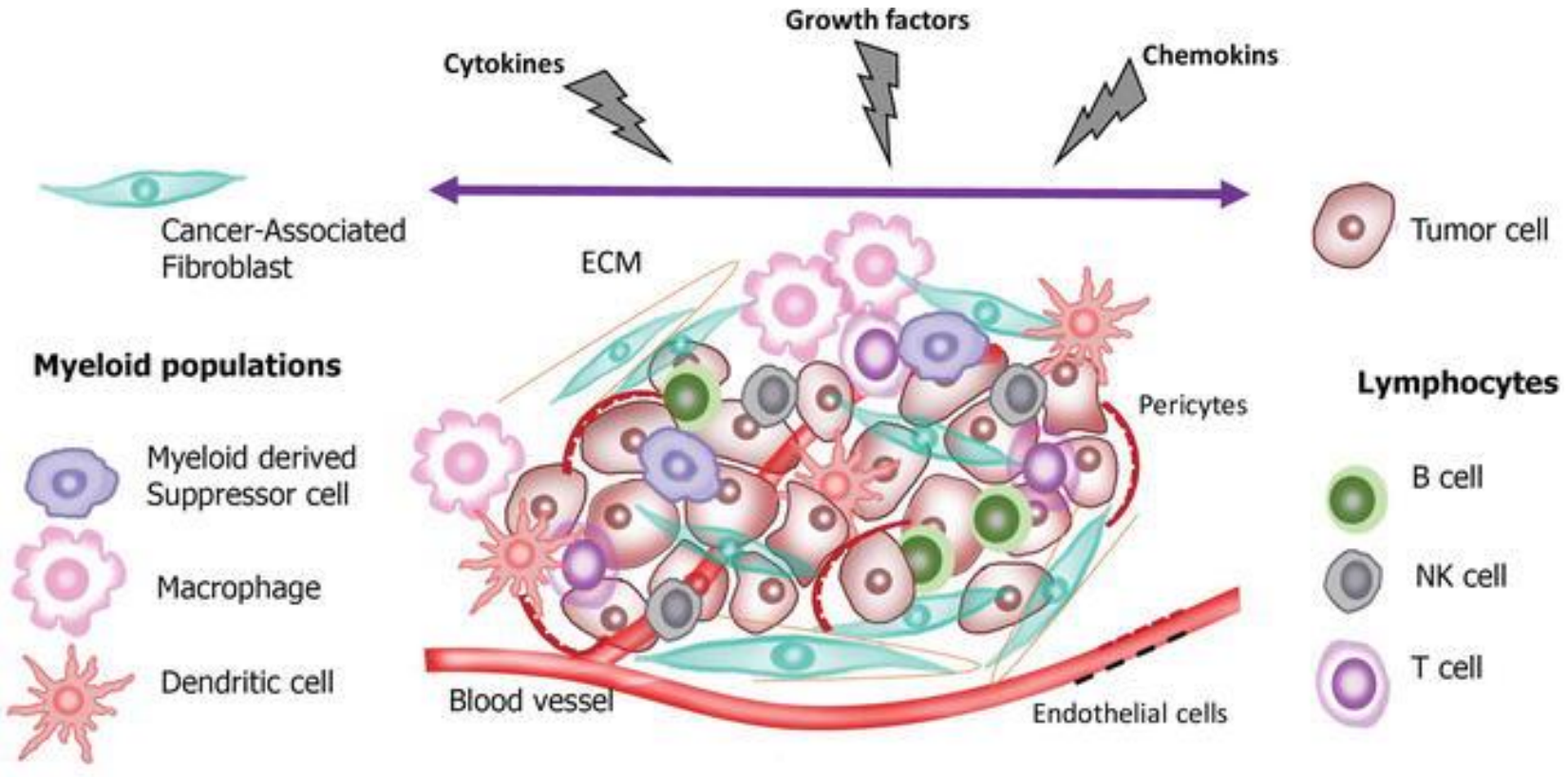
Poster available on ULTIVUE website www.ultivue.com
(Images used with permission)

Ultivue's multiplex IF panels enable simultaneous detection of multiple cell types involved in immune and TME biology

Offers an innovative and powerful approach for visualising and quantitating TIME modulation in preclinical (and clinical) IO studies

Novel Target Rationale

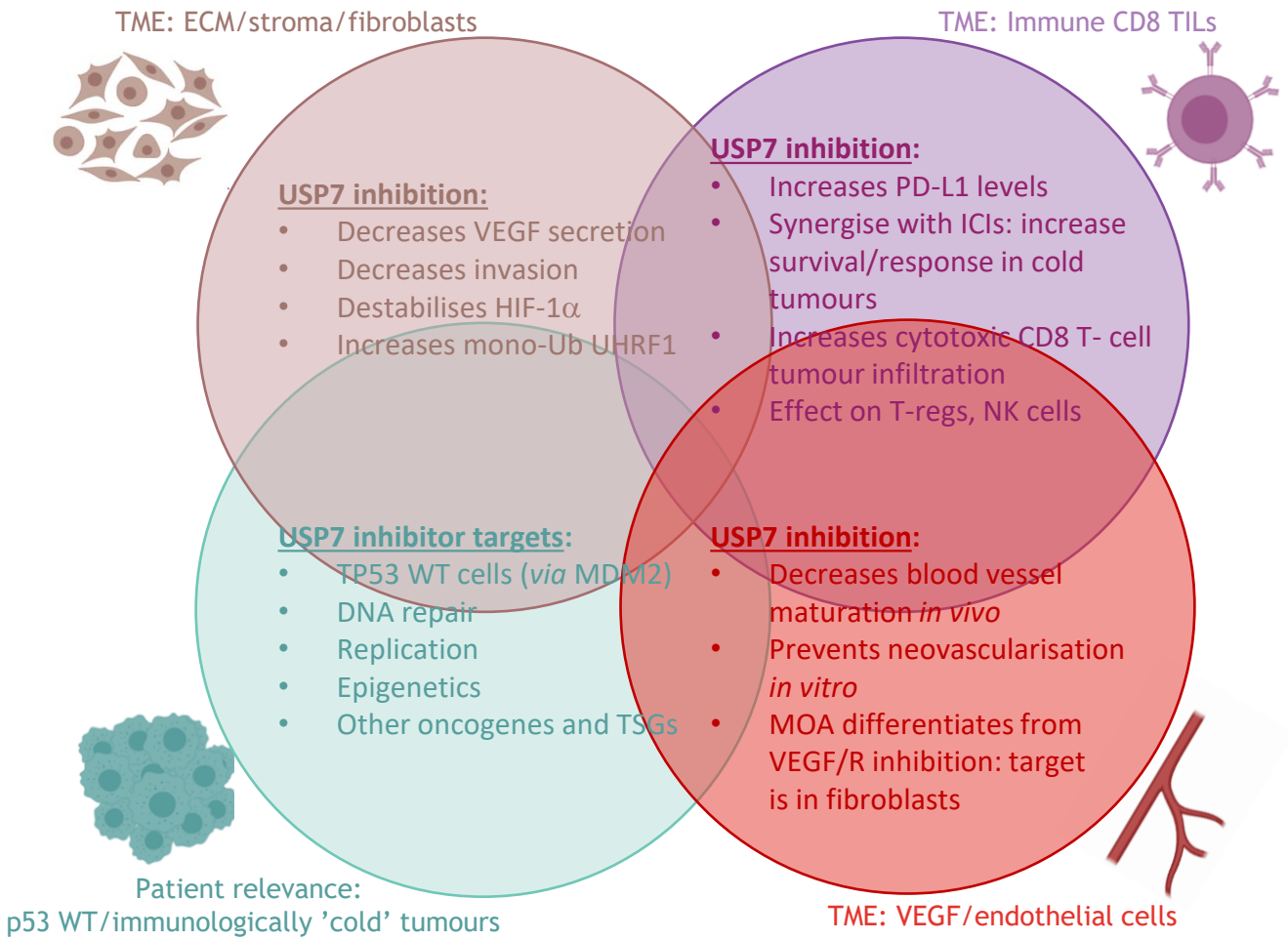
Summary: Reprogramming the Tumour Microenvironment (TME)



How do we convert the complexity of the tumour microenvironment into actionable/druggable biology?

Novel Target Rationale

Summary: Reprogramming the Tumour Microenvironment (TME)



USP7 = ubiquitin specific protease 7

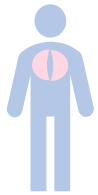
Looking Ahead…

Building a Tumour Microenvironment (TME) model for POC studies

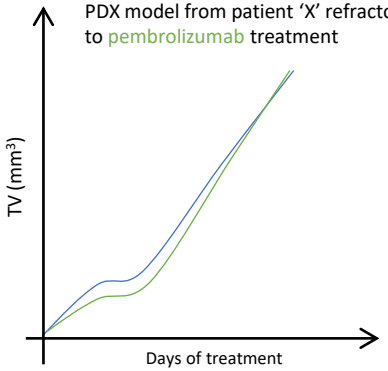
Models at CROs short-listed based on:

- Ability of CRO to perform humanisation esp. CD34 human umbilical cord blood
- No response to ICI (potential to potentiate)
- p53 activity score (in-house RNAseq 14-gene signature)
- Cold TME/low immune cell infiltrates (potential to turn cold tumours warm)

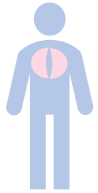
CRO ‘A’



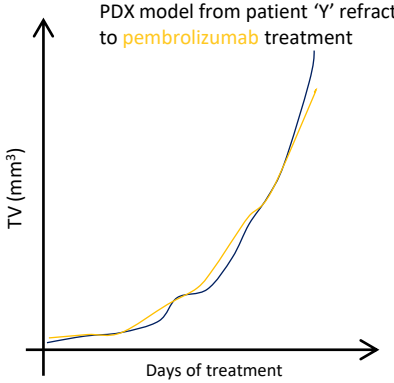
Male patient ‘X’
NSCLC
Progressed on pembrolizumab



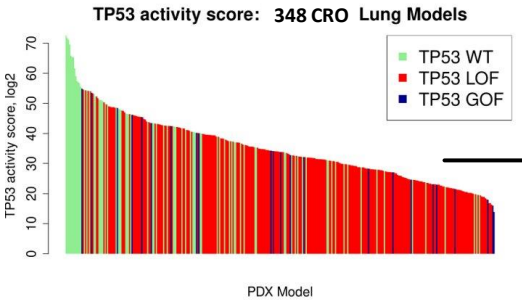
CRO ‘B’

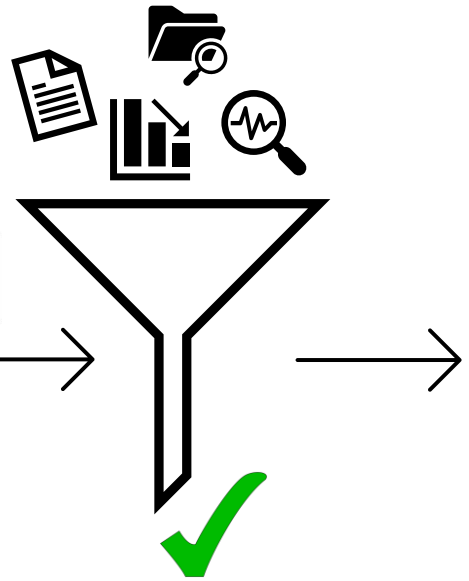


Patient ‘Y’
SCLC



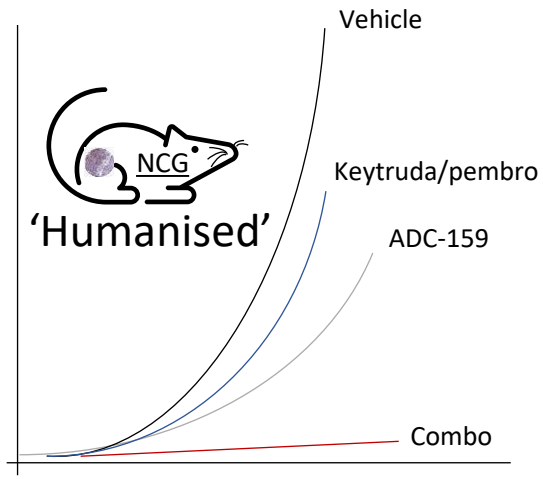
RNAseq 14-gene activity score signature





PDX model(s) selected

Expectations:



*Model information and response data for illustration only; used with permission but redrawn to protect confidentiality

Looking further ahead…

ELSEVIER

New Horizons in Translational Medicine

Volume 2, Issue 1, September 2014, Pages 1-4

Research Articles

Systems Patientomics: The virtual *in-silico* patient

D.V. Dimitrov

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https://doi.org/10.1016/j.nhtm.2014.08.002 Get rights and content

- We will undoubtedly continue to see improvements in host strains and humanisation approaches
- Datasets for 'hot' areas e.g. 2nd/3rd gen ADCs, CAR-T, IO cold/hot will continue to grow and inform predictability/utility of models
- There is a need for reverse-translation from clinical trials to better understand failings (*but appetite among biotechs & pharma companies is generally low*) - see slide S3 for example of where clinical success is informing preclinical models for IBD
- Likely to see a step-change in computational and systems biology (e.g. AI/ML) approaches and application to *in silico* patient avatar models

Acknowledgements

Ubi-Plex® DUB Drug Discovery Platform
Xavier Jacq Ph.D. (VP – Biology)
Anamarija Jurisic Ph.D.
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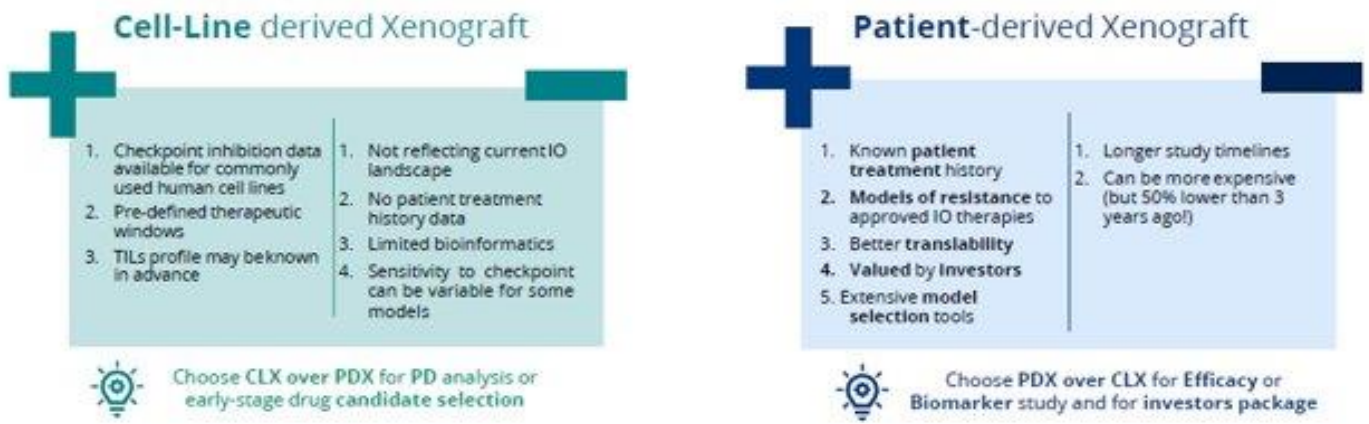
PREDICTINNOVATION: Leverage Novel Preclinical Oncology Approaches to Guide Clinical Success

In Partnership With CrownBio

Supplemental

S1. What have I learnt…?

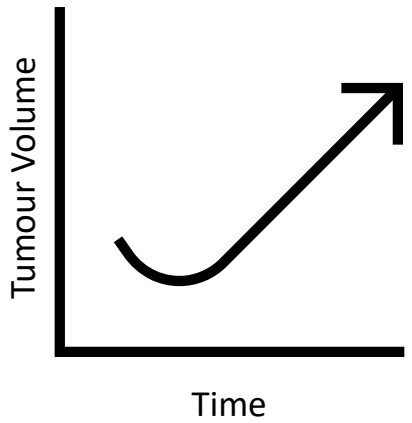
- Important to cross-validate models and/or approaches – take the time to do this! It seems obvious, but presence of target and disease biology is important
- Challenge your assumptions (challenge/stress-test published data)
- Work with the CRO to interrogate and understand the model – don't be afraid to ask!
- Perform power analyses upfront; ask CRO for historical data to help with this
- In our biotech world, we are mostly interested in demonstrating *in vivo* proof-of-concept effects in a couple of models (foothold/gain confidence in molecule/target biology → POC/hypothesis testing)
- Typically, on any given project, these models are complementary (each with their pros & cons):
  - High vs low target expression
  - Simple vs complex (syngeneic vs humanised PDX)
  - Overexpression vs KO
- Models most often used for:
  - Demonstrating efficacy
  - Modulation of target/MOA/downstream biological effects
  - Some toxicology readouts
- When possible, build a package of efficacy data in different models including different indications &/or types of model as this builds confidence (although not necessarily predictability)
- Just as 'one swallow does not make a summer', one good xenograft result does not make a successful clinical trial



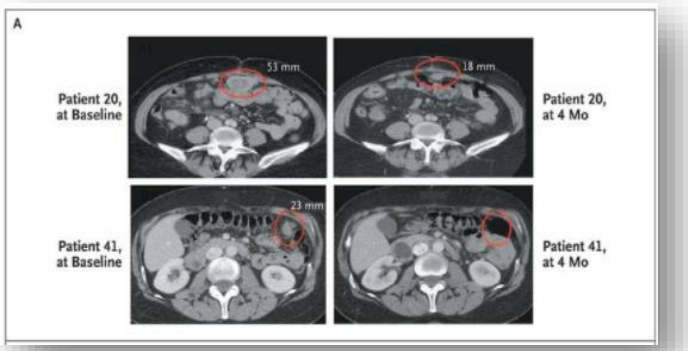
CHAMPIONS ONCOLOGY Xenograft 'pros & cons' taken from an IO presentation by Alex Moreau Ph.D., Champions Oncology Inc.
https://www.brighttalk.com/webcast/18129/444843?utm_source=Champions+Oncology&utm_medium=brighttalk&utm_campaign=444843

S2. Evaluating efficacy - Interpreting results, building confidence

- Preclinically, anti-tumour effects are often reported as changes in tumour volume over time (relative TV, absolute TV, mean, median, individuals, T/C, %TGI, mRECIST)
- Lack of standardisation/harmonisation between groups (although most published M&M are reasonably clear enabling some comparisons)
- Many published papers cite %TGI as the sole readout and it is often calculated on a day when results look best or on the day when the vehicle group is terminated; need for unbiased analyses/reporting (present best effect, biggest difference, best models)
- Some groups have tried to align tumour volume data from mouse xenograft studies with clinical RECIST* criteria. Tumour shrinkage and time to disease progression are important readouts to align with clinical parameters, objective response, time to event (progression), survival (*note: measurements e.g. 5mm increase over baseline = progression, and inclusion of lymph node assessment is technically limited in mouse studies*)
- Justice *et al.*, 2014 and Gao *et al.*, 2015 have presented the case for harmonising the criteria, analyses and reporting of mouse efficacy data (Best Average Response)
- One positive xenograft study ≠ a positive clinical trial result! A bank of positive data in different models provides increasing confidence – maybe even some predictability
- Given the complexity and many different cancers/patient disease state, there is unlikely to be a single 'best' model or approach – use the model that best replicates the disease, mechanism or target to build confidence



*RECIST = **R**esponse **E**valuation **C**riteria **I**n **S**olid **T**umours



Ultimately though, species differences and heterogeneity of many patients in a clinical trial with different disease biology, subtypes and stages of disease are most likely to drive the major contribution to the differences observed between clinical and non-clinical results

S3. Preclinical Models of IBD – Clinical success informing preclinical models

- In the field of Inflammatory Bowel Disease (IBD), tremendous progress has been achieved with the approval of anti-TNFs, integrin blockers, JAK inhibitors, anti-IL-12/23 agents for treating patients, with S1P antagonists potentially following soon
- However, the success in patients has actually highlighted the flaws in the predictability of preclinical models
- None of the approved treatments are efficacious in all of the rodent models of IBD
- In fact, many of the approved biologic treatments work only in one of the many rodent models
- The choice of the rodent model can avoid false negatives and add predictive value for preclinical drug discovery projects
- It seems obvious, but choice of model should represent the disease biology