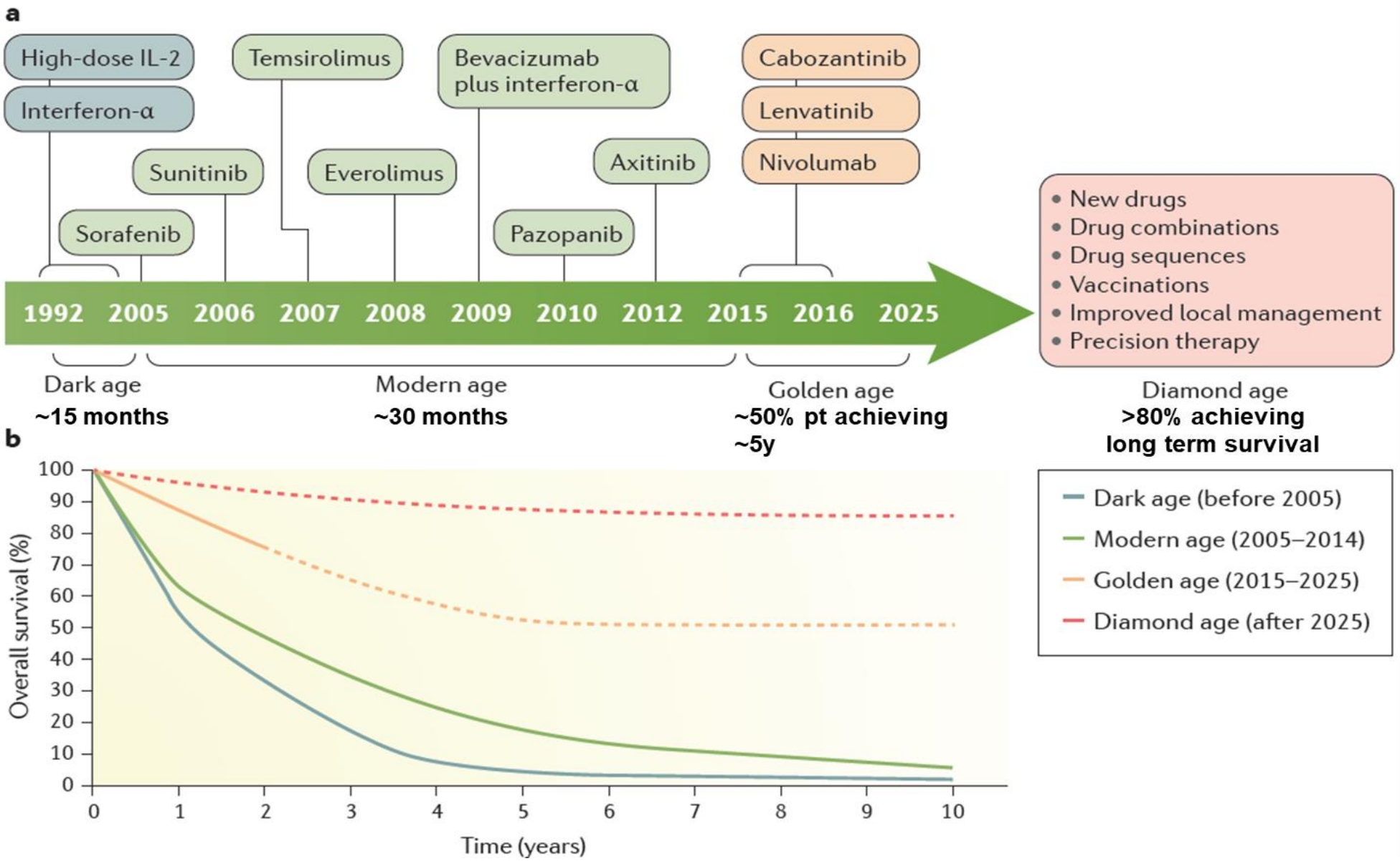
THE PROSTATE CENTRE
AT VANCOUVER GENERAL HOSPITAL

Department of
UROLOGIC SCIENCES
UBC

Translational Research Strategies to Understand Treatment Resistance in Renal Cell Carcinoma

Alan So, MD FRCSC
Department of Urologic Sciences
University of British Columbia
Chair, GU Tumour Group, BC Cancer
Head, Clinical Trials Unit Vancouver Prostate Centre
Fellowship Director, Uro-oncology Program

Therapeutic evolution and survival outcome of metastatic clear cell renal cell carcinoma (mRCC)



Hsieh et al, Nature Rev Dis Pri, 2017

Durable Responses to VEGF-Targeted Therapies Are Rare

Outcome	Sunitinib[1]	Sorafenib[2]	Pazopanib[3]	Bevacizumab/IFN[4]
mPFS, mo	11	5.5	9.2	10.2
ORR, %	47	10	30	31
CR, %	3	<1	<1	1
PR, %	44	10	30	30
SD, %	40	74	38	46
PD, %	7	12	18	20

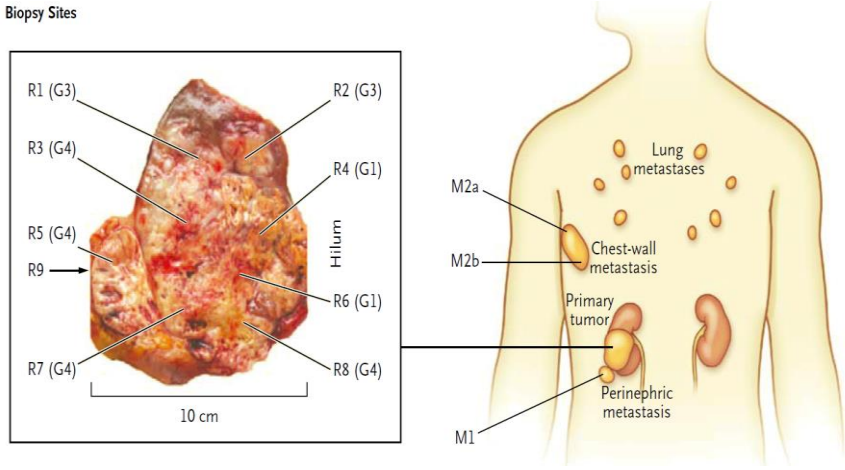
1. Motzer RJ et al. *J Clin Oncol.* 2009;27:3584-90.
2. Escudier B et al. *N Engl J Med.* 2007;356:125-134.
3. Sternberg C et al. *J Clin Oncol.* 2010;28:1061-1068.
4. Escudier B et al. *Lancet.* 2007;370:2103-2111.

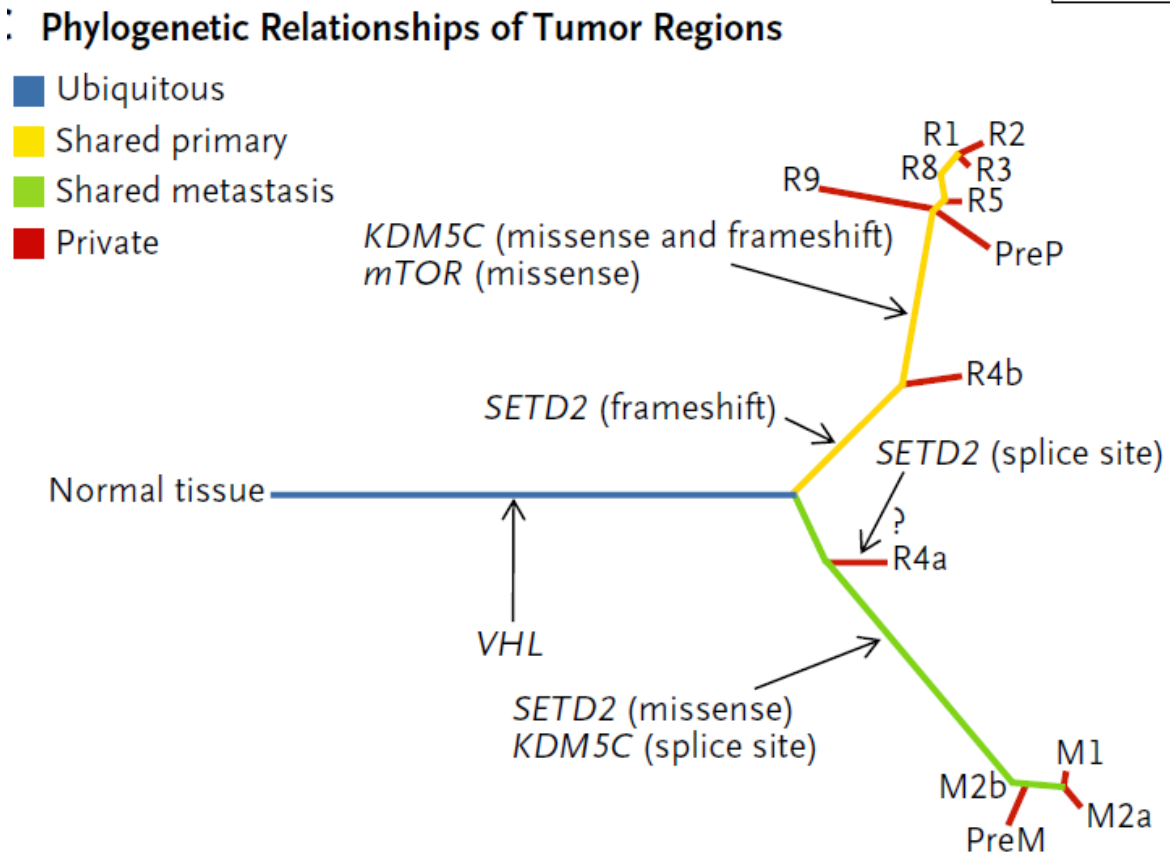
The NEW ENGLAND JOURNAL of MEDICINE

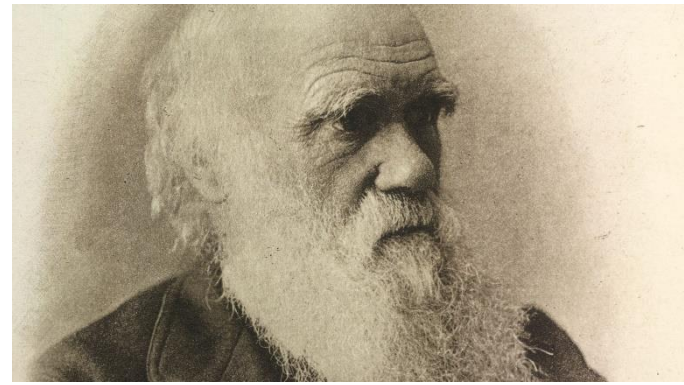
ESTABLISHED IN 1812 MARCH 8, 2012 VOL. 366 NO. 10

Intratumor Heterogeneity and Branched Evolution Revealed by Multiregion Sequencing

Marco Gerlinger, M.D., Andrew J. Rowan, B.Sc., Stuart Horswell, M.Math., James Larkin, M.D., Ph.D.,



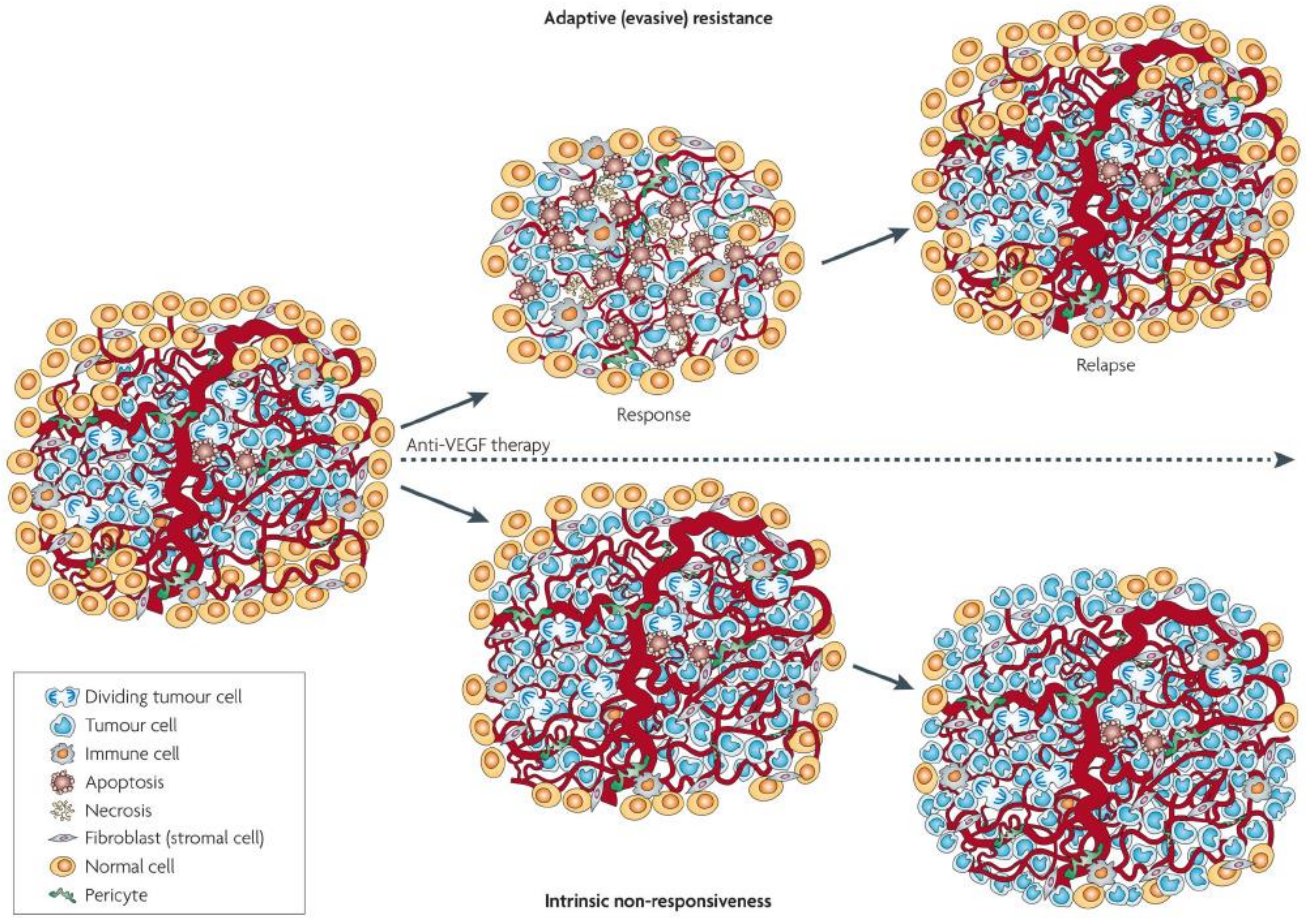




“Cancer is a process of genetic aberrations and clonal expansion subject to stochastic Darwinian selection within adaptive cellular ecosystems”

Miller et al, Clin Tran Med, 2017

Resistance to VEGF-Targeted Therapy: Adaptive vs Intrinsic Phenomenon

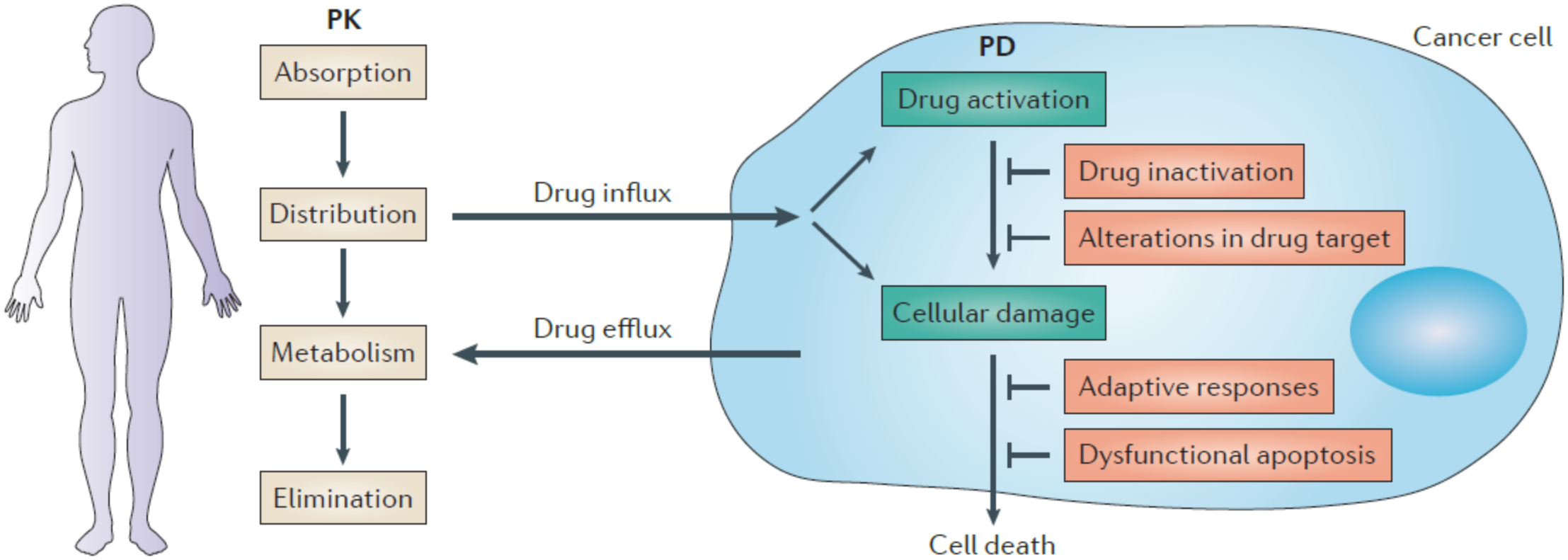


Adaptive resistance:
Progression after benefit represents an adaptive resistance to TKI therapy

Intrinsic resistance:
No response at all, immediate progression

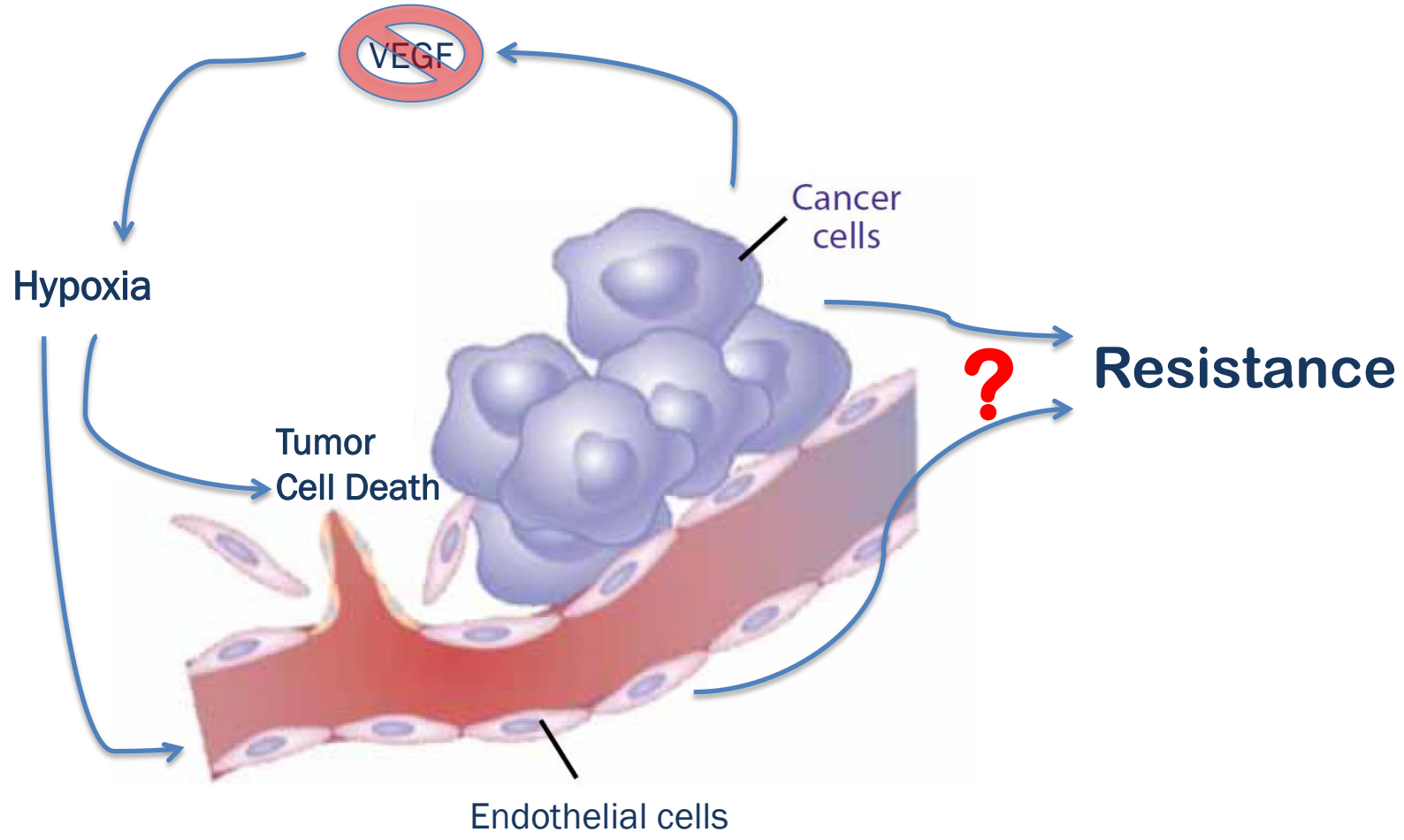
General principle of ‘adaptive’ drug resistance

Pharmacometrics



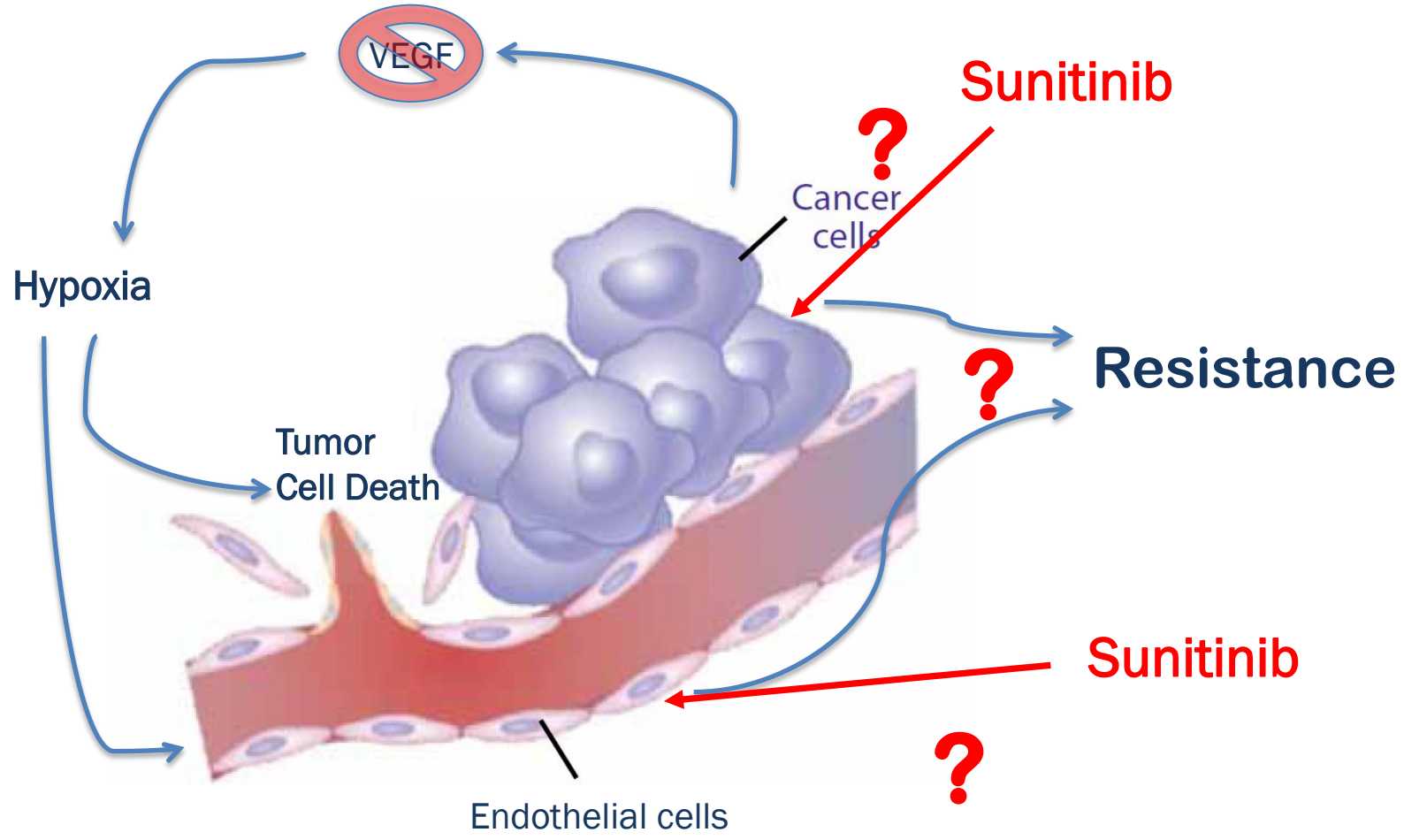
Cancer drug resistance: an evolving paradigm, Nature 2013

Treatment with Therapies Targeting Angiogenesis:



So 2019, Tamaskar 2011, Yao 2011, Loges 2010, Bergers 2008

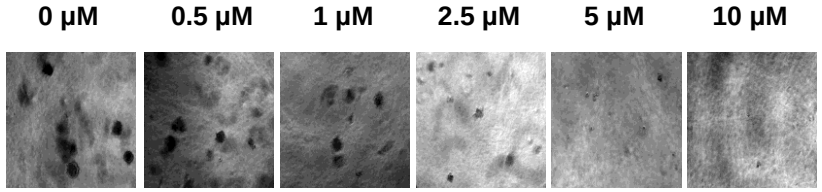
Treatment with Tyrosine Kinase Inhibitor



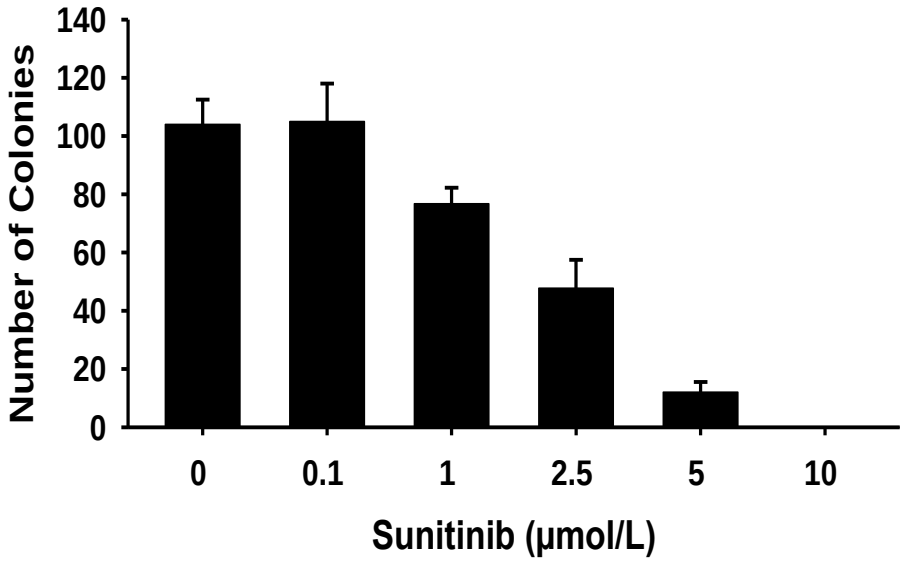
So 2019, Tamaskar 2011, Yao 2011, Loges 2010, Bergers 2008

In vitro effect of sunitinib- RCC vs Endothelium

RCC Cells

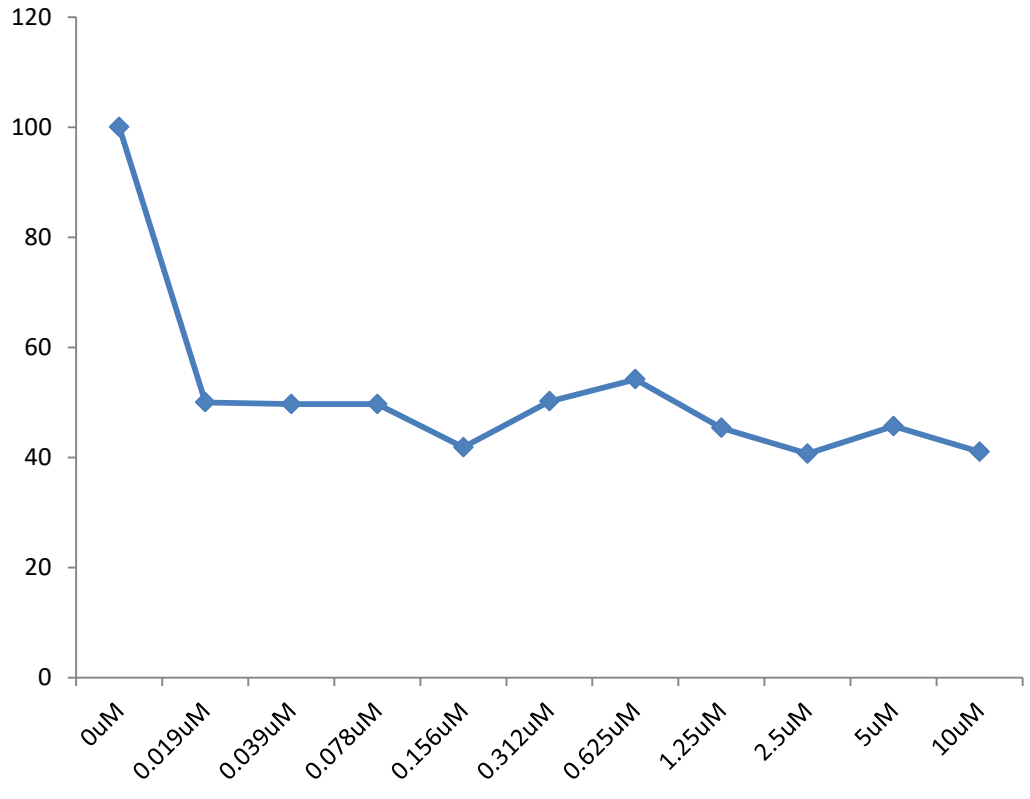


Anchorage-independent cell growth assay



CaKi-1 Cells

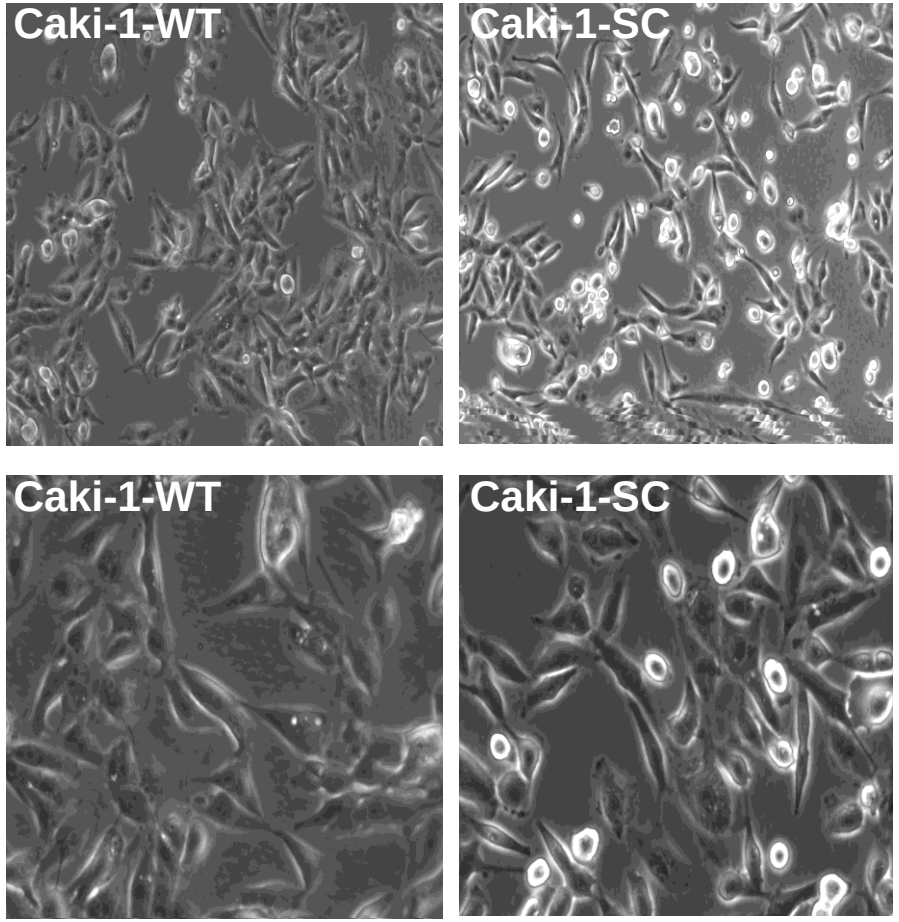
Endothelium

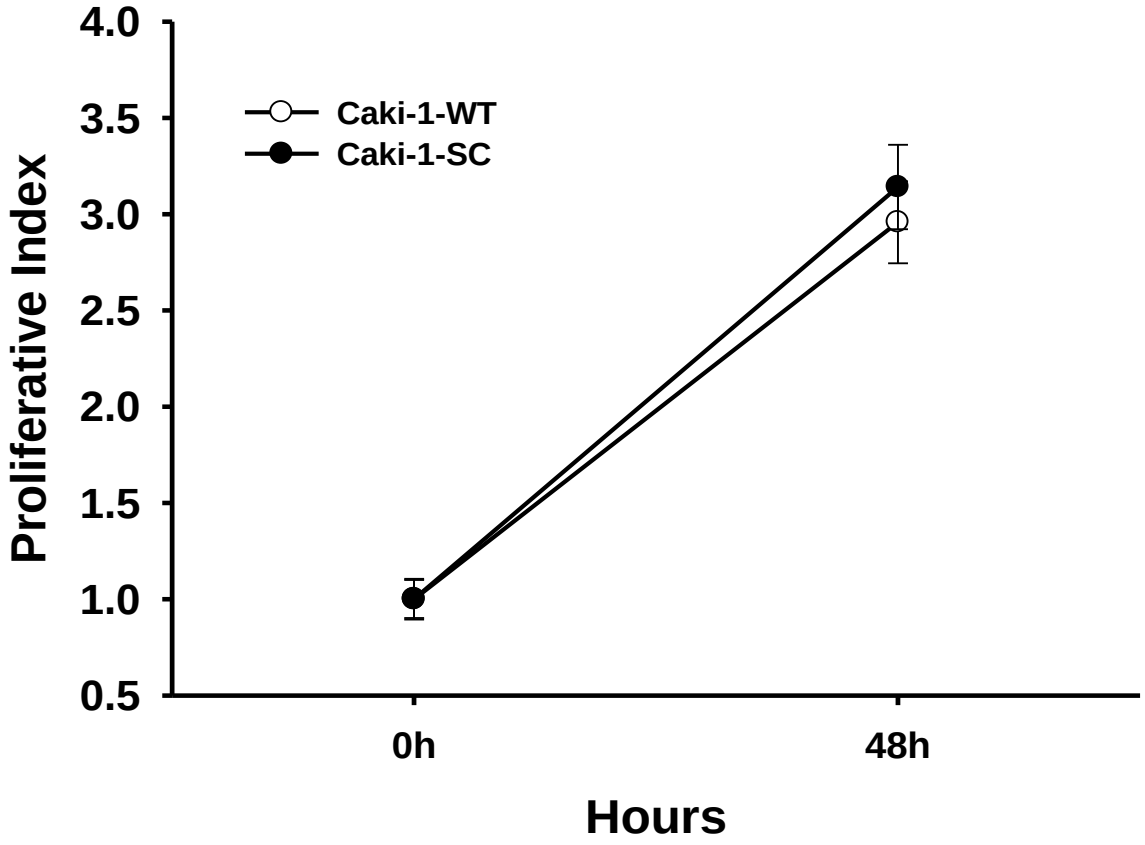


HUVEC Cells

Han KS., et al. Neoplasia (2015)

Development of sunitinib ‘resistant’ cell line

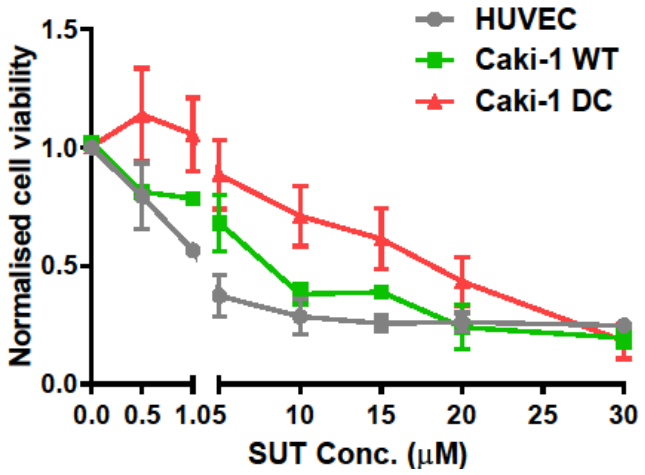




Han KS., et al. Neoplasia (2015)

Phenotypic differences between sunitinib-resistant and sensitive mRCC

A



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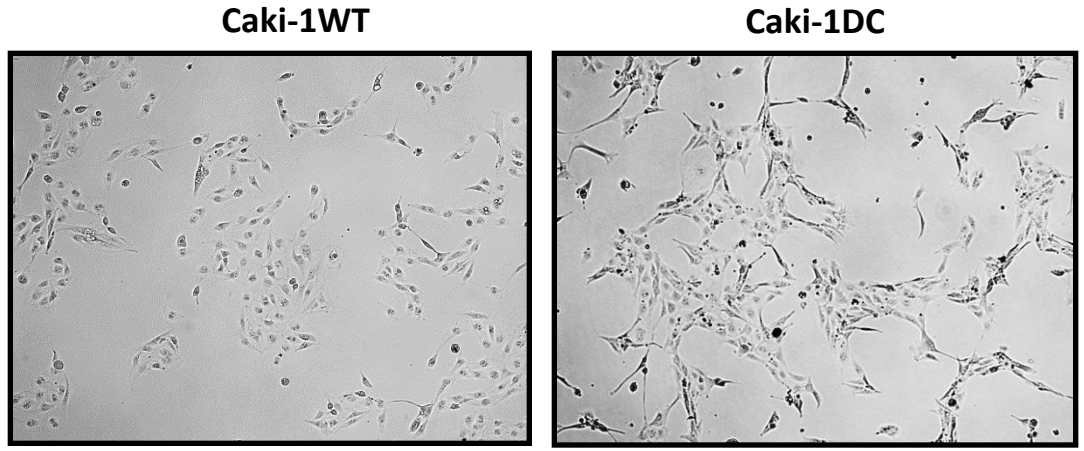

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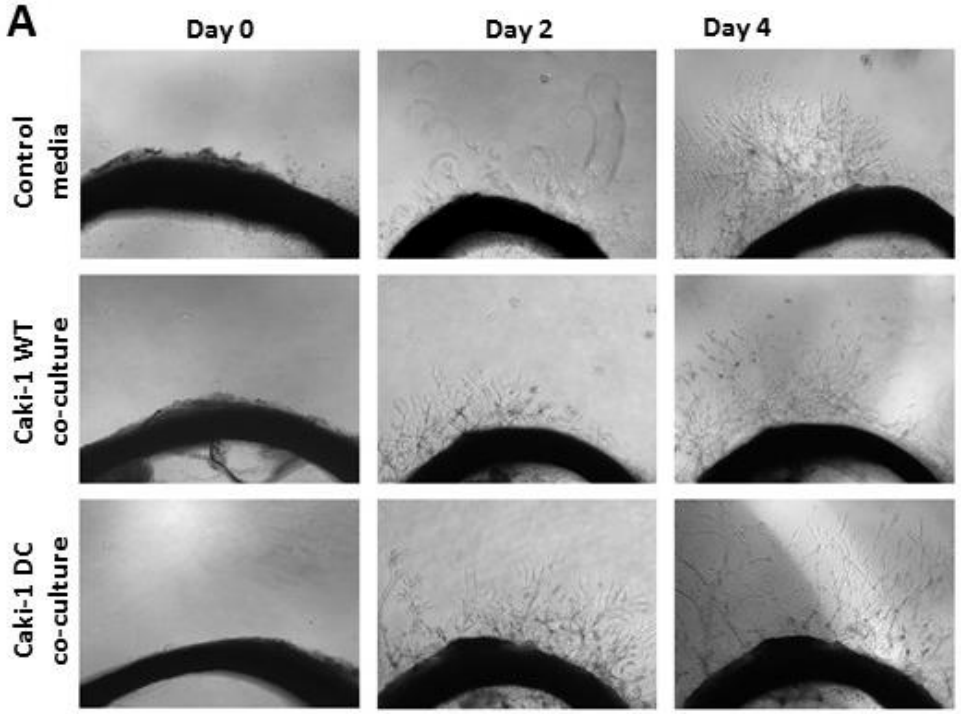

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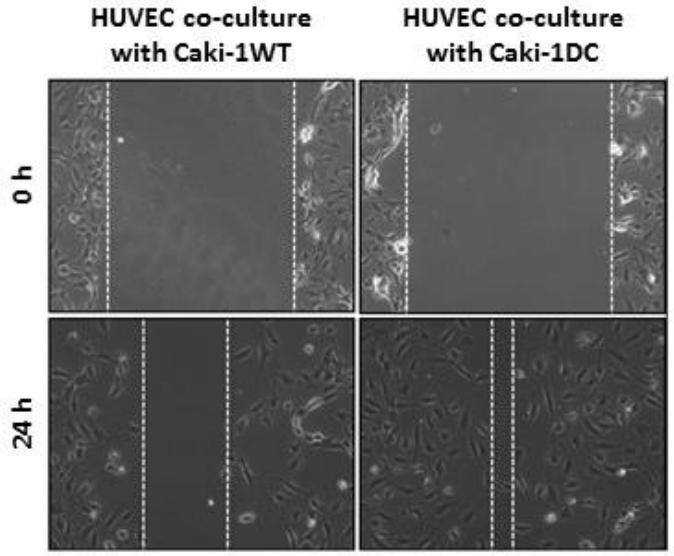
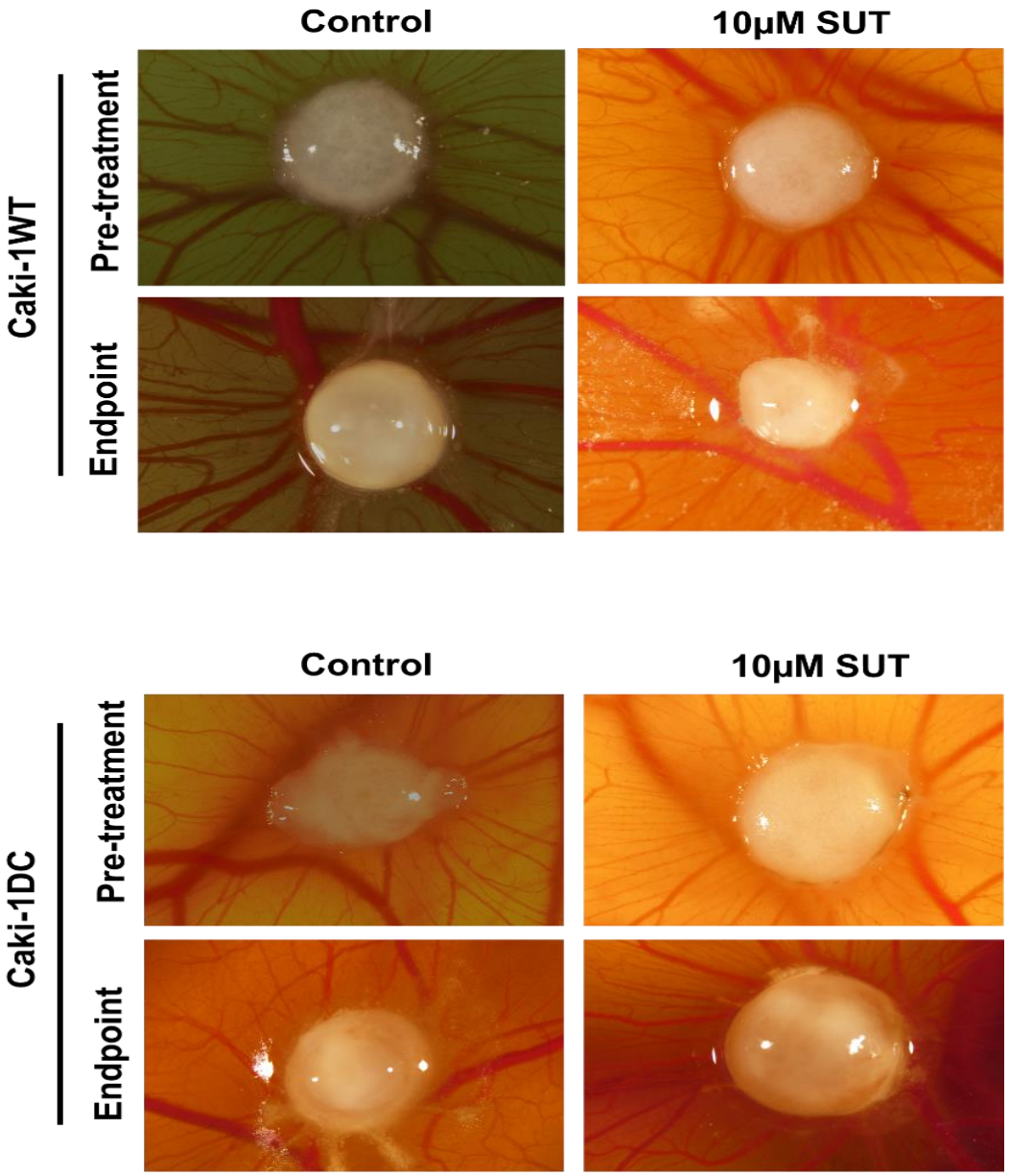
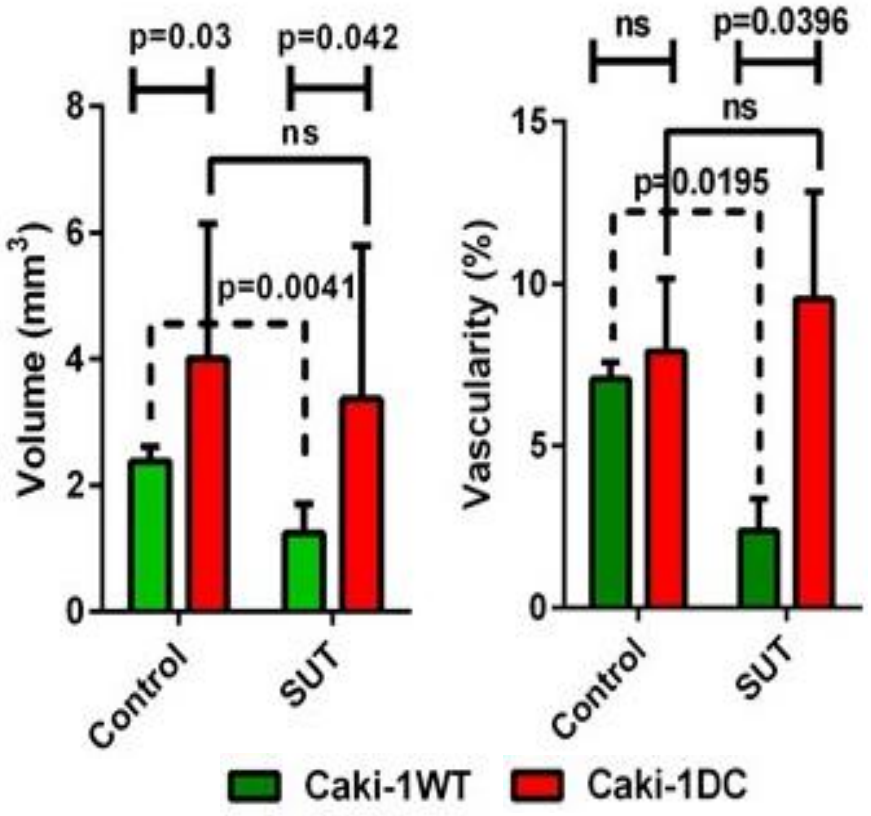
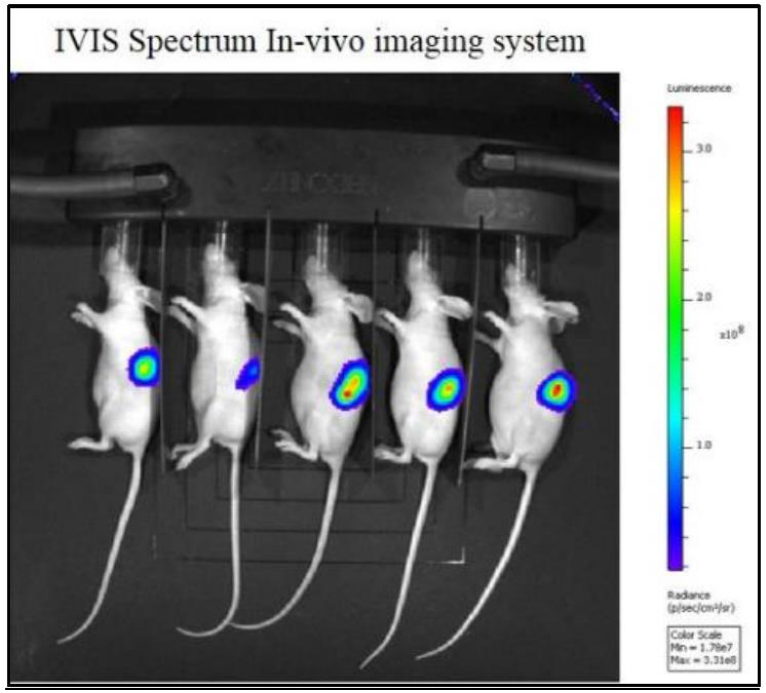


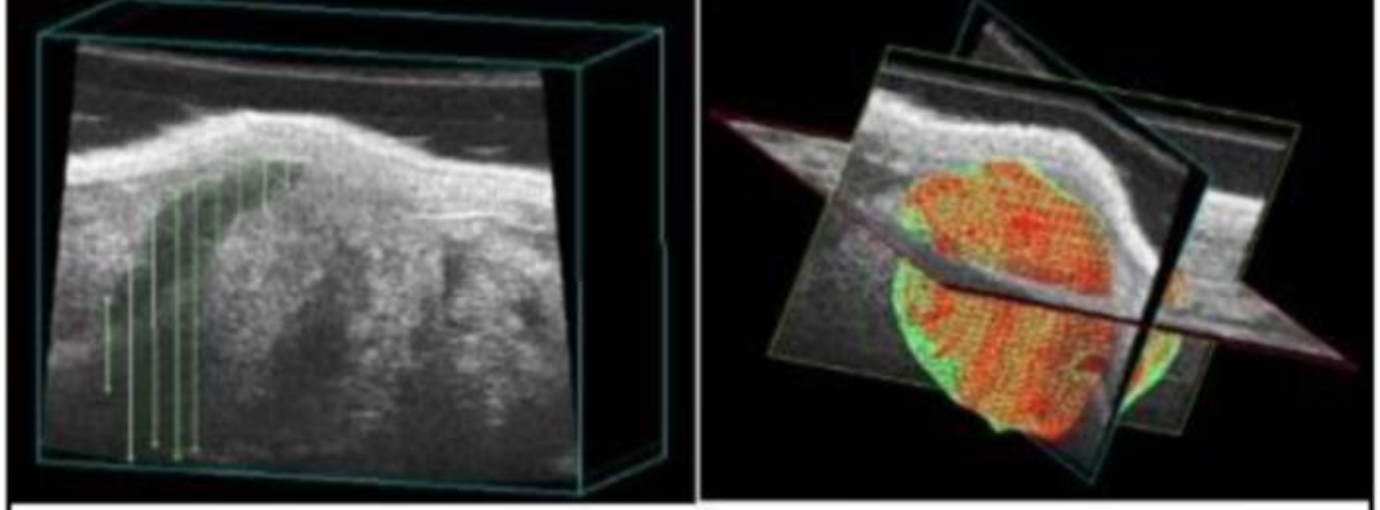
Phenotypic differences between sunitinib-resistant and sensitive mRCC

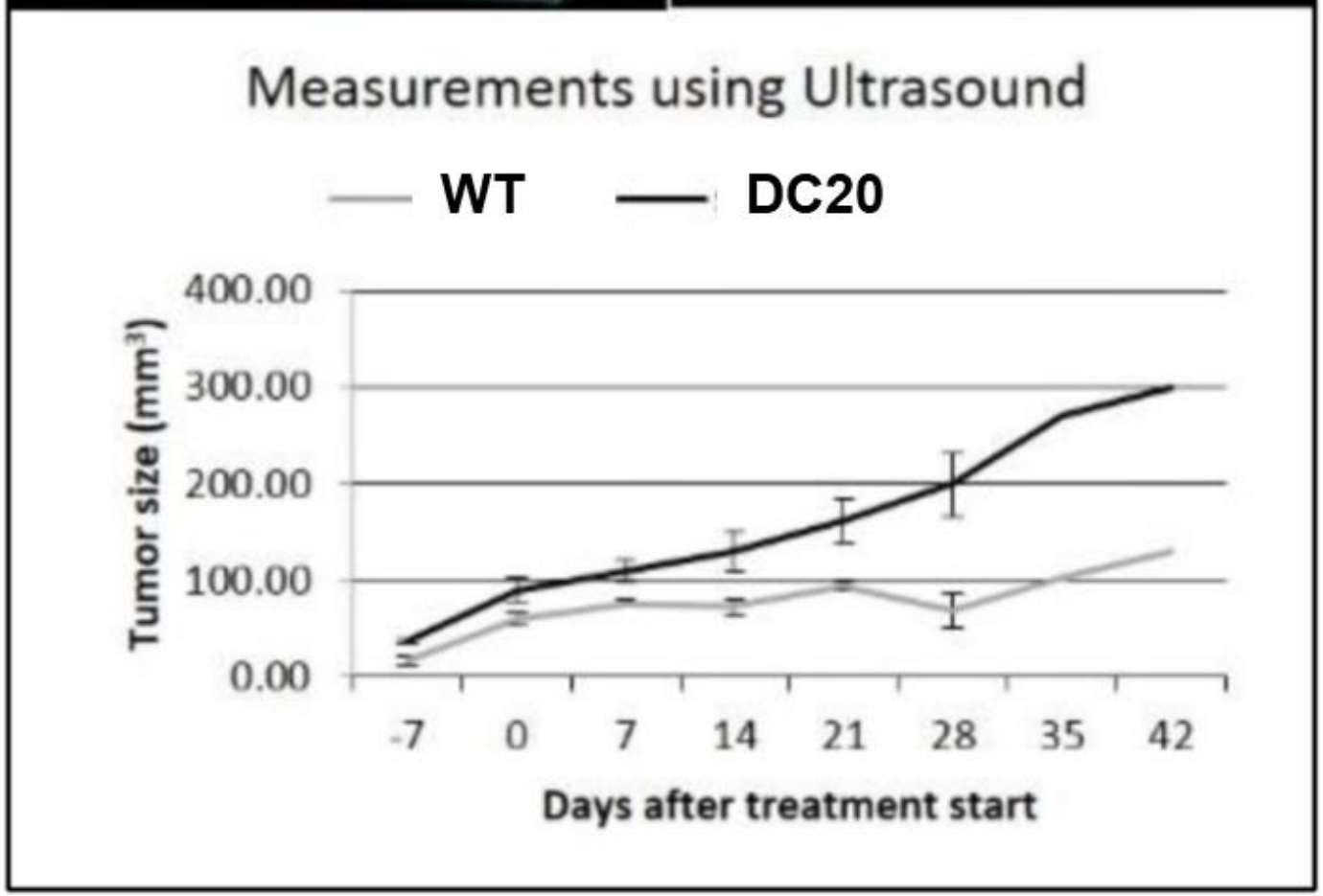






Sunitinib Tx: 60 mg/kg





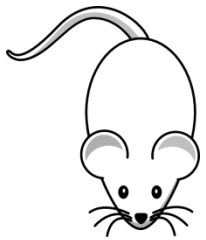
In vivo resistance model

Timeline

Drop out rate

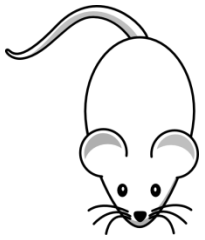
One week — **Ultrasound guided injection of CAKI-1 LUCP1 under renal capsule**

2-3 weeks — **When tumours reach about 0.5 cm^3 (day 21), mice will be randomly assigned (2:1) to receive the following: sunitinib 40 mg kg^{-1} daily and placebo**



10

sunitinib 40 mg kg^{-1} daily



5

placebo

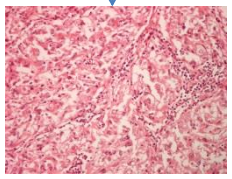
Draw blood and urine once a week to check for secreted factors (netrin-1, Reelin)

2 weeks before harvesting — **Order Mice – Adaption time**

Harvest when tumor are in exponential phase

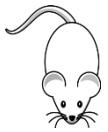
Fresh Frozen

Cell Culture



Formalin Fixed / TMA

Recycle most aggressive Tumors

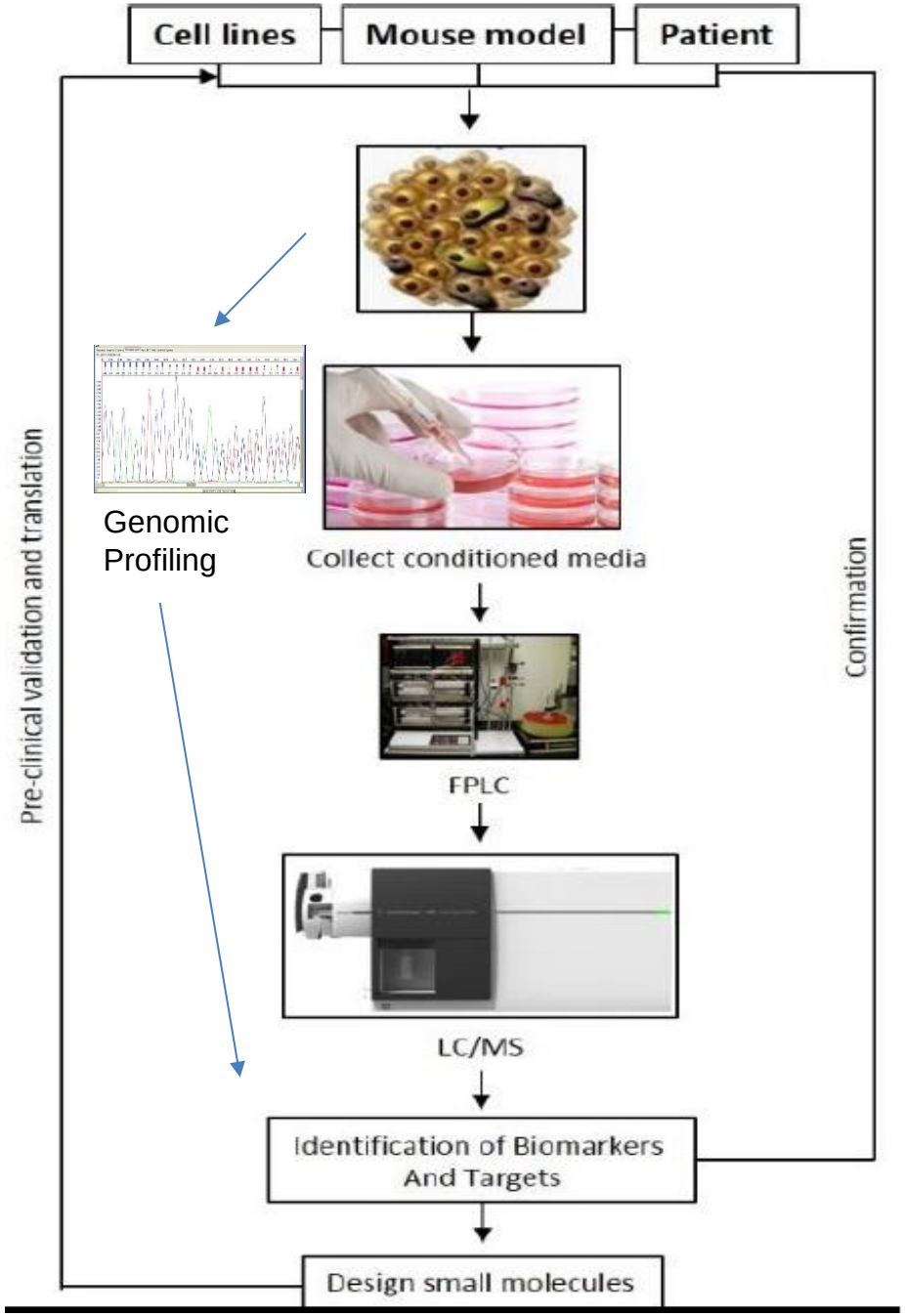


10

sunitinib 40 mg kg^{-1} daily

5

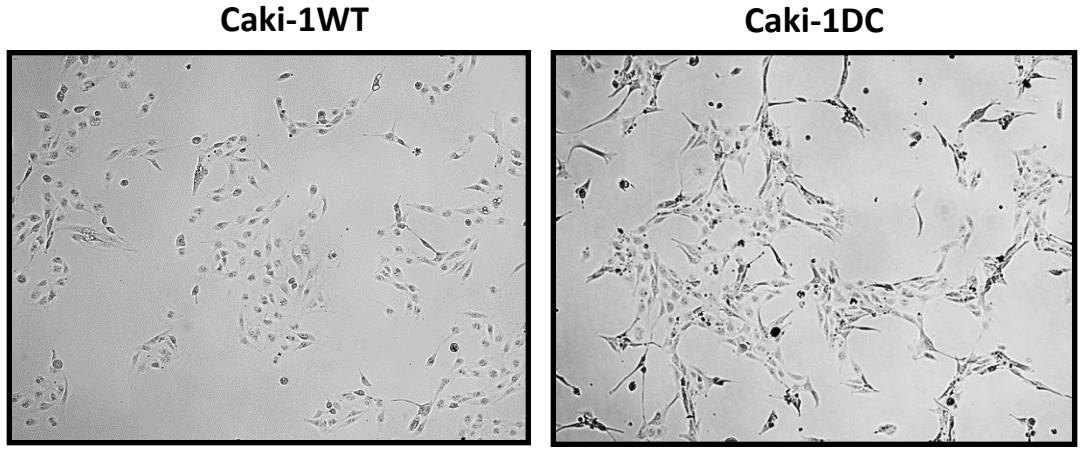
placebo



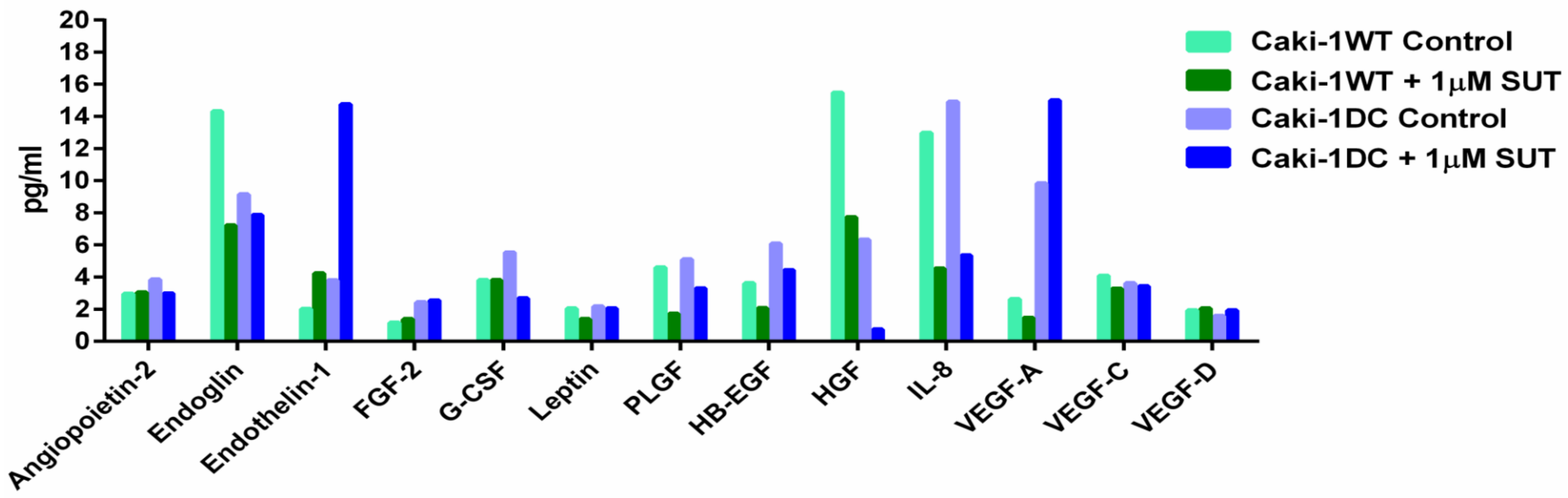
Identification of Novel Therapeutics

- Confirmation of mechanisms of resistance:
  - Human Samples, *in vitro* resistance model, *in vivo* resistance models
- Identification of small molecules that can target these alterations

Protein Expression in Media of Sunitinib-resistant and Sensitive mRCC

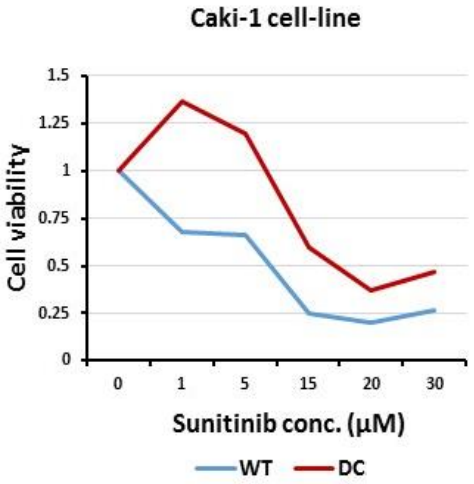


Angiogenic Factors



Differences between sunitinib-resistant and sensitive mRCC

A



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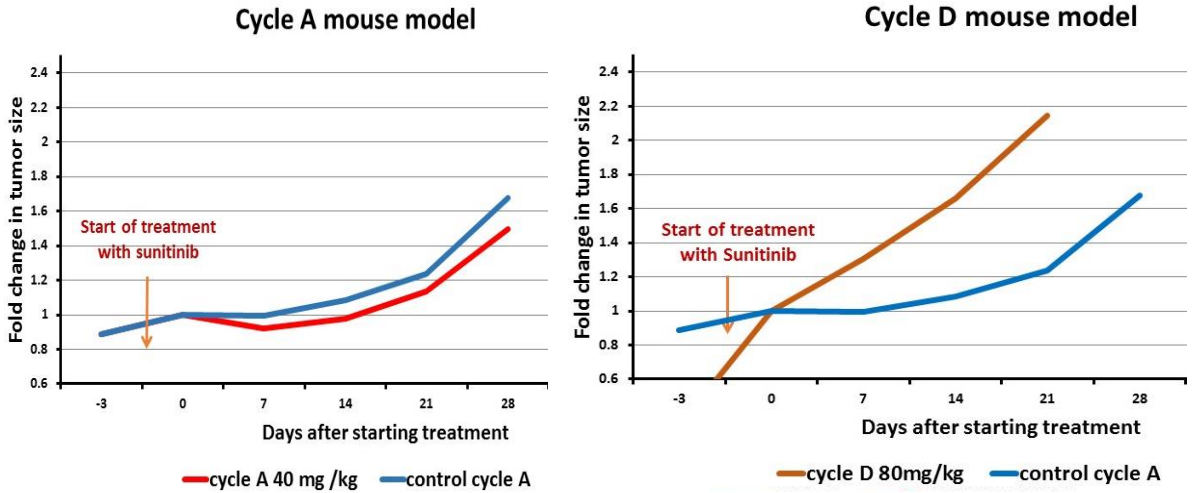

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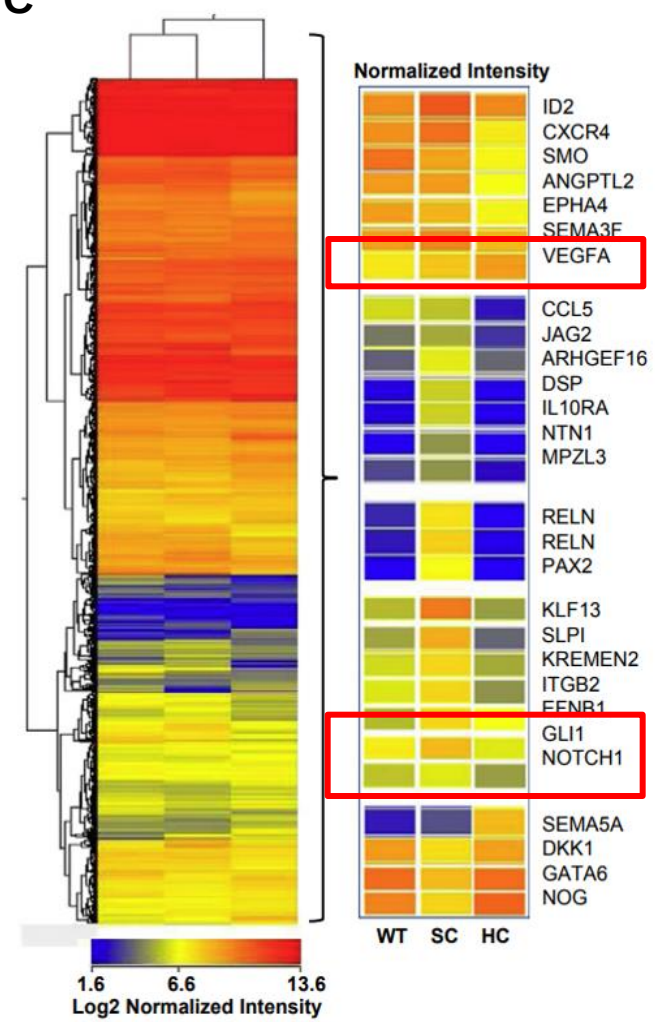
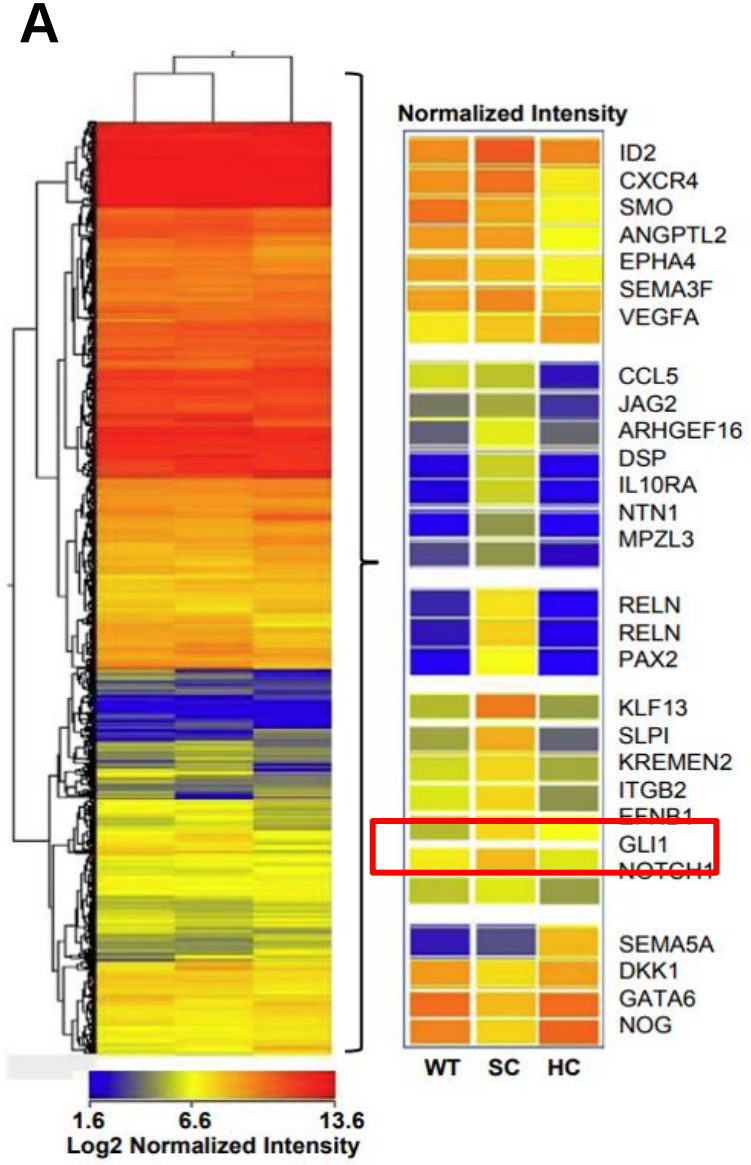


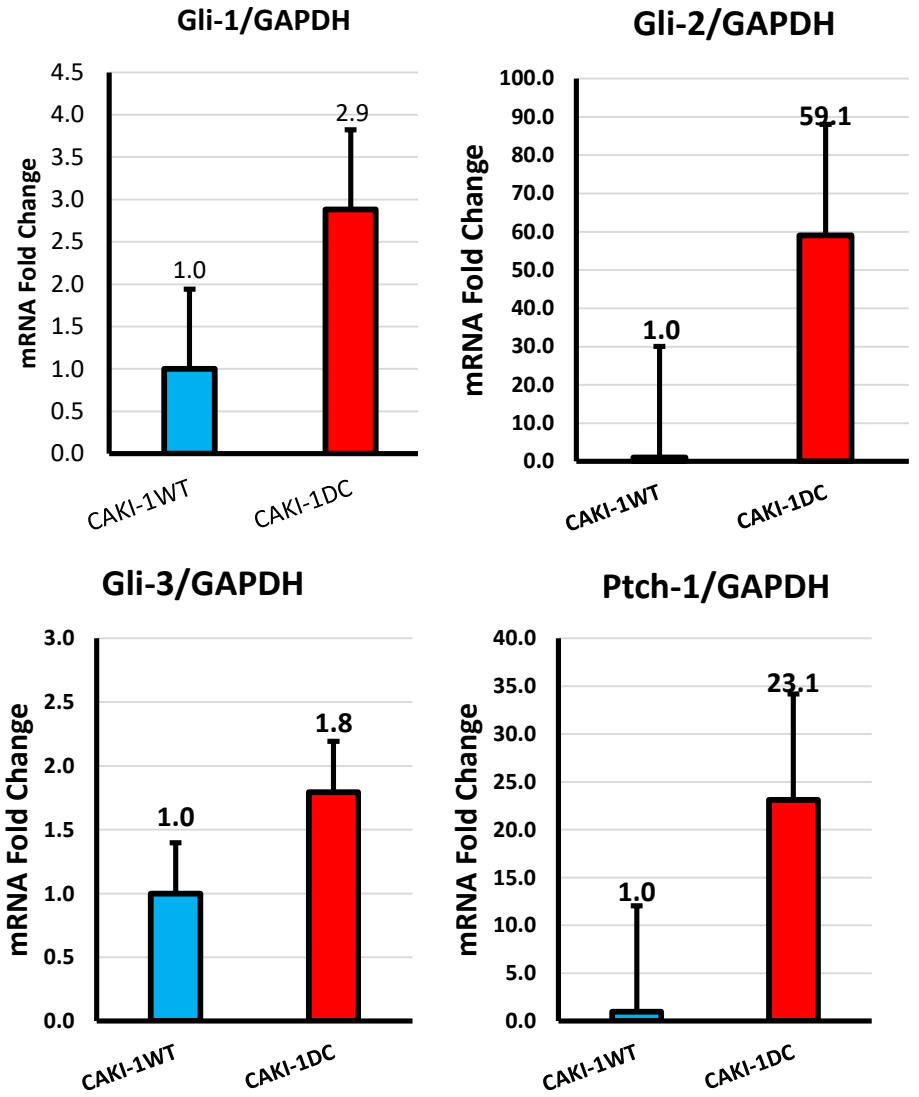
D

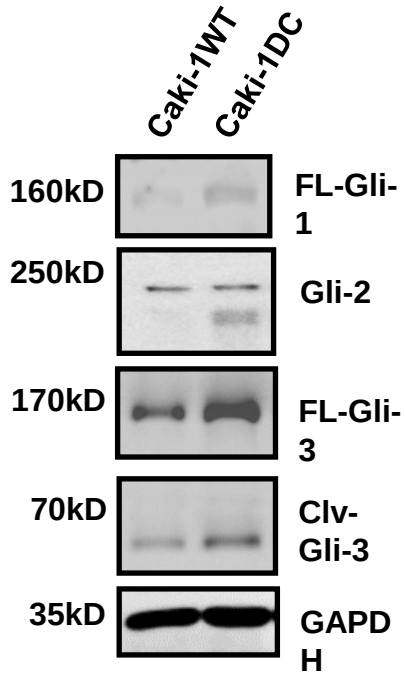
Gene	Accession#	Name	Peptide#	Log FC_80mg/kg	T	score	p-value	FDR
ABCB1	P08183	Multidrug resistance protein 1	26	-0.45323	-2.29642	0.053957	4.1E-11	9.55E-09
ABCB10	Q9NRK6	ATP-binding cassette sub-family B member 10, mitochondrial	5	0.267708	1.8302	0.108369	0.010748	0.152629
ABCC4	O15439	Multidrug resistance-associated protein 4	12	0.473752	2.73992	0.027898	6.12E-08	7.98E-06
ABCD1	P33897	ATP-binding cassette sub-family D member 1	6	-0.30498	-1.41874	0.197421	0.042111	0.34299
ABCD3	P28288	ATP-binding cassette sub-family D member 3	12	-0.22344	-1.26589	0.244637	0.02586	0.262226
ABCE1	P61221	ATP-binding cassette sub-family E member 1	16	0.195736	1.60078	0.151901	0.000465	0.016489
ABCF2	Q9UG63	ATP-binding cassette sub-family F member 2	20	0.23689	1.651535	0.14105	5.74E-05	0.003047

Han KS., et al. Neoplasia (2015)

Upregulation of Gli-proteins in sunitinib-conditioned cell-line compared to wild-type



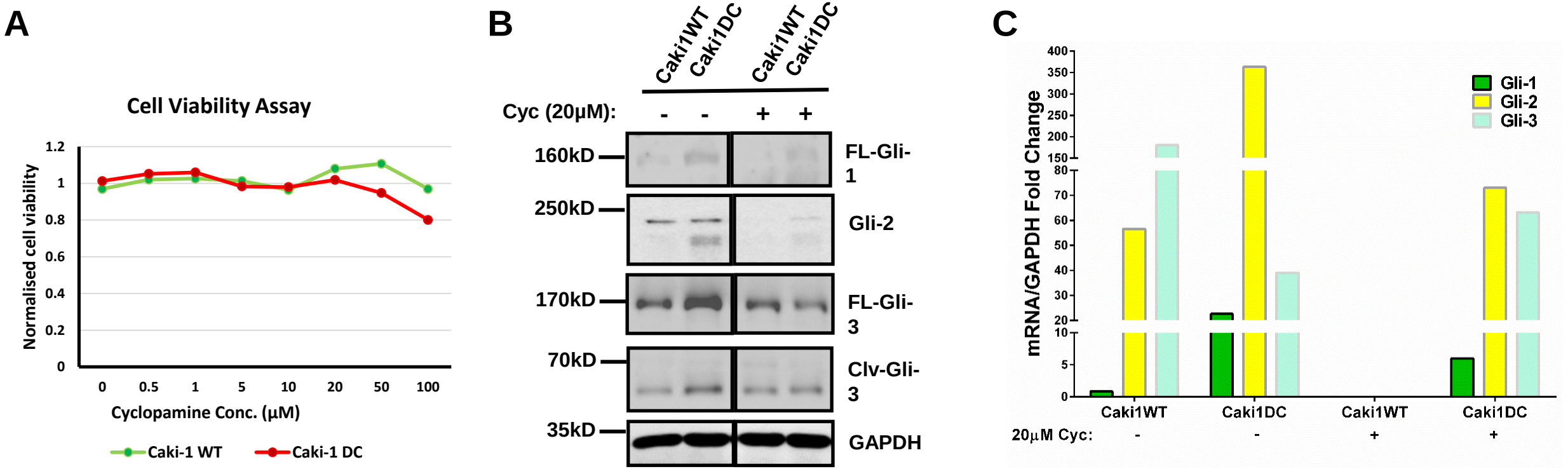




Han KS., et al. Neoplasia (2015)

D'Costa NM, et al. (In preparation)

Cycloplamine inhibits *Gli-pathway in sunitinib-sensitive and resistant mccRCC cell-lines*



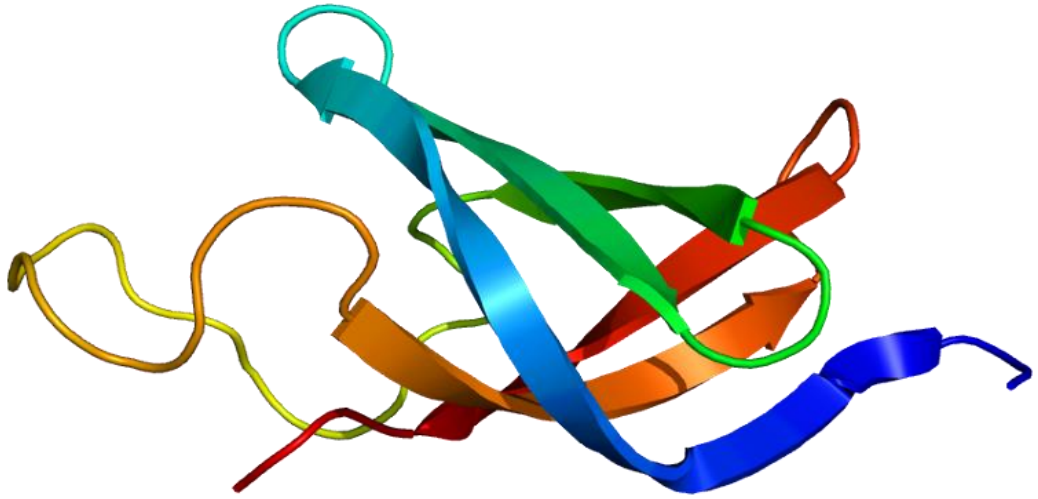
Novel Gli inhibitors currently being tested:

- **GLI ASO**
- **UBC Drugs Screens (Dr. Art Cherkasov)**

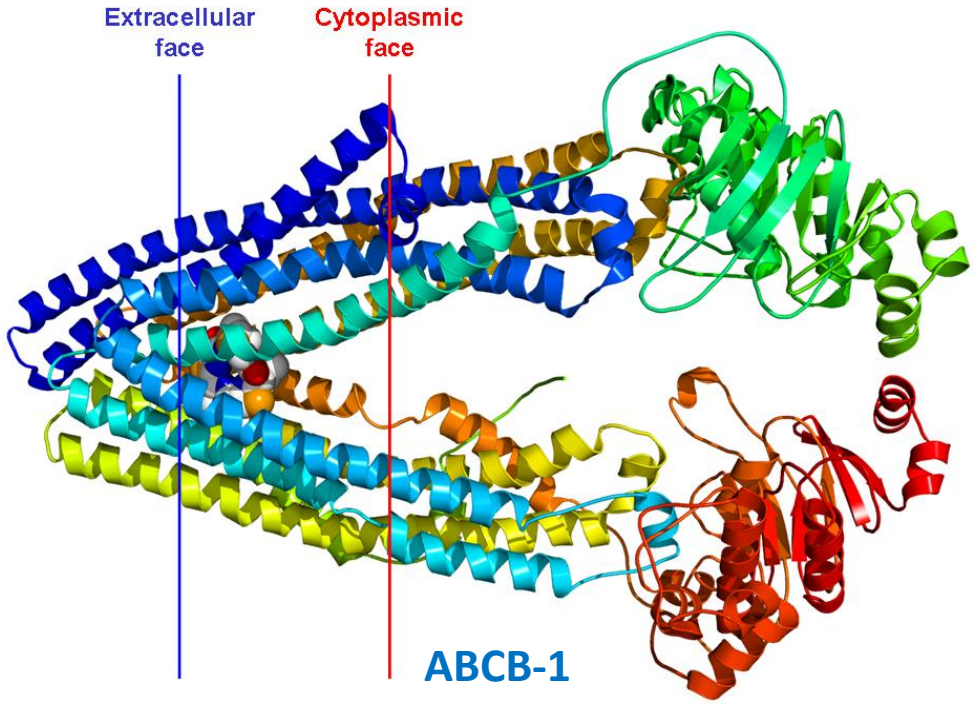
D'Costa NM, et al. (In preparation)

YB-1 and ABCB-1 in sunitinib resistant mRCC

- **YB-1**
  - a multifunctional protein.
  - found in cell cytoplasm, nucleus and can also be secreted.
  - involved in DNA and mRNA dependent processes and treatment resistance.
  - expression is correlated with RCC pathogenic stage.

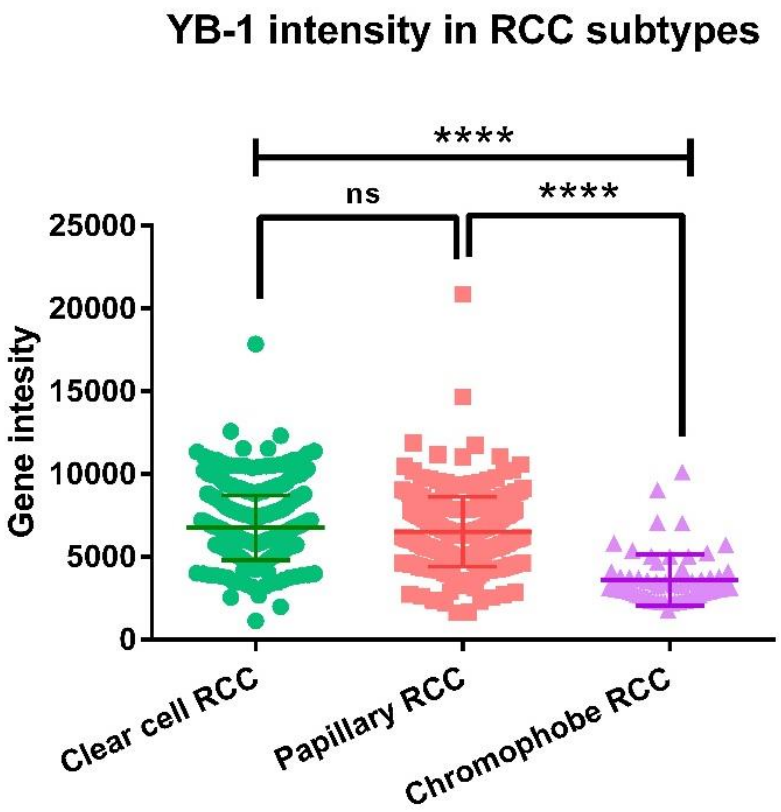


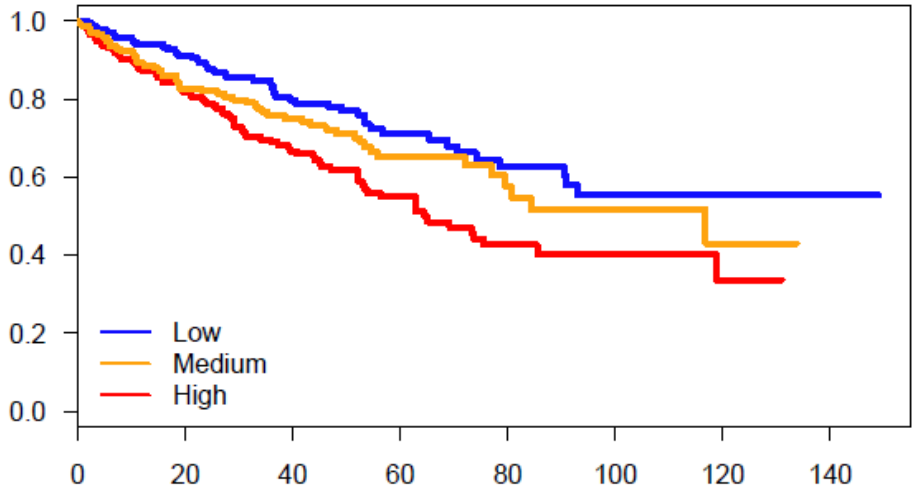
YB-1

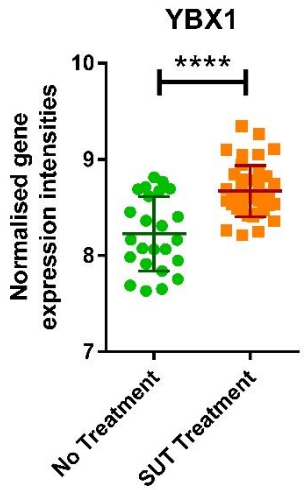


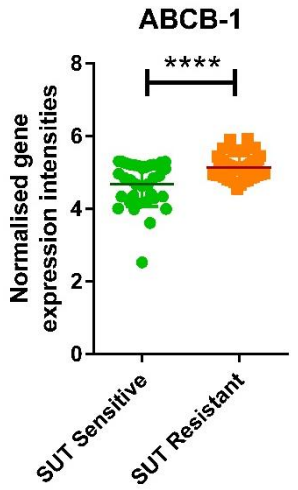
- **ABCB-1 (MDR1 or P-gp)**
  - actively efflux compounds from the cell.
  - thought to be central in drug resistance development of many therapies.
  - Sunitinib was found to be a substrate for ABCB-1.
  - ABCB-1 is a known downstream target of YB-1.

Significance of YB-1 Expression in ccRCC – TCGA Analysis



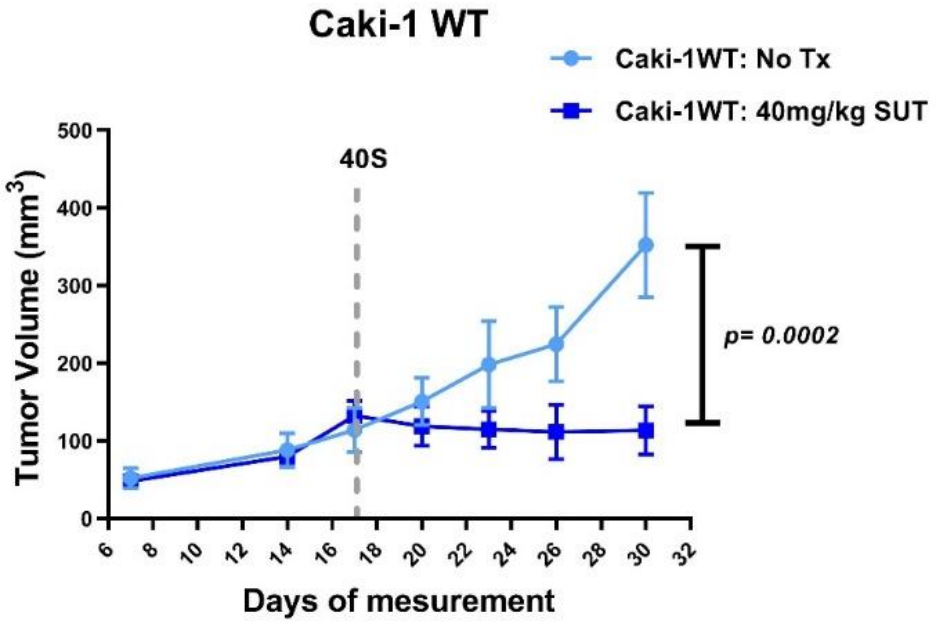


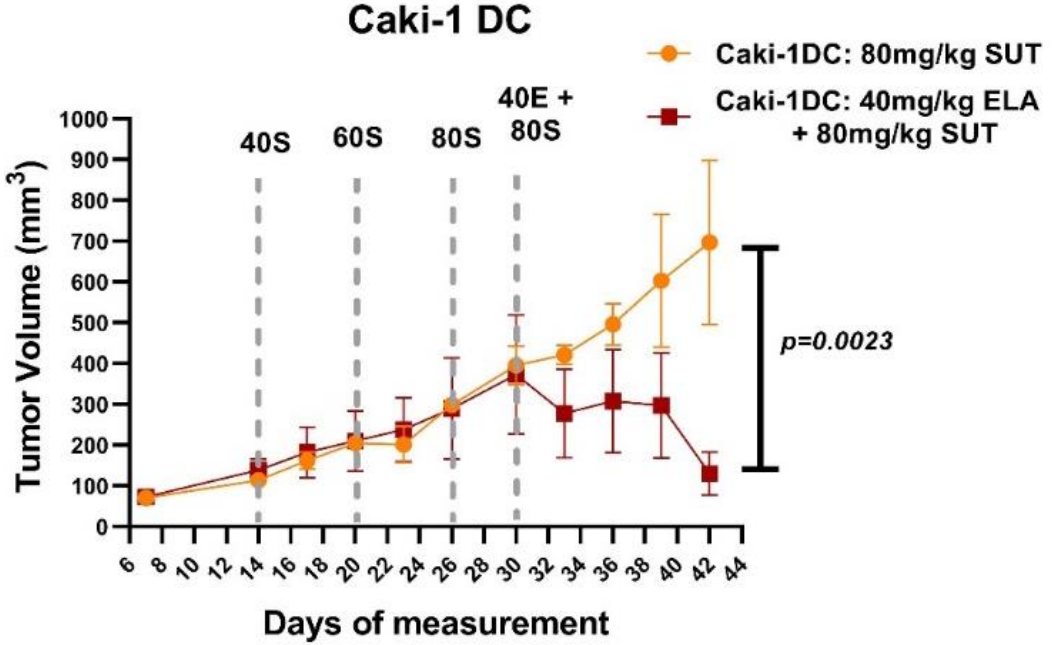




D'Costa NM, et al.

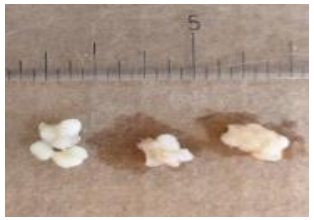
Resensitization of sunitnib conditioned Caki-1 cells with inhibition of Yb-1





Caki-1WT: No treatment

Caki-1WT: 40mg/kg SUT

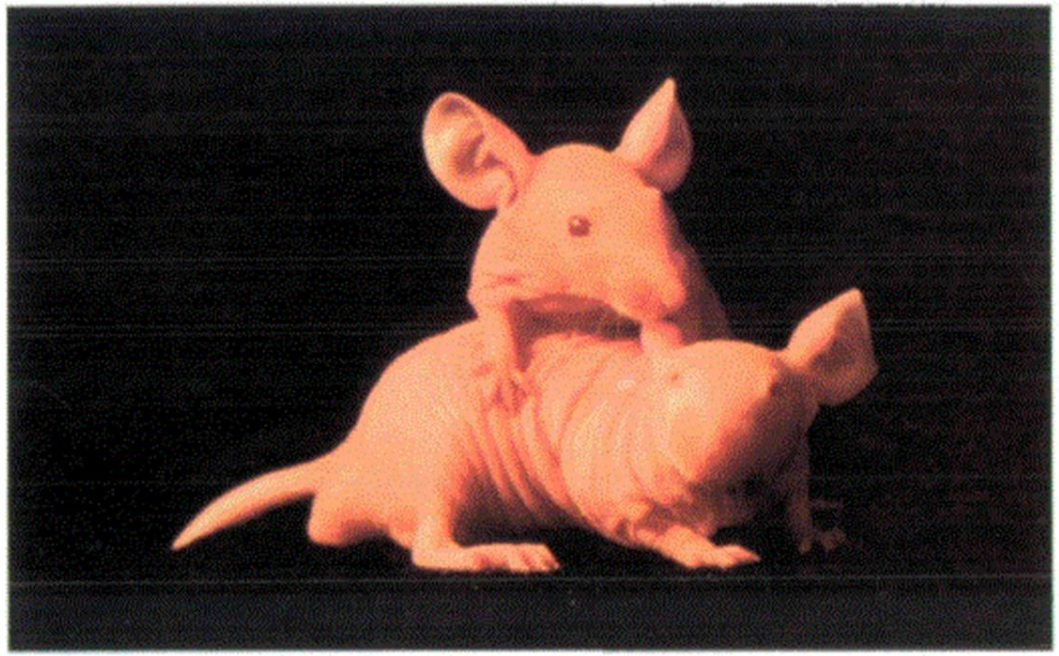


Caki-1 DC: 80mg/kg SUT

Caki-1 DC: 40mg/kg ELA
+ 80mg/kg SUT

Limitations of Current "Models"

***Side Glance:* Laboratory-Bred Mice**

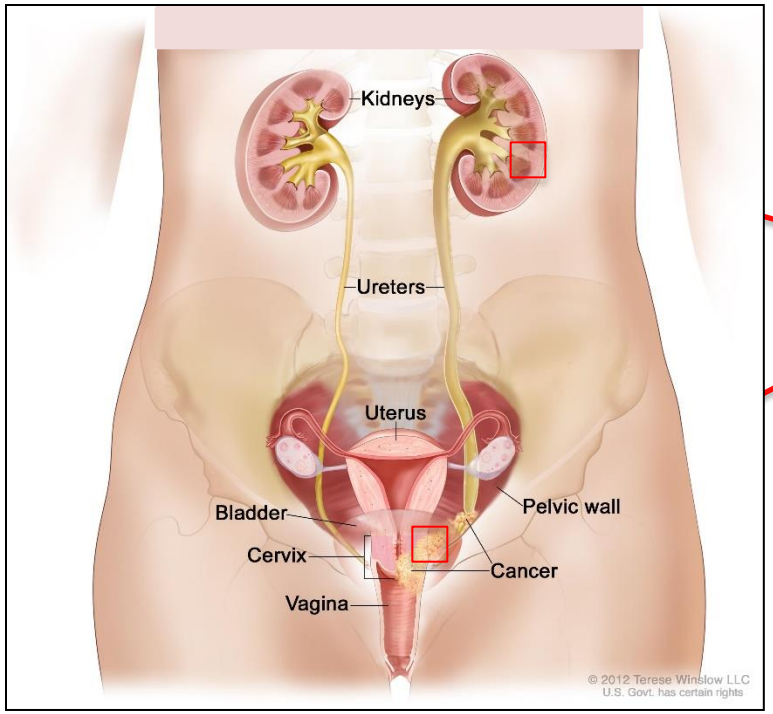


When the National Cancer Institute changed its initial drug screening system to a cell assay, the number of mice needed for drug screening fell from 6 million to about 300,000 each year. Studies in nude (athymic) mice are necessary to confirm in vitro results before trials in humans can be considered. About 10,000 compounds are screened each year in the cell assays; 200 to 400 compounds show enough anti-tumor activity to move into animal studies.

Journal of the National Cancer Institute, Vol. 87, No. 4, February 15, 1995

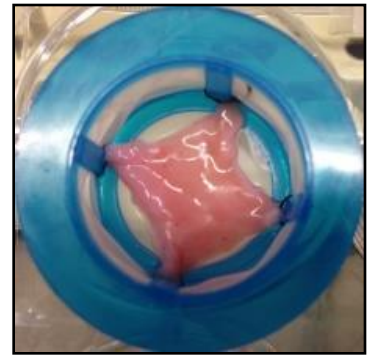
- How do we select the best treatments to develop?
- How do we select the best treatments to treat our patients?

3D Patient-derived tumor model



Tumor biopsy

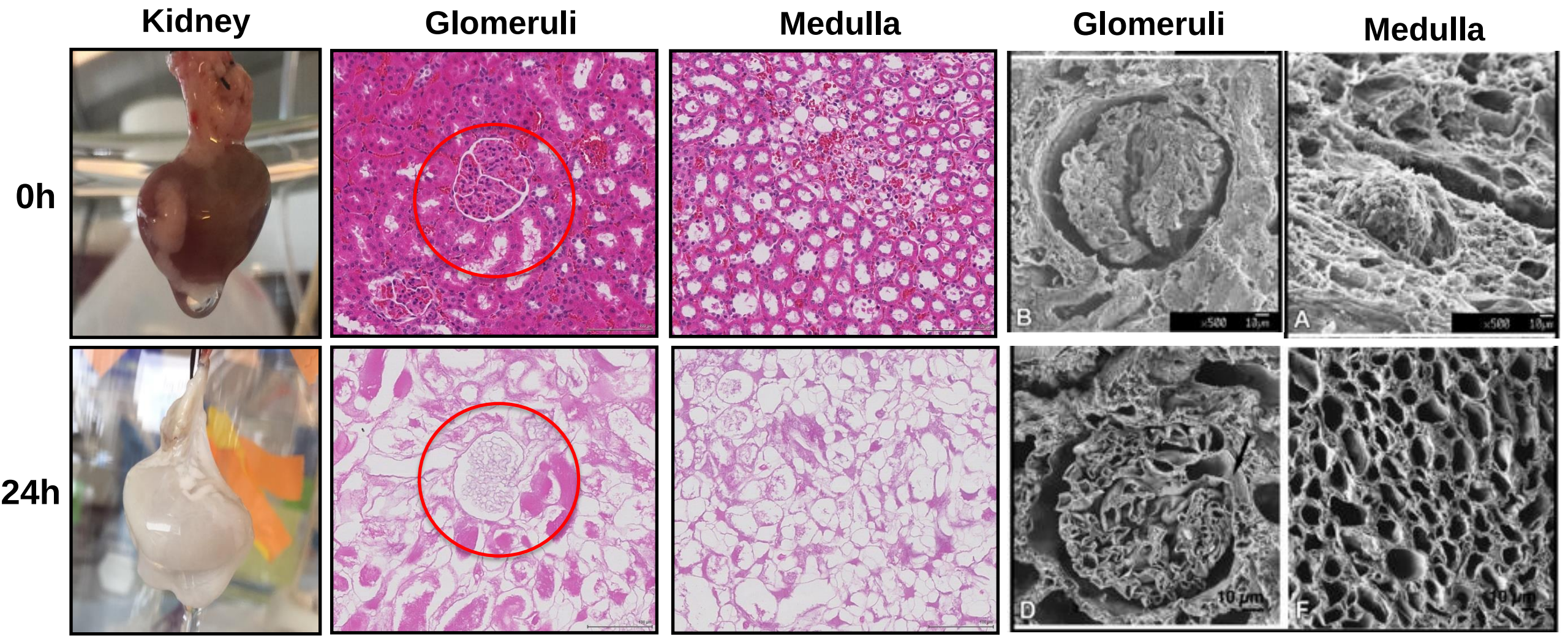
Decell organ matrix



Drug testing

Most efficacious treatment

3D – Tumor Models: Decellularization



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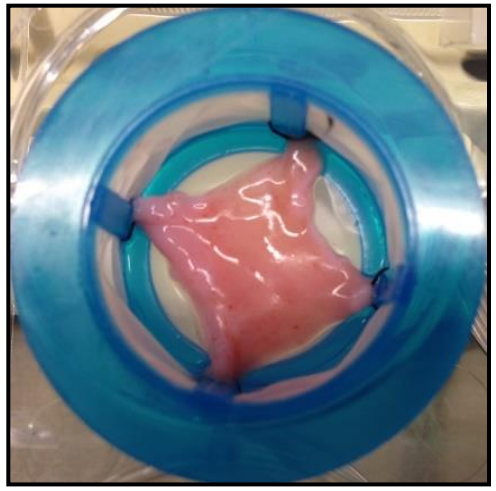
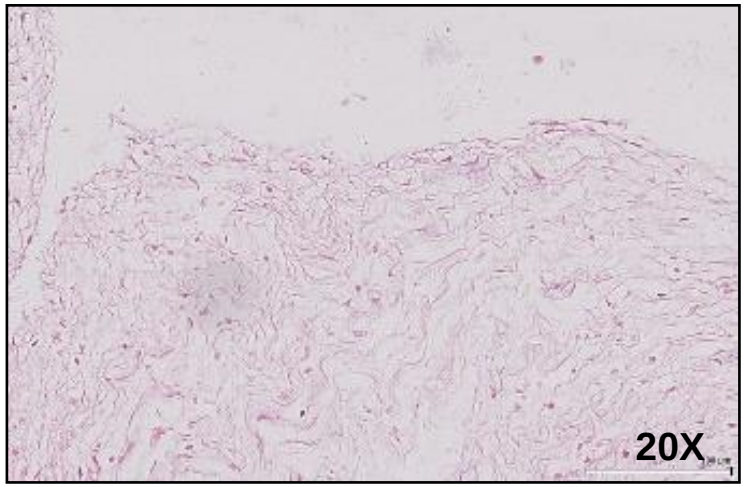
Reseeding process of decellularised tissue

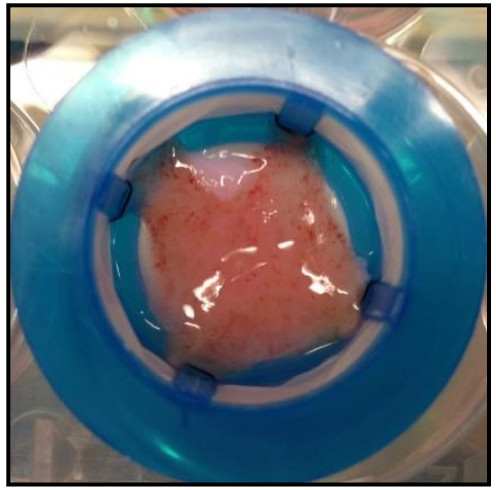
Pig Bladders

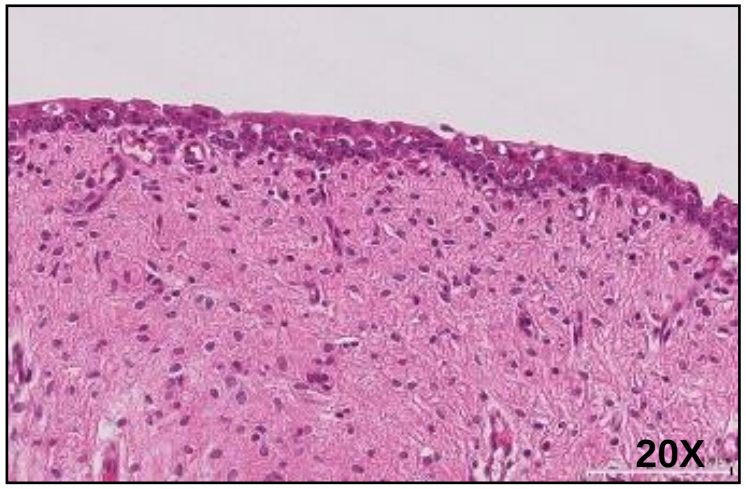
Decellularized





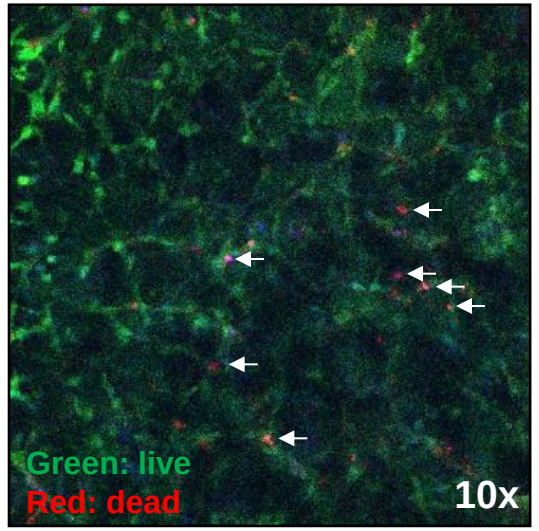
Reseeded

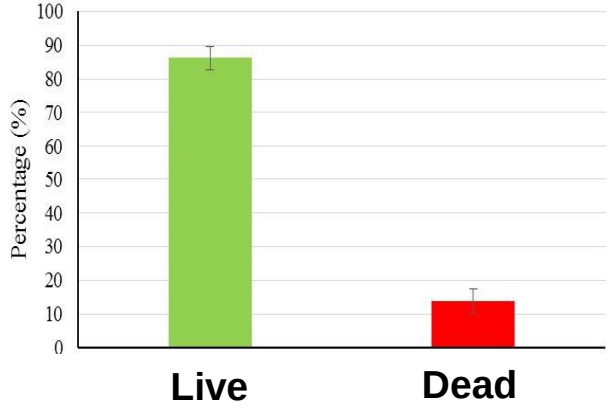




UM-UC-3, HUVEC, SV-HUC, Fibroblast

Cell Viability Assay

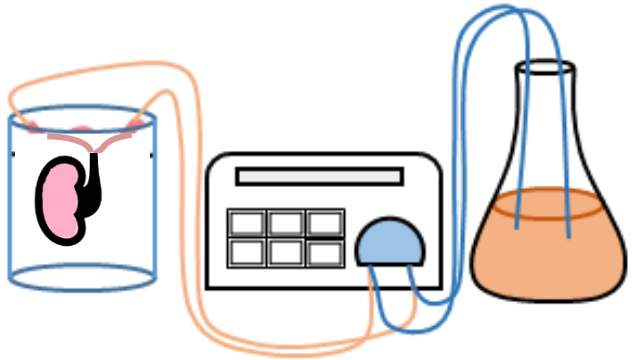




Tan Z., et al., (In preparation)

Schematic Diagram of Renal Cancer Avatar Program

Decellularization process

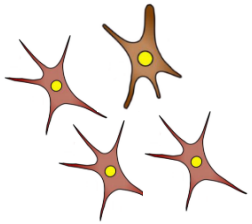


Decellularization of rat kidney by perfusion

Decellularized rat kidney

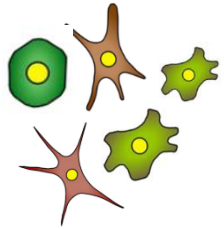
Decellularized kidney sections

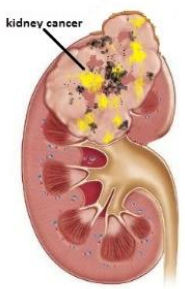
Reseeding process



mccRCC cell line (CAKI-1DC)

+

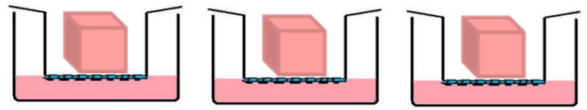




mccRCC patient tumor

High throughput treatment process

Treatment A | Treatment B | Treatment C



Reseeded kidney sections treated with Gli-inhibitors

Inhibitors against specific targets

SHH: 5E1

SMO: Cyclopamine, Vismodegib, etc.

Gli-1: GANT-61, ATO, etc.

Elacradar

Summary:

- Treatment resistance in RCC is complex: mechanisms are multifactorial with significant interaction between endothelial cells and cancer cells.
- Models of resistance allow for biomarker and treatment development.
- Validation of treatments can be streamlined with novel 3D- *in vitro* models that minimize cost and also better recapitulate tumor microenvironment compared to traditional models.

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