charles river

Research models and target identification in oncology

Julia Schüler

01 Intro drug development process

02 Available in vivo models

03 Example study PDX

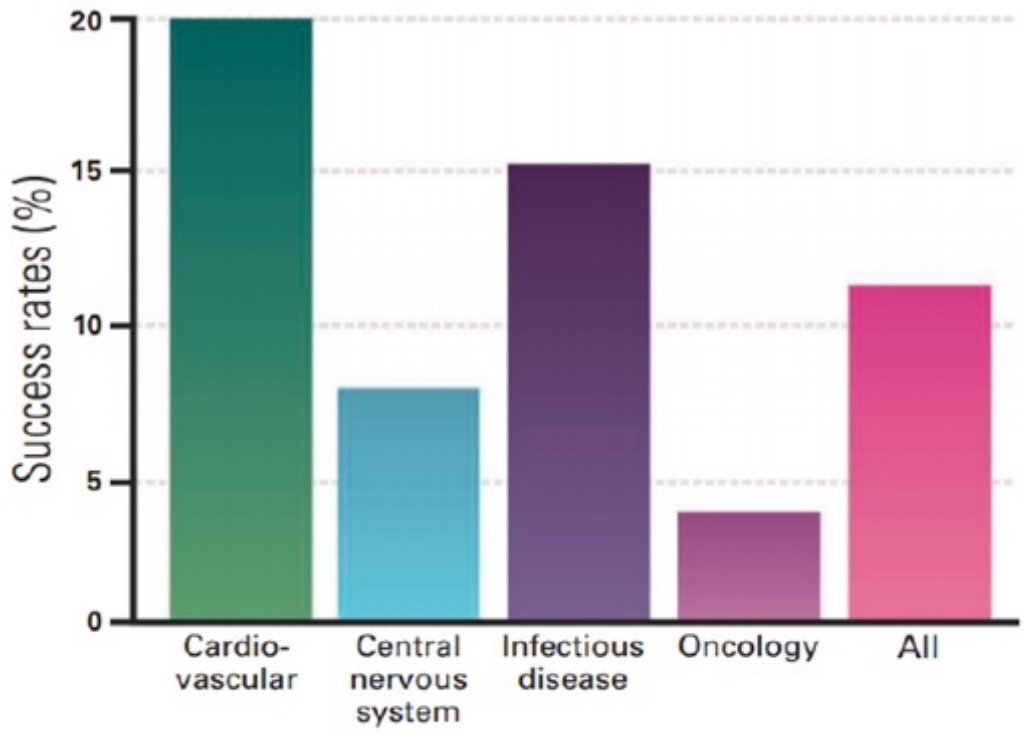
04 Example study mouse tumor model

charles river

charles river

Intro drug development process

Conversion rate in oncology

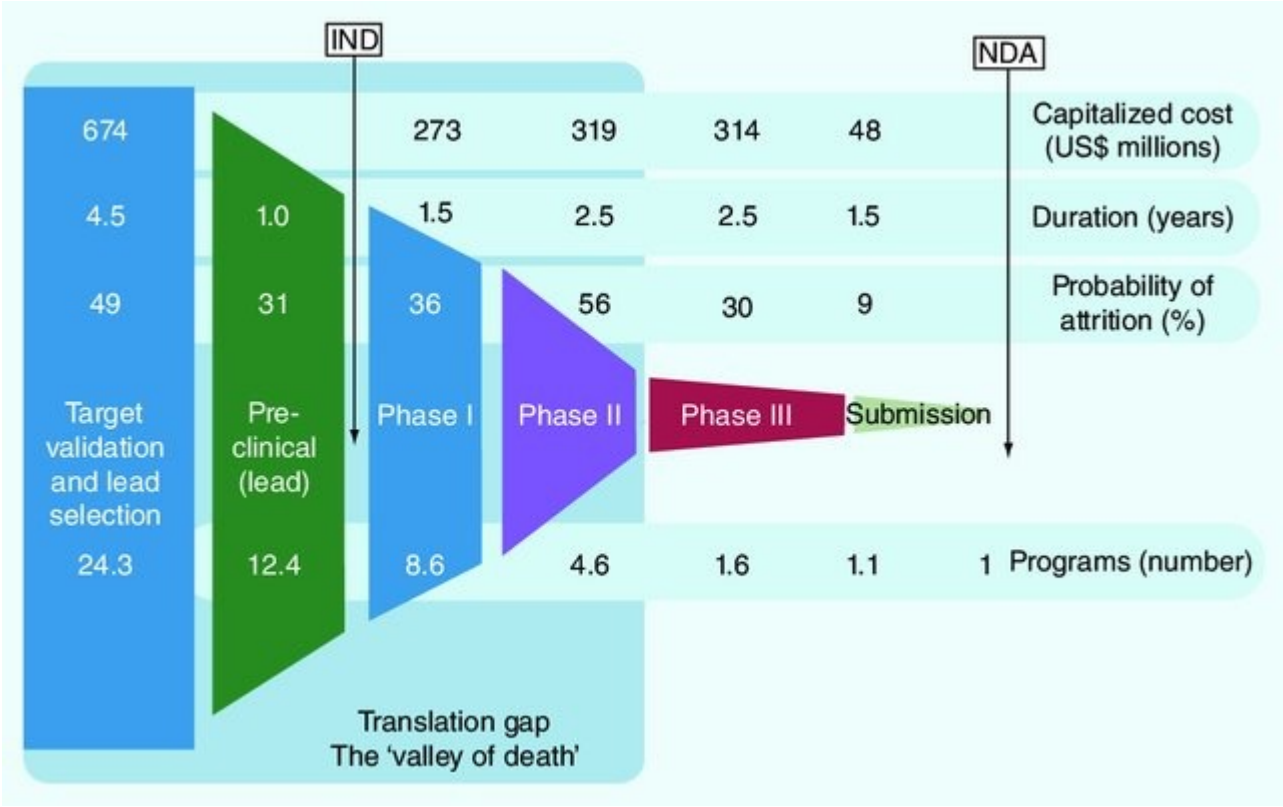


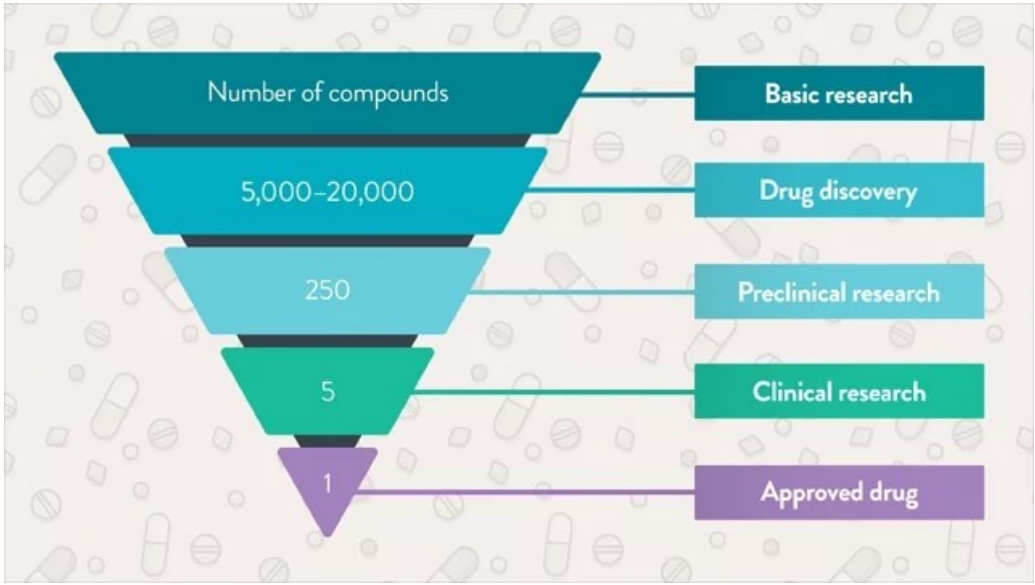
adapted from: Bhattacharjee Y (2012) Biomedicine. Science 338: 29

charles river

Drug development process

R&D efficiency and effectiveness in oncology



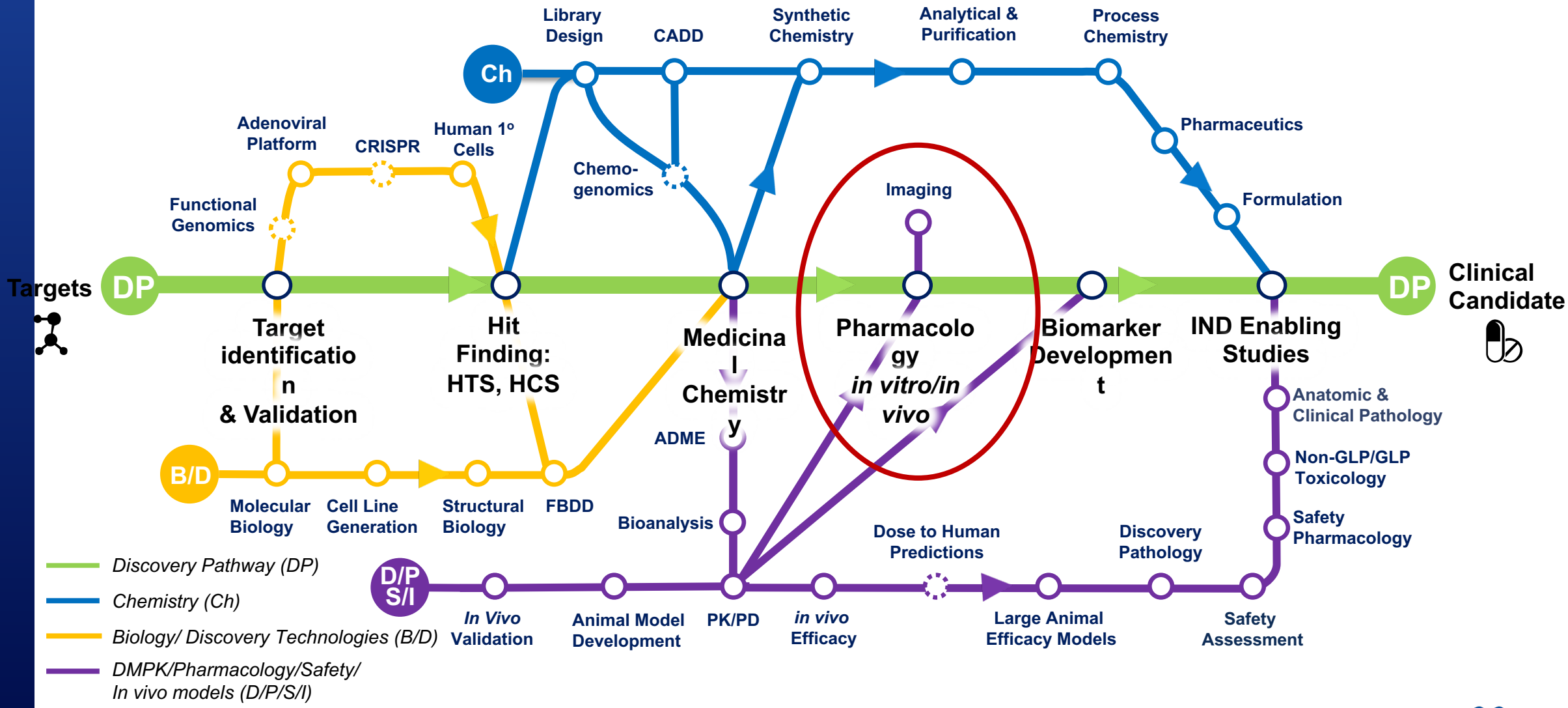


*Zurdo J et at al, pharmaceutical Bioprocessing, 2013

*Lansdowne LE et at al, https://www.technologynetworks.com/ ; 2020

charles river

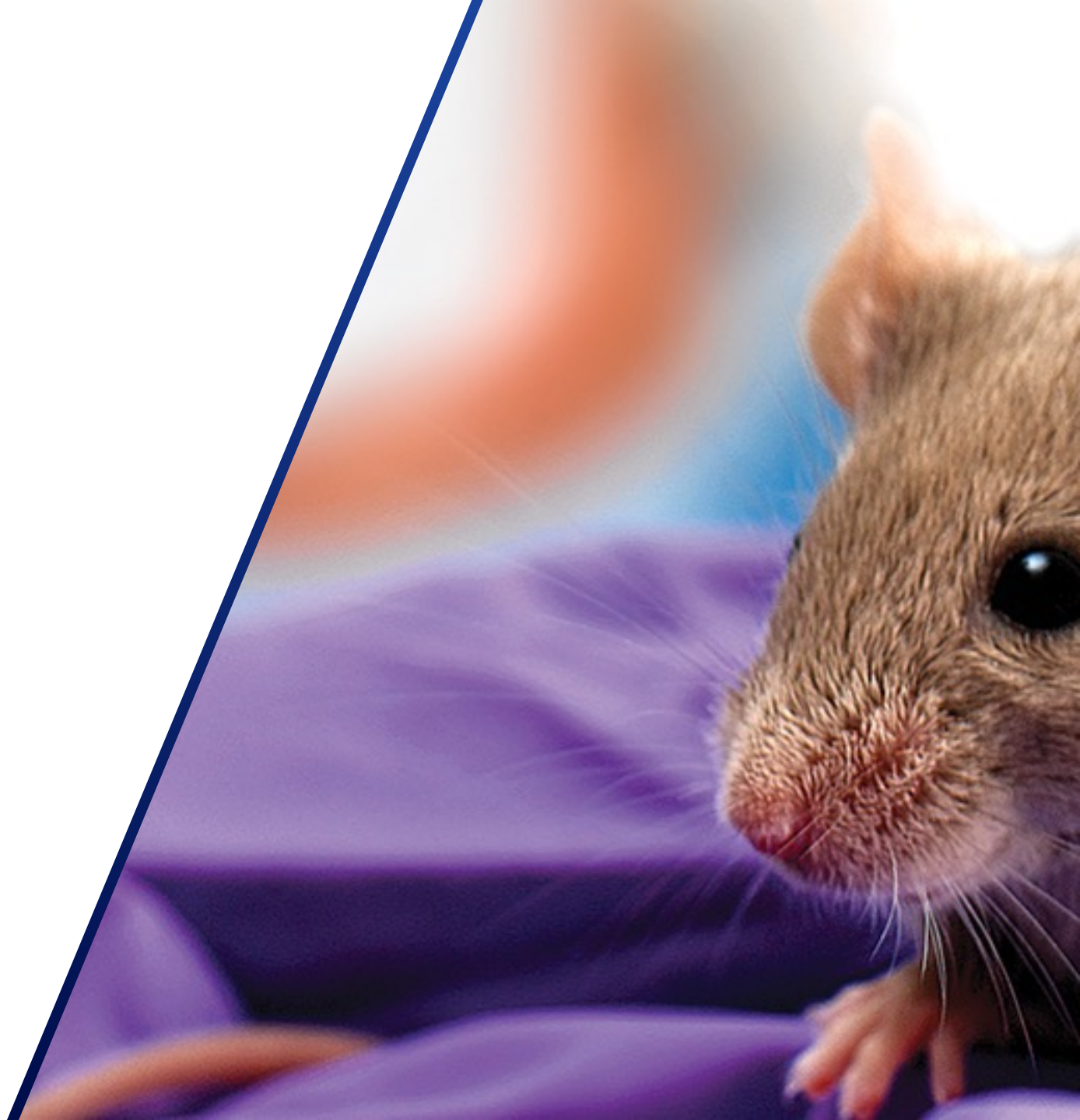
Drug development process



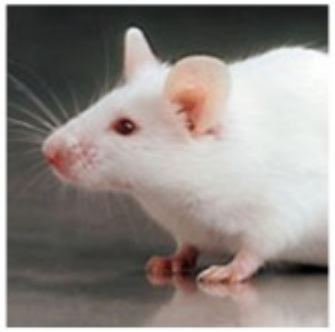
charles river

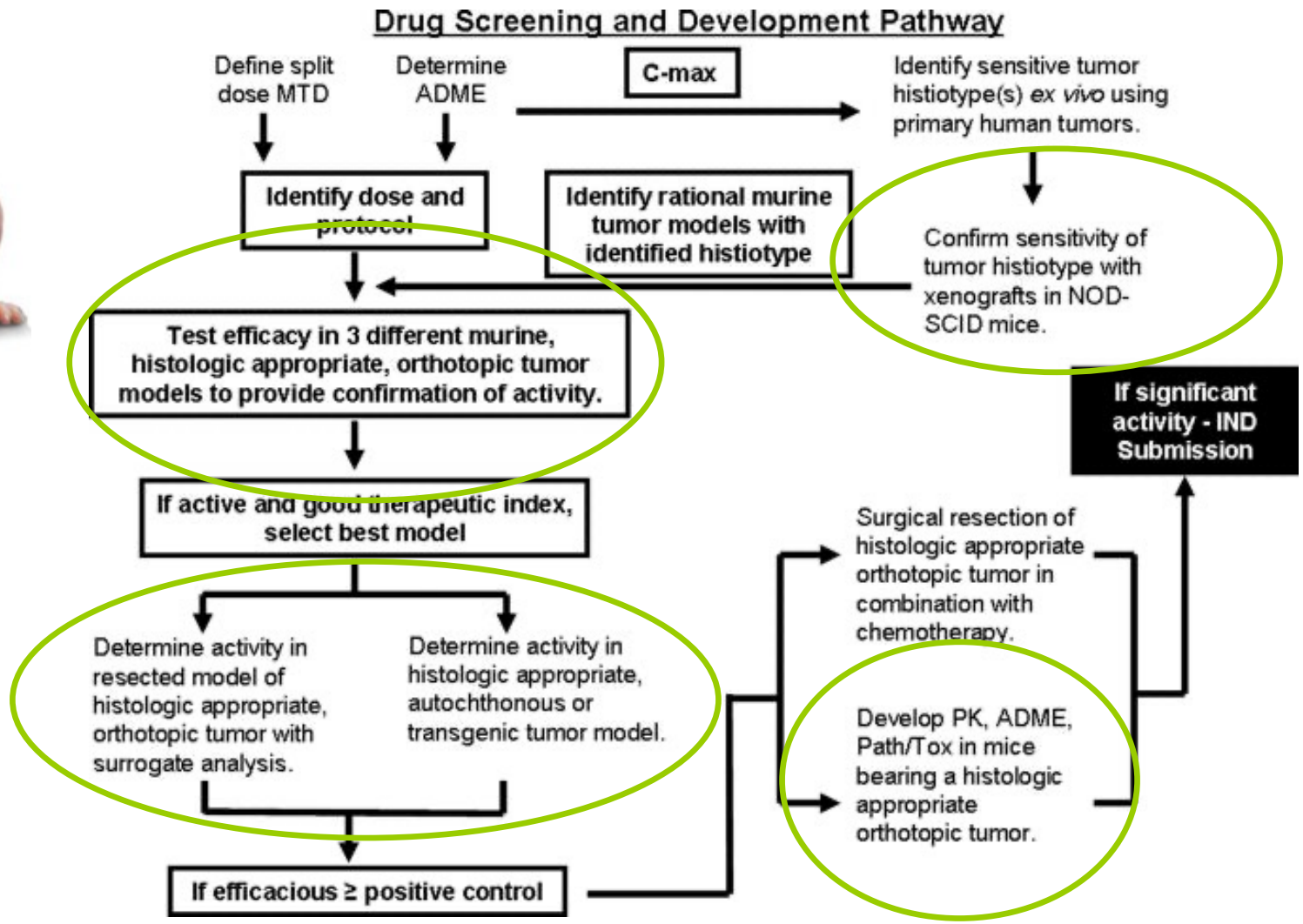
charles river

Available in vivo models



Use of animals/mice in the oncology drug pipeline



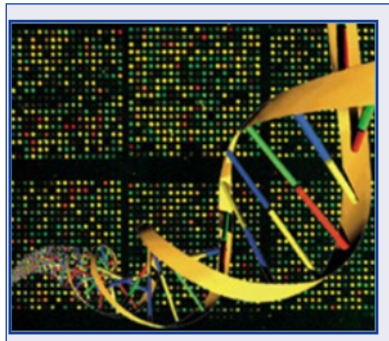


*James E. Talmadge Am J Pathol. 2007 March; 170(3): 793–804.

J. Folkman: "If you are a mouse and have cancer, we can take good care of you"

charles river

Platforms reflect key Oncology trends



Fast-Evolving Science

DNA, RNA, Protein Platforms

Functional Genomics

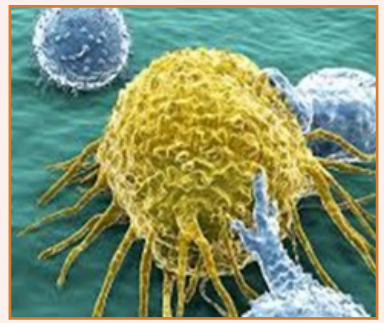
Biomarkers

Patient Stratification

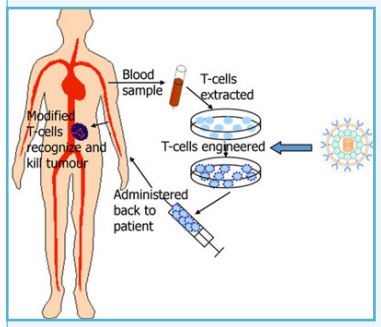
Complex Study Designs

Protocol Flexibility

Workflow



Complex and Flexible Studies



Modality Explosion

Small/large Molecule Combos

Cell Therapy

Modalities

Gene / Nucleotides

Cancer Vaccines

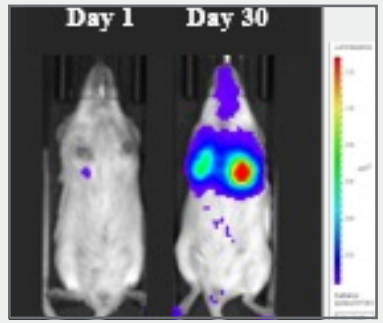
Humanized Models

Assays

Imaging

Immunology assays

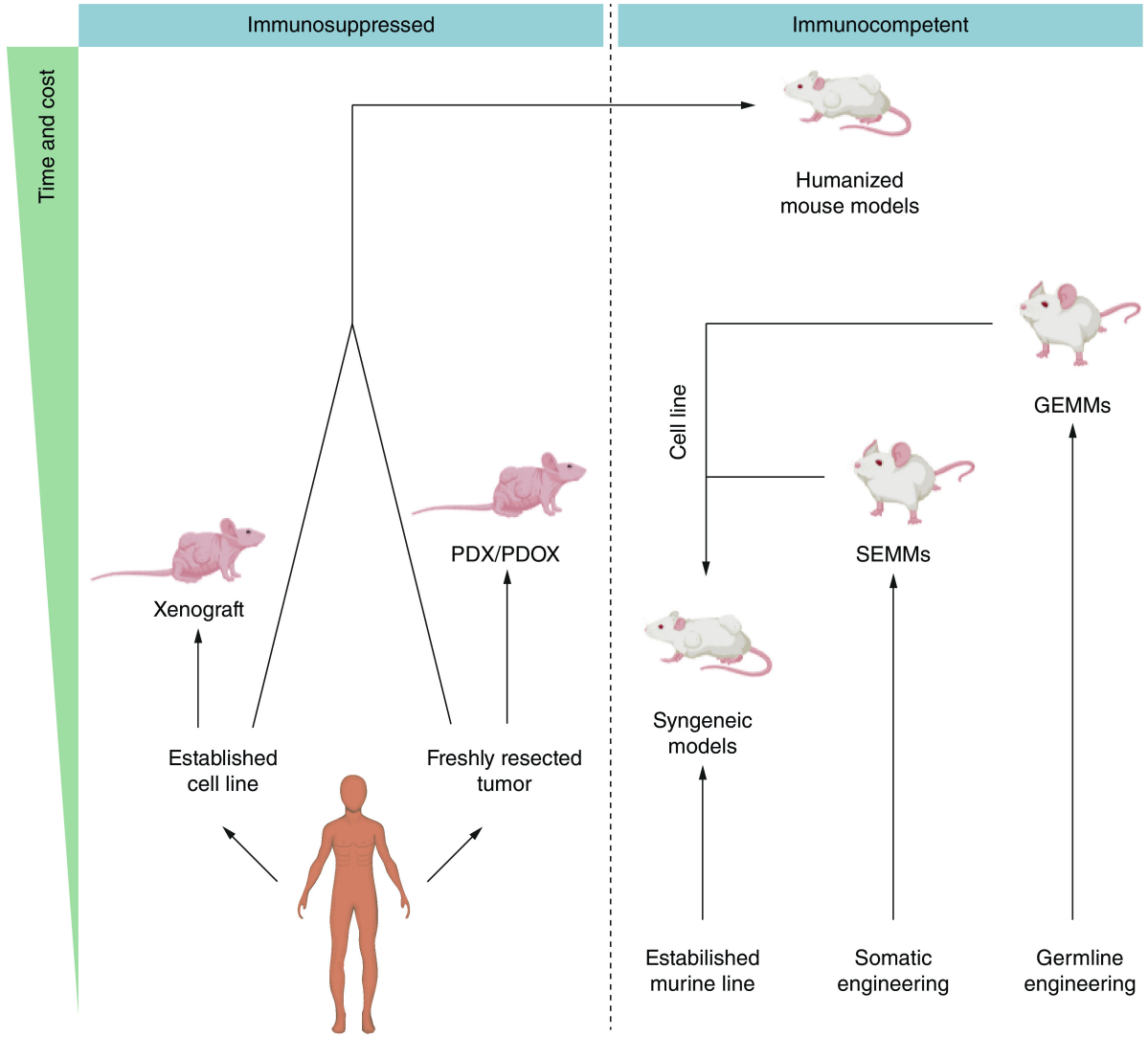
Microdialysis



Big data Endpoints

charles river

Different animal models in drug discovery



*Long, J et al, Future Sci OA. 2021 Jun 23;7(8):FSO737

charles river

Immunity is key

Different mouse strains for different questions

	Immunodeficient		Immunocompetent
	Non Humanized	Humanized	
Cells 	Human xenograft (CDX)		Mice allograft (syngeneic CDA)
Fragments 	Human fragments (PDX)		Mice GEM fragments (GDA)
Spontaneous 	-		GEM / Chemically induced / Natural

Suitability of the model for

- Cytotoxic or targeted agents
- Immune-modulating agents

Immunodeficient mice and rats

Model features and degree of immunodeficiency

	MICE								RATS	
	NSG	NRG	NODSCID	SCIDbeige	SCID	B6Rag1	Inbred nude	Outbred Nude	SRG	RNU nude
Mature B cells	Absent	Absent	Absent	Absent	Absent	Absent	Present	Present	Absent	Present
Mature T cells	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Dendritic cells	Defective	Defective	Defective	Present	Present	Present	Present	Present	Present	Present
Macrophages	Defective	Defective	Defective	Present	Present	Present	Present	Present	Present	Present
Natural killer cells	Absent	Absent	Defective	Defective	Present	Present	Present	Present	Absent	Present
Hemolytic complement	Absent	Absent	Absent	Present	Present	Present	Present	Present	Present	Present
Leakiness	Very low	Absent	Low	Low	Low	Absent	N/A	N/A	Very low	Low
Radiation tolerance	Low	High	Low	Low	Low	High	High	High	High	High
Spontaneous tumor incidence (type)	Low	Low	High (thymic lymphoma)	High (thymic lymphoma)	High (thymic lymphoma)	Low	Low	Low	Low	Low

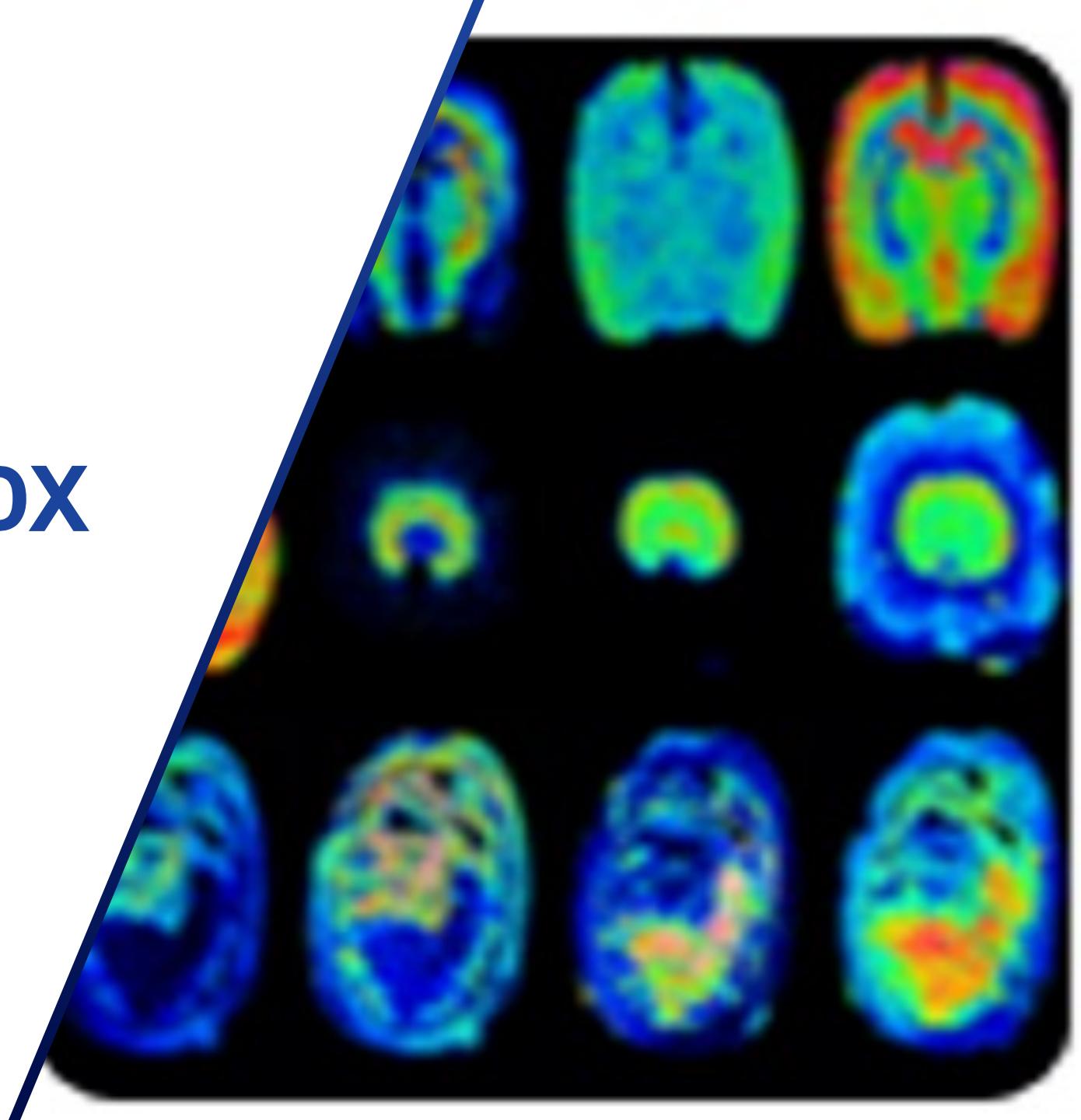
most — least

Degree of immunodeficiency

charles river

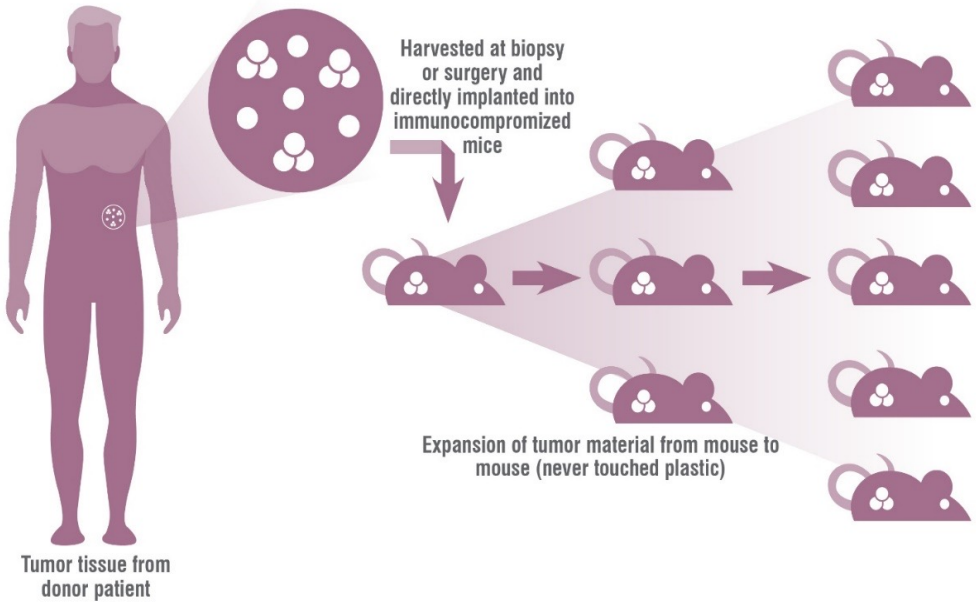
charles river

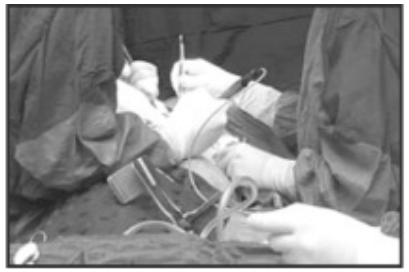
Example study PDX



Patient derived xenograft - PDX

Current gold standard for preclinical drug development



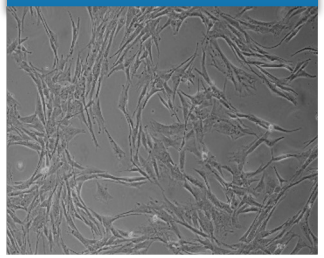


Patient tumor resection

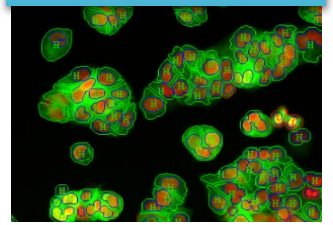
PDX development & storage in liquid nitrogen,

Low passage numbers Long term availability

In vitro 2D



In vitro/ex vivo 3D

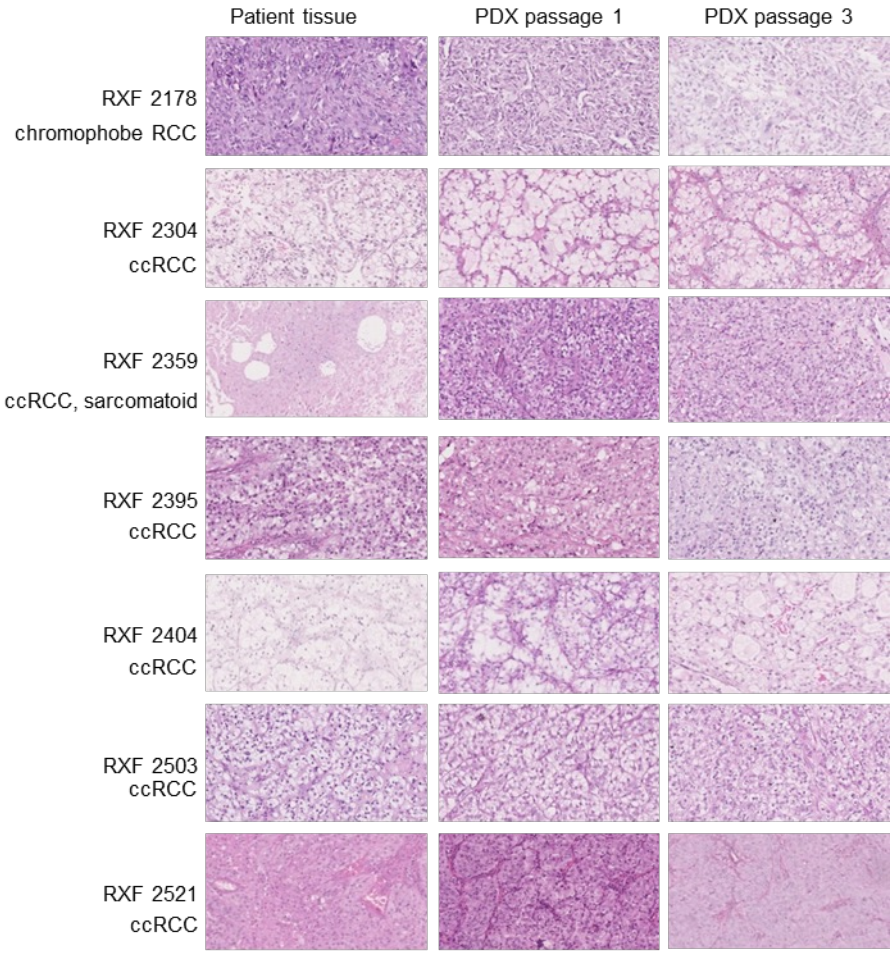


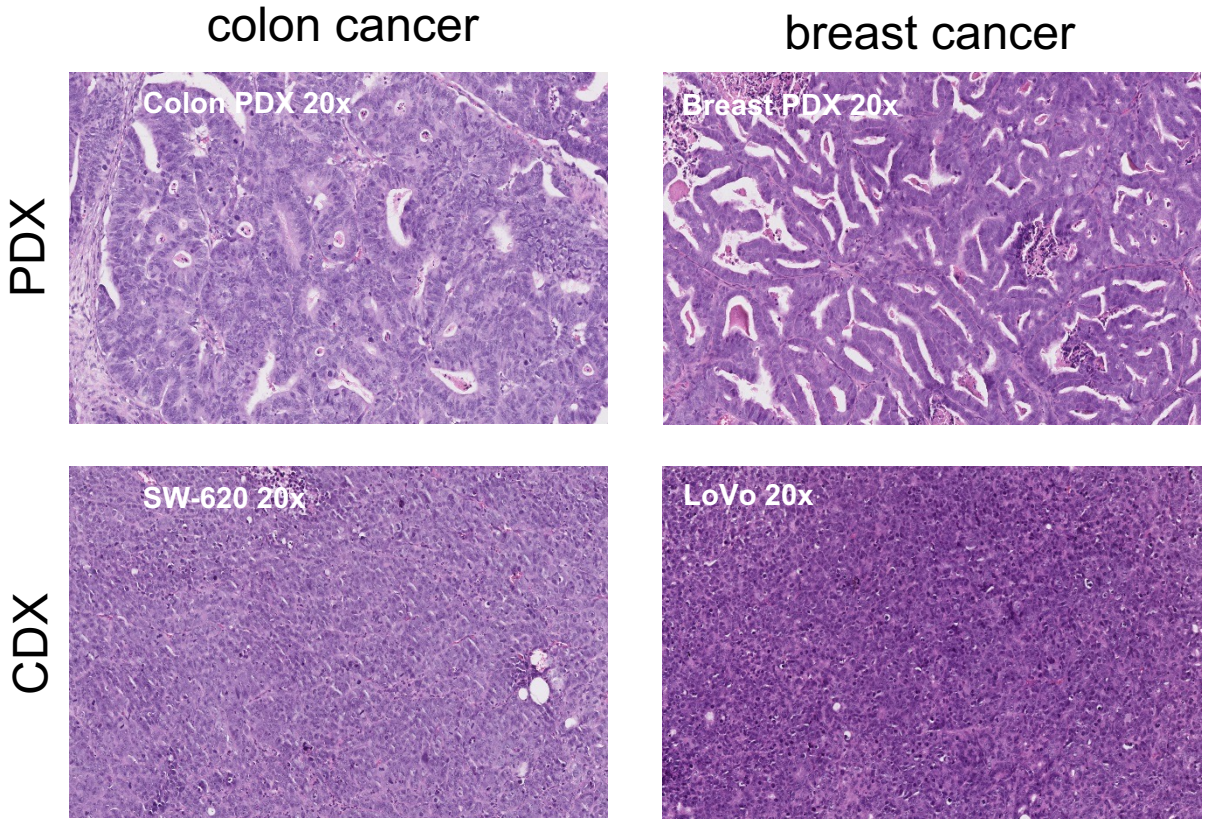
In vivo

charles river

PDX preserve tumor architecture

Histology and heterogeneity are preserved in PDX

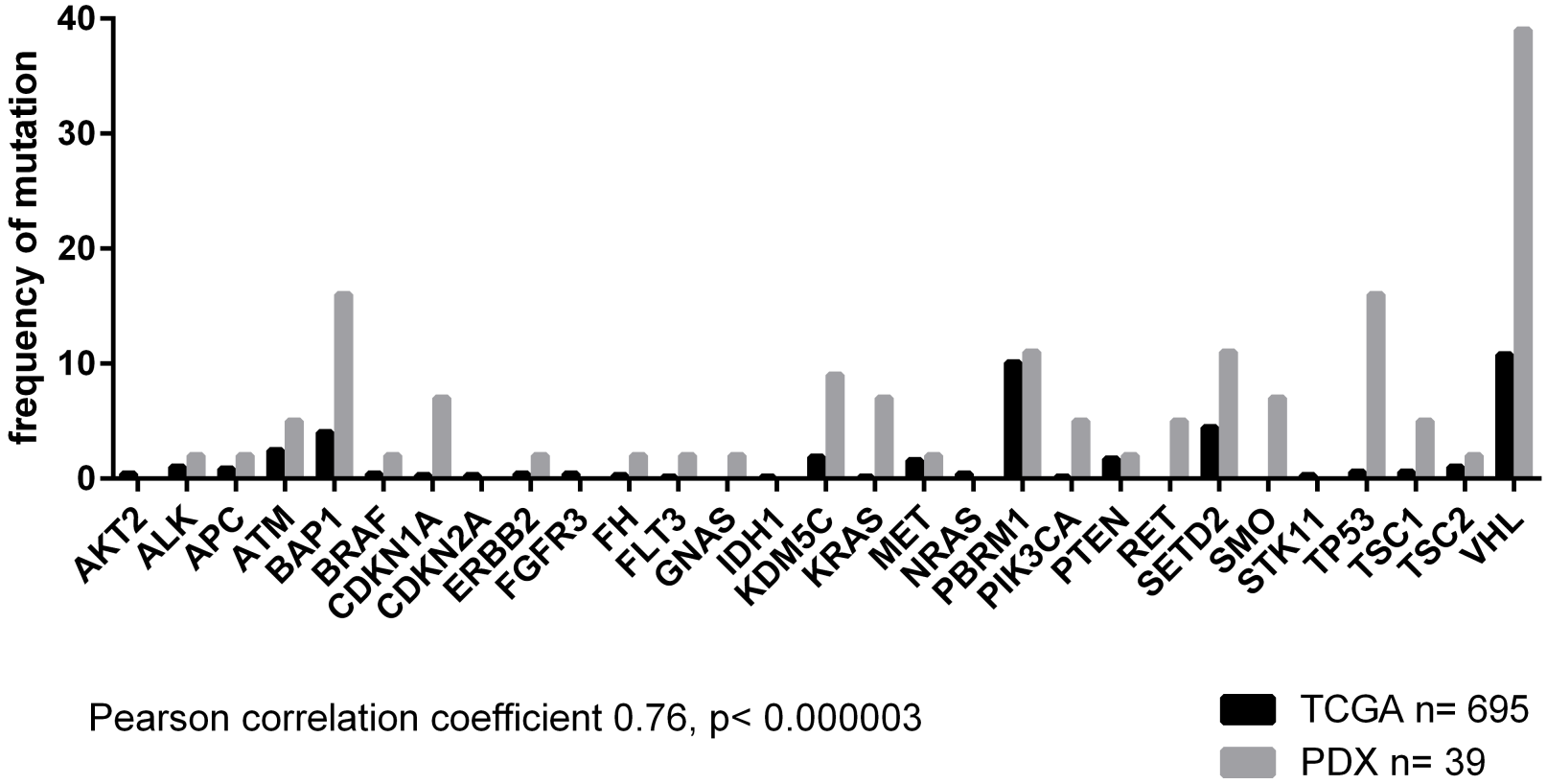




*Schueler et al, Oncotarget 2018, 9; 57; p.30946-30961

charles river

PDX represent (largely) the molecular landscape

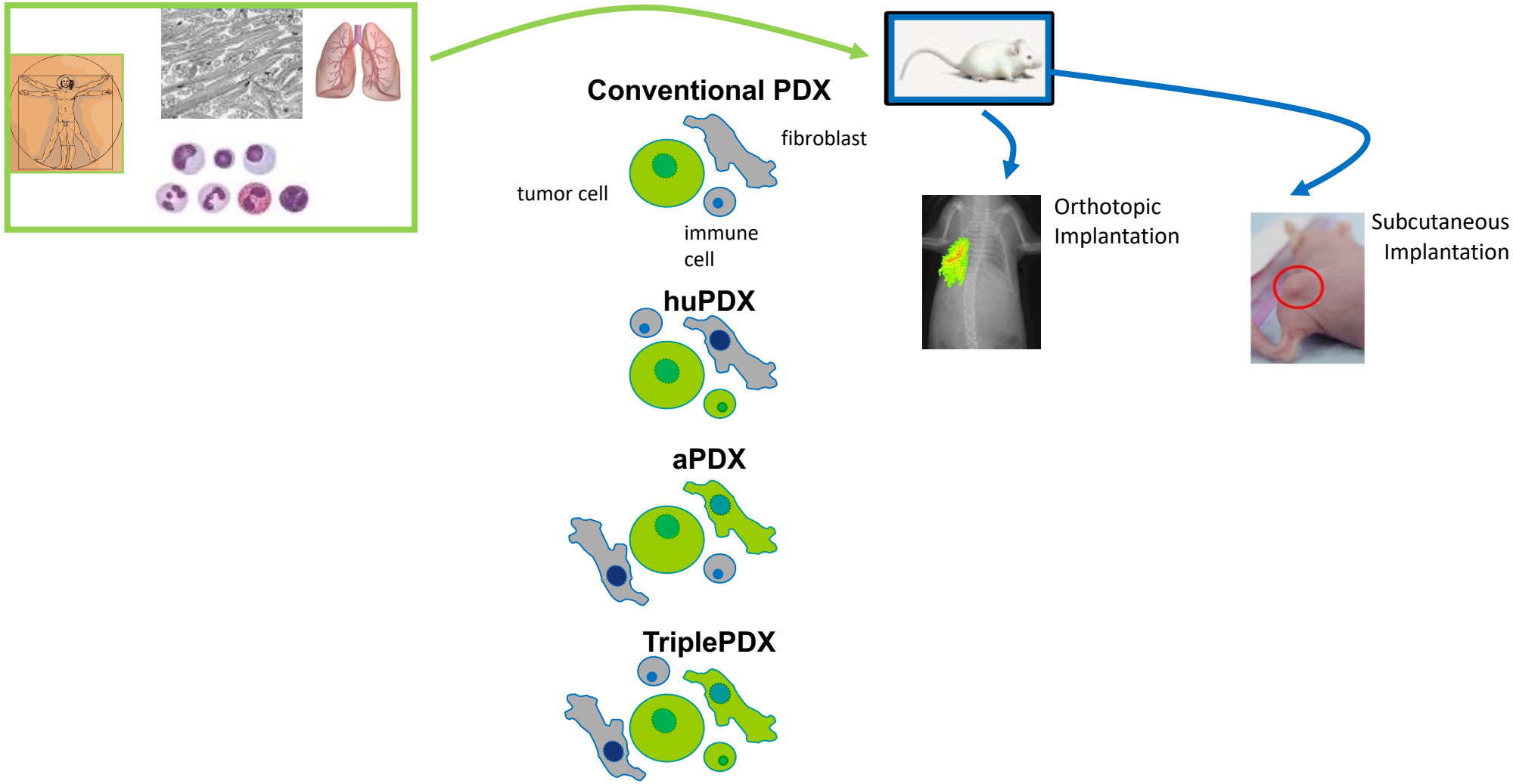


*Schueler et al, Oncotarget 2018, 9; 57; p.30946-30961

charles river

PDX and the tumor microenvironment

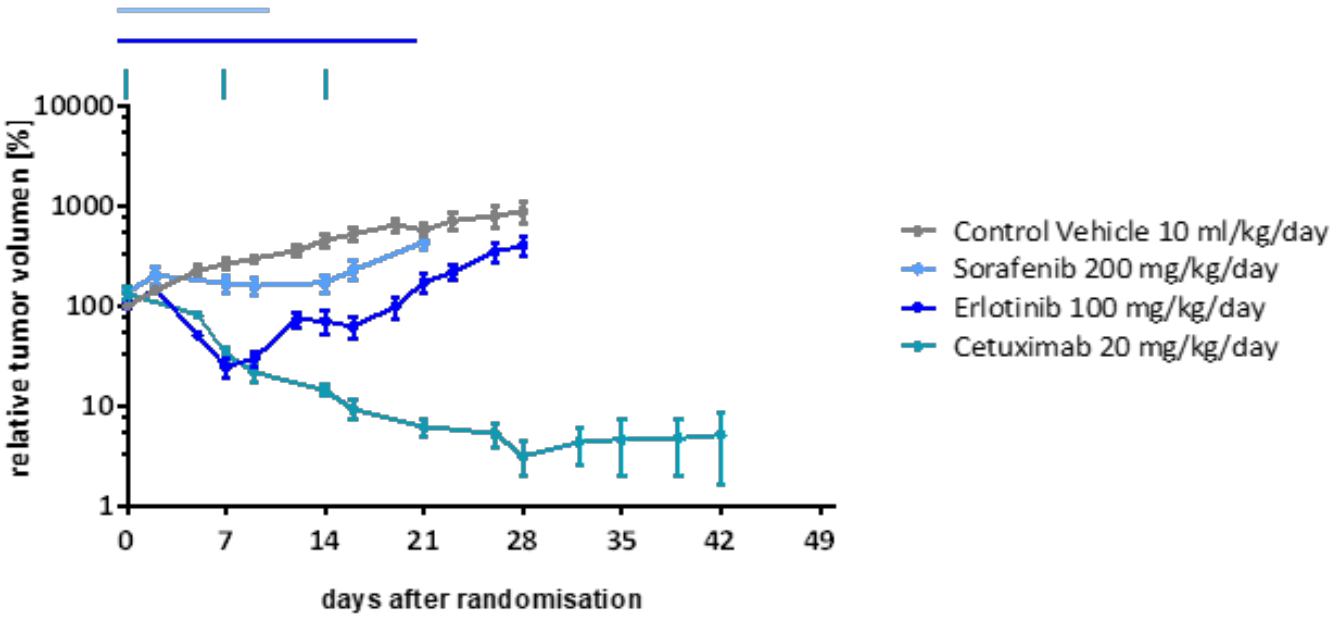
human tumor cells interacting with the murine host



charles river

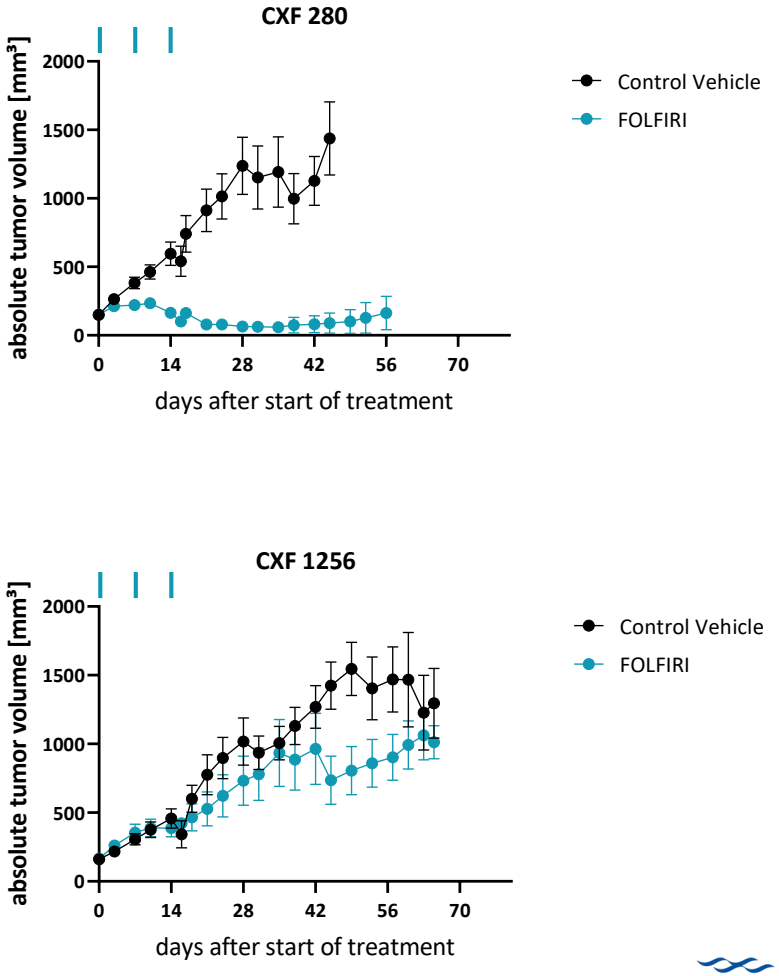
PDX for drug development

Tumor growth over time of NSCLC PDX model under treatment with 3 different targeted agents



*Schueler et al, Cells 2019, 8, 740

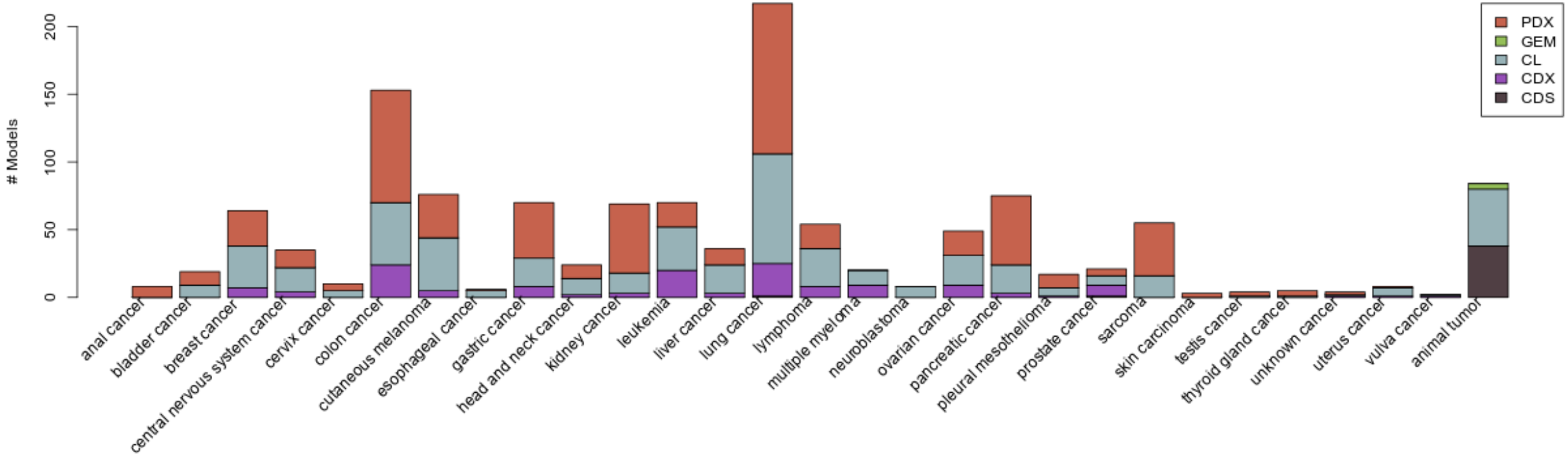
Tumor growth over time of CTC PDX model under treatment with combination SoC



charles river

Model selection for drug testing

breadth and depth of the collection is key

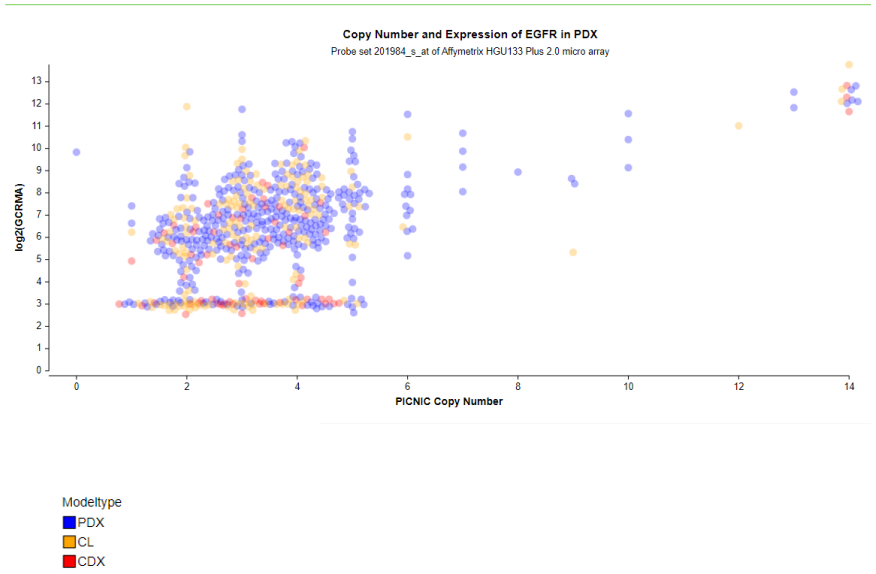


charles river

Model selection for drug testing

Identify models that express your target (or not)

RNA expression



RNAseq

Protein expression

IHC on TMA

Protein quantification

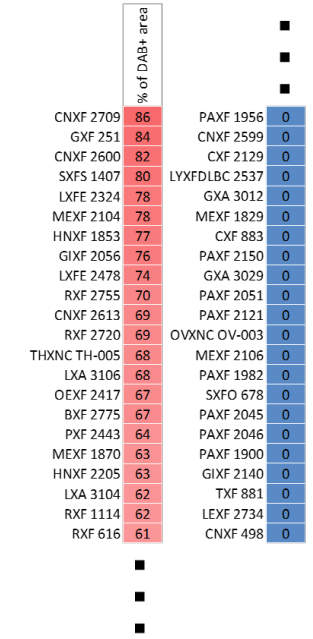
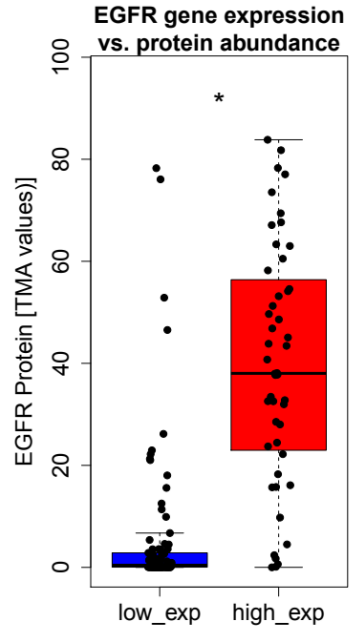


Image analysis

RNA/protein correlation

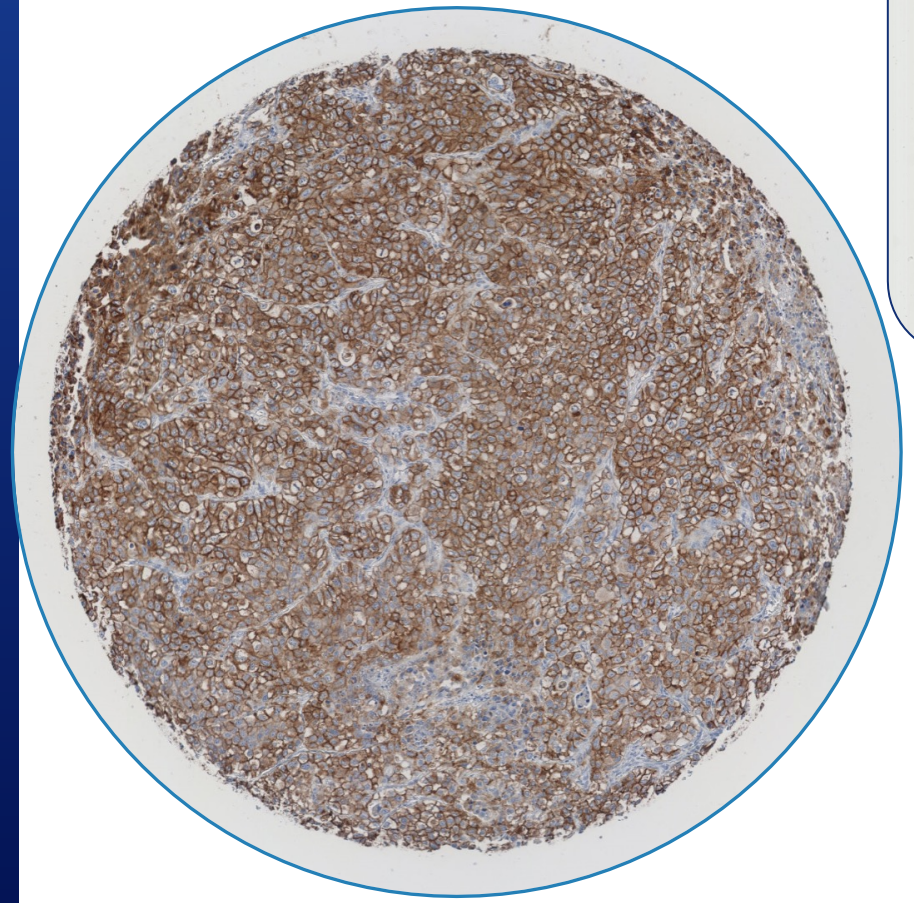


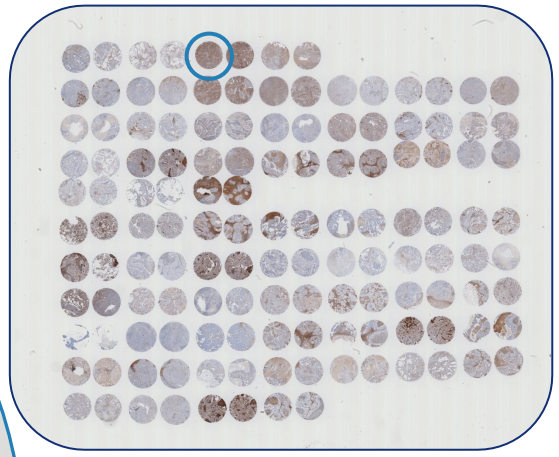
bioinformatics

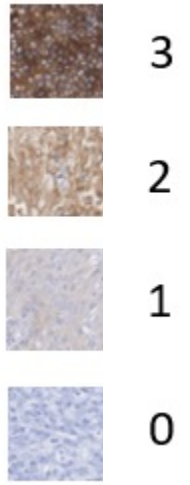
charles river

Tissue microarrays for model selection

Anti-EGFR

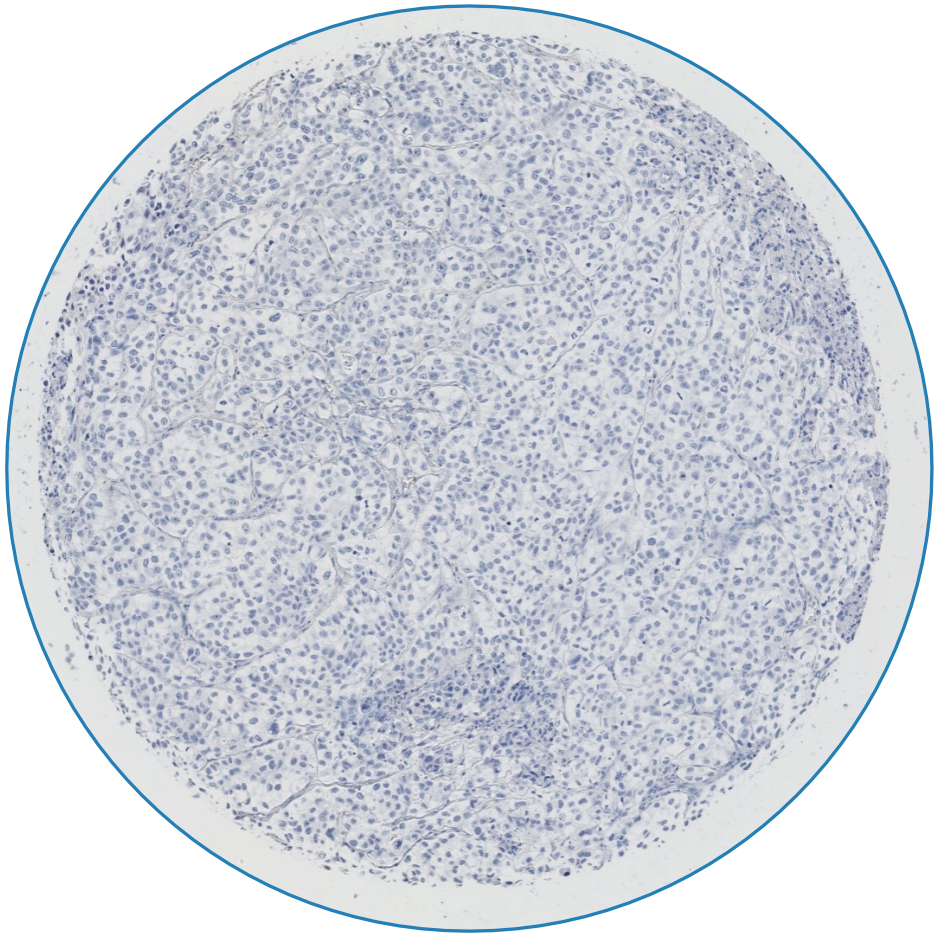






250 µm

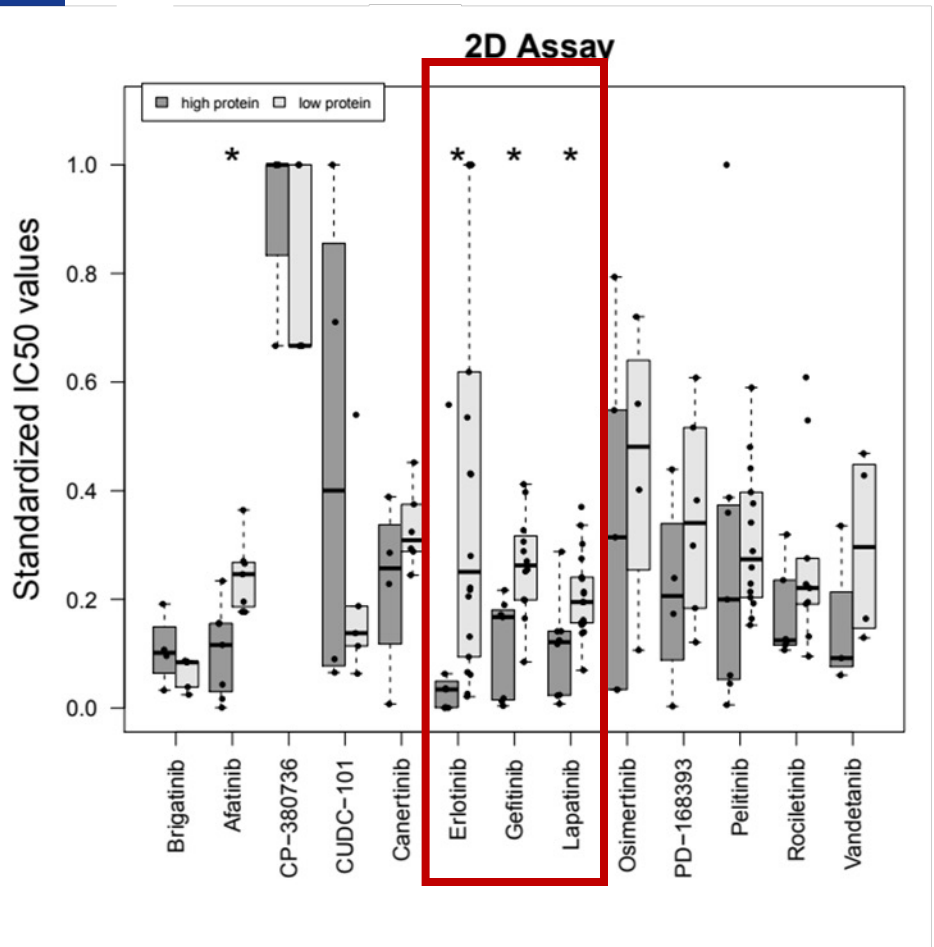
$IgG1_K$



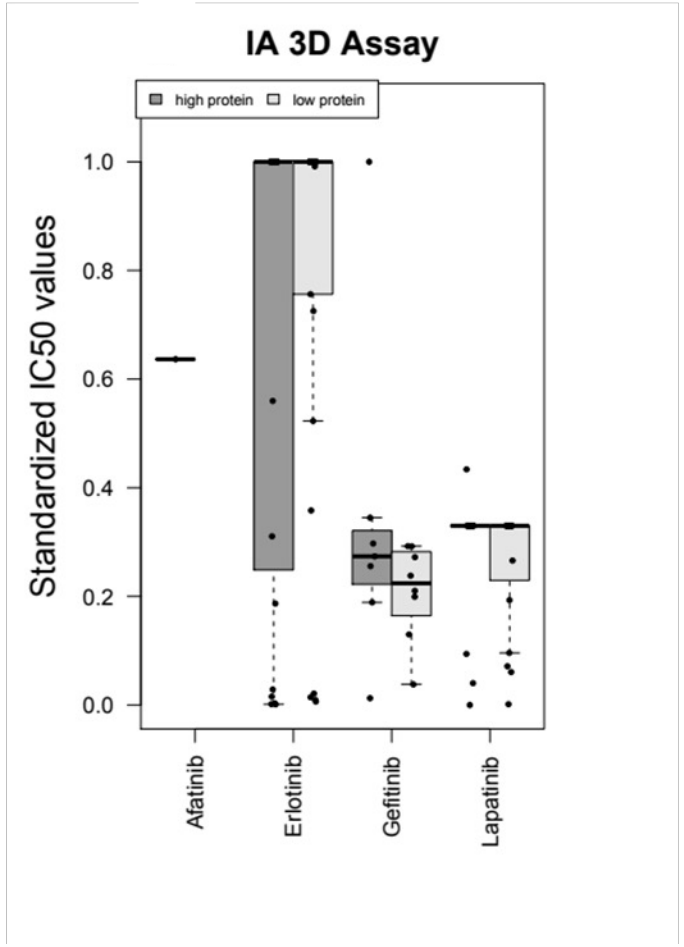
charles river

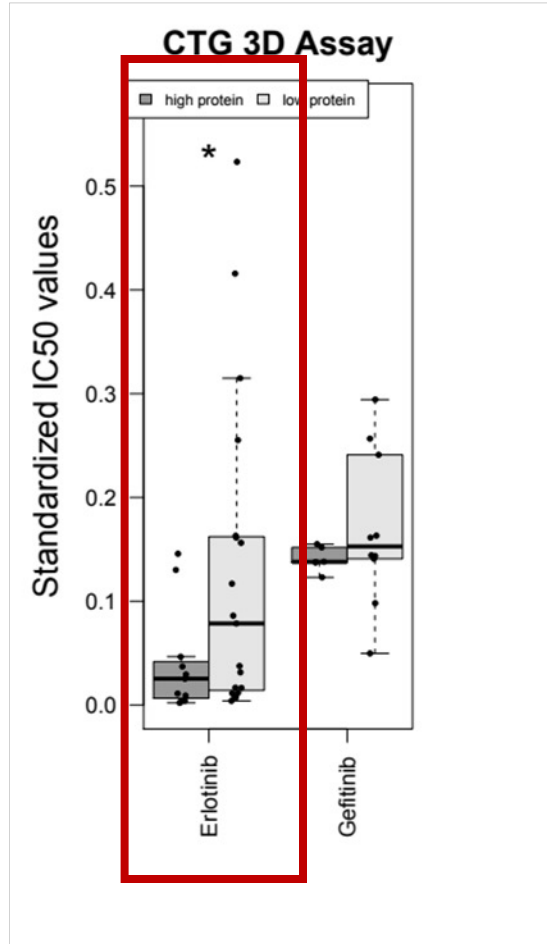
In vitro screening

Determine activity and specificity



selection of most specific compounds

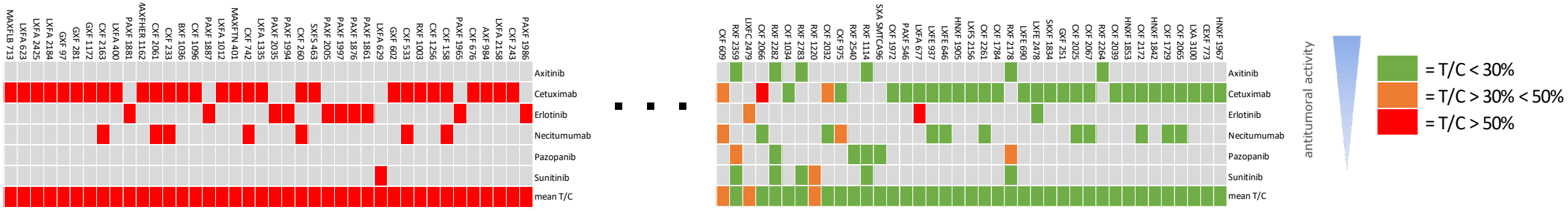


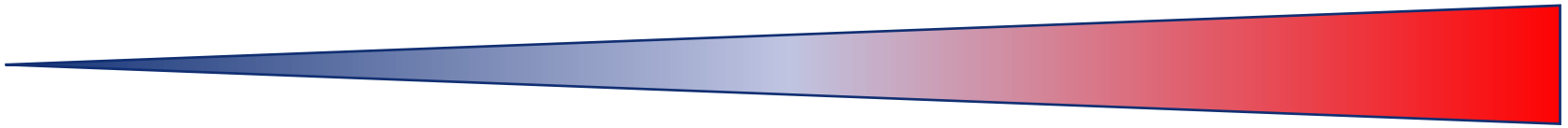


charles river

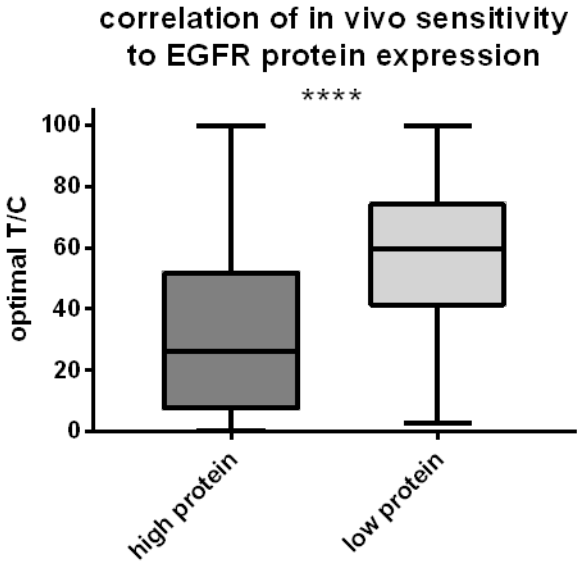
In vivo screening

Screening of seven EGFRi across 169 subcutaneously implanted PDX models *in vivo*





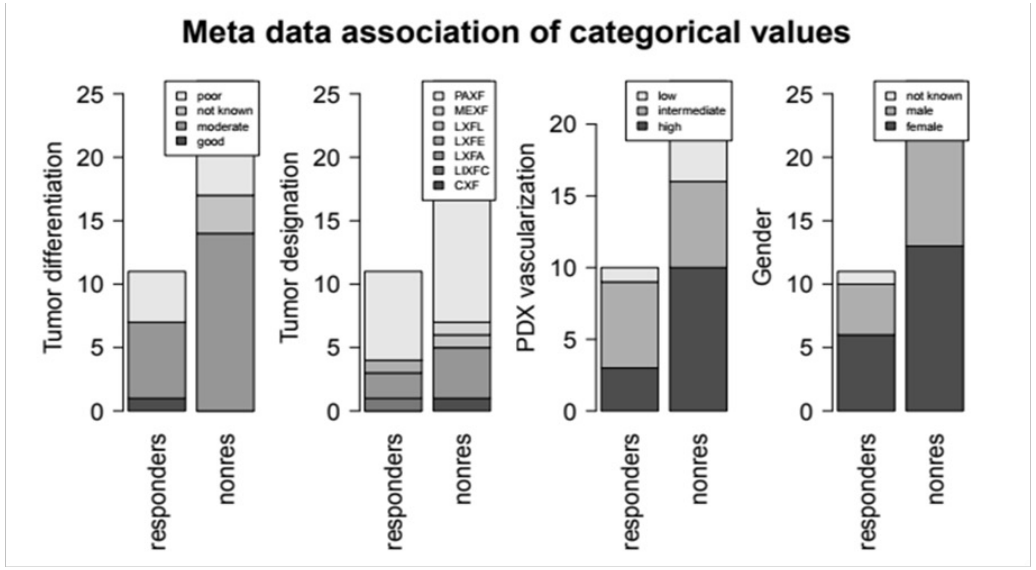
EGFR expression determined by IHC

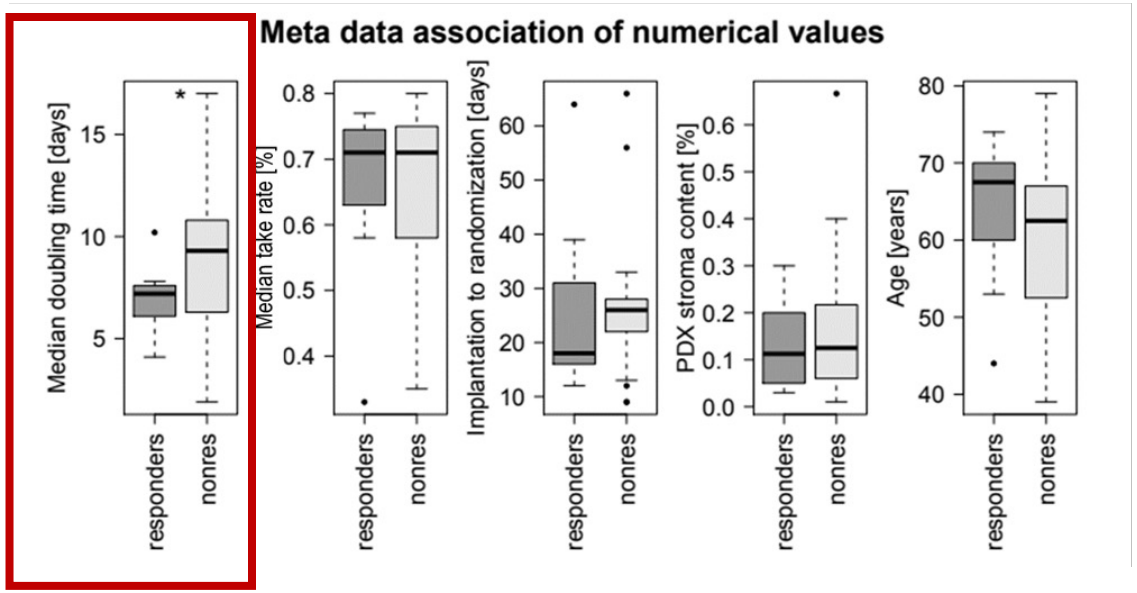


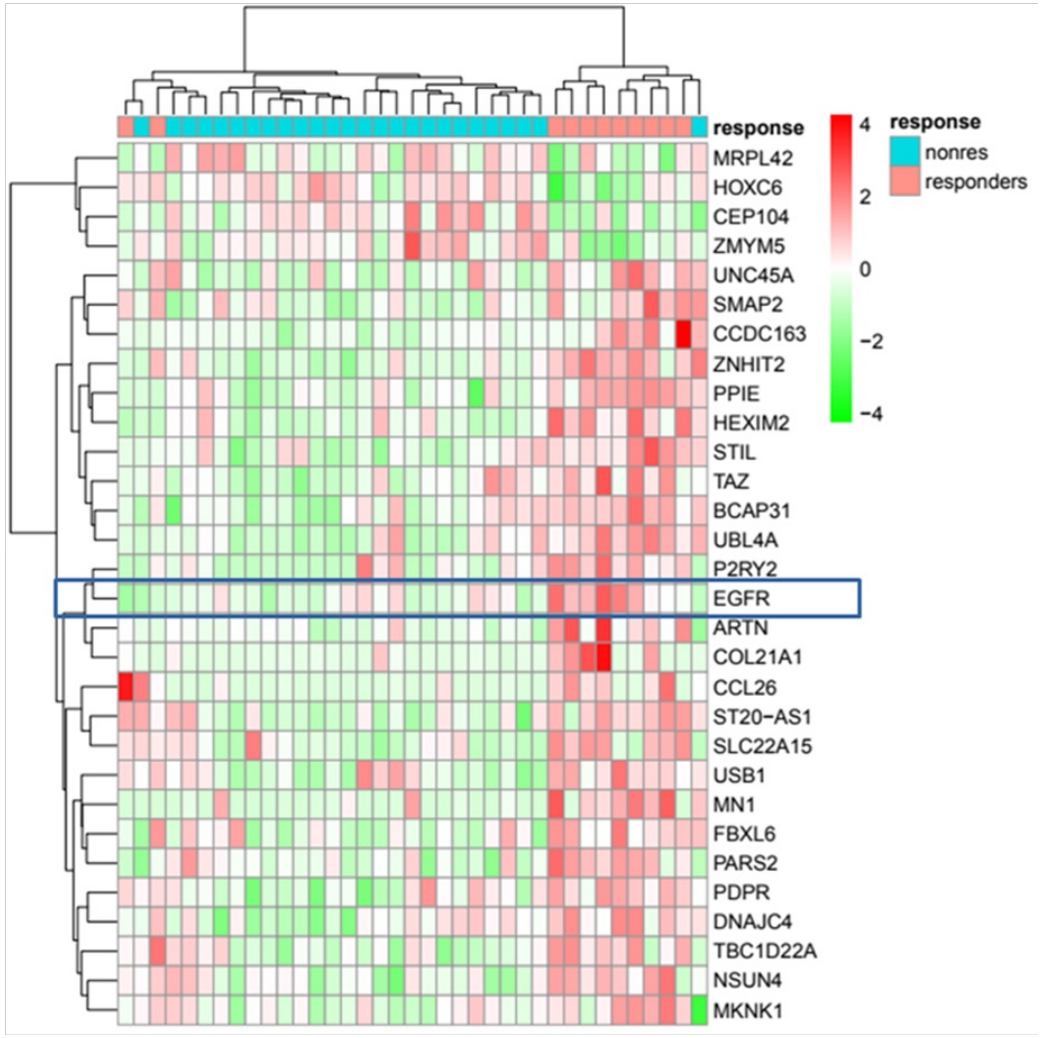
**Adapted from Klett et al, Precision Medicine, March 2019*

charles river

Biomarker identification



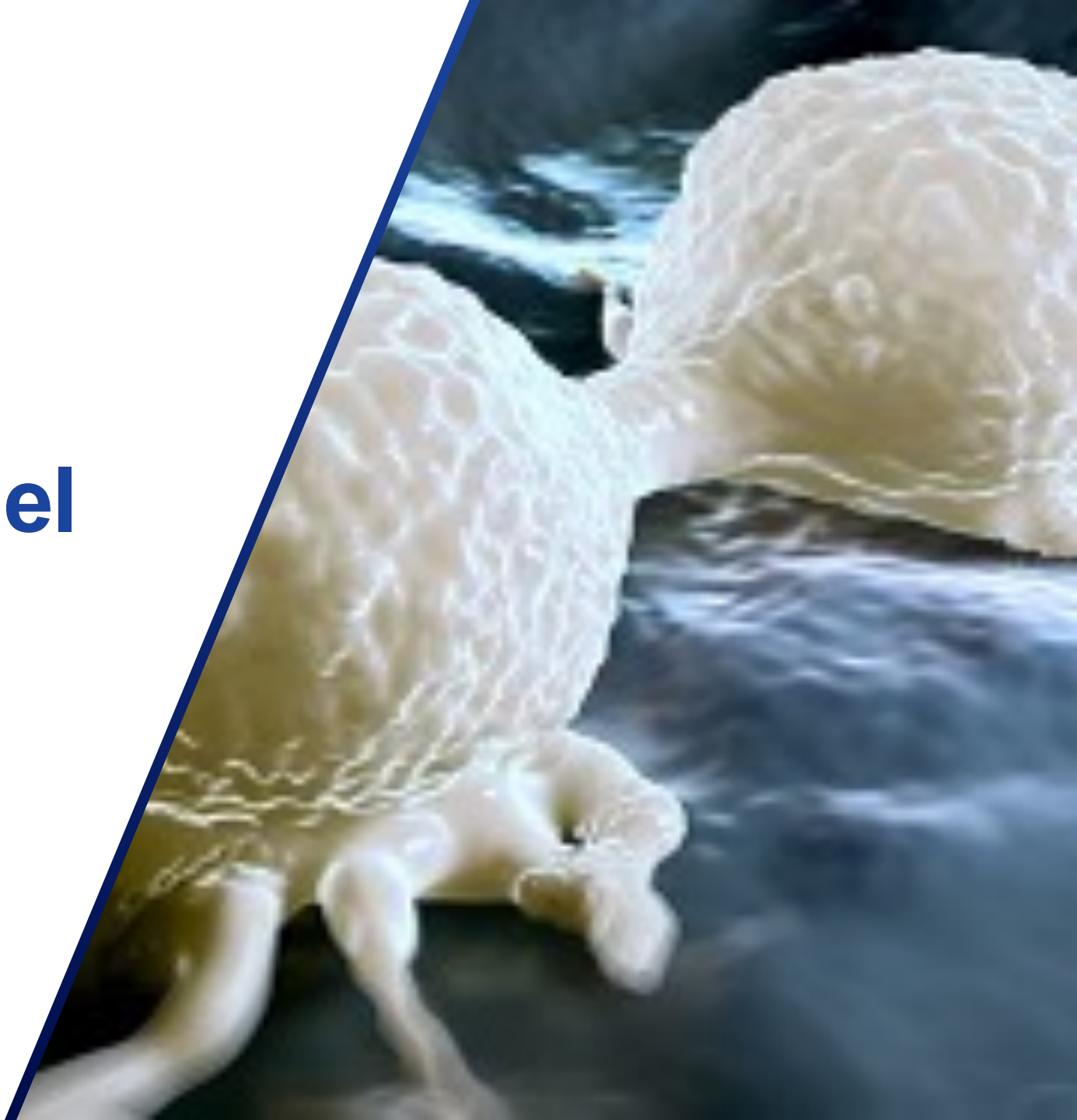




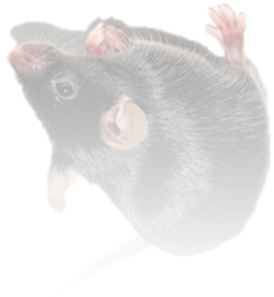
charles river

charles river

Example study mouse tumor model



Pre-Clinical *in vivo* Models for Immuno-Oncology



GEMMs & syngeneic models

Pro`s

Complete immune system

Immune & tumor cells from the same host

Preserved tumor development

Con`s

Murine tumor cells

Murine immune cells

Humanized mice

Pro`s

Human tumor cells

Human immune cells

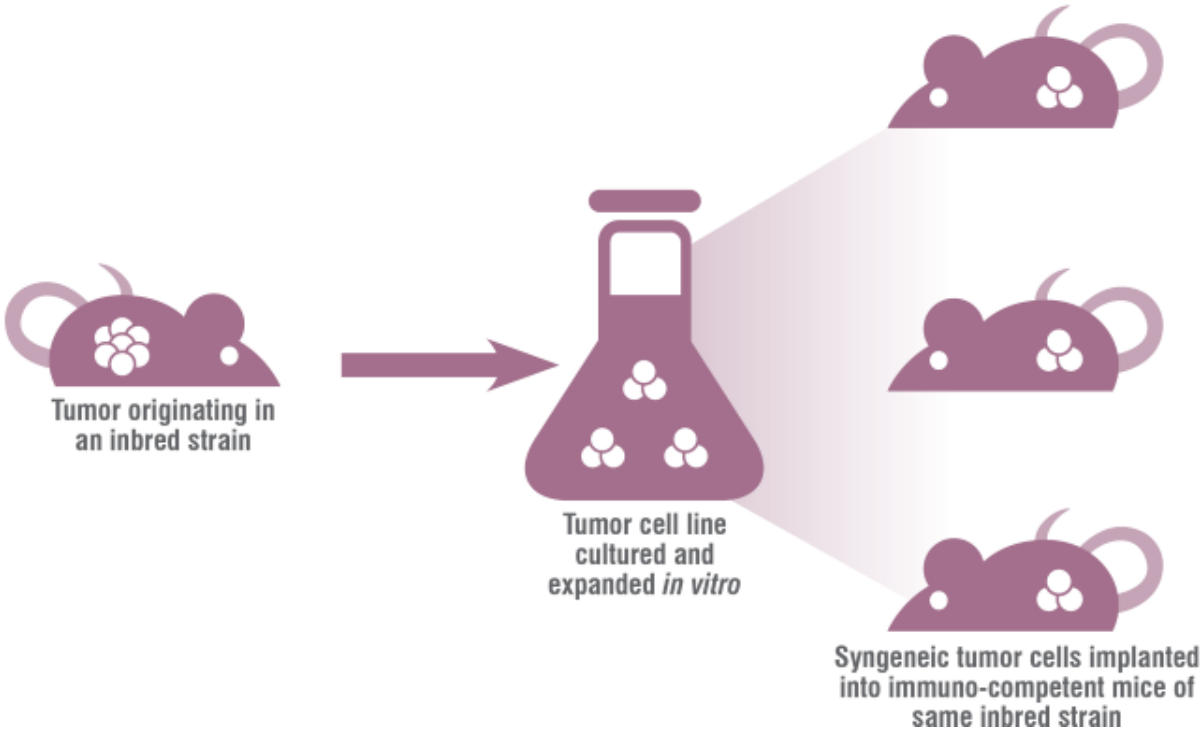
Preserved Tumor heterogeneity

Con`s

Chimeric organism

(still) incomplete immune system

Syngeneic mouse models



Characterized by

- molecular phenotype (WES and RNAseq)
- efficacy testing towards CPi

charles river

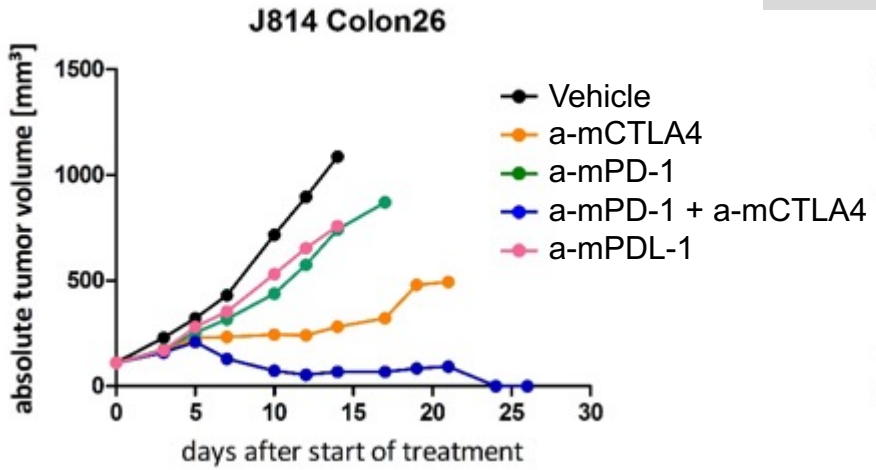
Syngeneic Models

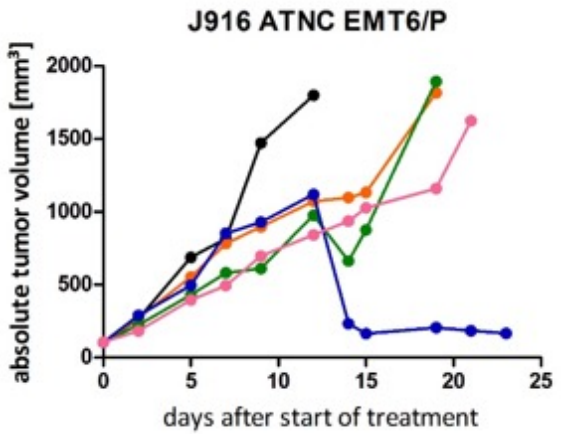
Established models by CHK-i response

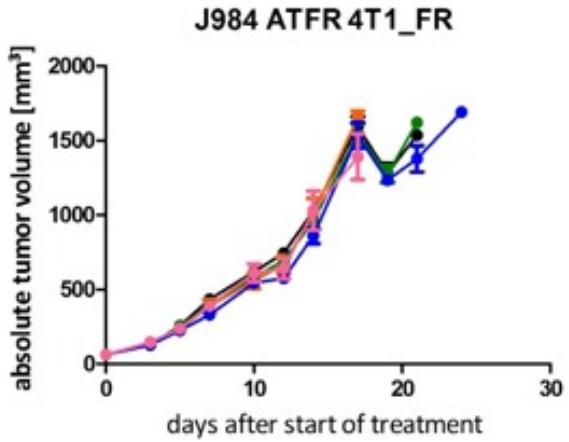
RESPONSIVE	
HISTOTYPE	CELL LINES
Colon	Colon26, CT26, MC38
Lymphoma	A20
Brain	GL261
Bladder	MBT-2

MODERATE RESPONSIVE	
HISTOTYPE	CELL LINES
Breast	EMT-6
Lymphoma	E.G7-OVA
Melanoma	CloudmanS91
Pancreatic	Pan02-HA
Renal	Renca

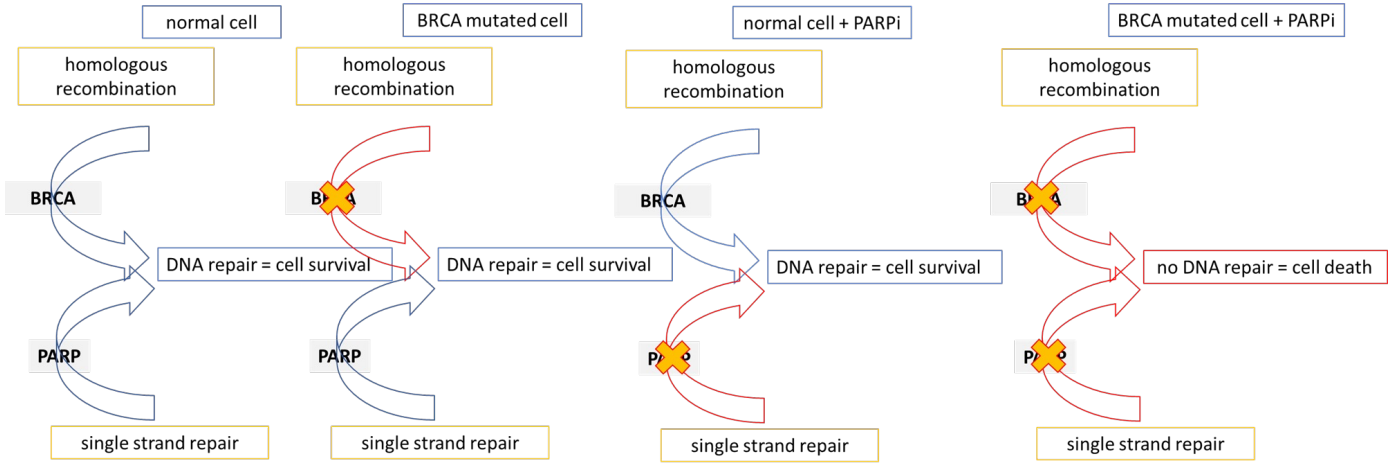
REFRACTORY	
HISTOTYPE	CELL LINES
Breast	4T-1
Lung	Lewis Lung, Madison 109
Melanoma	B16F10
Pancreatic	Pan02



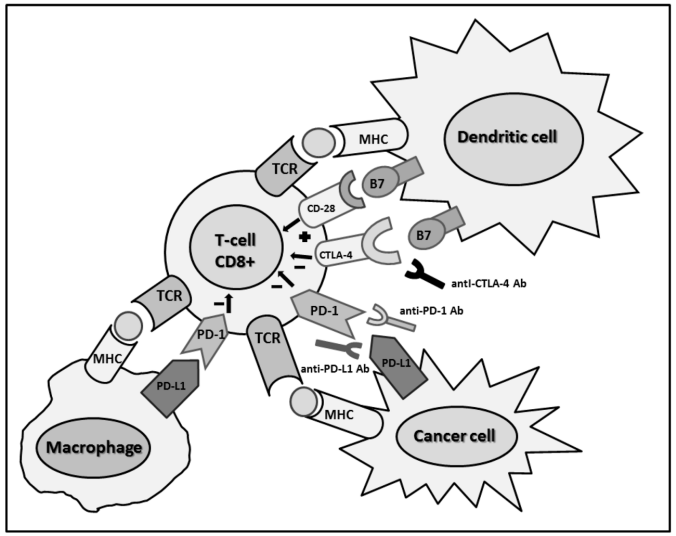




Combination therapy screen in vivo



Synthetic lethality

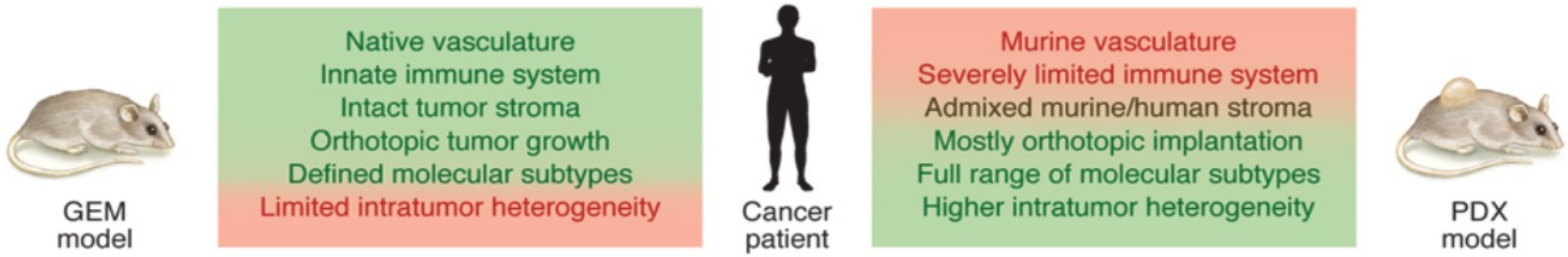


Checkpoint inhibition

charles river

Combination therapy screen in vivo

Lack of preclinical model for combination therapy



lack of BRCA mutated model

lack of fully functional immune system

charles river

Creation of homozygous EMT6 BRCA1 ko line

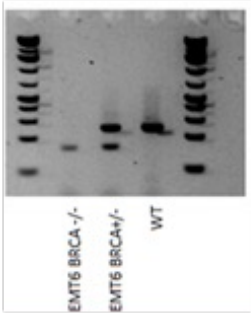
Generation of a Brca1 knock-out in mouse breast cancer cell line EMT6 mice using the HDR pathway

EMT6

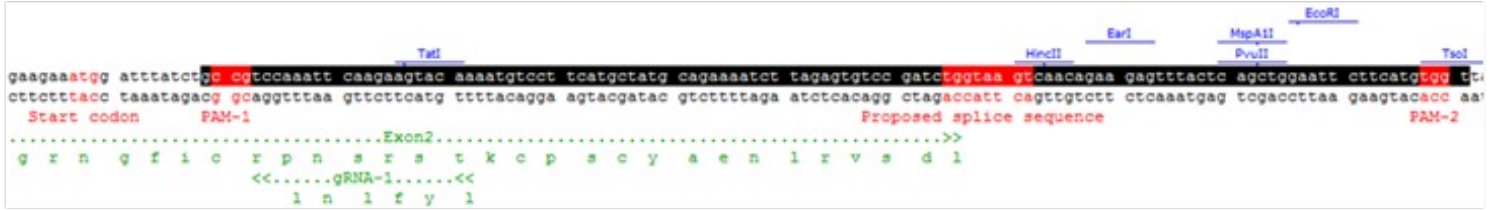
Strategy for full *Brca1* knock-out:

- Exon 2 removal using two gRNAs
- Introduction of frameshift mutation
- Removal of splice signal

PCR analysis of clones



Sequence verification of homozygous *Brca1* knock-out



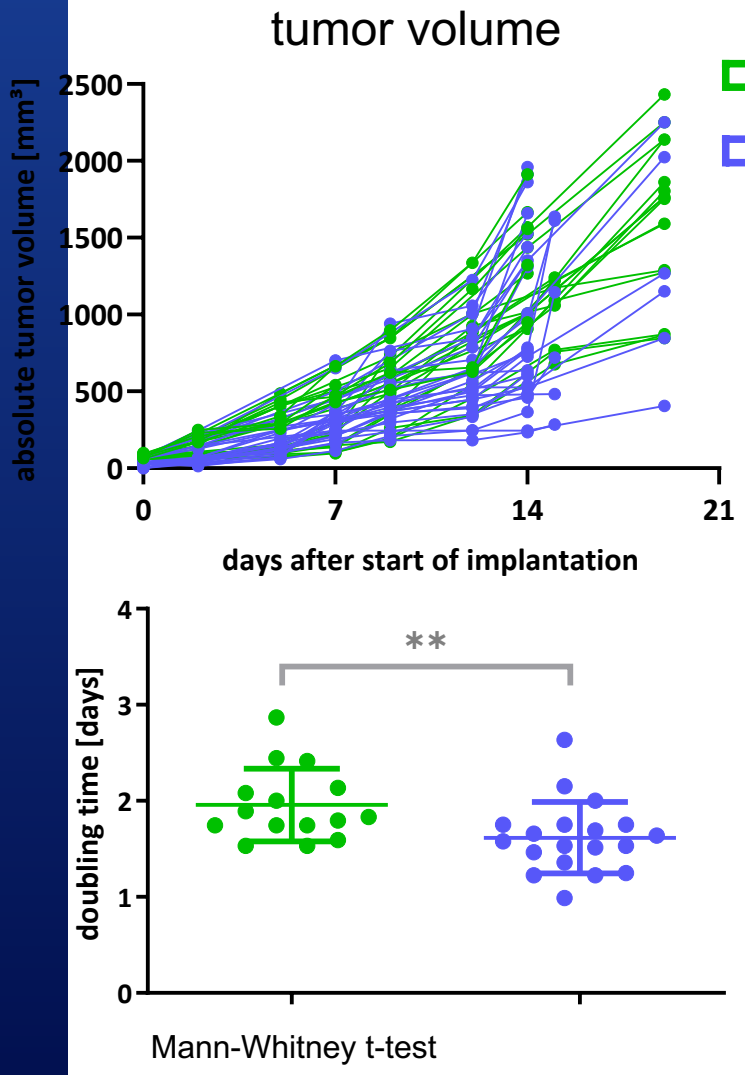
127 bp deleted (*black)

Tumor growth after transplantation of *Brca1* KO EMT6 cells

charles river

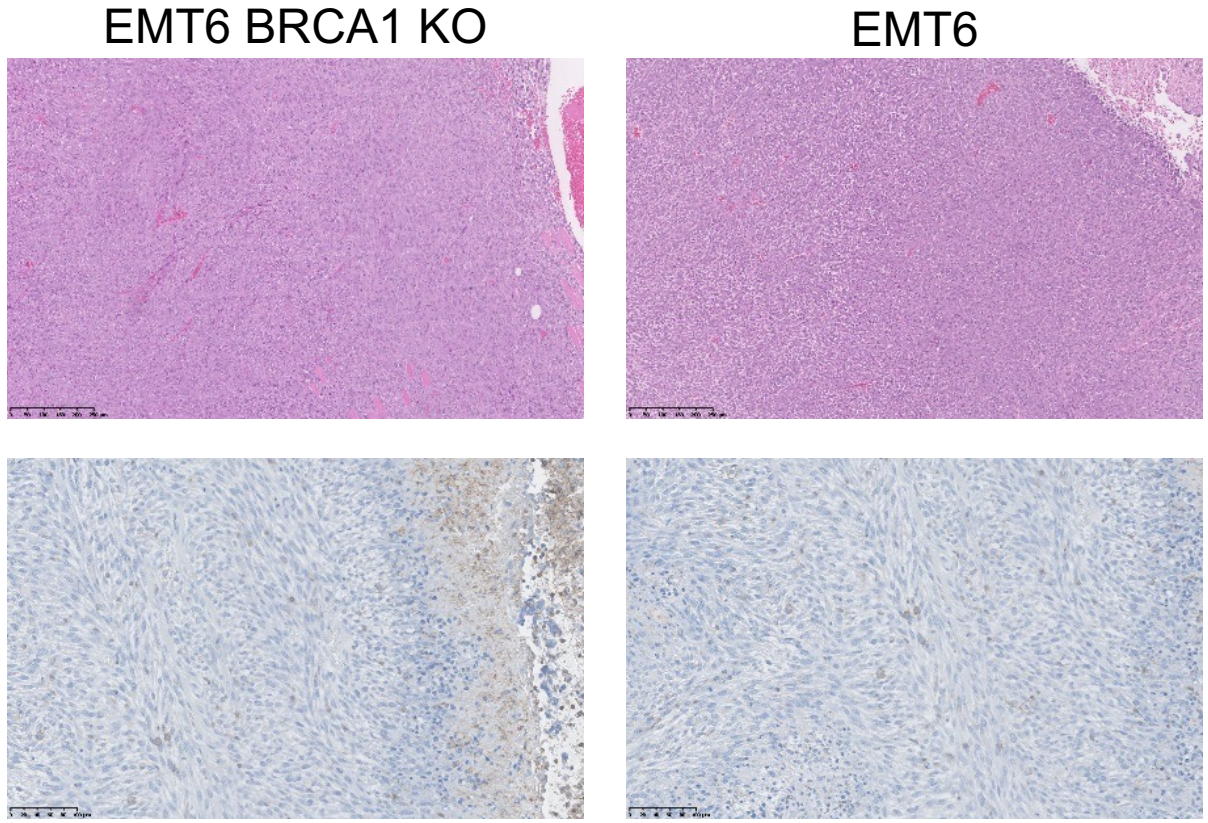
Basic tumor biology characteristics

Subcutaneously implanted into female balb/c



H&E 10x

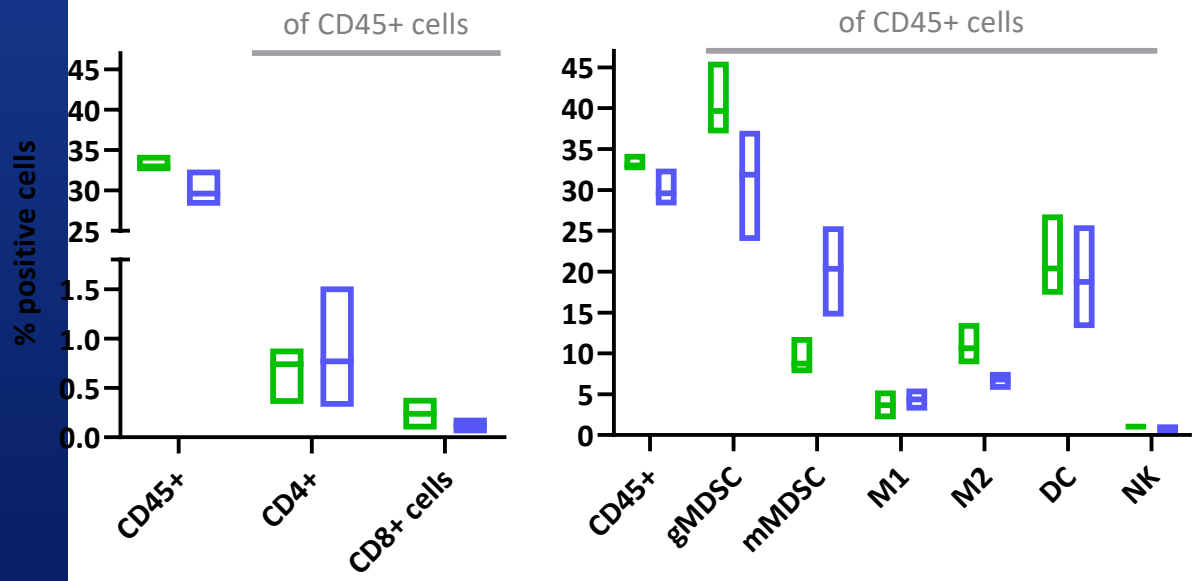
mCD45 IHC 20x



charles river

Basic tumor biology characteristics

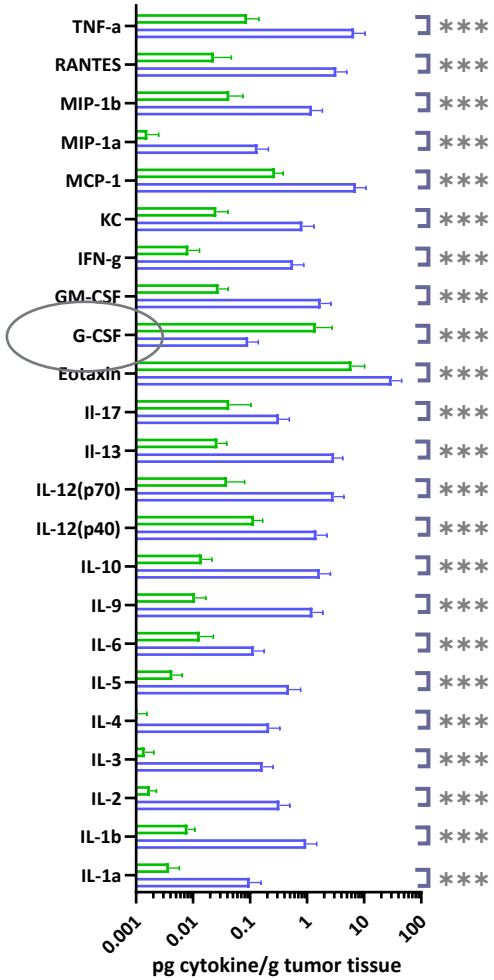
tumor infiltrating lymphocytes



EMT6 BRCA1 KO

EMT6

cytokine profile



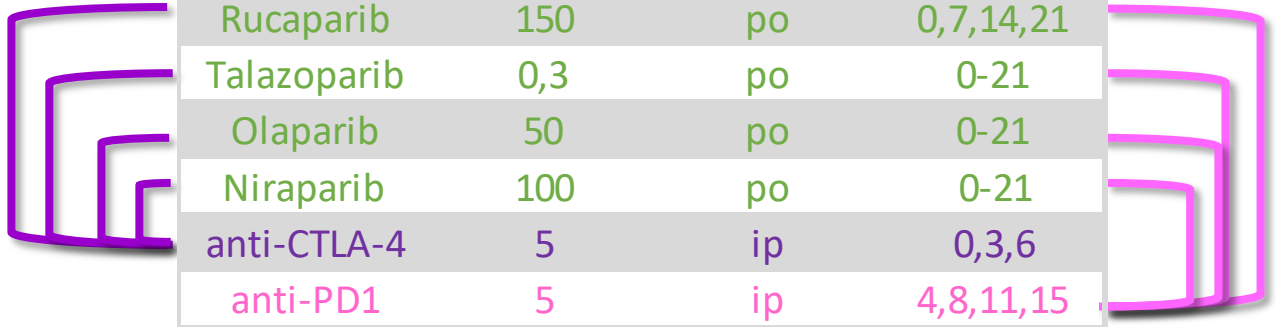
multiple t-test´s

Phenotypic differences EMT6 vs EMT6 BRCA1 KO

- The creation of a murine breast cancer cell line bearing a homozygous frame shift mutation was successfully conducted.
- The comparison of the mutated vs the wt EMT6 cell line in vivo revealed significant differences in the tumor doubling time. The mutated cell line grew significantly slower (1.96 ± 0.38 vs 1.61 ± 0.37 d).
- The histological architecture was similar in both lines, depicting an undifferentiated carcinoma.
- The percentage of tumor infiltrating lymphocytes (TILs) was similar for CD45 (determined by FC and IHC).
- The subtyping of TILs revealed higher percentages for gMDSC and M2 macrophages in the EMT6 BRCA1 KO line. Nevertheless, those differences were not statistically significant.
- The cytokine profile of the two lines differed significantly with higher cytokine levels of 22/23 analytes in the non-modified cell line. G-CSF is the only determined cytokine expressing higher levels in the serum of EMT6 BRCA1 KO animals.

Treatment regimen & study layout

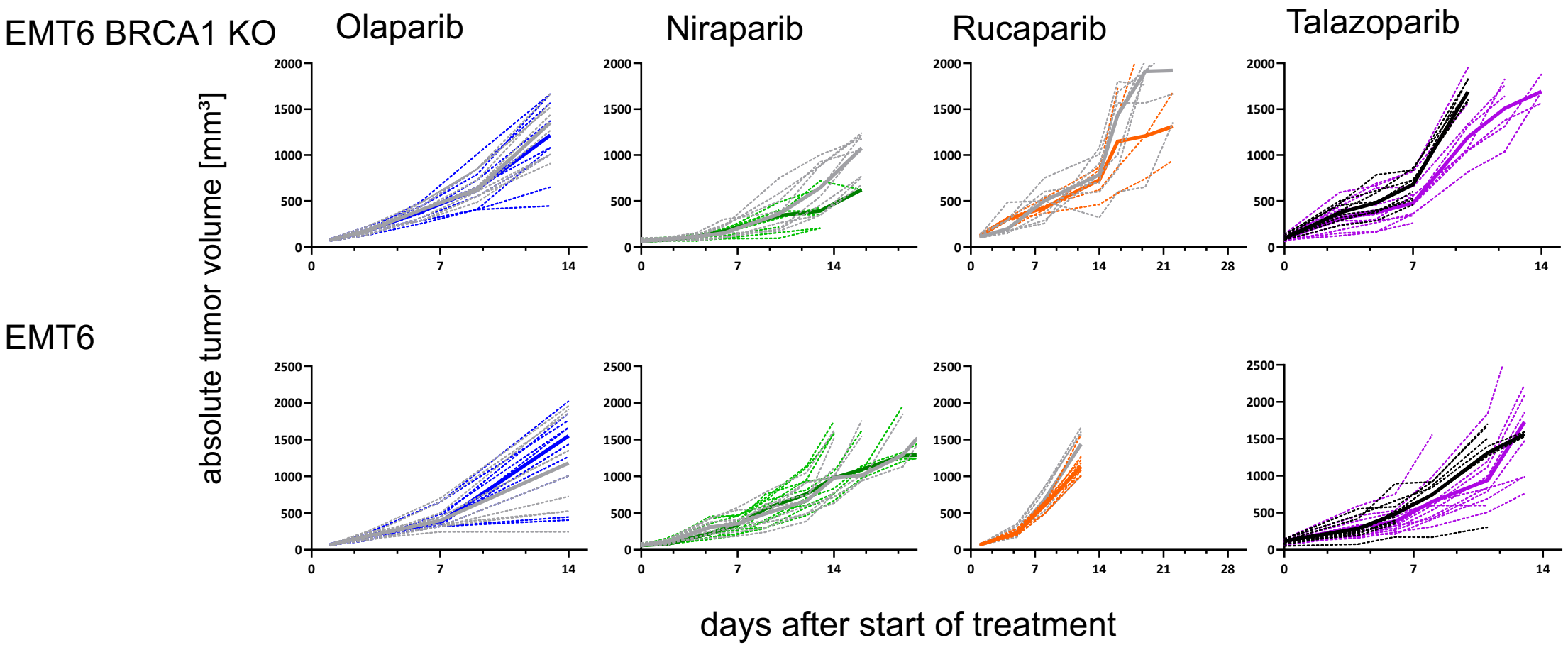
Agent	Dose [mg/kg]	Route	Schedule [d]
vehicle	10 ml/kg	po	0-21
Rucaparib	150	po	0,7,14,21
Talazoparib	0,3	po	0-21
Olaparib	50	po	0-21
Niraparib	100	po	0-21
anti-CTLA-4	5	ip	0,3,6
anti-PD1	5	ip	4,8,11,15



Read out:

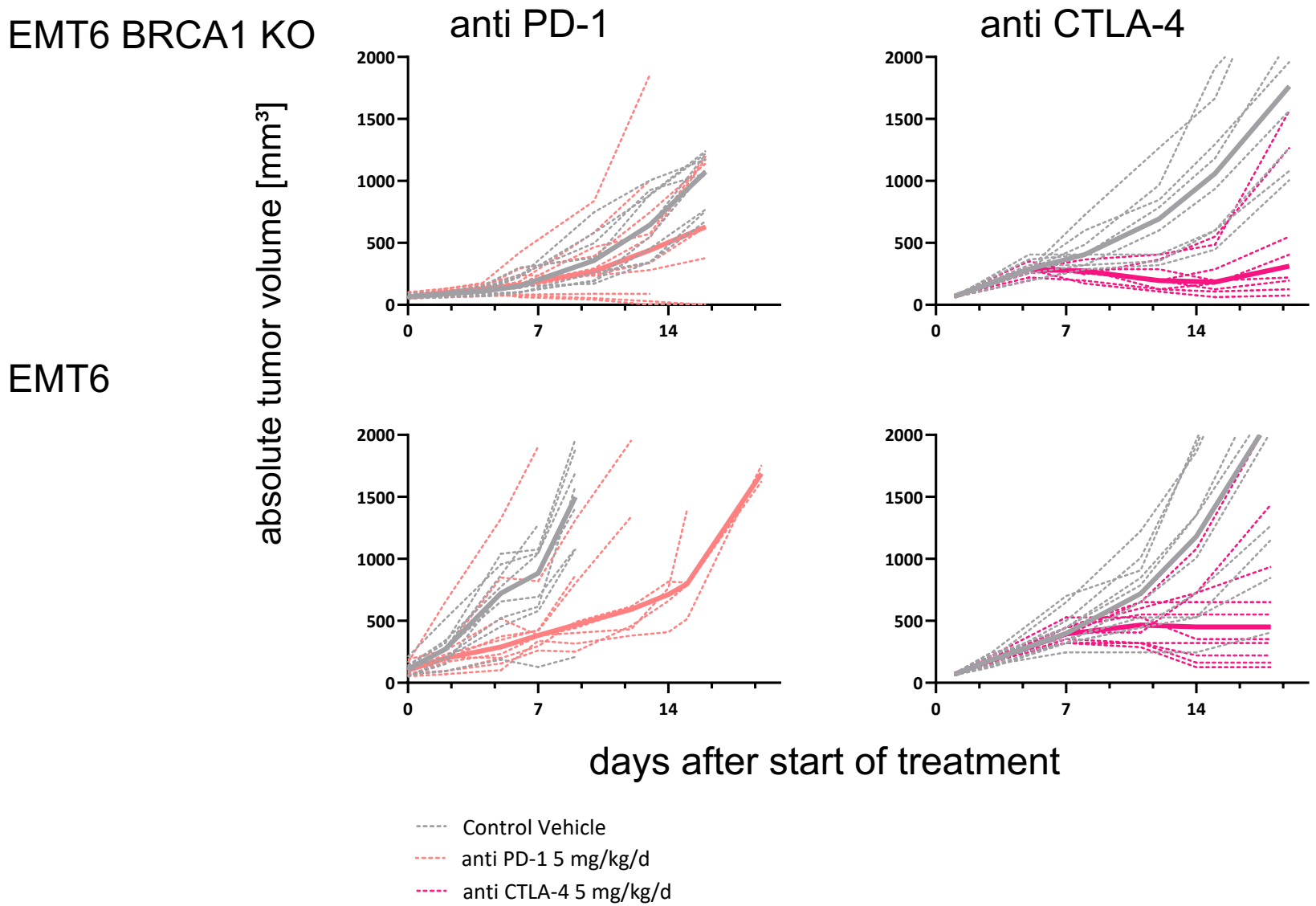
- Tumor volume over time
- TIL analysis at end point
- Cytokine analysis in serum under treatment

Sensitivity towards PARPi in monotherapy



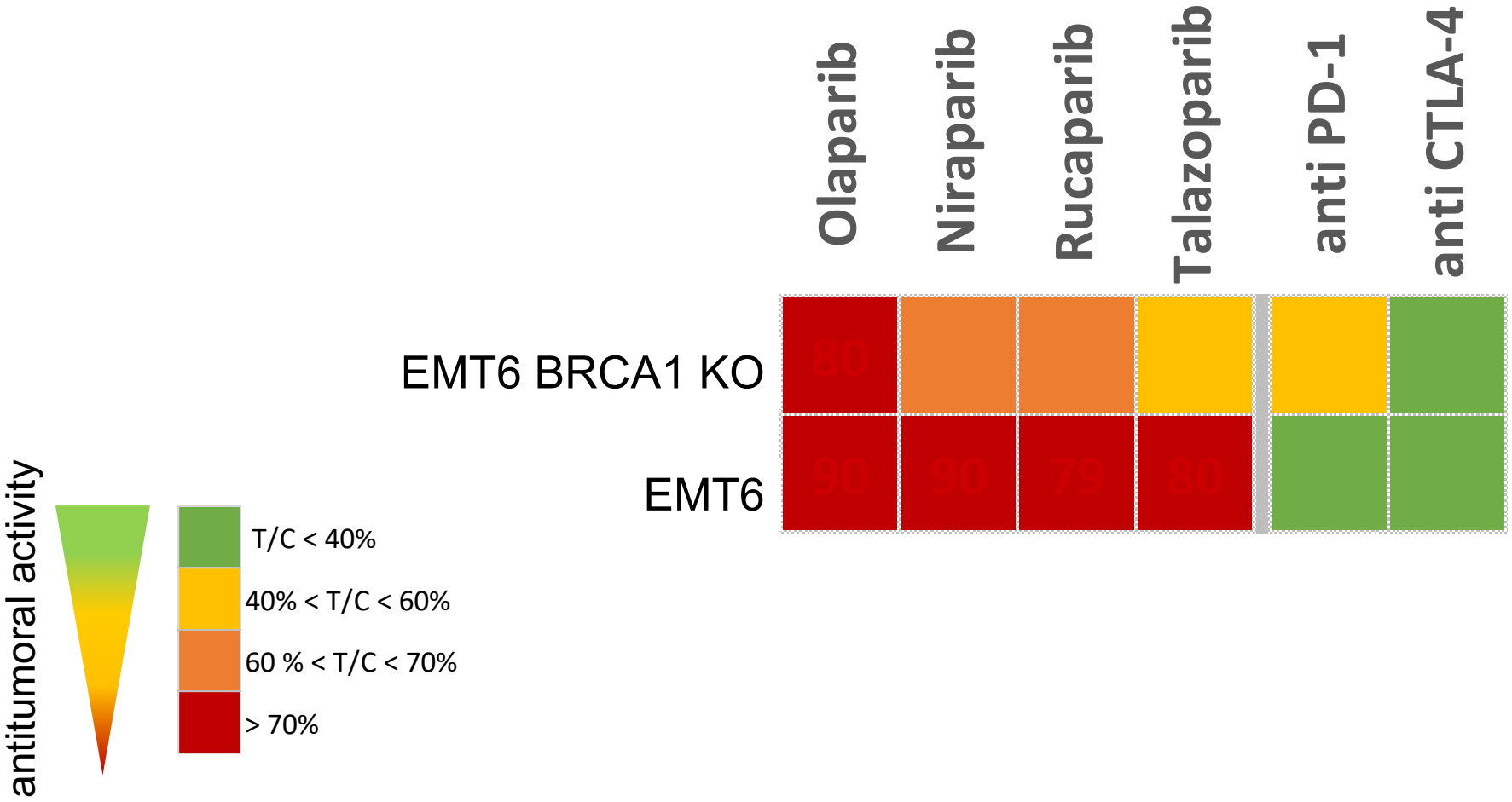
charles river

Sensitivity towards CPi in monotherapy



charles river

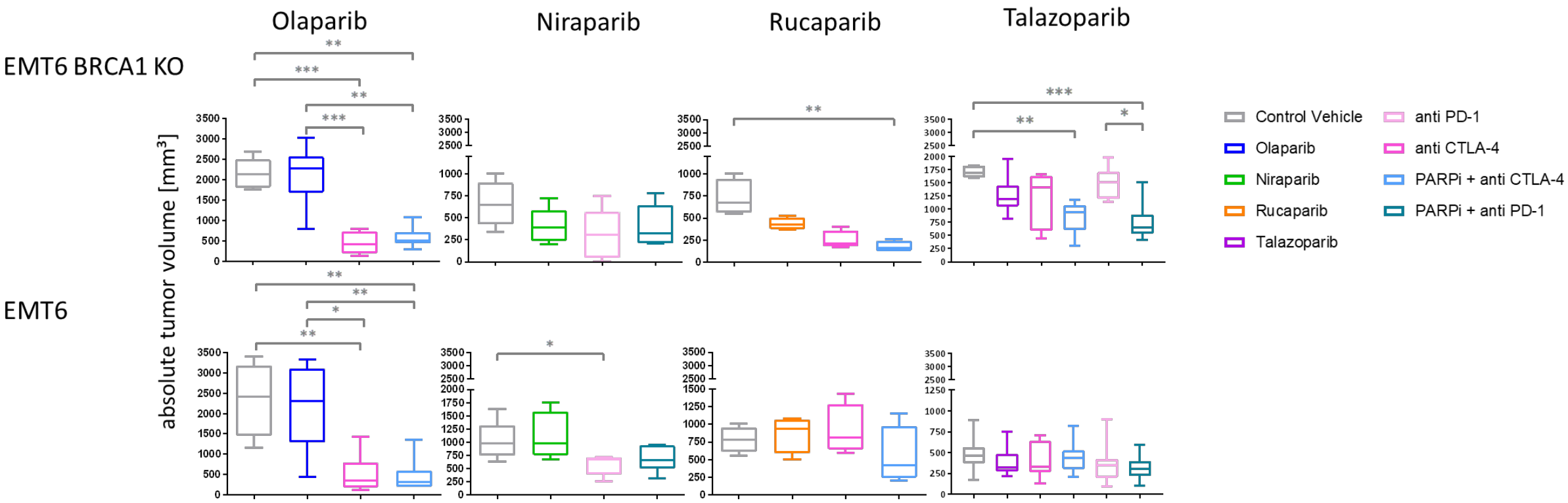
Overview monotherapy



charles river

Combination therapy PARPi and CPi

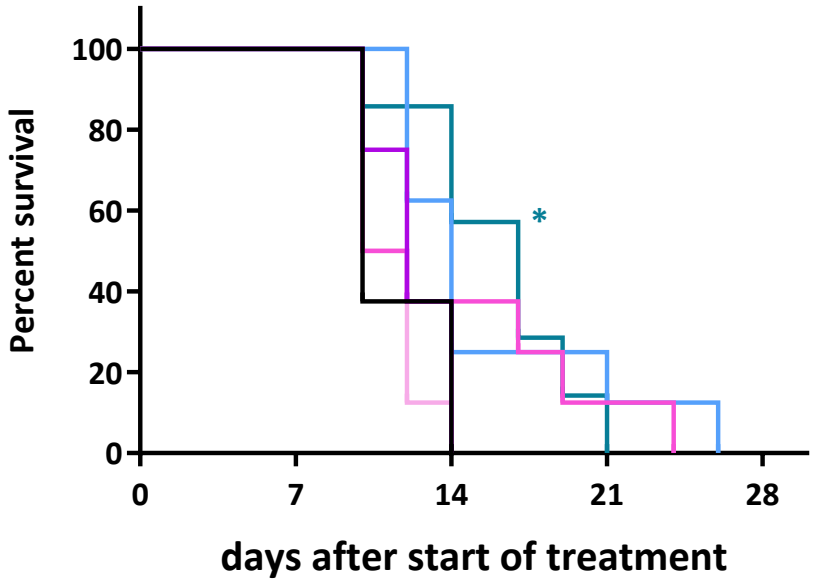
Comparison of PARP inhibitors in absence and presence of a BRCA1 KO



charles river

Combination therapy with talazoparib

Overall survival in EMT6 BRCA1 KO



Control Vehicle
Talazoparib
anti CTLA-4
Talazoparib + anti-CTLA-4
anti PD-1
Talazoparib + anti PD-1

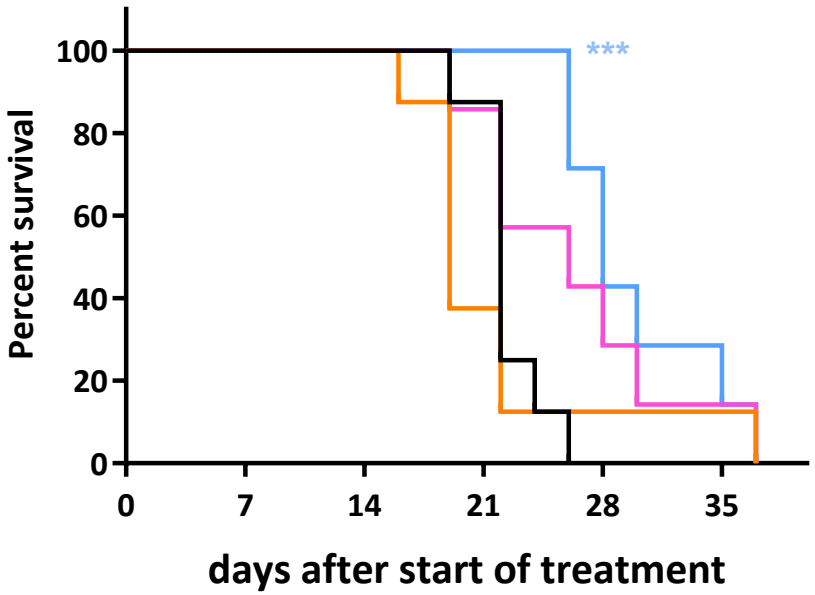
	Control Vehicle	Talazoparib	anti CTLA-4	Talazoparib + anti-CTLA-4	anti PD-1	Talazoparib + anti PD-1
Median survival in days	10	12	11	14	11	**17**

*p< 0.011; Log-rank test

charles river

Combination therapy with Rucaparib

Overall survival in EMT6 BRCA1 KO



Control Vehicle
Rucaparib
anti CTLA-4
Rucaparib + anti CTLA-4

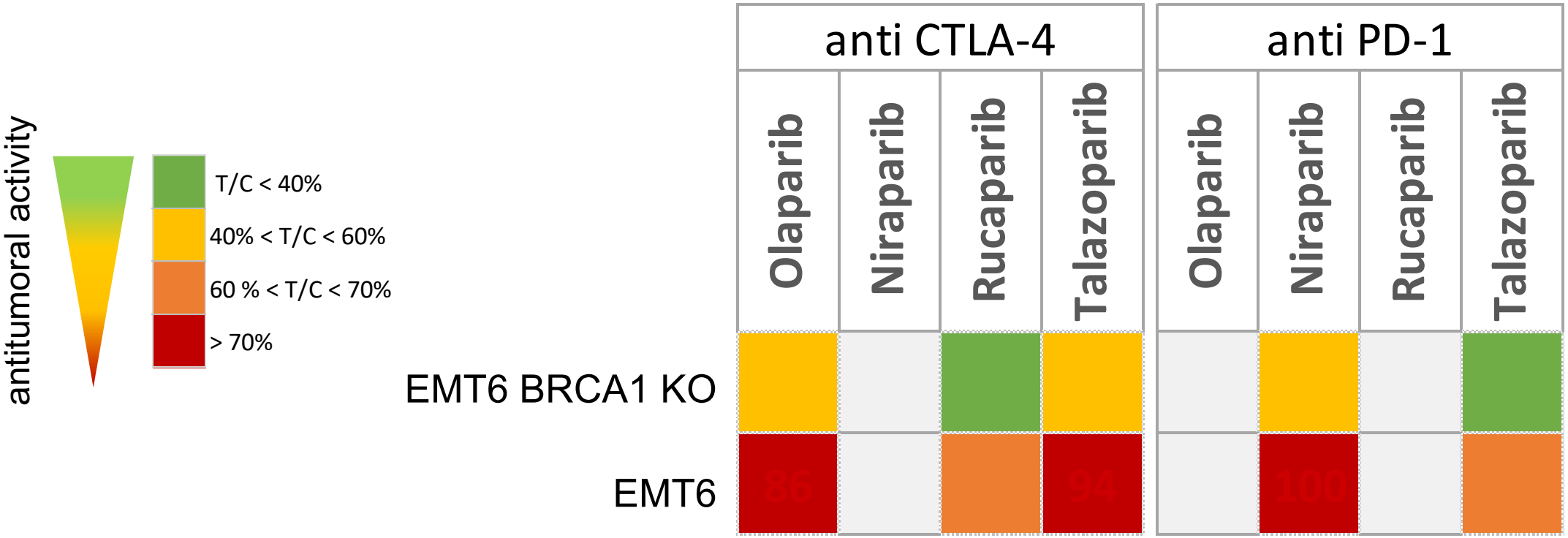
	Control Vehicle	Rucaparib	anti CTLA-4	Rucaparib + anti CTLA-4
Median survival in days	22	19	26	**28**

***p< 0.004; Log-rank test

charles river

Overview combination therapy

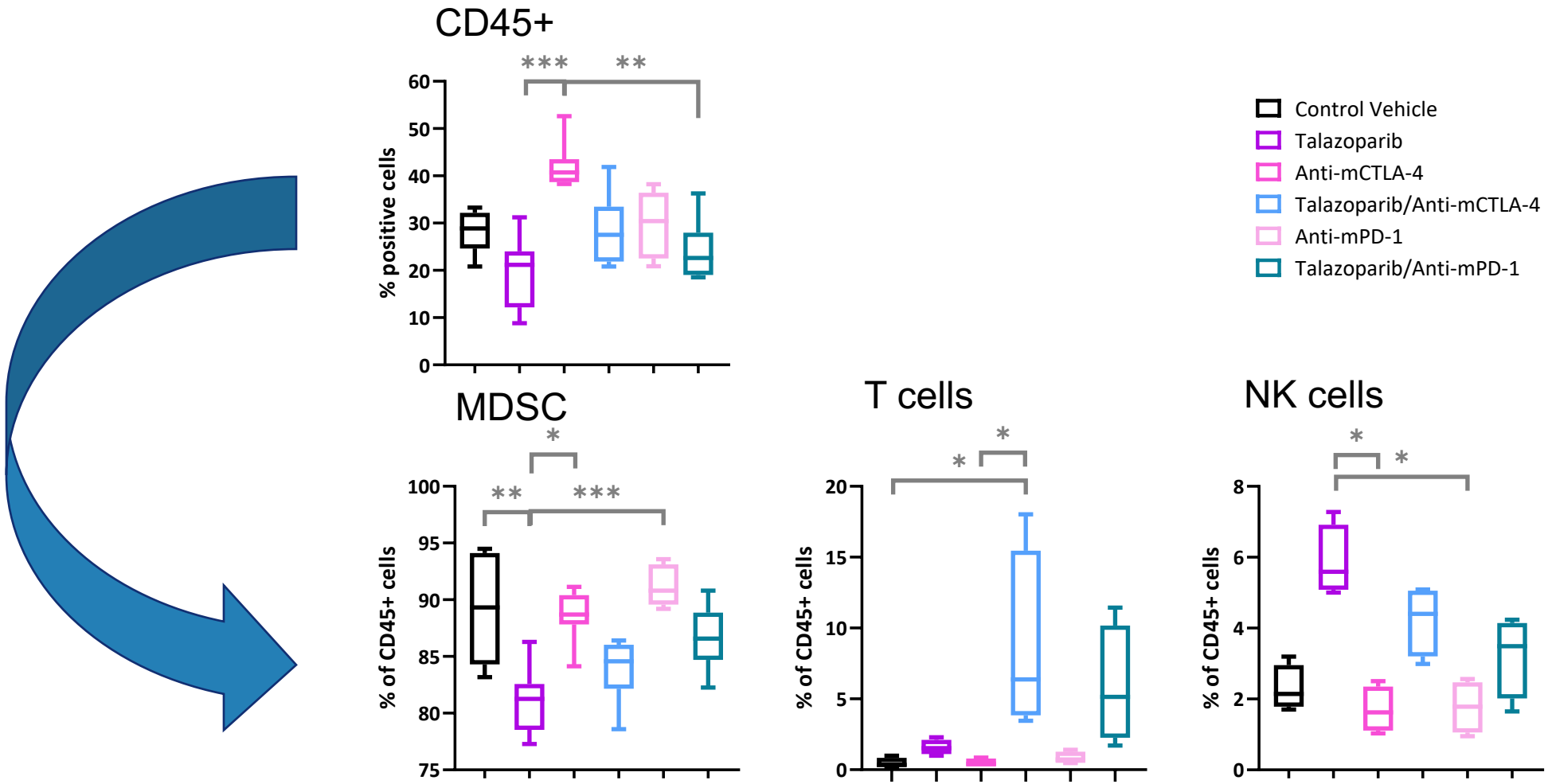
PARPi and checkpoint inhibitors in a syngeneic breast cancer model



charles river

TIL analysis of EMT6 BRCA1 KO

Influence of different treatments on composition of diverse TIL subpopulations

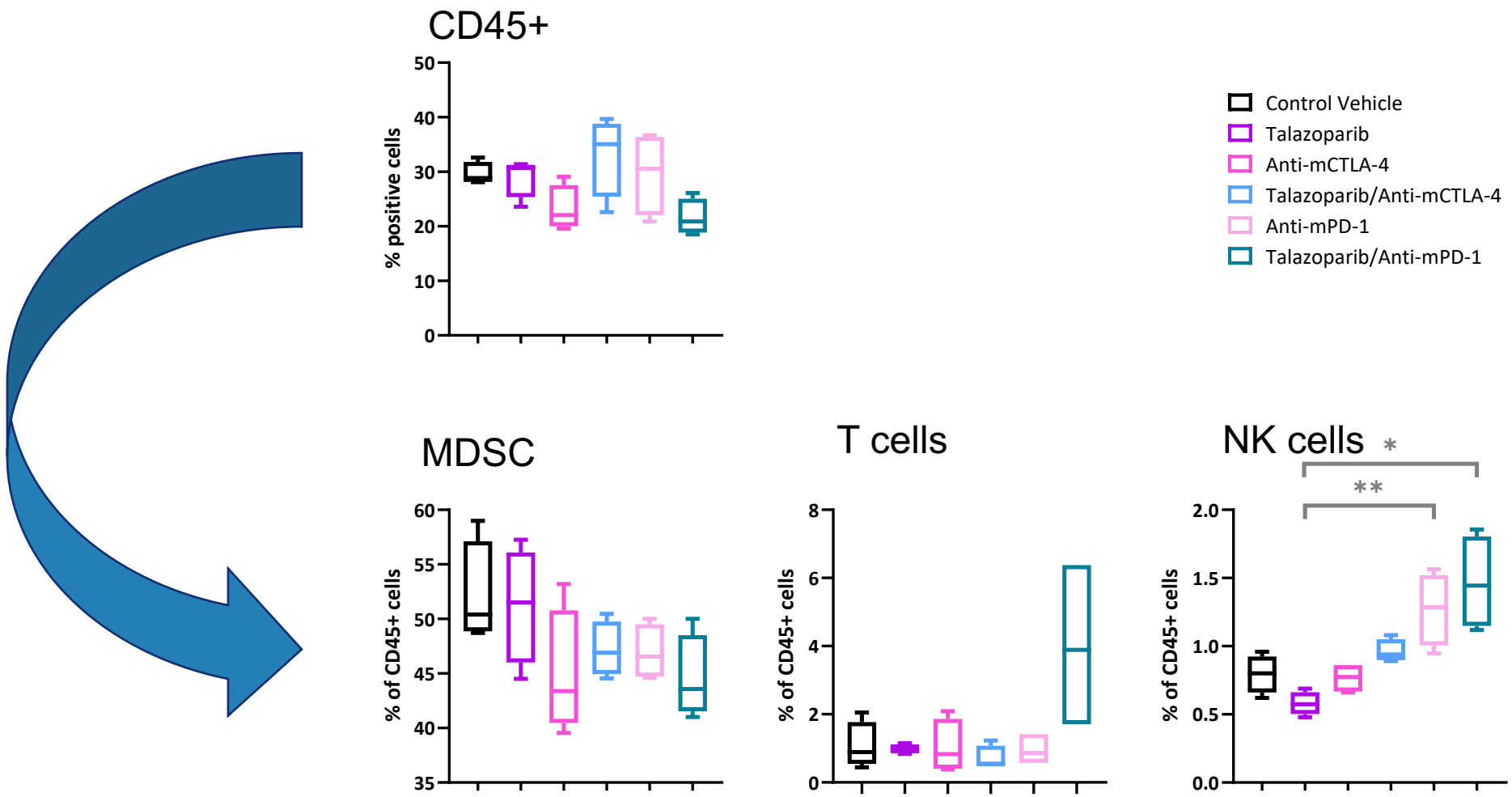


Kruskal-Wallis test

charles river

TIL analysis of EMT6

Influence of different treatments on composition of diverse TIL subpopulations

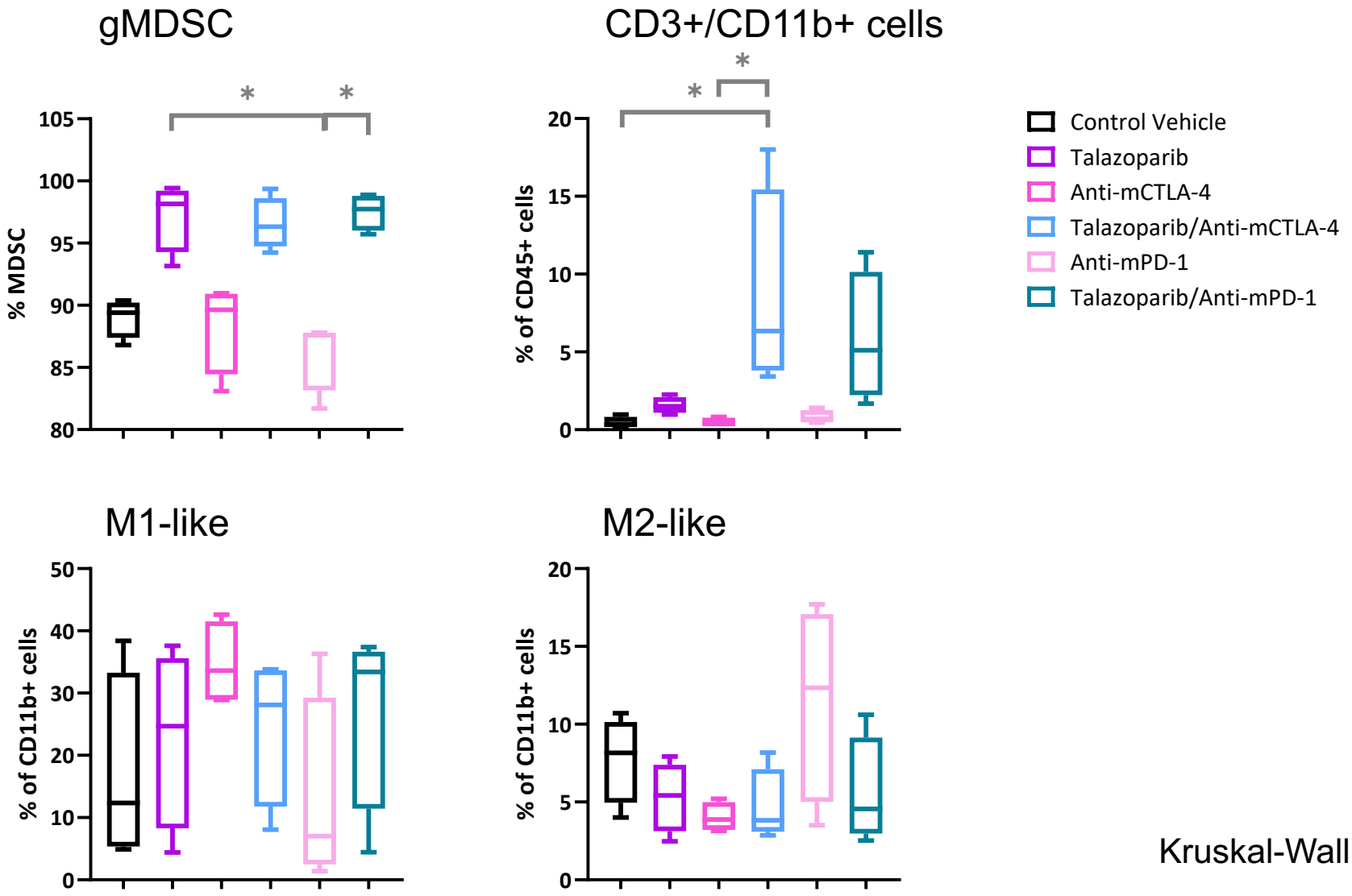


Kruskal-Wallis test

charles river

TIL analysis of EMT6 BRCA1 KO

Further subtyping of TILs under treatment

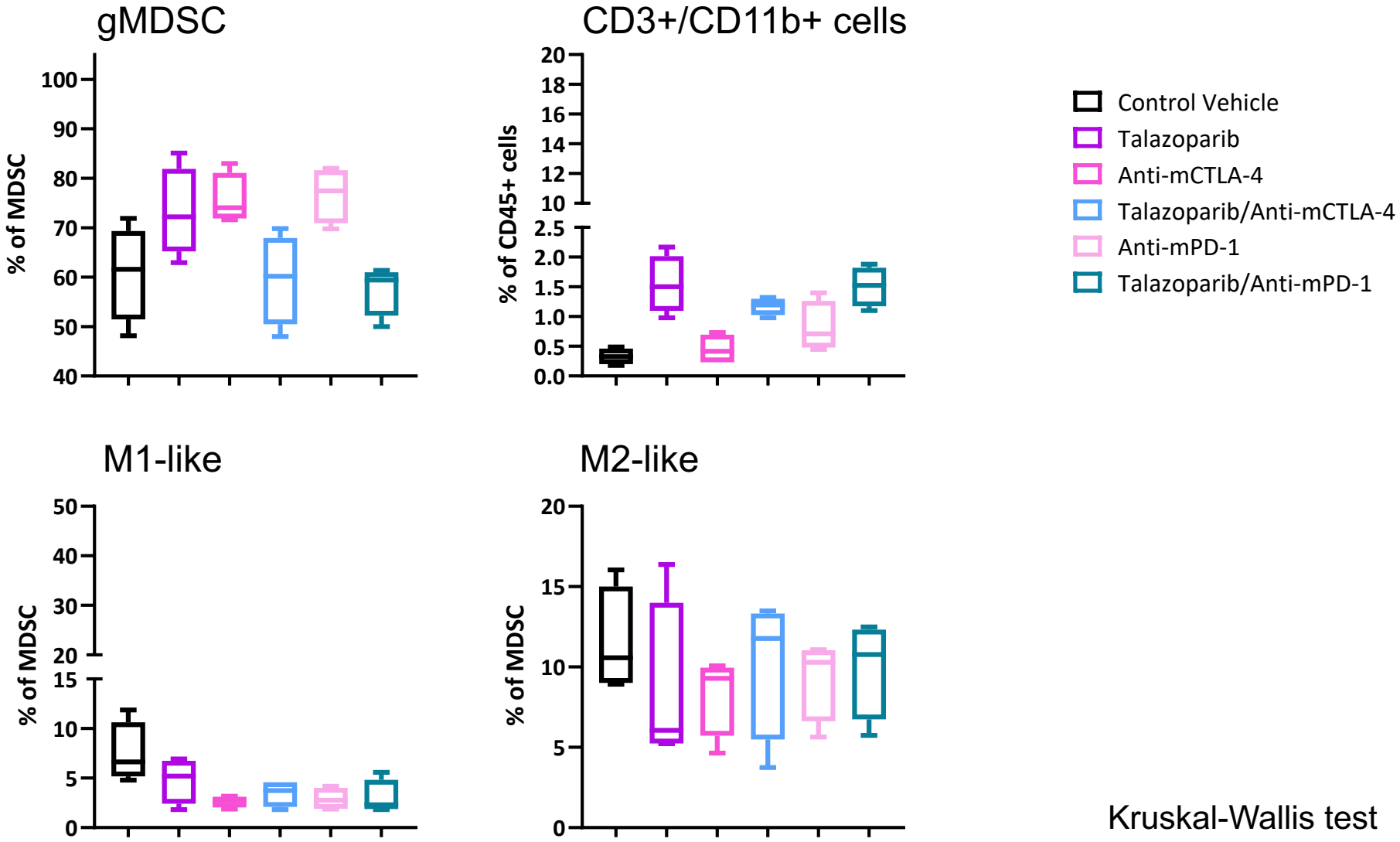


Kruskal-Wallis test

charles river

TIL analysis of EMT6

Further subtyping of TILs under treatment

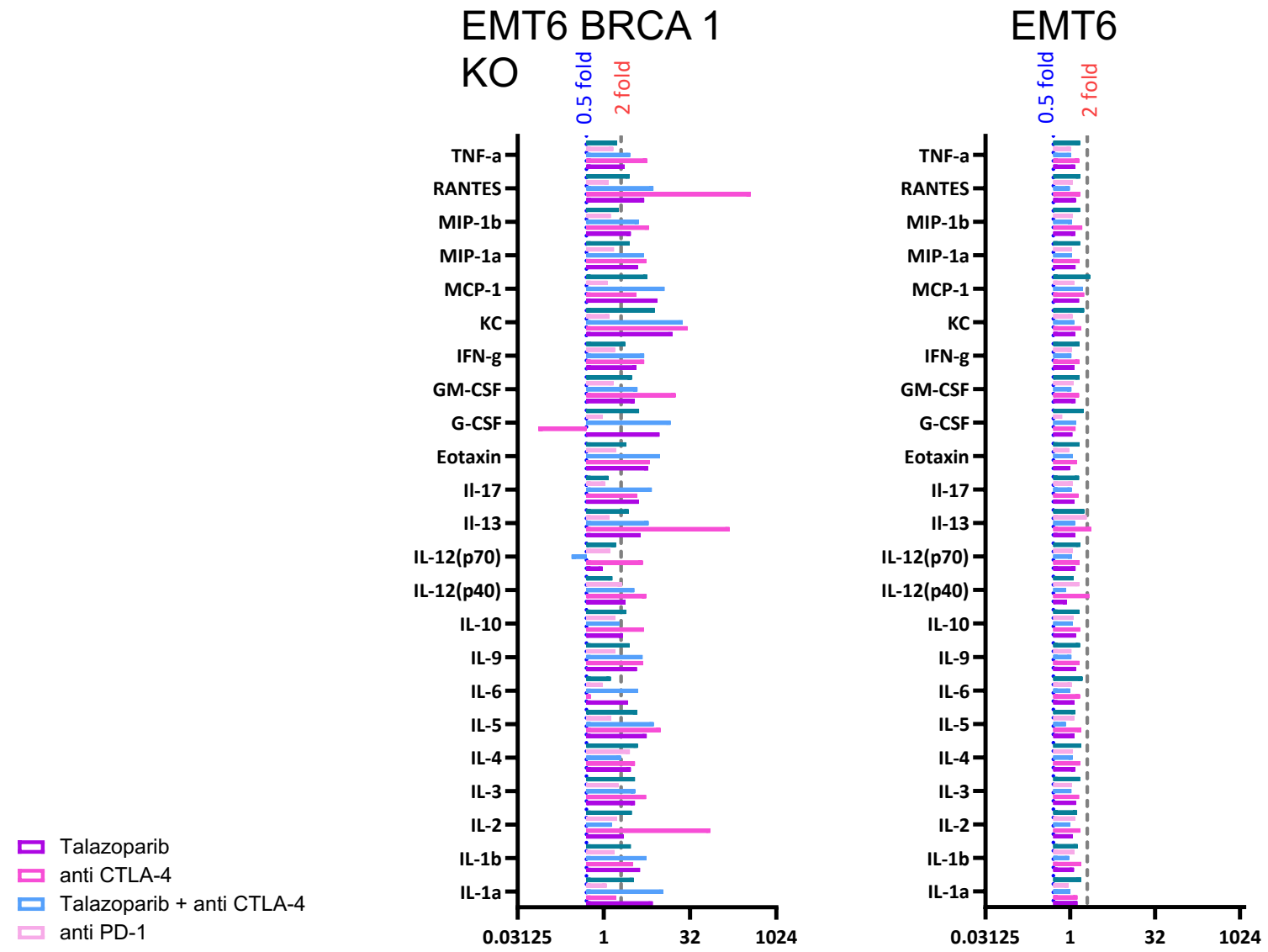


Kruskal-Wallis test

charles river

Cytokine secretion under therapy

EMT6 and EMT6 BRCA1 KO show distinct cytokine profile under therapy



charles river

Conclusion

- Talazoparib was the most active compound in the EMT6 BRCA1 KO model, followed by Niraparib and Rucaparib. Olaparib was considered inactive.
- The EMT6 model was resistant against all tested PARPi.
- The EMT6 BRCA1 KO model turned out to be sensitive towards anti CTLA-4 treatment but showed mild tumor growth delay under anti PD-1 treatment in monotherapy.
- The EMT6 model was sensitive towards both checkpoint inhibitor treatments.
- Combination therapy was more effective in all tested settings. However, Rucaparib + anti CTLA-4 as well as Talazoparib + anti-PD-1 induced a significant prolongation of the life span of the treated animals.
- TIL analysis revealed that significant differences under different treatment regimen specifically in the EMT6 BRCA1 KO model.
- The secreted cytokine profile supported the TIL data by upregulation of multiple pro-inflammatory cytokines specifically in the EMT6 BRCA1 KO line. In contrast, none of the treatment regimen had a major impact on the cytokine profile of EMT6 bearing mice.

summary

- *In vivo* models in oncology are a key component for the drug discovery process.
- No model is a perfect fit throughout the drug development workflow.
- Each scientific question can be addressed with a specific *in vivo* model.
- The introduction of genome editing technologies such as the CRISPR/Cas9 system reduced cost and time for the generation of a broad range of preclinical models.

In the end

The choice of your model depends on the scientific question

The quality of the results is indispensably related to the quality of your experiment

The read-out and interpretation of your data must withstand clinical requirements

"Data don't make any sense,
we will have to resort to statistics."

charles river