Modelling of the RAS-RAF-MEK-ERK pathway in hepatocellular carcinoma cells

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Joint work with
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Antoine Galmiche

SORAMIX

Outline

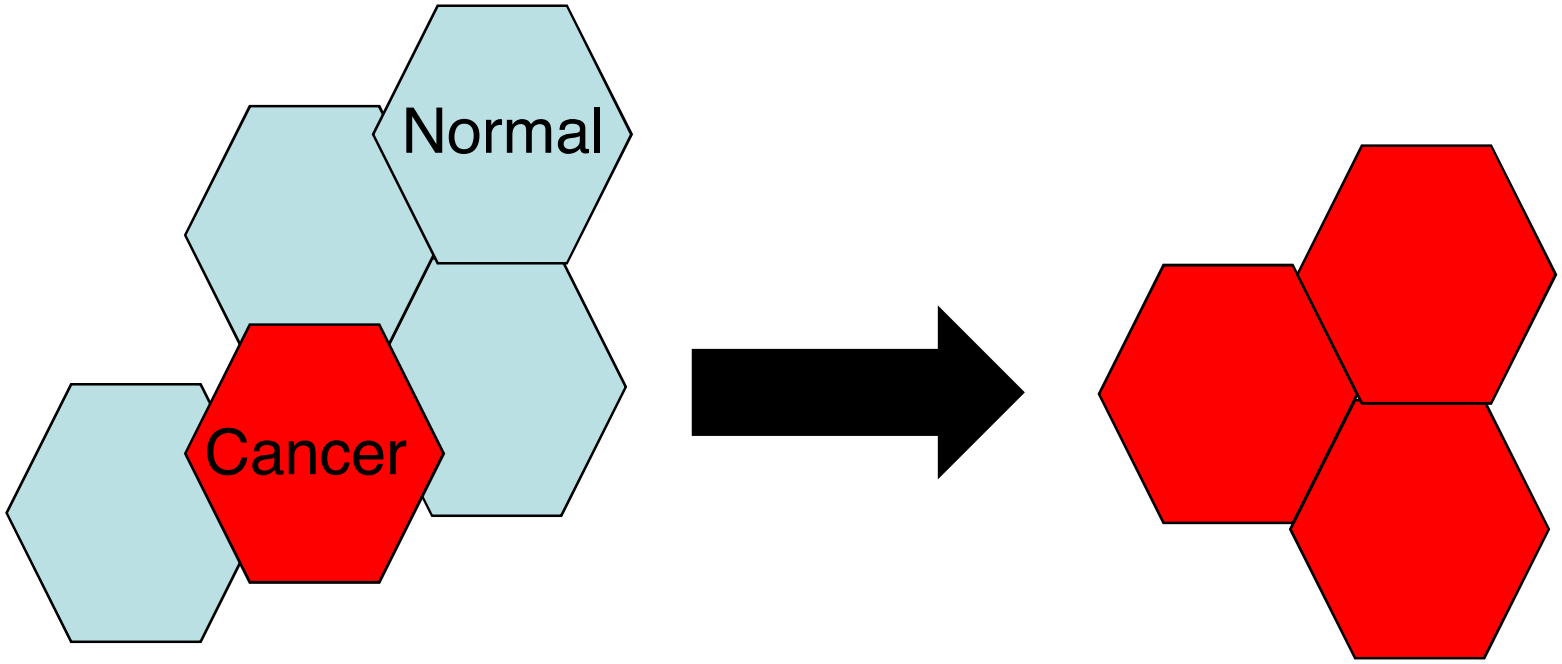
1. General informations about cancer

2. Therapeutic target: the ERK pathway

3. Modelling of the pathway

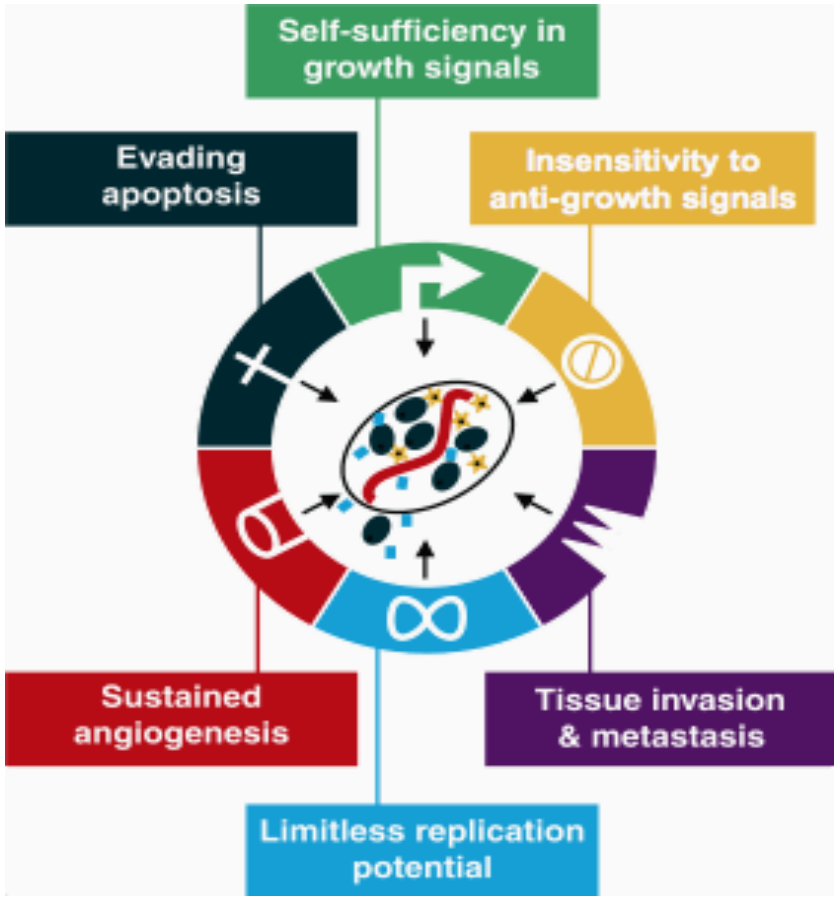
4. Conclusion

Cancer



Onset of a tumor is a clonal process + inheritable cell phenotype

Tumor cells



Hanahan & Weinberg, Cell 2000

increased proliferation, resistance to apoptosis, invasion of tissues, ability to form metastases ....

Complex disease

Up to several tens of thousands of mutations in the genome of cancer cells

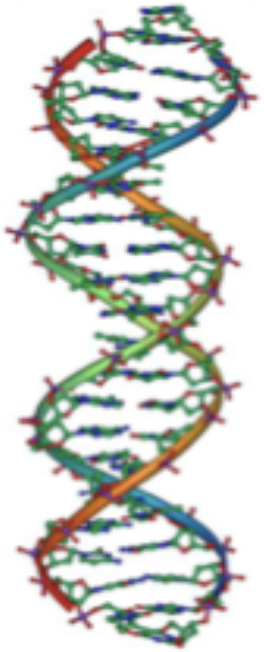
Each cancer is different

Sequencing compared lung cancer cell / blood cells:
23000 mutations
(10 cigarettes smoked = 1 mutation)

Nature 463, 2010.

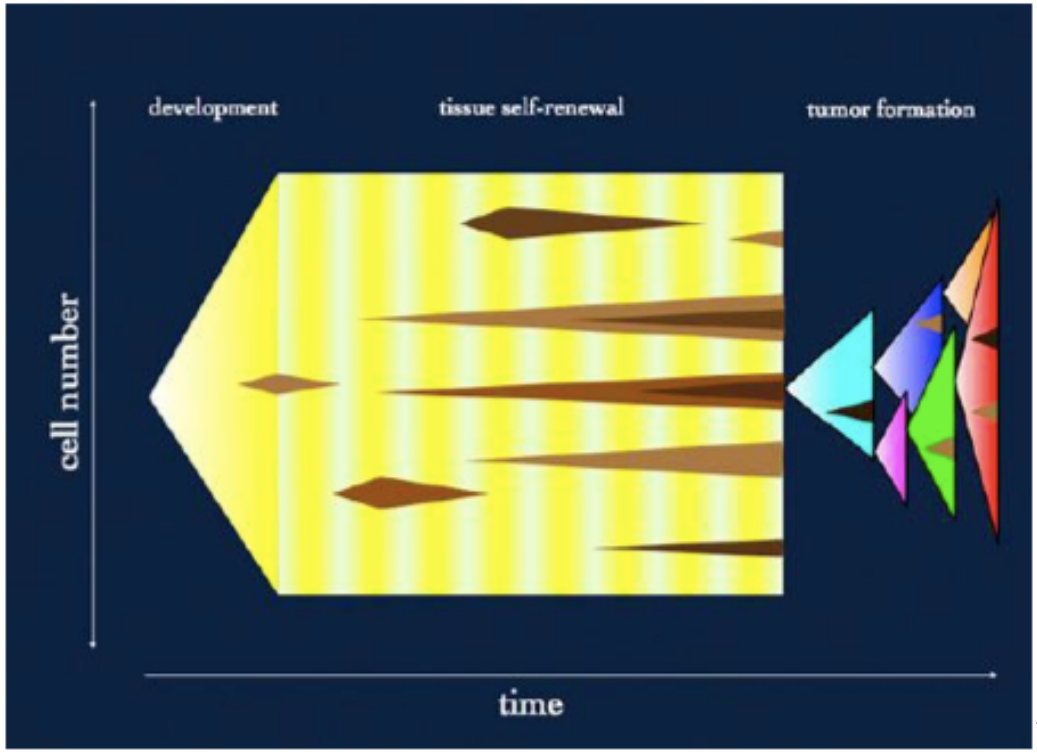
An idea of the problem

Bible:
3 million of characters



Genome:
3 billion of characters

Mutations accumulation

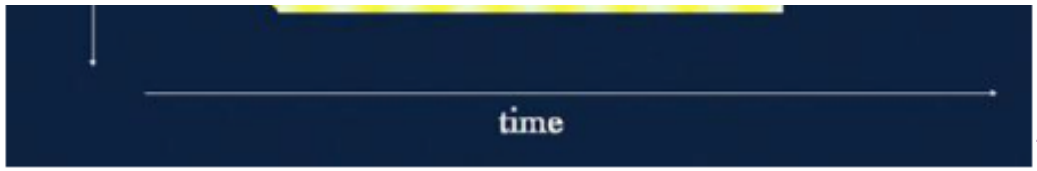


Proc Natl Acad Sci USA 110, 2013.

Mutations accumulation

Fraction of somatic mutations before tumor initiation

Tumor type	25-y-old	Median age at diagnosis	85-y-old
CLL	(10.09 – 5.86)/10.09 = 0.42	(18.53 – 5.86)/18.53 = 0.68	(24.16 – 5.86)/24.16 = 0.76
Uterine cancer	(45.96 – 33.33)/45.96 = 0.27	(77.95 – 33.33)/77.95 = 0.57	(96.48 – 33.33)/96.48 = 0.65
Colorectal cancer	(50.2 – 50.2)/50.2 = 0	(102.4 – 50.2)/102.4 = 0.51	(121.38 – 50.2)/121.38 = 0.59



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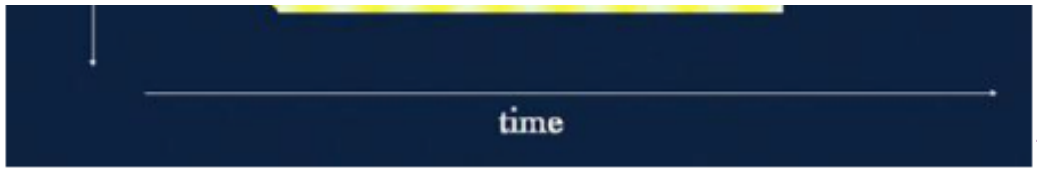
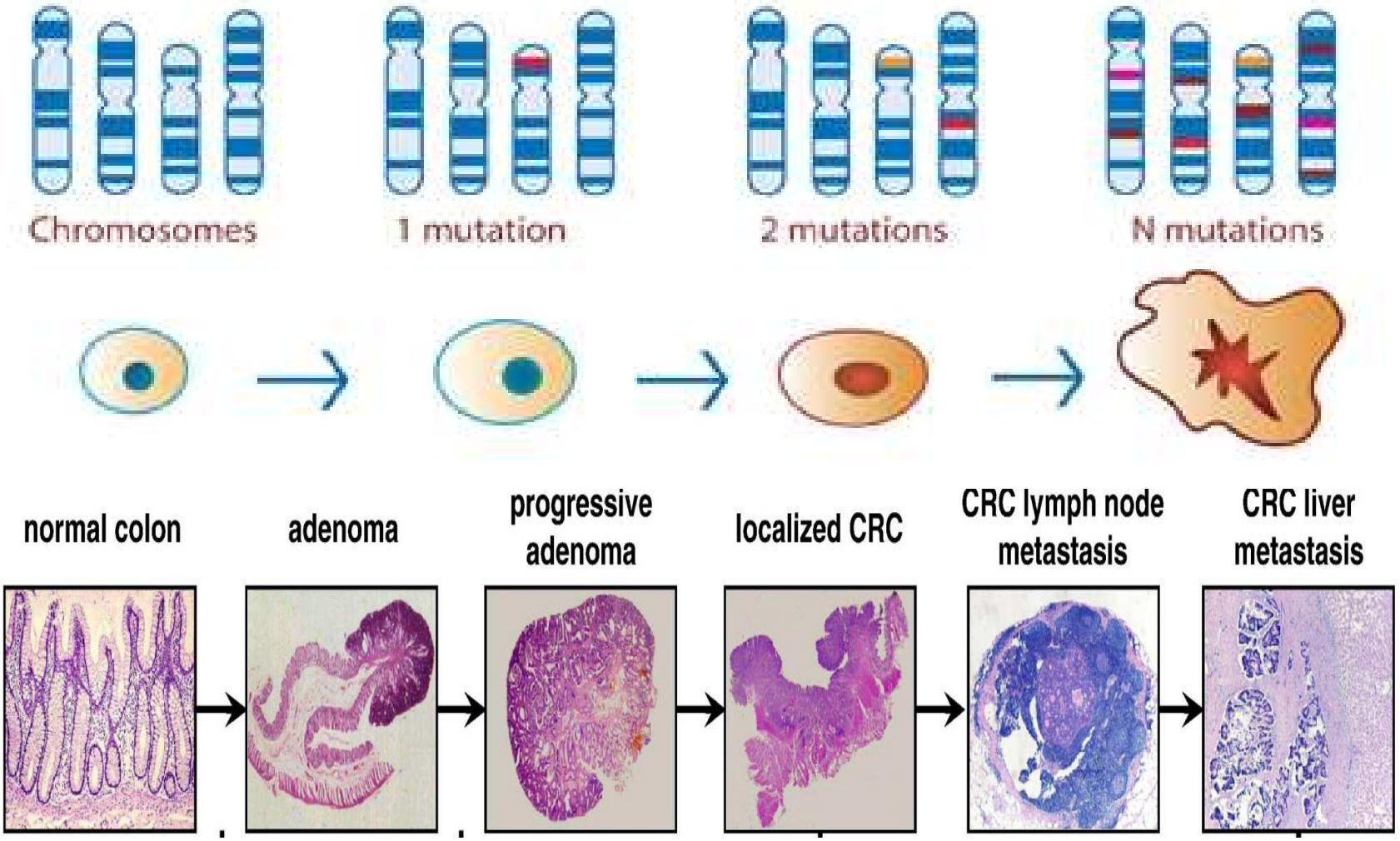


Proc Natl Acad Sci USA 110, 2013.

Most mutations precede the onset of cancer!

Mutations accumulation



Hepatocellular carcinoma cells

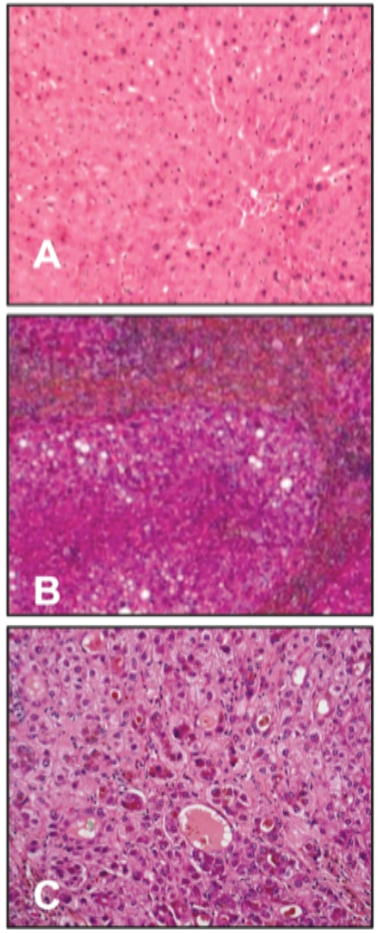
HCC the most common form of primary liver tumor

HBV/HCV

Alcohol

Alterations in intermediary metabolism

Few mutations



Normal liver

↓

Cirrhosis

↓

HCC

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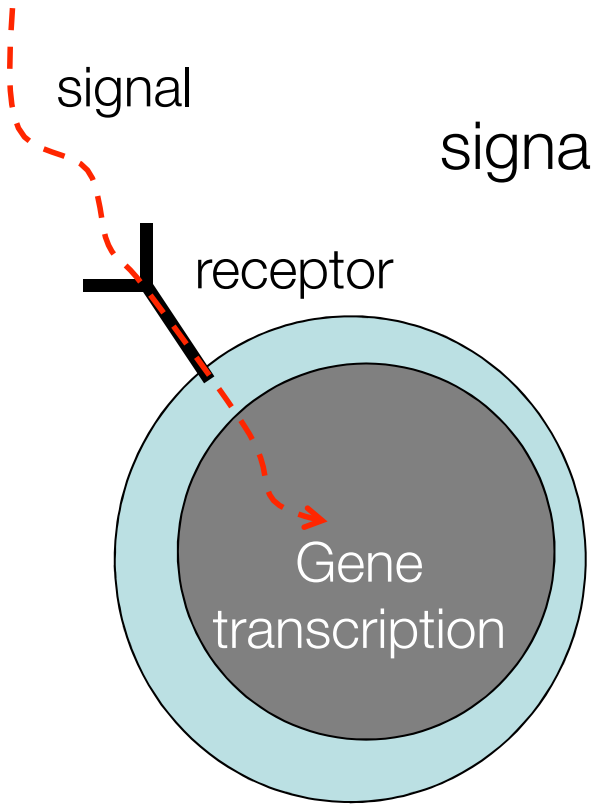
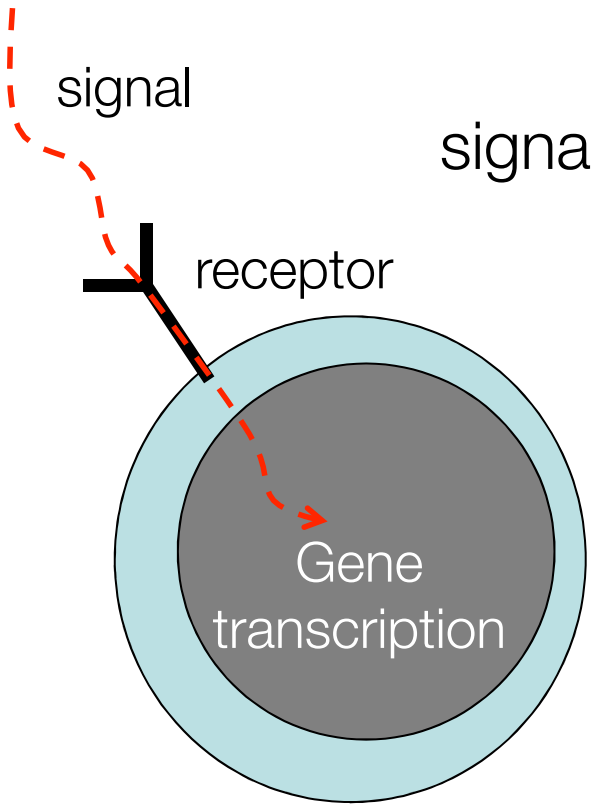
Cell signaling

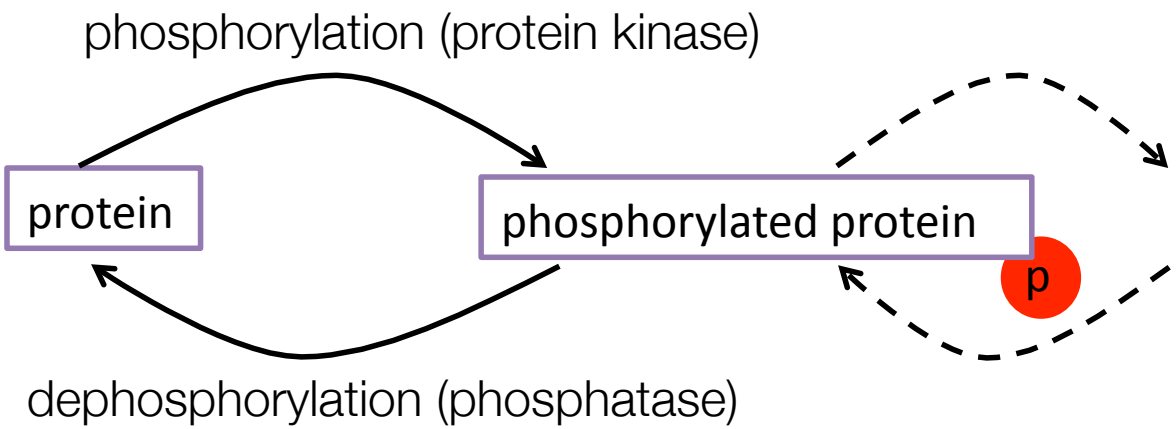


signal from membrane receptors

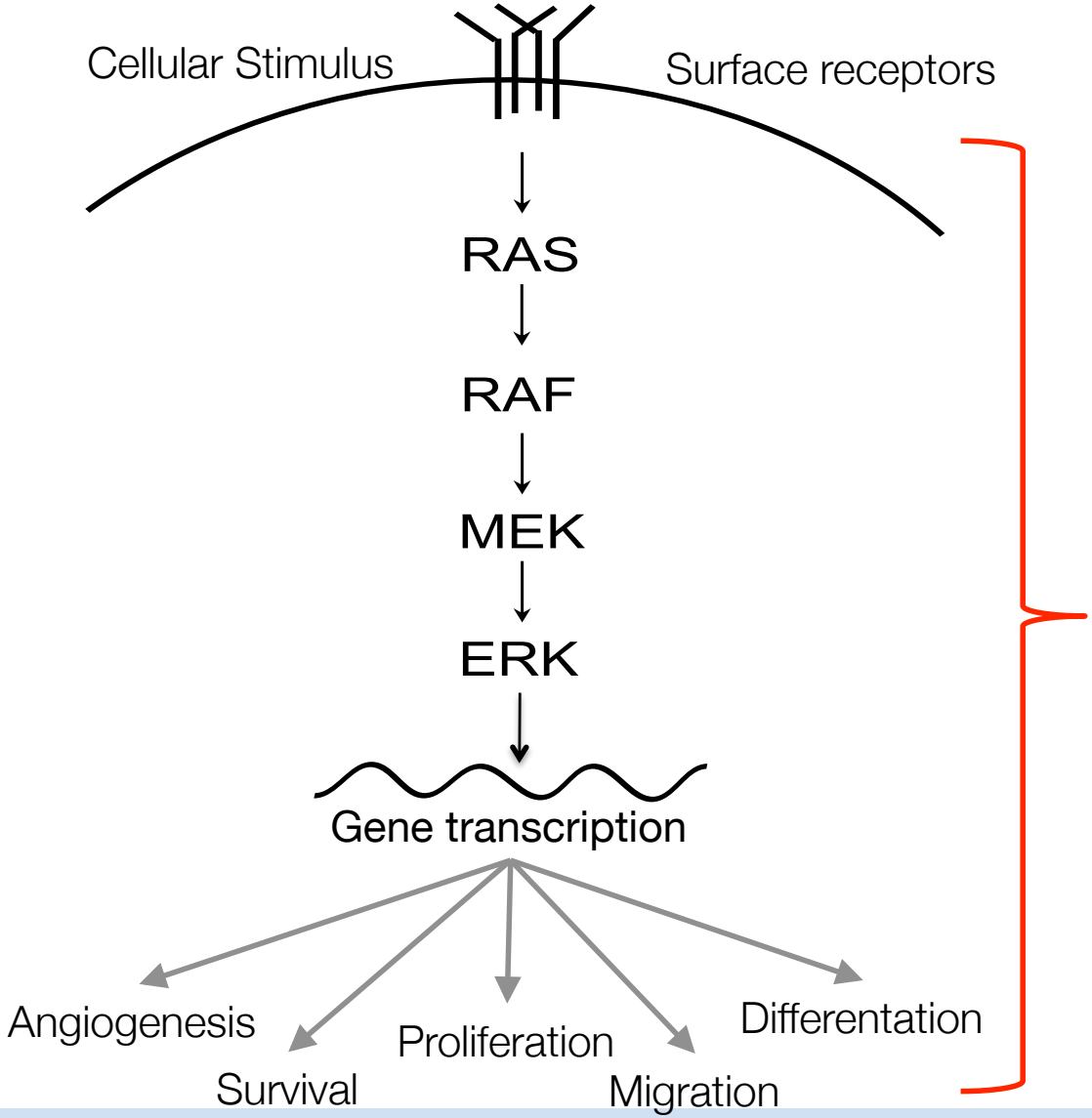
Cell signaling



signal from membrane receptors

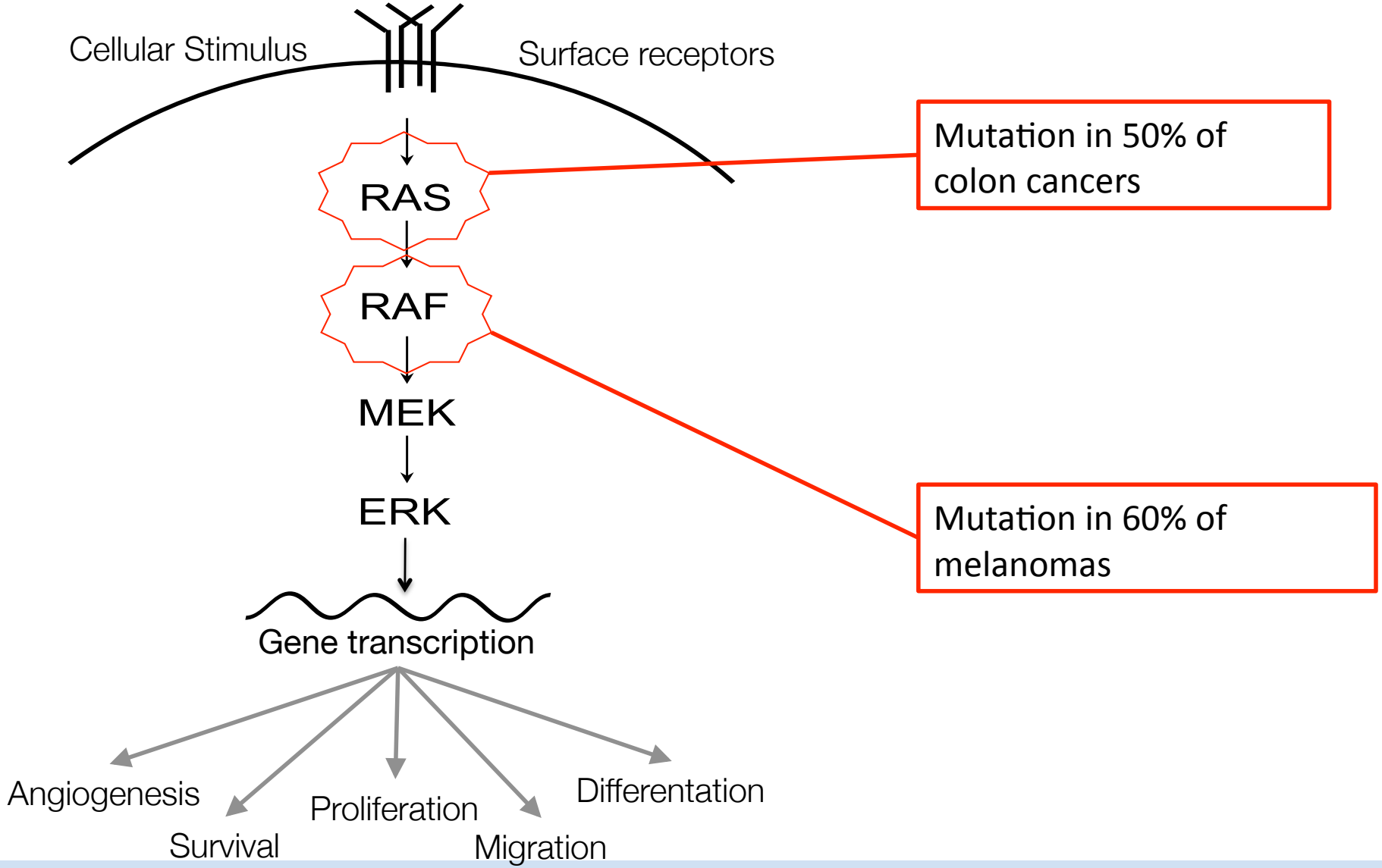


Basic pathway



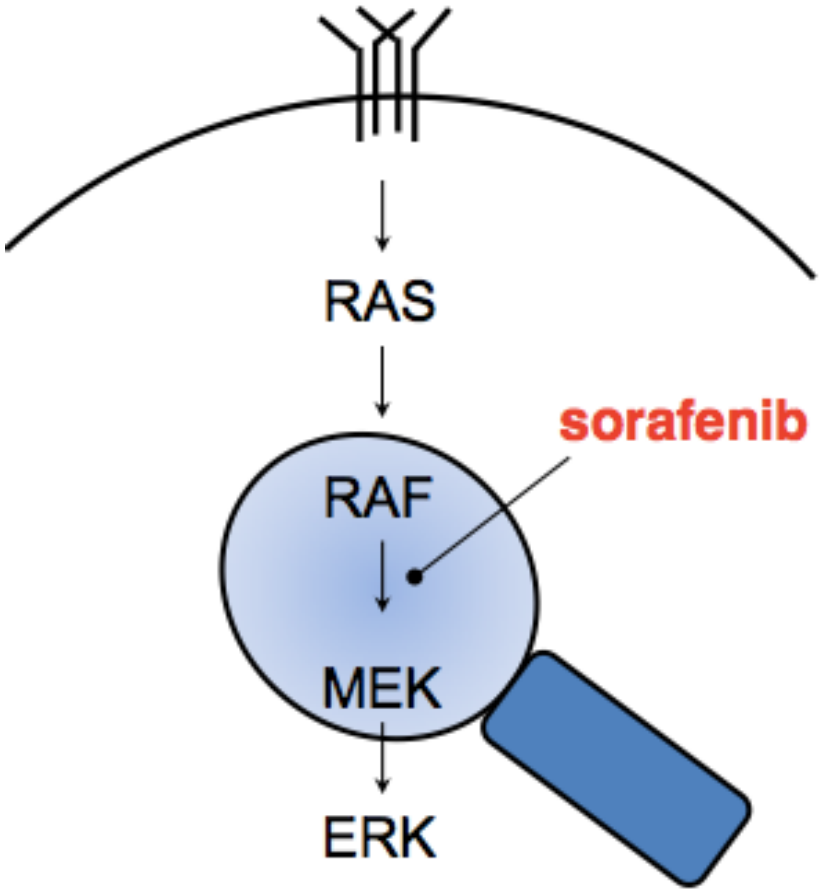
A normal but often deviated transduction pathway in cancers

Basic pathway

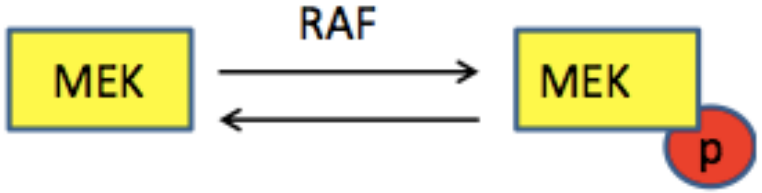


Therapeutic targeting

New medical treatments designed rationally:



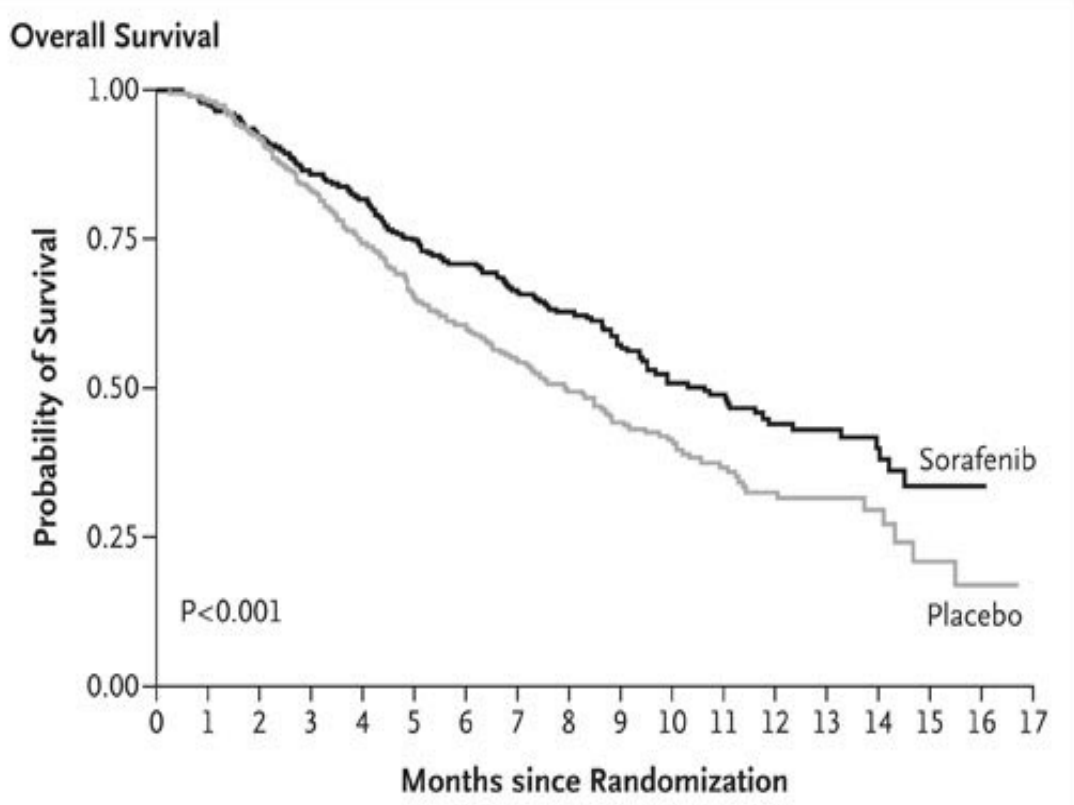
Enzyme inhibitors (kinases)



Goal: turn off oncogenic signaling

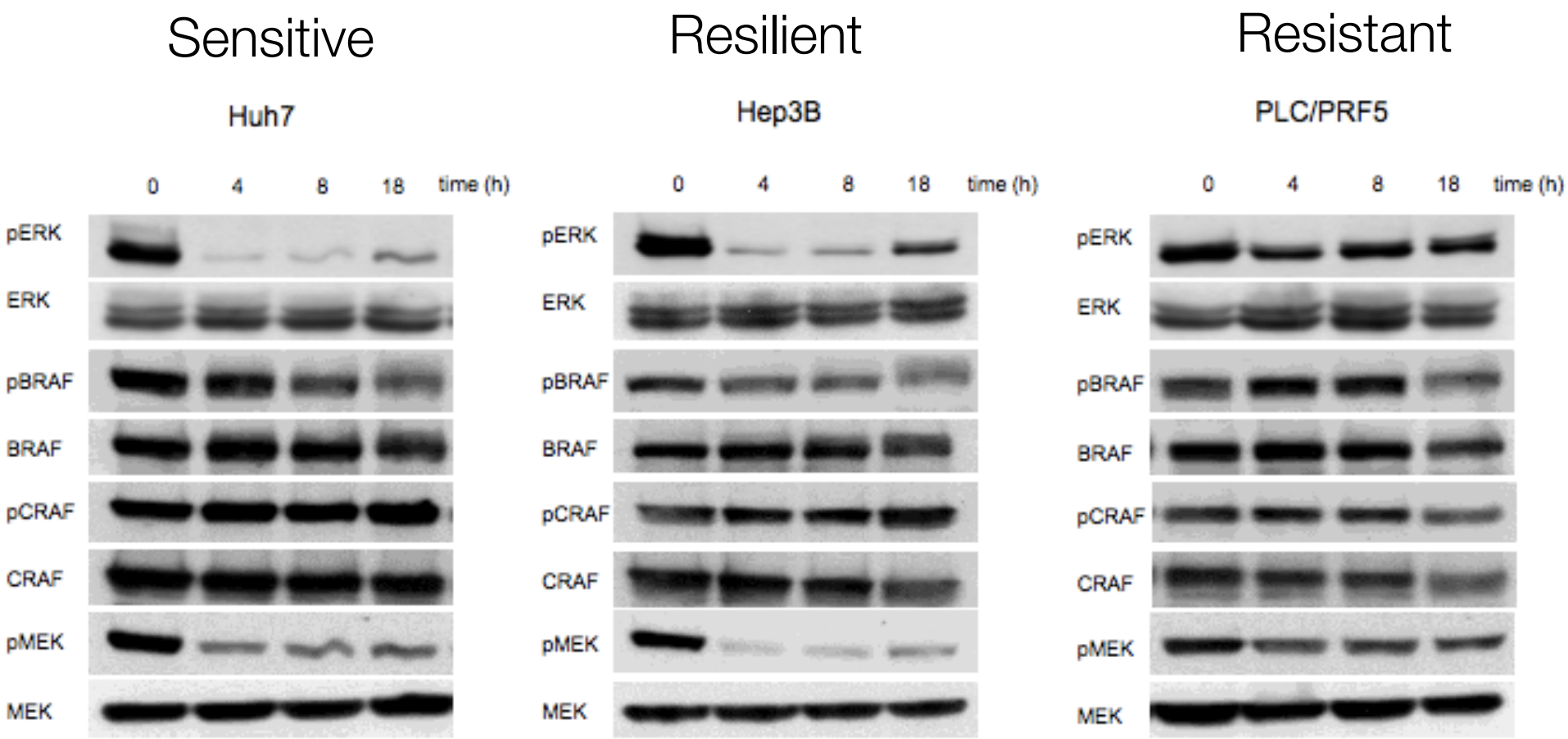
Sorafenib in HCC

- Mortality +++
- 80% of patients are diagnosed at a late stage of the disease
- Until 2008, no medical treatment of proven efficacy on survival for these advanced stages



Llovet et al. N Engl J Med 2008

Heterogeneous dynamic



Saidak et al. Cancer Letters 2017

Motivation

Improve the knowledge of the ERK pathway regulation

Understanding how the medicine works

Identify and control the pathway

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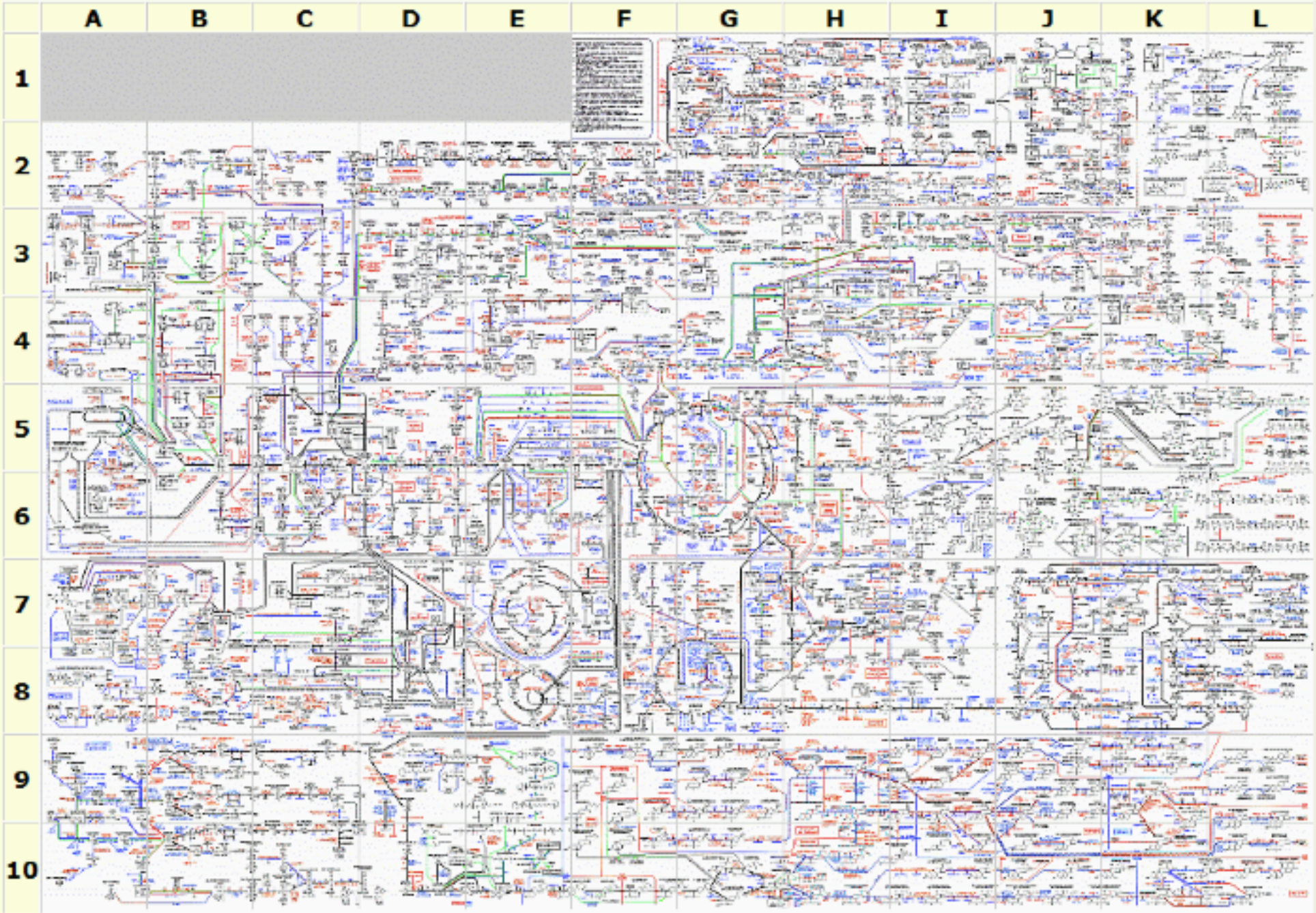
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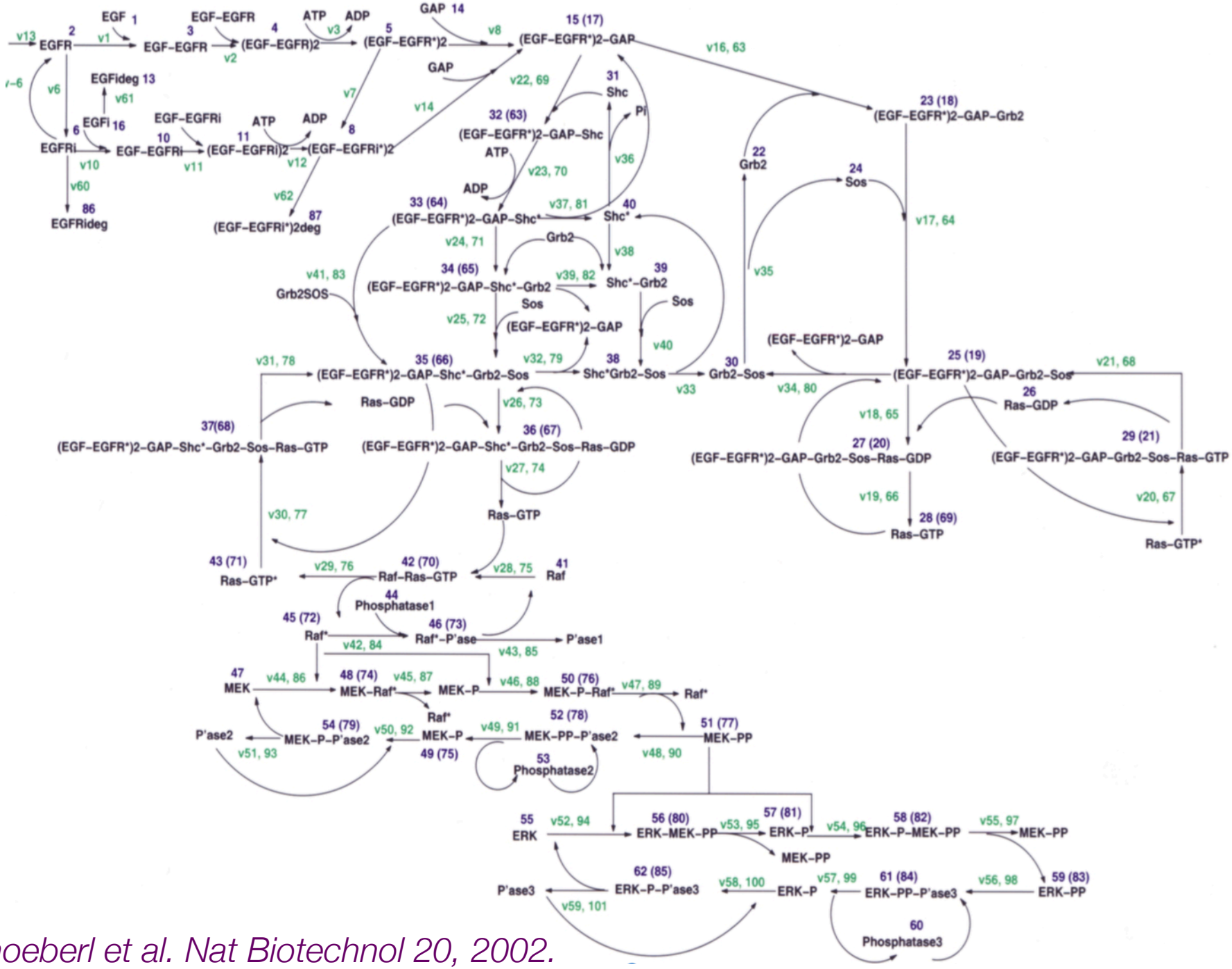
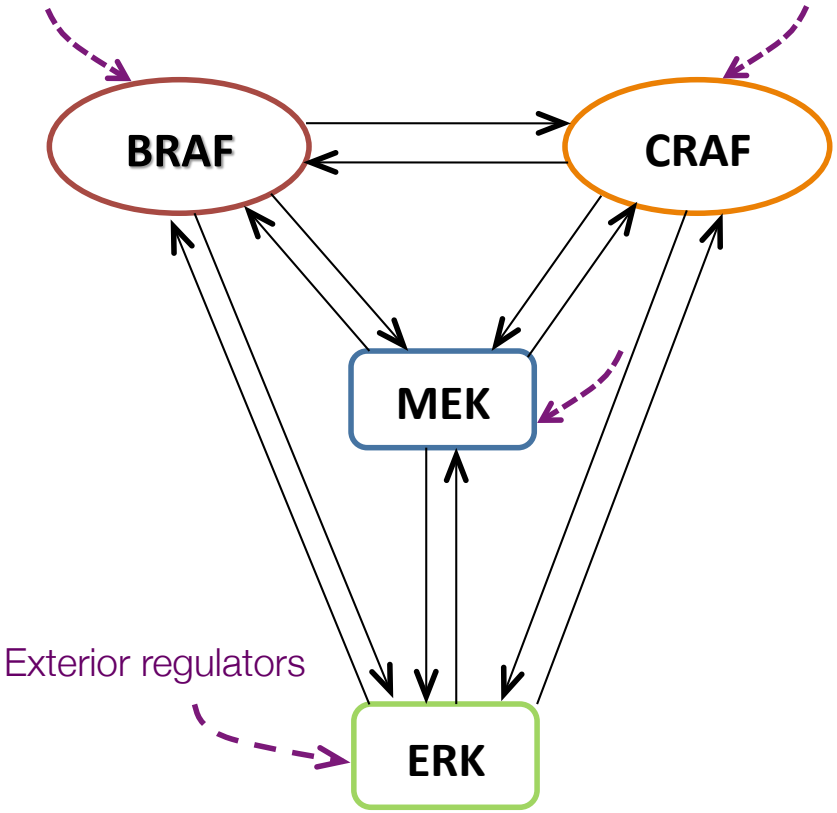


Schoeberl et al. Nat Biotechnol 20, 2002.

Average



Material support

3 cell lines : Hep3b (sensitive), Huh7 (resilient) and PLC\PRF5 (resistant),

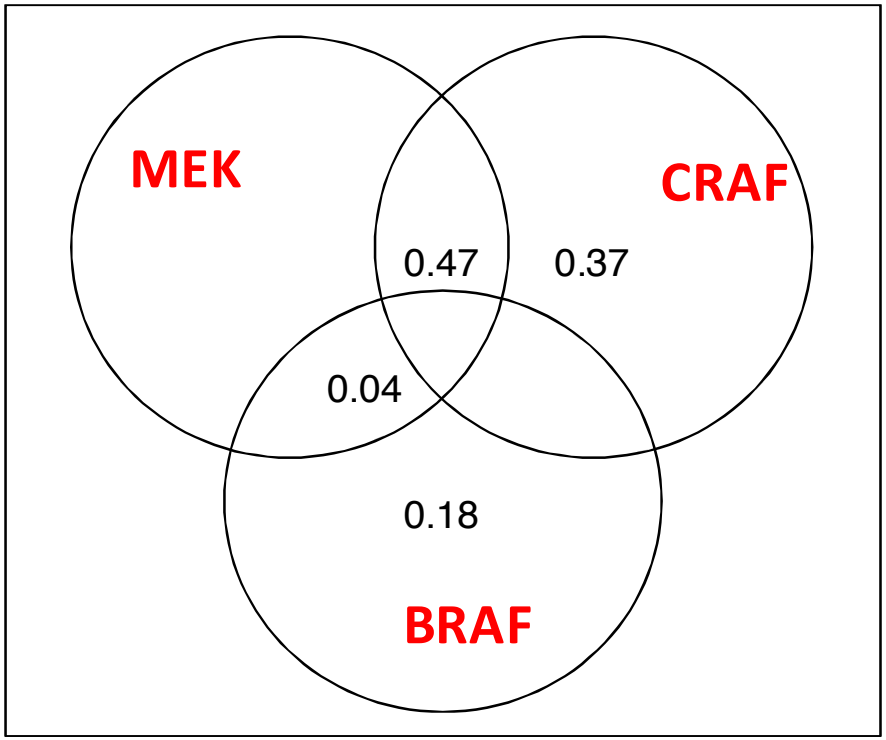
Western blot analysis

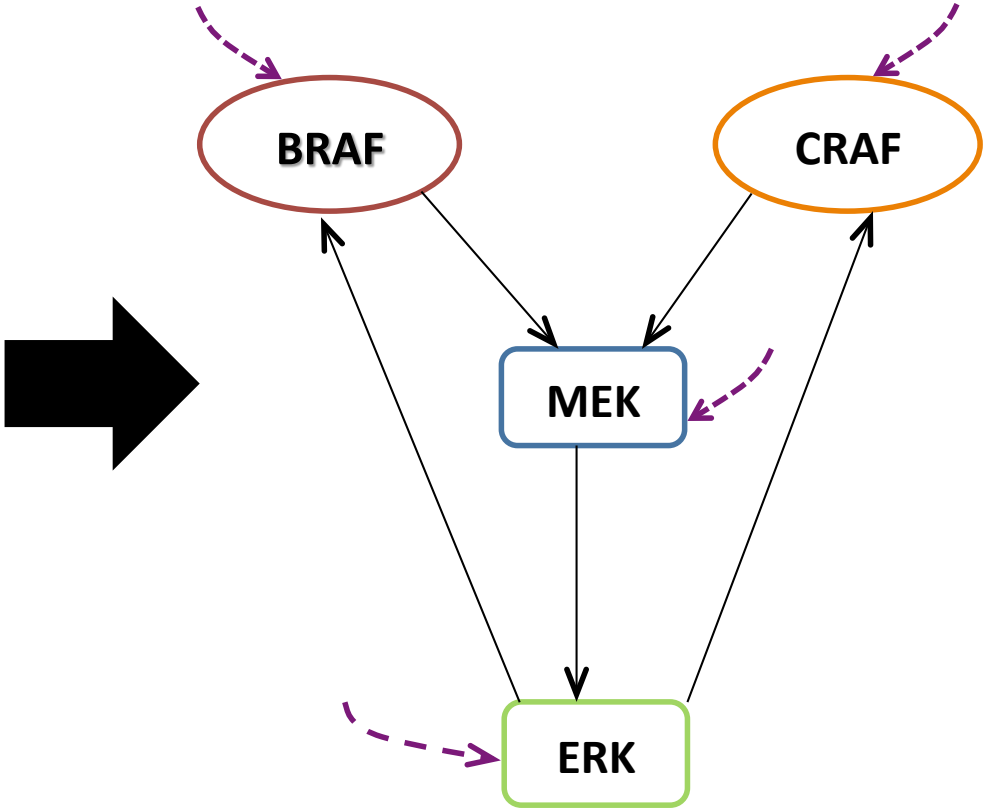
Quantification of each phosphorylated protein and their total at three measure times after sorafenib treatment (4, 8 and 18h).

Experiments repeated three times.

Data analysis

ERK at 18 hours





Model a reaction

The general chemical reaction

$$aA + bB \underset{k_o}{\overset{k_i}{\rightleftharpoons}} cC + dD$$

defines a reaction rate

$$v = -\frac{1}{a}\frac{d[A]}{dt} = -\frac{1}{b}\frac{d[B]}{dt} = \frac{1}{c}\frac{d[C]}{dt} = \frac{1}{d}\frac{d[D]}{dt}$$

where [X] denotes the concentration of the chemical species X (usually in mol/L).

Model a reaction

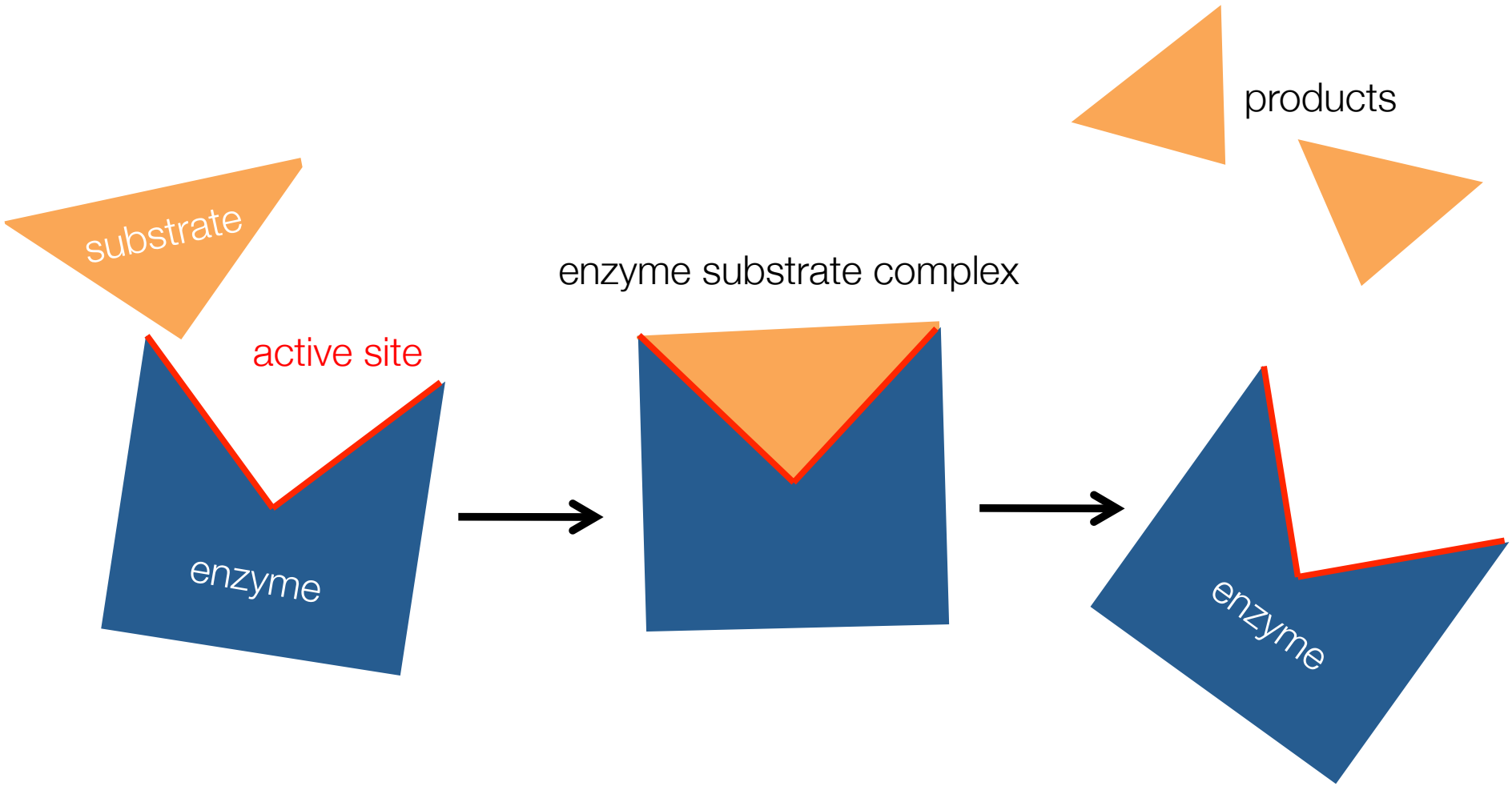
Consider the simple reaction

$$A + B \underset{k_o}{\overset{k_i}{\rightleftharpoons}} C$$

the change in amount of [A] = production – degradation

$$\frac{d[A]}{dt} = \frac{d[B]}{dt} = -\frac{d[C]}{dt} = k_o[C] - k_i[A][B]$$

Enzyme kinetics



Enzyme kinetics

$$\mathrm{A} + \mathrm{E} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{AE} \xrightarrow{k_3} \mathrm{A}^\star + \mathrm{E}$$

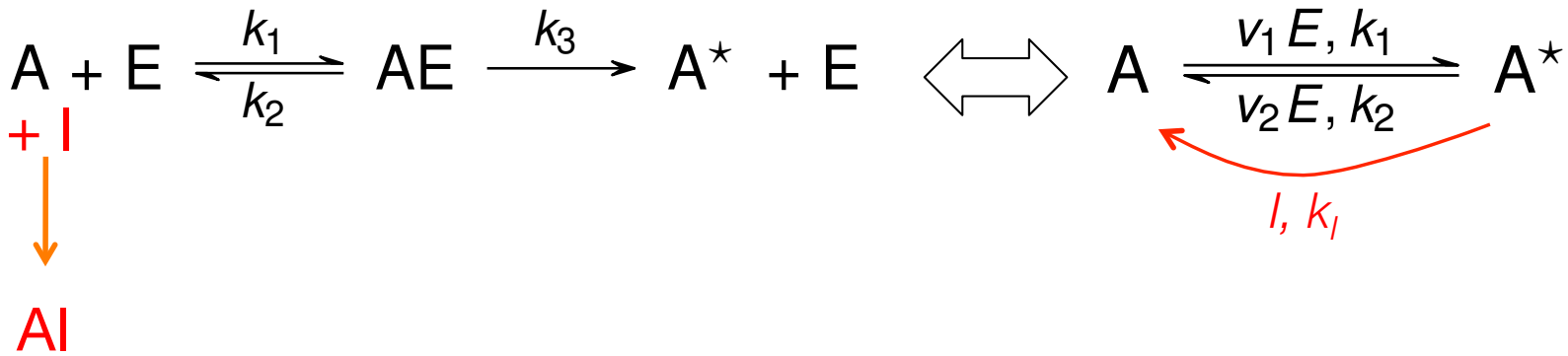
$$\begin{aligned}
\frac{d[A]}{dt} &= -k_1[A][E] + k_2[AE] \\
\frac{d[E]}{dt} &= -k_1[A][E] + k_2[AE] + k_3[AE] \\
\frac{d[AE]}{dt} &= k_1[A][E] - k_2[AE] - k_3[AE] \\
\frac{d[A^\star]}{dt} &= k_3[AE]
\end{aligned}$$

Enzyme kinetics

$$\mathrm{A} + \mathrm{E} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{AE} \xrightarrow{k_3} \mathrm{A}^\star + \mathrm{E} \quad \Longleftrightarrow \quad \mathrm{A} \underset{v_2 E,\, k_2}{\overset{v_1 E,\, k_1}{\rightleftharpoons}} \mathrm{A}^\star$$

$$\begin{aligned} \frac{d[A^\star]}{dt} &= \frac{v_1[E]([A_{total}] - [A^\star])}{k_1 + ([A_{total}] - [A^\star])} - \frac{v_2[A^\star]}{k_2 + [A^\star]} \\ A_{total} &= A + A^\star. \end{aligned}$$

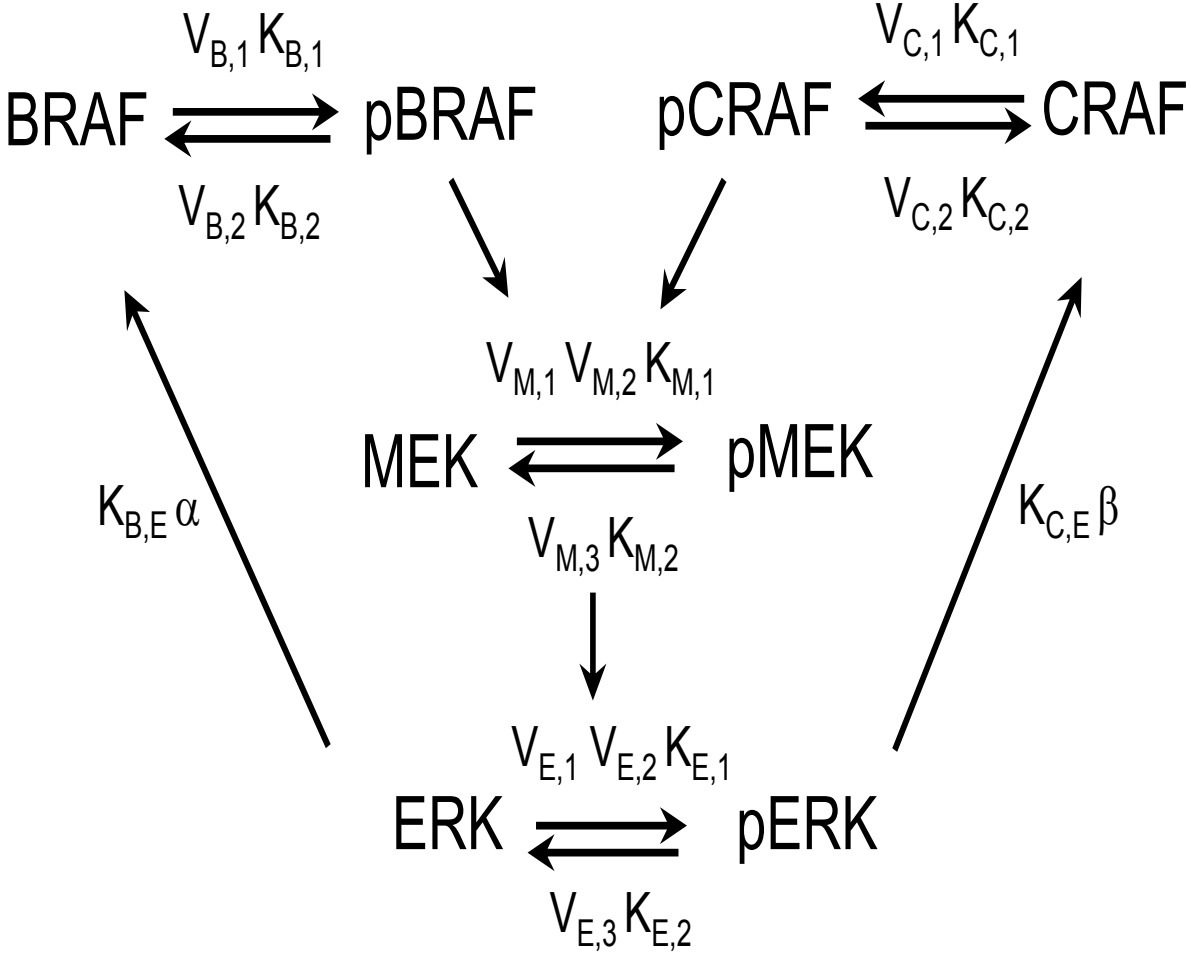
Enzyme kinetics



$$\frac{d[A^\star]}{dt} = \frac{v_1[E]([A_{total}] - [A^\star])}{k_1 + ([A_{total}] - [A^\star])}\left(1 + \frac{[I]}{k_I}\right)^{\alpha_I} - \frac{v_2[A^\star]}{k_2 + [A^\star]}$$

$$A_{total} = A + A^\star.$$

ERK pathway


$V_{B,1}$ $K_{B,1}$
BRAF
pBRAF
$V_{B,2}$ $K_{B,2}$
$V_{C,1}$ $K_{C,1}$
pCRAF
CRAF
$V_{C,2}$ $K_{C,2}$
$V_{M,1}$ $V_{M,2}$ $K_{M,1}$
MEK
pMEK
$V_{M,3}$ $K_{M,2}$
$K_{B,E}$ α
$K_{C,E}$ β
$V_{E,1}$ $V_{E,2}$ $K_{E,1}$
ERK
pERK
$V_{E,3}$ $K_{E,2}$

ERK pathway

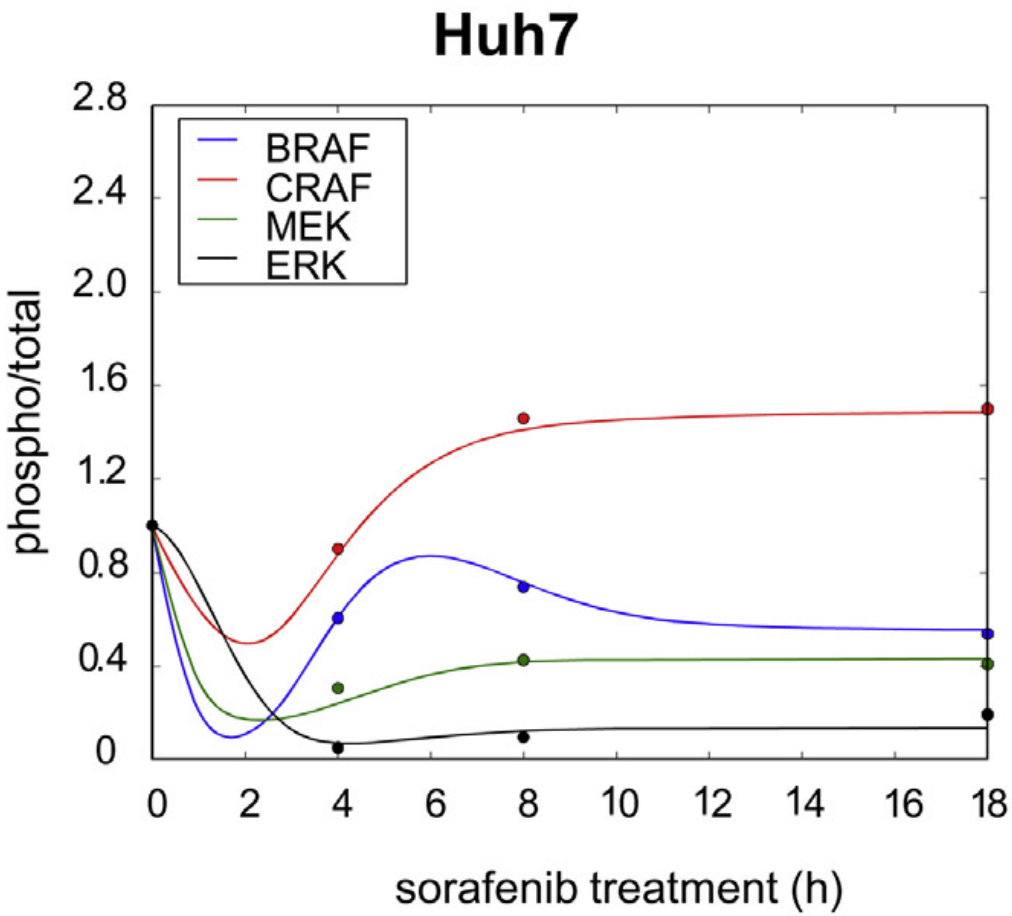
$$\frac{d[pBRAF]}{dt} = \frac{V_{B,1}([BRAF_{tot}] - [pBRAF])}{(K_{B,1} + ([BRAF_{tot}] - [pBRAF]))}\left(1 + \frac{[pERK]}{K_{B,E}}\right)^{-\alpha} - \frac{V_{B,2}[pBRAF]}{(K_{B,2} + [pBRAF])}$$

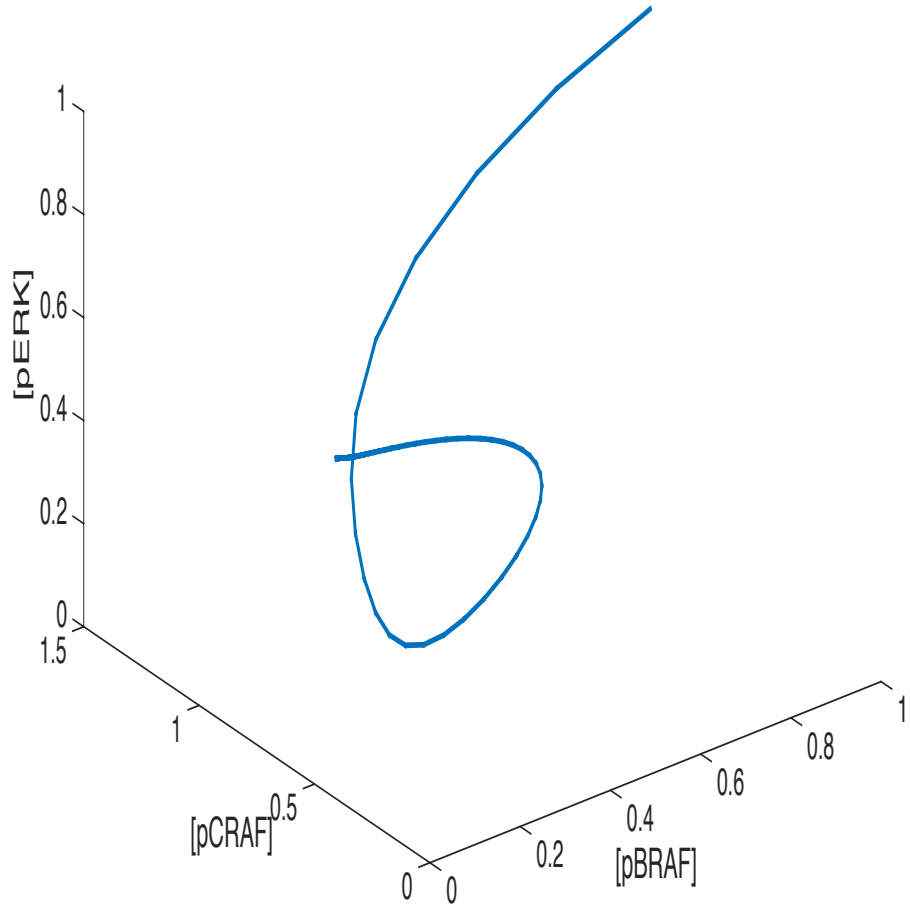
$$\frac{d[pCRAF]}{dt} = \frac{V_{C,1}([CRAF_{tot}] - [pCRAF])}{(K_{C,1} + ([CRAF_{tot}] - [pCRAF]))}\left(1 + \frac{[pERK]}{K_{C,E}}\right)^{-\beta} - \frac{V_{C,2}[pCRAF]}{(K_{C,2} + [pCRAF])}$$

$$\frac{d[pMEK]}{dt} = \frac{(V_{M,1} + V_{M,2}[pBRAF] + (1 - V_{M,2})[pCRAF])([MEK_{tot}] - [pMEK])}{K_{M,1} + ([MEK_{tot}] - [pMEK])} - \frac{V_{M,3}[pMEK]}{(K_{M,2} + [pCRAF])}$$

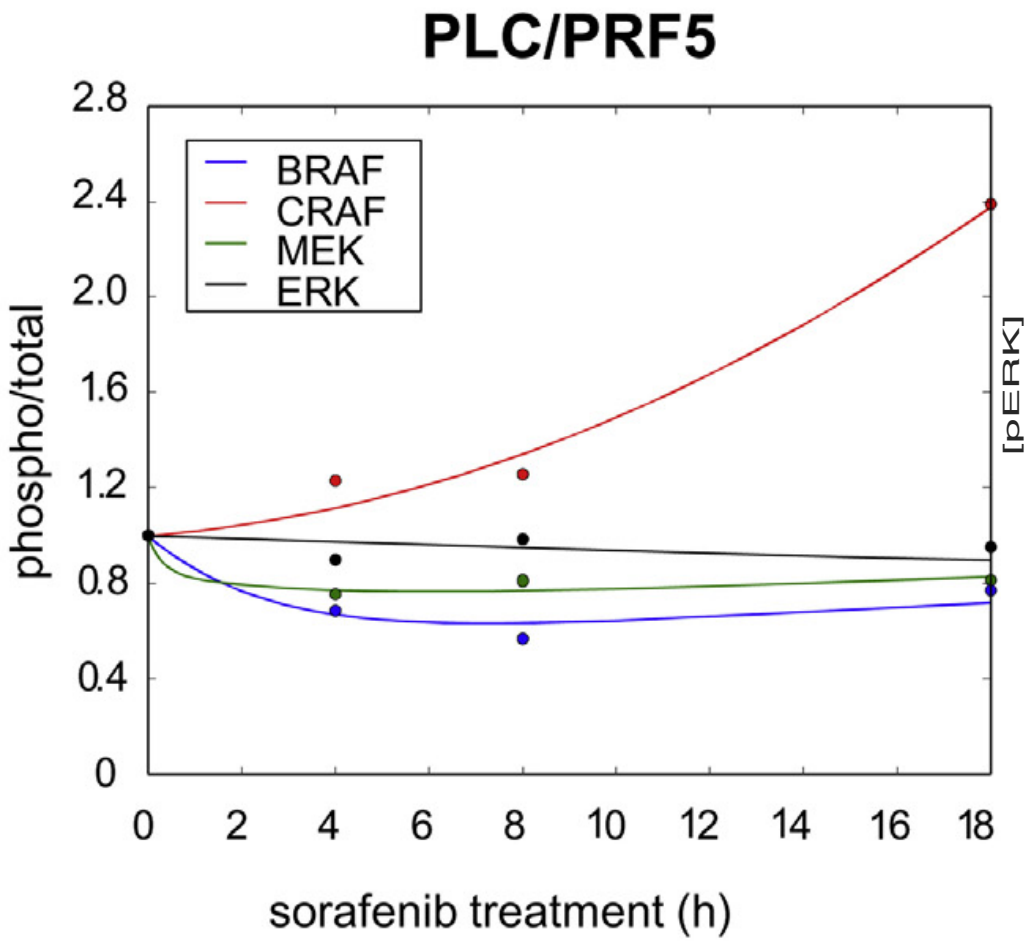
$$\frac{d[pERK]}{dt} = \frac{(V_{E,1} + V_{E,2}[pMEK])([MEK_{tot}] - [pMEK])}{K_{E,1} + ([ERK_{tot}] - [pERK])} - \frac{V_{E,3}[pERK]}{(K_{E,2} + [pERK])}$$

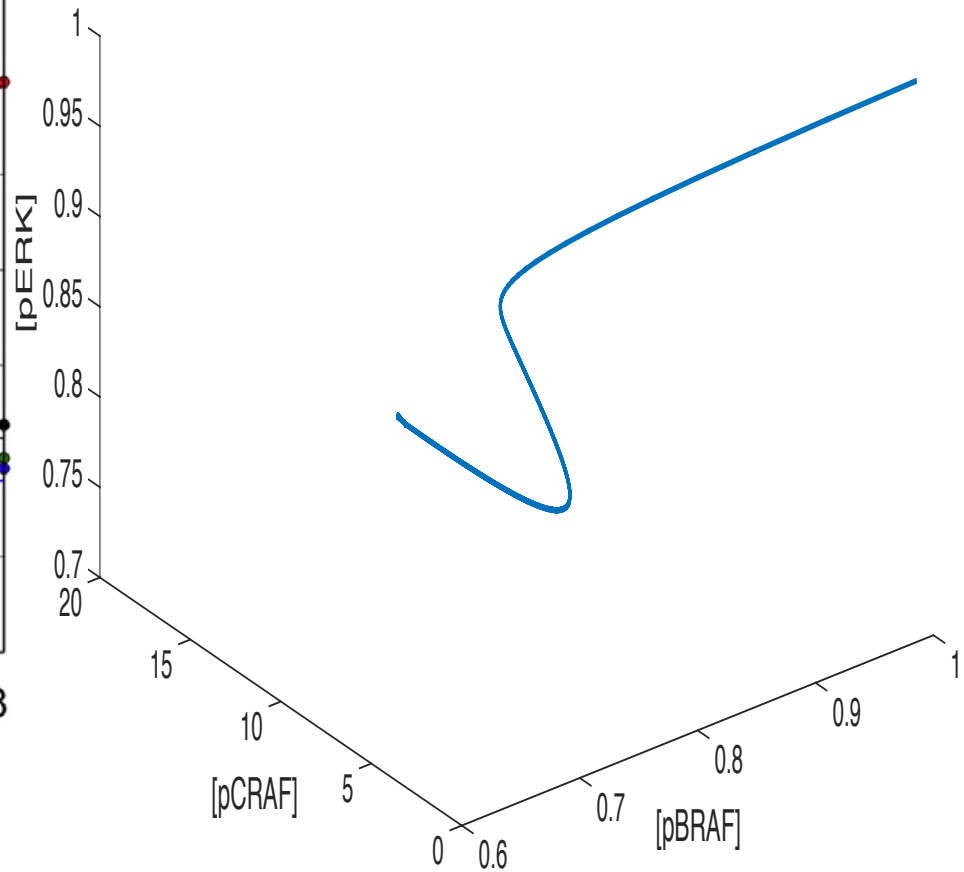
Sensitive cells



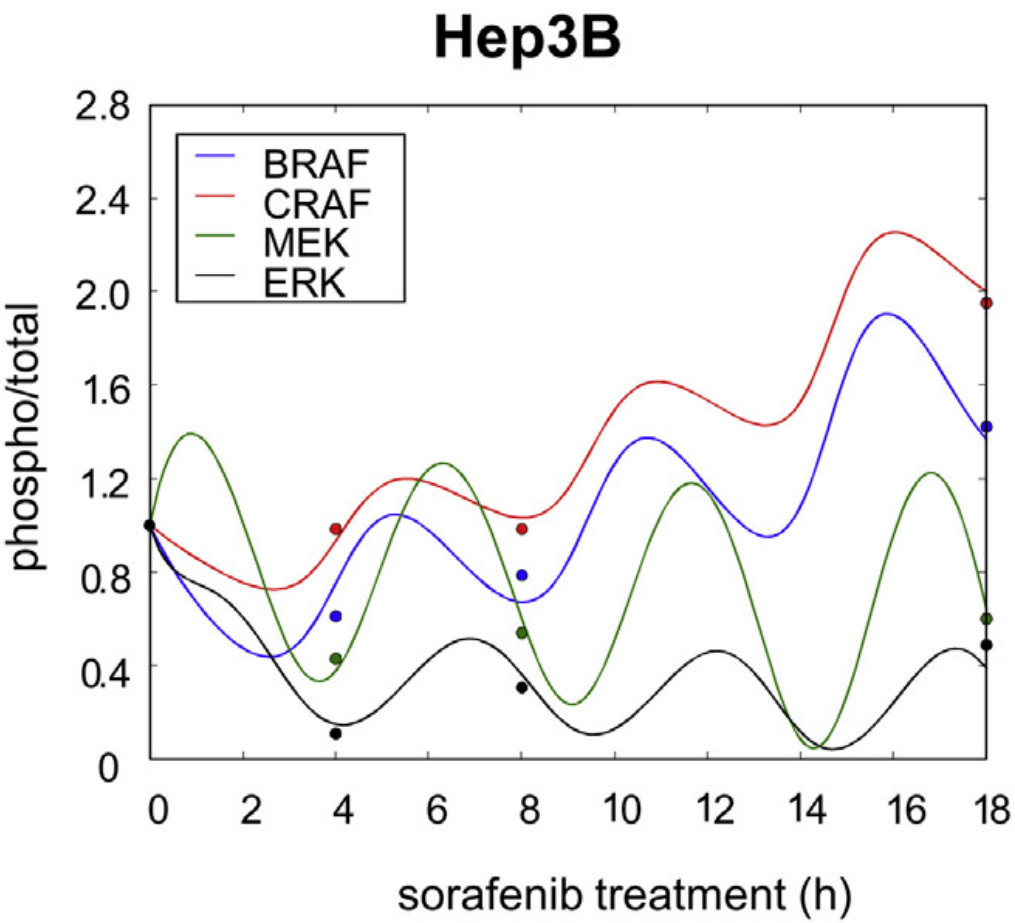


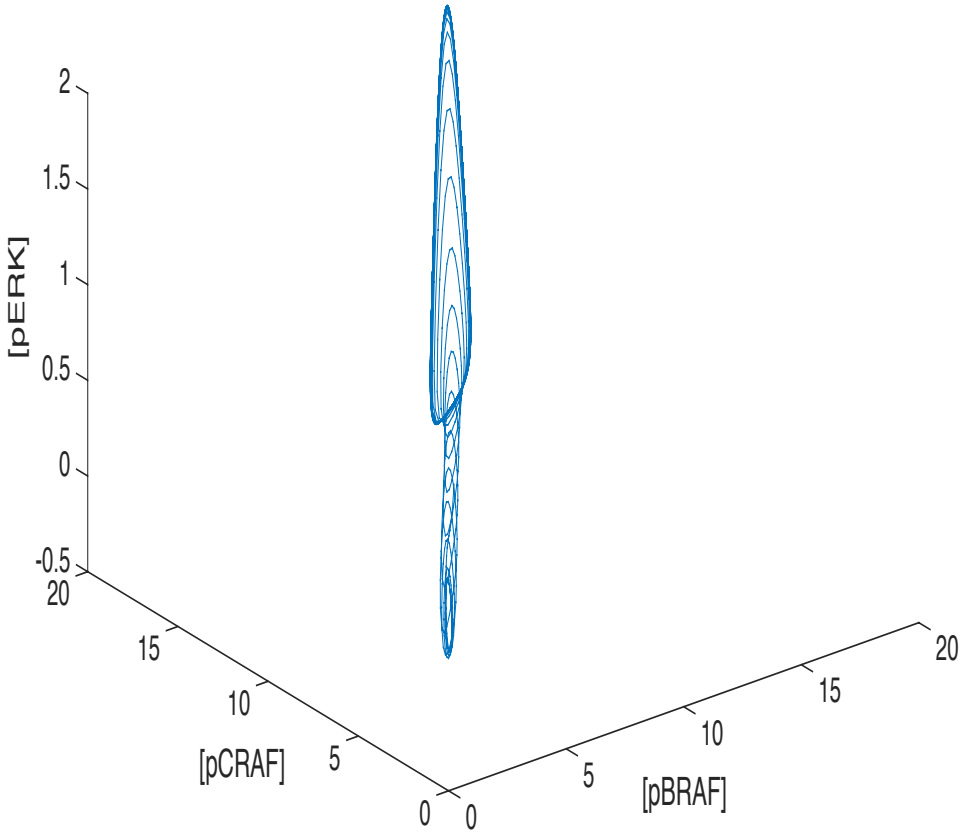
Resistant cells





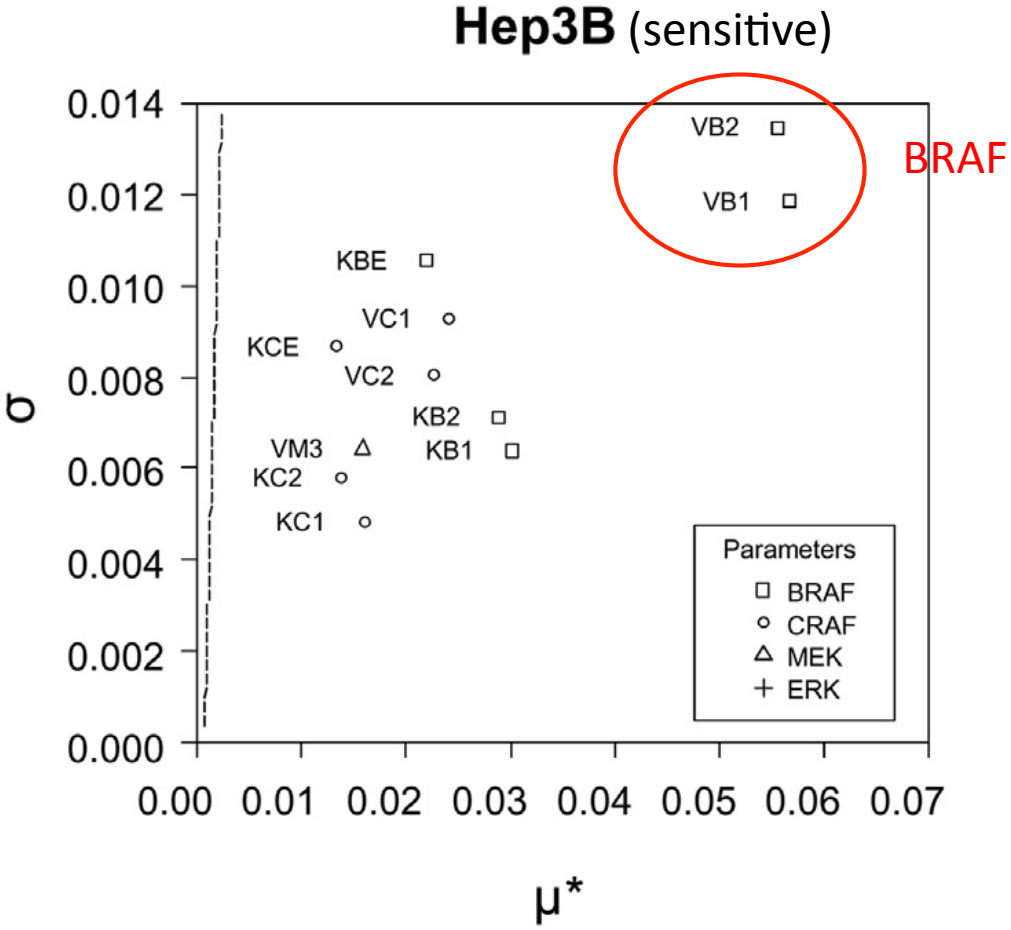
Resilient cells





Sensitivity analysis

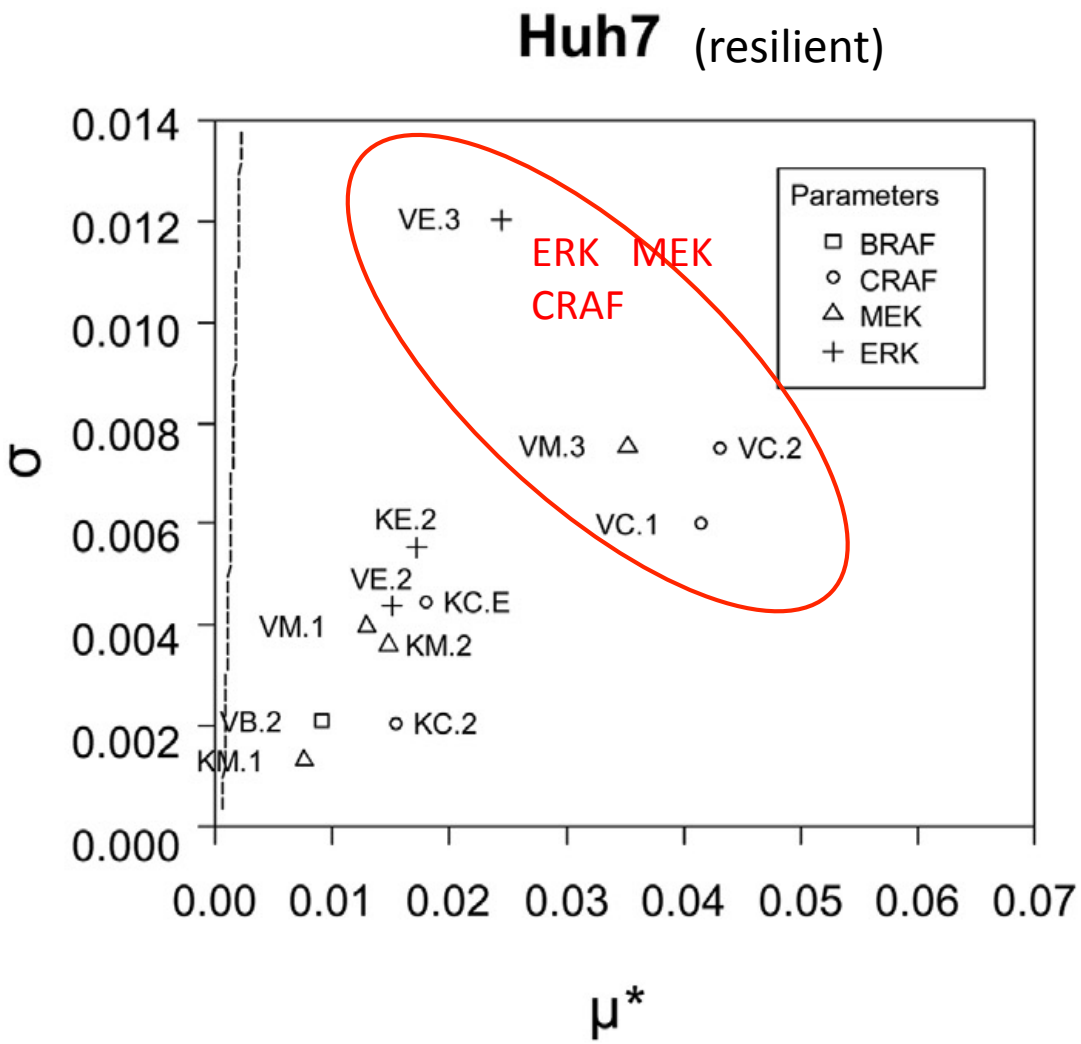
Identify the most significant effects provided by the parameters on the phosphorylated ERK amount.



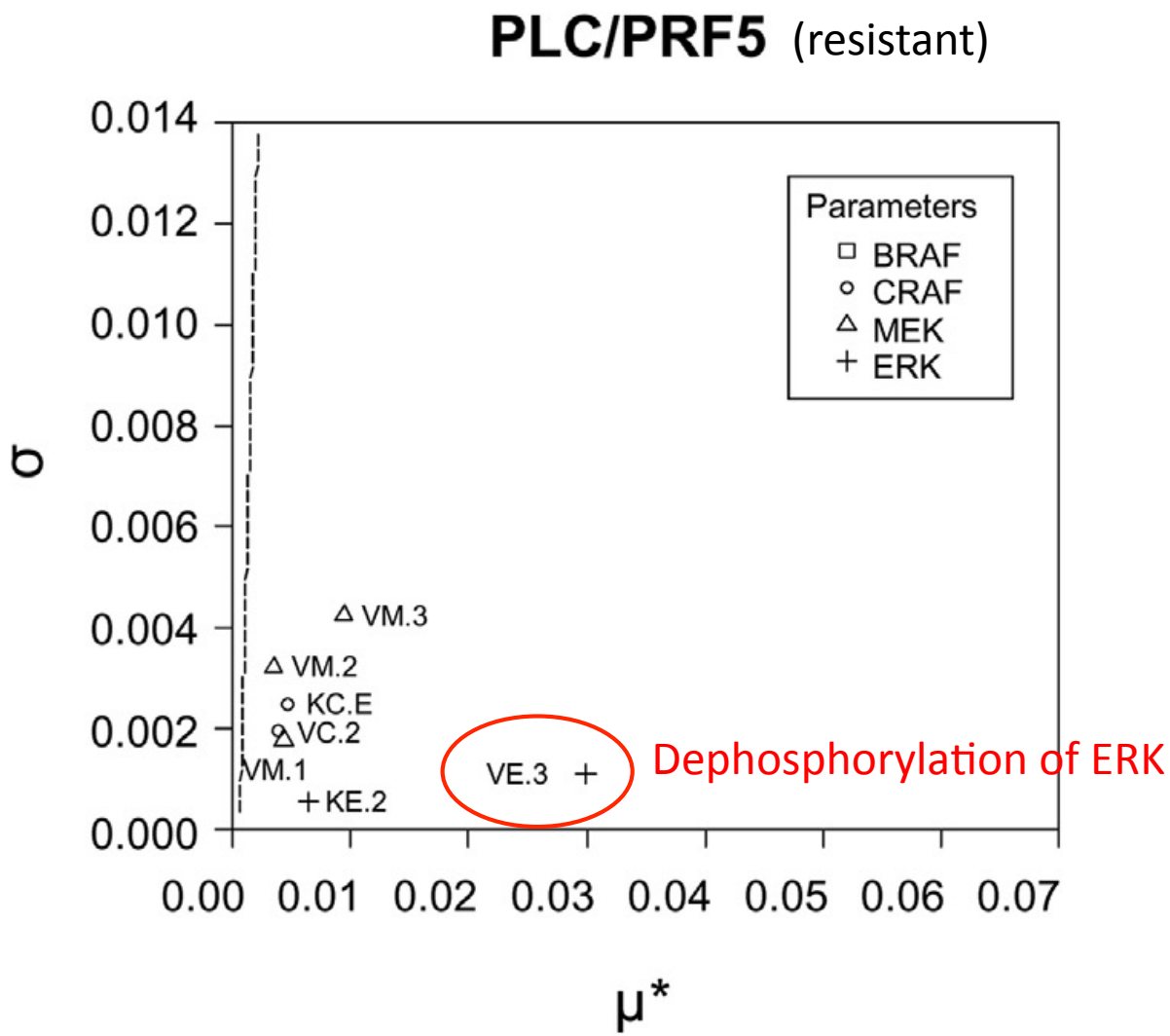
μ^* absolute mean tendancy

σ nonlinear effects with interactions

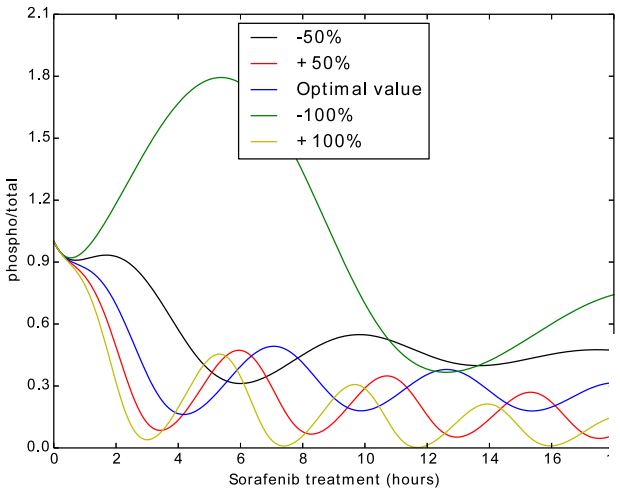
Sensitivity analysis



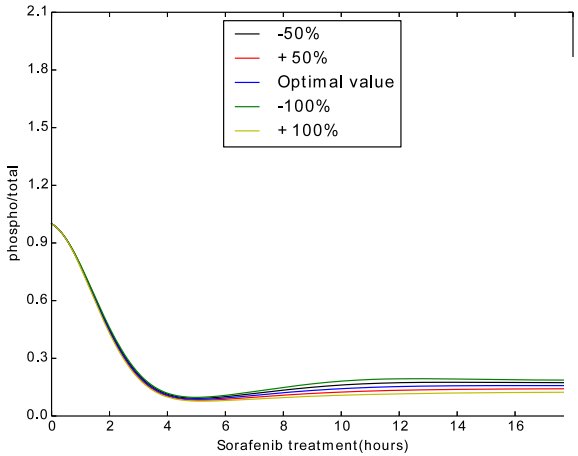
Sensitivity analysis



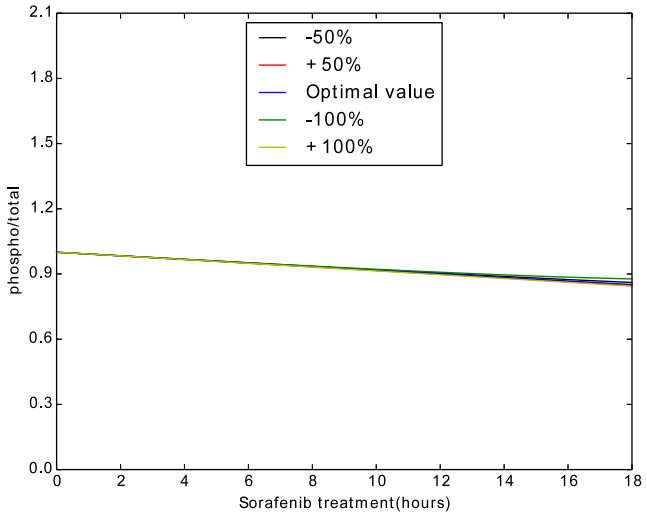
Target: RAF production?



Hep3b

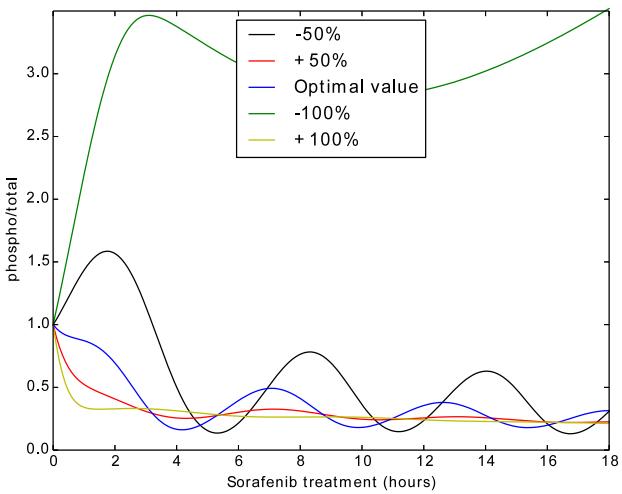


Huh7

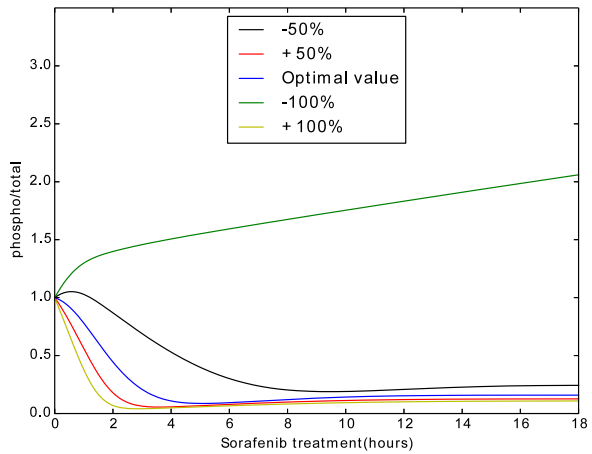


PLC\PRF5

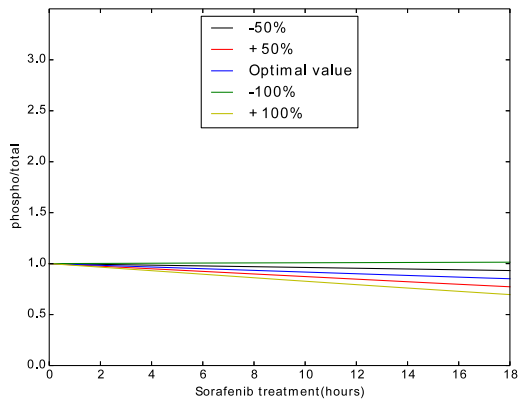
Target: ERK dephosphorylation?



Hep3b



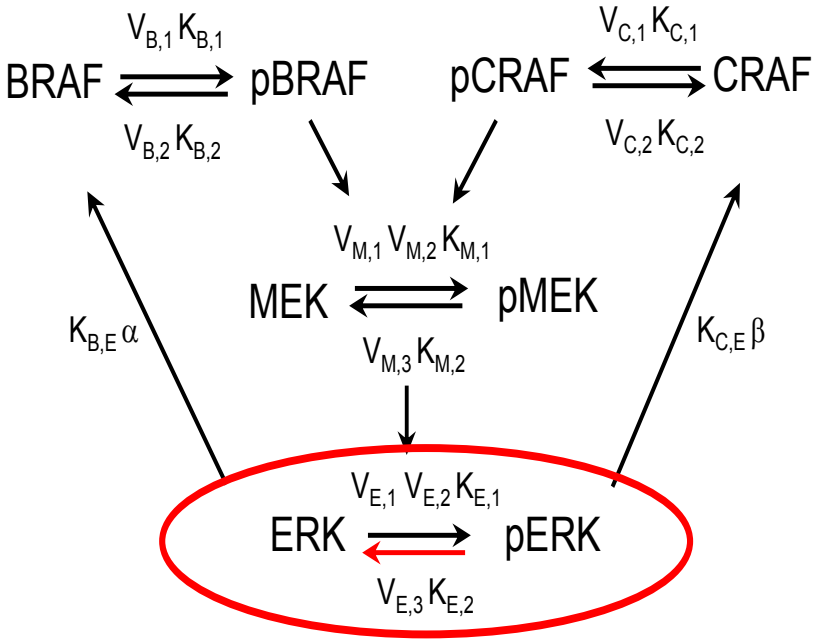
Huh7

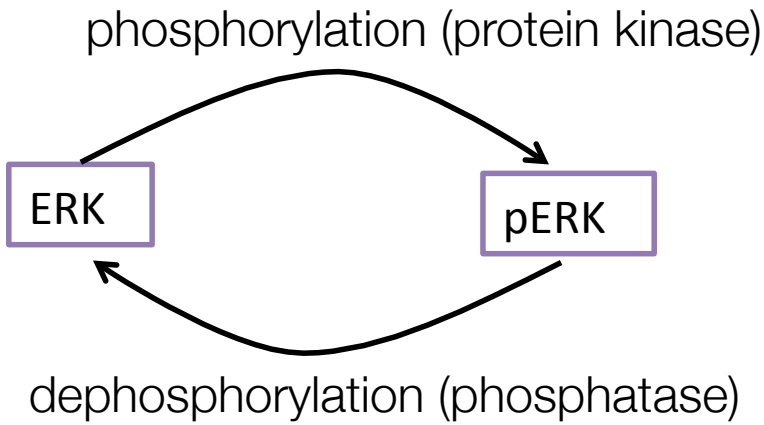


PLC\PRF5

Phosphatases

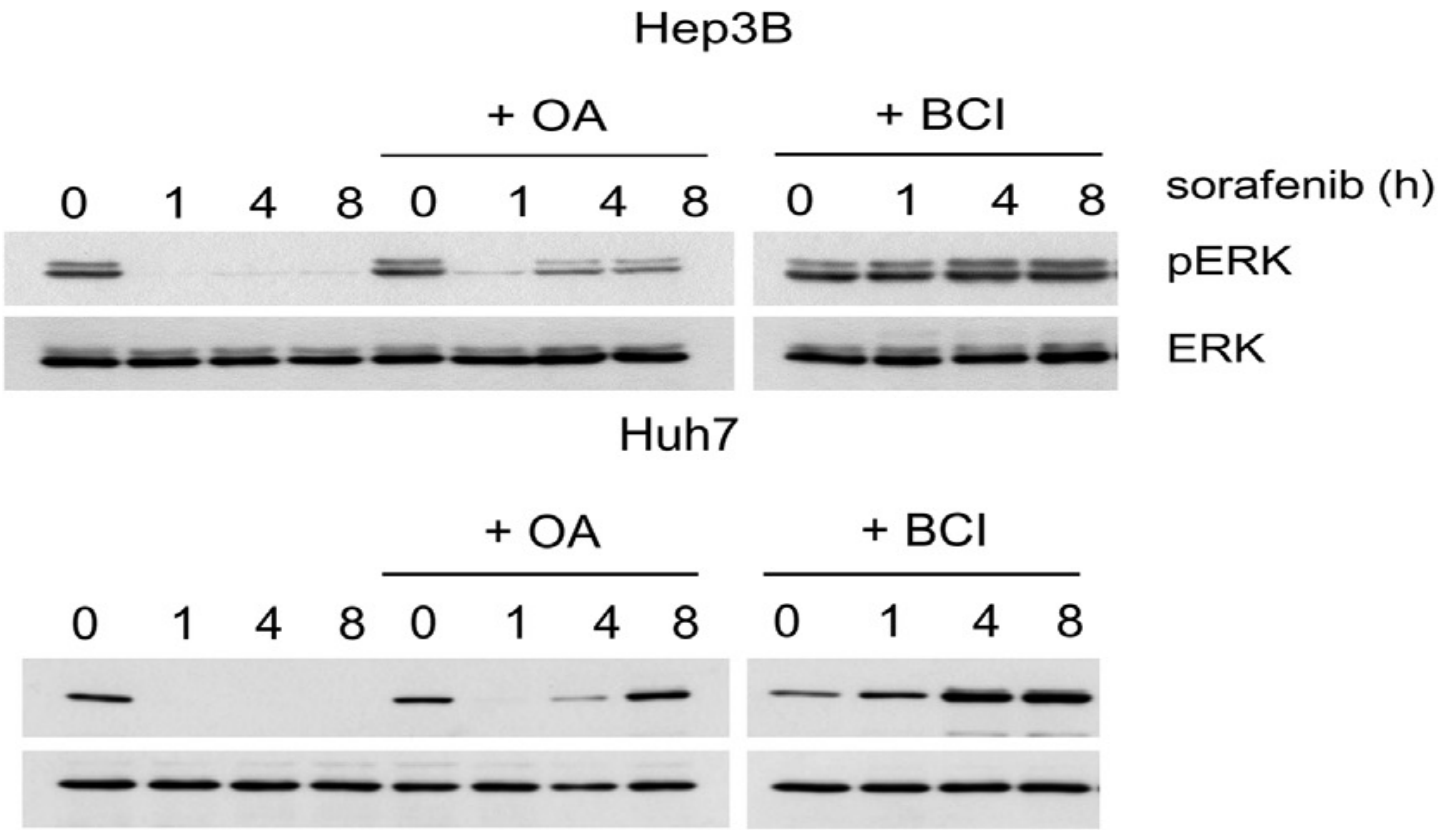
An essential actor in blocking ERK pathway by sorafenib!





Phosphatases

An essential actor in blocking ERK pathway by sorafenib!



Outline

1. General informations about cancer

2. Therapeutic target: the ERK pathway

3. Modelling of the pathway

4. Conclusion

Conclusion

Mathematics allows the study of all aspects of oncology from the most practical to the most fundamental:
non-reductionist approaches adapted to complex problems

unexpected action mode of sorafenib phosphatases
activate the pERK degradation

Each tumor is different ➔ Adjust individually the treatment of the patient

Towards a predictive oncology

Most anti-cancer drugs fail in Phase 2 clinical development

Better design clinical trials to increase the chances of success and promote therapeutic innovation

Need to develop tools to integrate this complexity and to predict the impact of each therapeutic intervention

Confirm on cells

↓↑

Transpose to animal

↓↑

Study for patient

including modeling in clinical studies

Thank for your attention