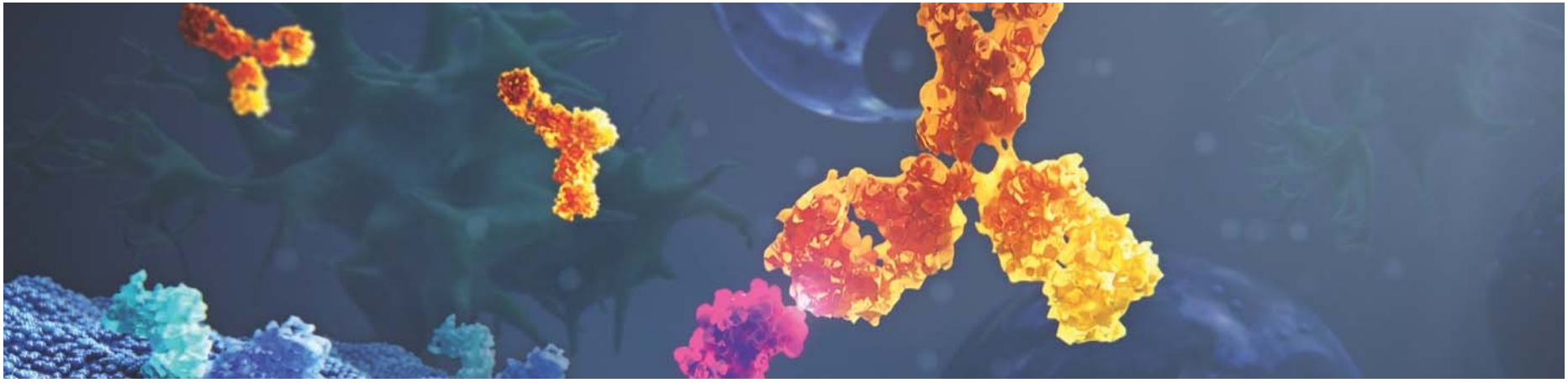
Attack of the Clones: Understanding the kinetics of resistance to cancer treatment

Dr James Yates, Oncology R&D, AstraZeneca



Introduction

Treatment failure is a barrier to cure or at least long term disease control in cancer patients.

The evolution of drug resistant cancer cells is a predominant cause.

How can we understand this quantitatively?

What do we know about drug resistance in the clinic?

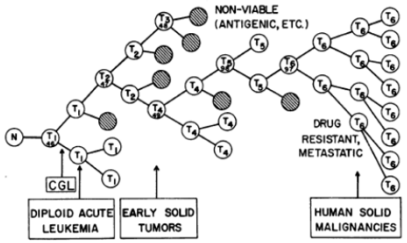
By the end of the 1970s there was evidence of clonal selection

The Clonal Evolution of Tumor Cell Populations

Acquired genetic lability permits stepwise selection of variant sublines and underlies tumor progression.

1 OCTOBER 1976 Science

Peter C. Nowell



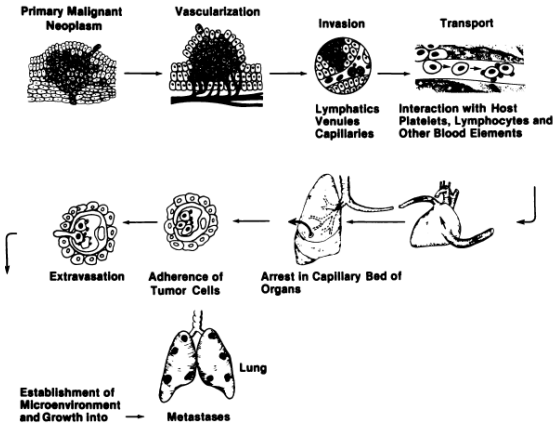
The acquired genetic instability and associated selection process, most readily recognized cytogenetically, results in advanced human malignancies being highly individual karyotypically and biologically. Hence, each patient's cancer may require individual specific therapy, and even this may be thwarted by emergence of a genetically variant subline resistant to the treatment. More research should be directed toward understanding and controlling the evolutionary process in tumors before it reaches the late stage usually seen in clinical cancer.

Cancer Research 38:2651-2660. 1978

Tumor Heterogeneity and the Biology of Cancer Invasion and Metastasis[1]

Isaiah J. Fidler

Cancer Biology Program, National Cancer Institute Frederick Cancer Research Center, Frederick, Maryland 21701

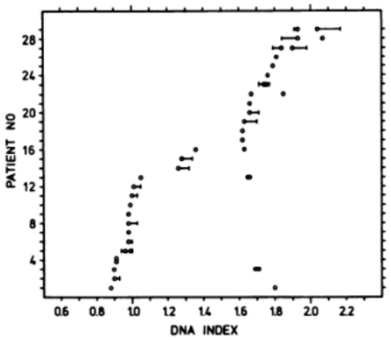


Cancer Research 40:4295-4300. 1980

Clonal Heterogeneity of Small-Cell Anaplastic Carcinoma of the Lung Demonstrated by Flow-Cytometric DNA Analysis[1]

Lars L. Vindeløv,[2] Heine H. Hansen, Ib J. Christensen, Mogens Spang-Thomsen, Fred R. Hirsch, Mogens Hansen, and Nis I. Nissen

The Finsen Institute [L. L. V., H. H. H., I. J. C., F. R. H., M. H., N. I. N.] and the University Institute of Pathological Anatomy [M. S.], Copenhagen, Denmark



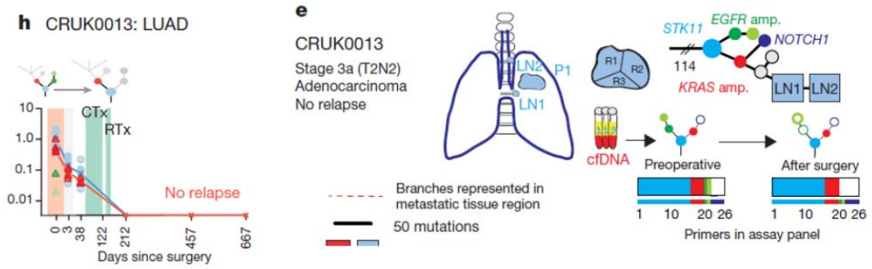
of the clones. To reach 10^9 to 10^{12} cells (1 g to 1 kg of tumor), 30 to 40 tumor volume doublings have occurred. If the 2 clones have identical TD's, they must both have been present within the first 4 TD's of the tumor. Otherwise, one of them would constitute less than 6.3% (one-sixteenth) of the total mass and would therefore escape detection. Similar doubling times there-

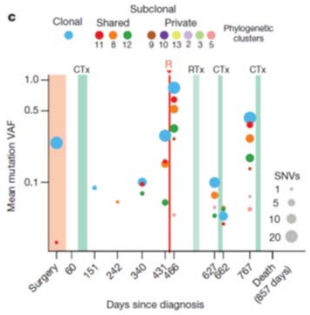
TracerX trial: C21st Tracking clonal evolution in NSCLC

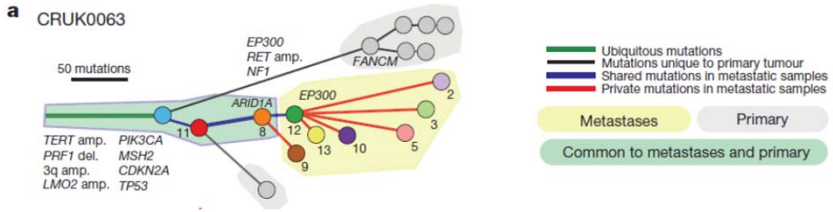
ARTICLE Nature 545: 446. 2017
doi:10.1038/nature22364

Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution

A list of authors and their affiliations appears in the online version of the paper.







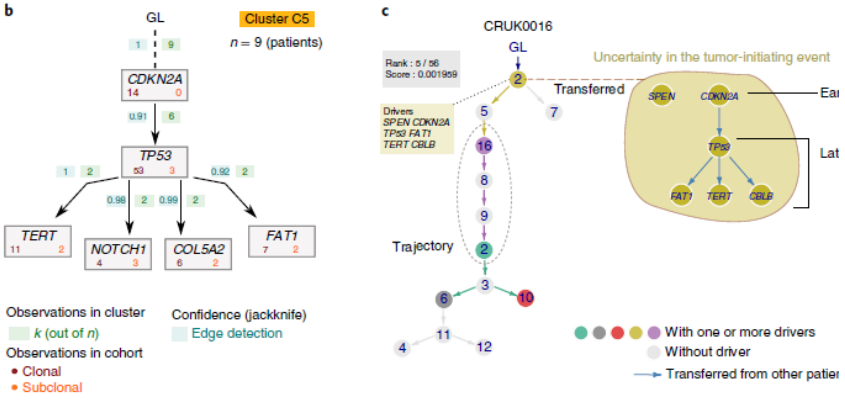
Longitudinal and multisite biopsies coupled with bioinformatic techniques unveiled the clonal evolution that results in heterogeneous tumour populations

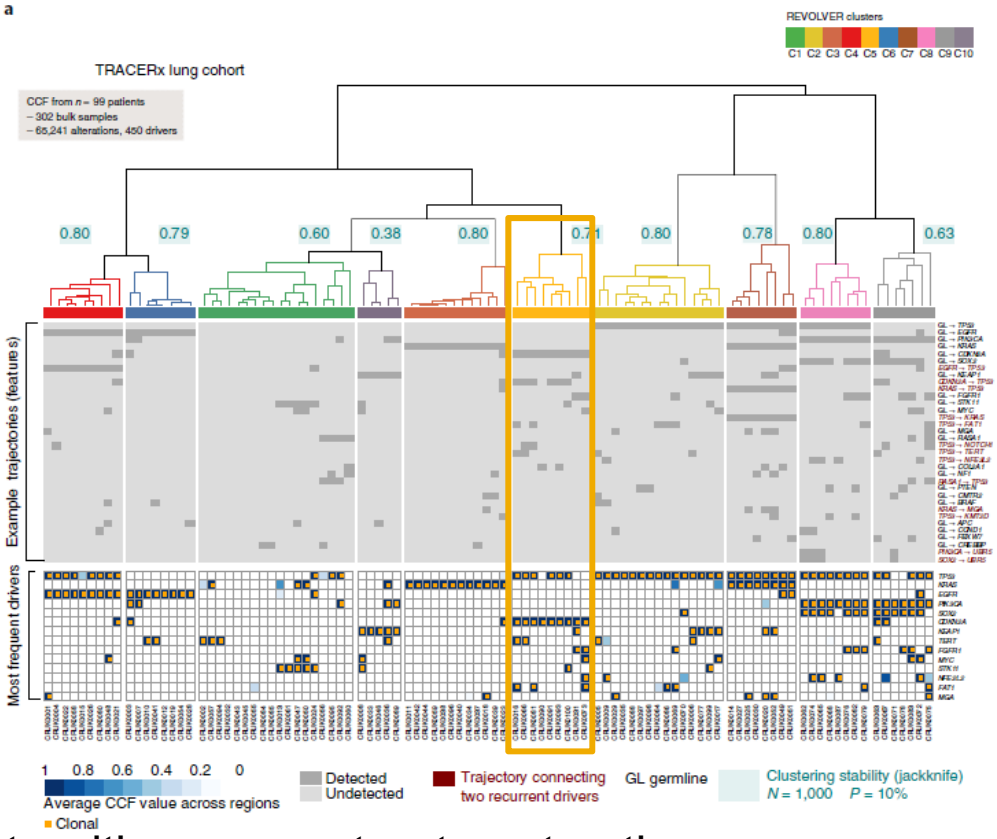
TracerX: patient clusters of evolutionary history

nature methods Vol 15. 2018 ARTICLES
https://doi.org/10.1038/s41592-018-0108-x

Detecting repeated cancer evolution from multi-region tumor sequencing data

Giulio Caravagna[1,2*], Ylenia Giarratano[2,3], Daniele Ramazzotti[4], Ian Tomlinson[5], Trevor A. Graham[6], Guido Sanguinetti[2*] and Andrea Sottoriva[1*]

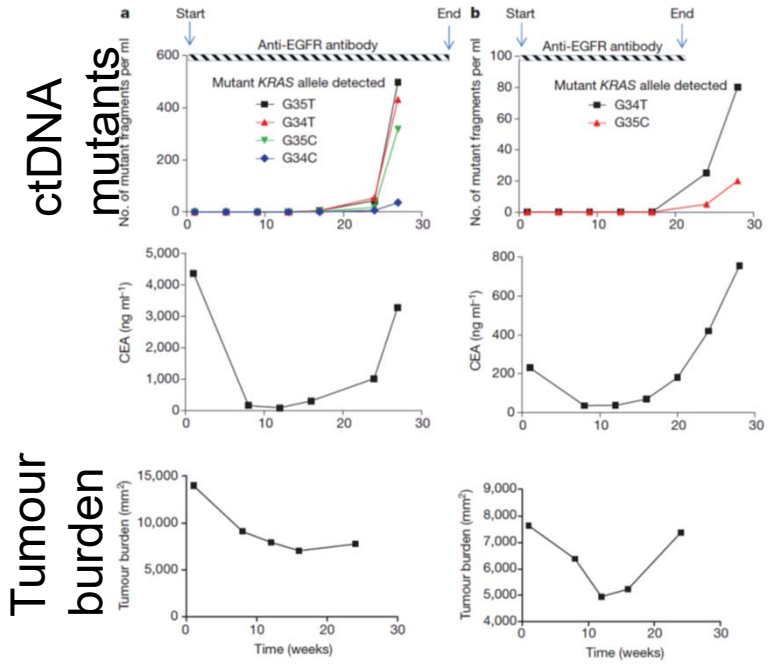




Suggests there are subgroups of patients with common treatment options
Anticipating this before treatment is key

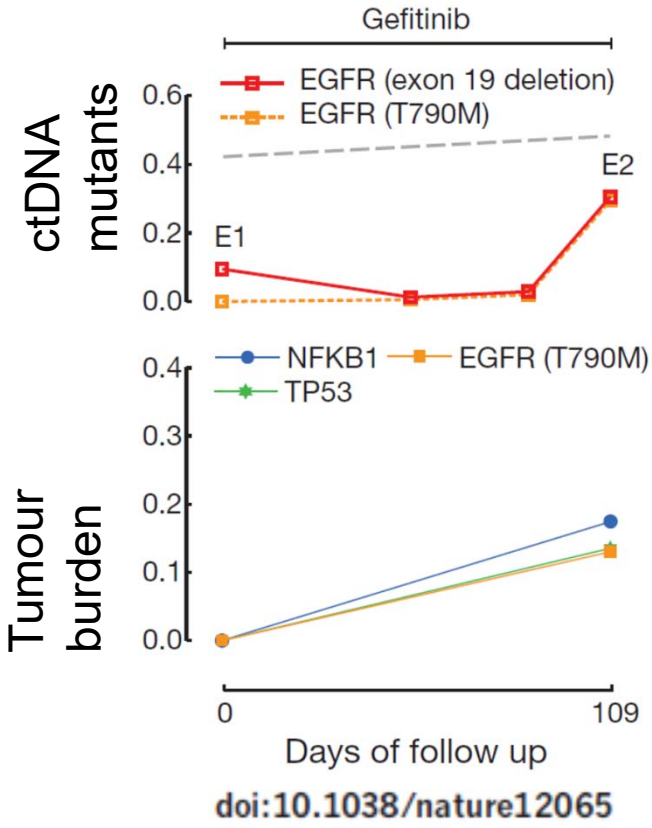
Resistance kinetics observed in Circulating tumour DNA

Colorectal cancer



doi:10.1038/nature11219

LUAD



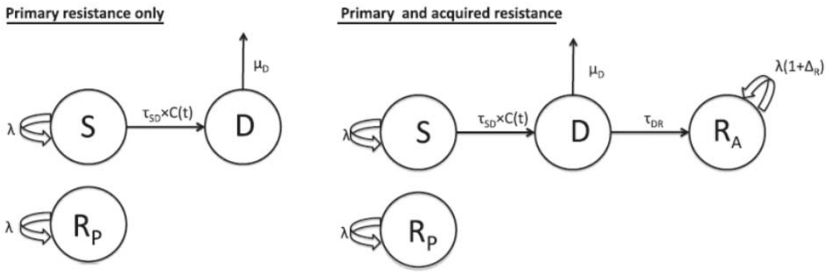
doi:10.1038/nature12065

CPT:PSP review on resistance models. Models reflecting tumour heterogeneity

Citation: CPT Pharmacometrics Syst. Pharmacol. (2019) XX, 1–18; doi:10.1002/psp4.12450

doi: 10.1111/jcp.12259

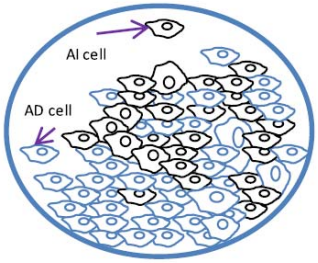
Analysis of temozolomide resistance in low-grade gliomas using a mechanistic mathematical model



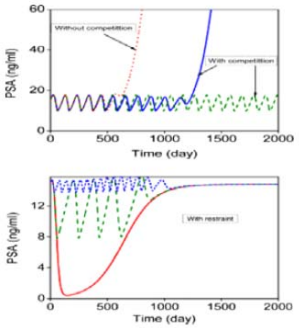
A nonlinear competitive model of the prostate tumor growth under intermittent androgen suppression

Jing Yang, Tong-Jun Zhao *, Chang-Qing Yuan, Jing-Hui Xie, Fang-Fang Hao

Journal of Theoretical Biology 404 (2016) 66–72



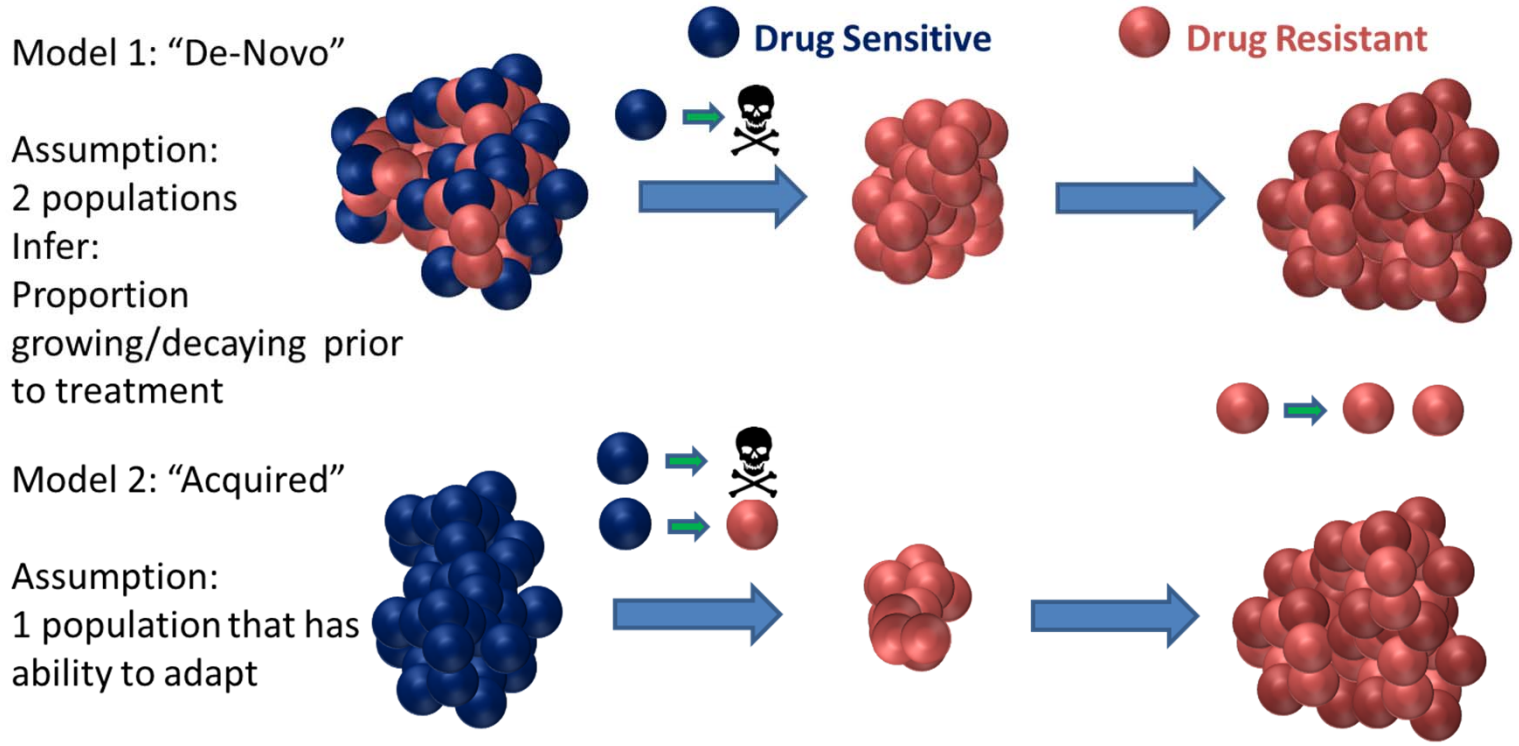
Cancer cells compete with each other in a limited environment



Common motif is delineation between drug Sensitive and Resistant Cells
Various assumptions on whether resistance pre-exists treatment
Incorporation of both mechanisms + reversal of resistant phenotype to sensitive raises question of parameter identifiability

Application: early or late onset resistance for EGFRi?

- Tested 2 models that describe baseline “de novo” resistant fraction vs on treatment mutation.



Cancer Chemotherapy and Pharmacology
https://doi.org/10.1007/s00280-019-03840-3

Application: early or late onset resistance for EGFRi?

- Tested 2 models that describe baseline “de novo” resistant fraction vs on treatment mutation.

De Novo $$Y(t) = Y(0)((1-\phi)e^{-dt}+\phi e^{gt})$$

Acquired $$Y(t) = Y(0)e^{-(d+c)t} + \frac{cY\,(0)}{d+c+g}(e^{gt} - e^{-(d+c)t}),$$

Initial size $Y(0)$

Fraction resistant ϕ

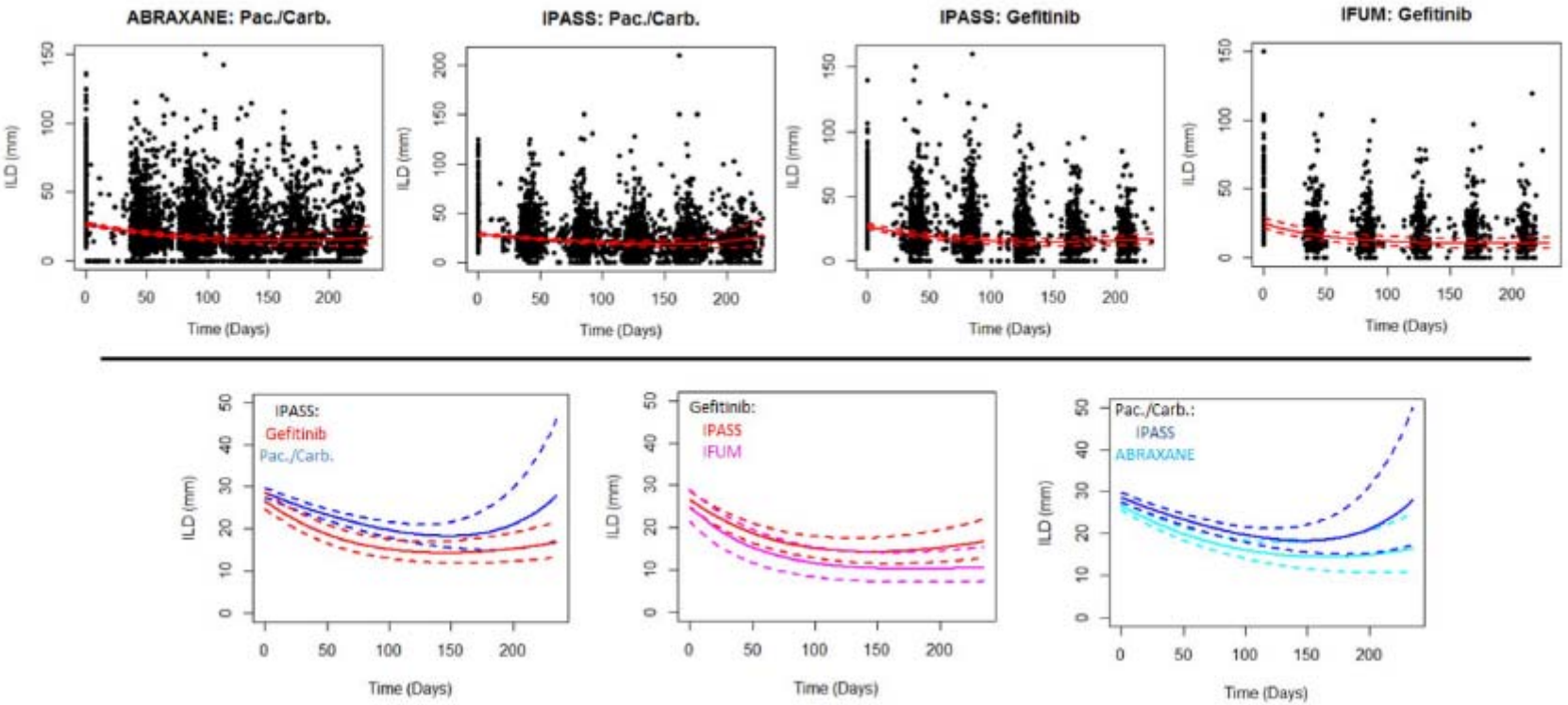
Kill rate d

Outgrowth rate g

“Mutation rate” c

Can we distinguish between these two models?

Early onset “de novo” resistance kinetics observed

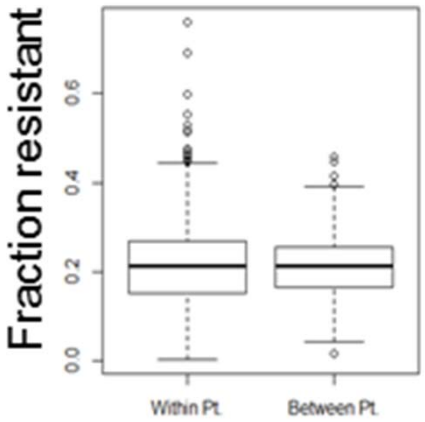


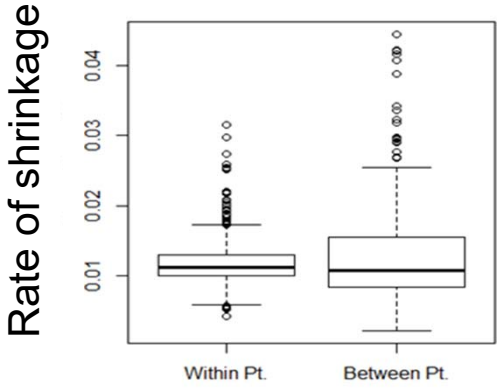
We see early “de novo” resistance and provides evidence that pre-clinical clone mixing approach is valid.

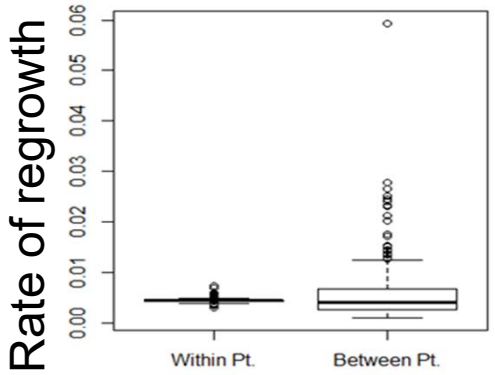
Early onset “de novo” resistance kinetics observed

Gefitinib data described by “de novo” model

Individual lesion size modelled: within patient variability< between patient except for fraction resistant







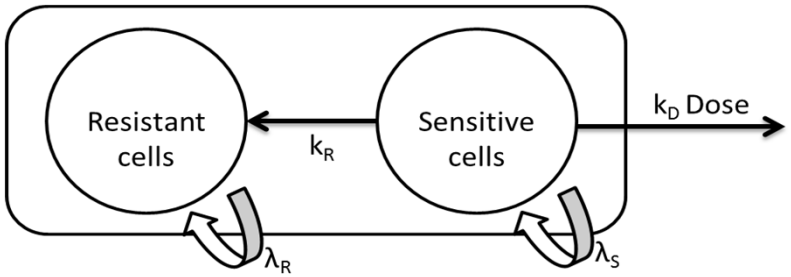
We see early “de novo” resistance and provides evidence that pre-clinical clone mixing approach is valid for investigating resistance.

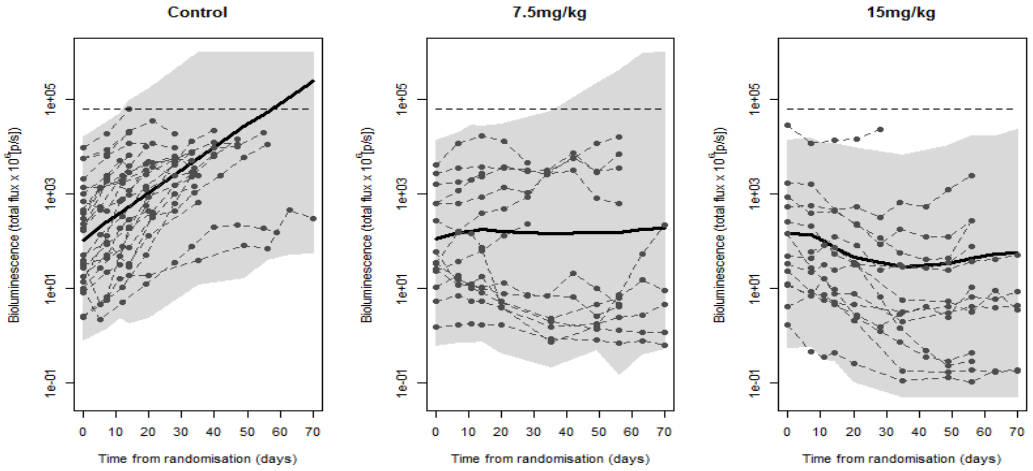
Resistance kinetics to EGFRi in mouse models

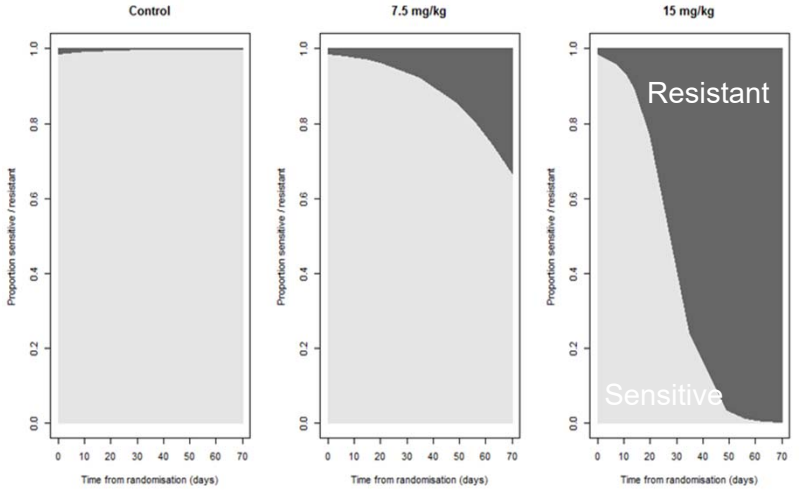
Cancer Chemotherapy and Pharmacology (2018) 82:669–675
https://doi.org/10.1007/s00280-018-3630-8

ORIGINAL ARTICLE

Pharmacodynamic modelling of resistance to epidermal growth factor receptor inhibition in brain metastasis mouse models







- Again: Baseline resistance better explanation
- Resistant cells have reduced proliferation rate
- Lower dose gives greater disease control

The impact of doubling time on resistance fraction

Bull Math Biol (2012) 74:1379–1395
DOI 10.1007/s11538-012-9717-1

ORIGINAL ARTICLE

On the Probability of Random Genetic Mutations for Various Types of Tumor Growth

Cristian Tomasetti

Model

$$S'(t) = \left[L(1-u)(1-a-b) - \left(D + bL + \frac{uaL}{2}\right)\right]\left(1 - \frac{S(t)}{K}\right)S(t),$$

$$R'(t) = \left[\left[L(1-a-b) - (D+bL)\right]R(t) + uL\left(1 - \frac{a}{2} - b\right)S(t)\right] \times \left(1 - \frac{S(t)}{K}\right). \quad (7)$$

$$P_R = 1 - \exp\left(-uM\left(\frac{1-\frac{a}{2}-b}{1-a-b}\right)\frac{1}{C}\ln\left(\frac{1}{1-C}\right)\right), \quad C = \frac{D+Lb}{L(1-a-b)}$$

$$E(T \mid resistance) \approx \frac{M}{P_R}\left(\frac{u(1-\frac{a}{2}-b)}{(1-a-2b) - D/L}\right)\ln(M).$$

Probability of resistance at diagnosis independent of law of growth kinetics

It is dependent on underlying balance of proliferation (L) vs death (D)

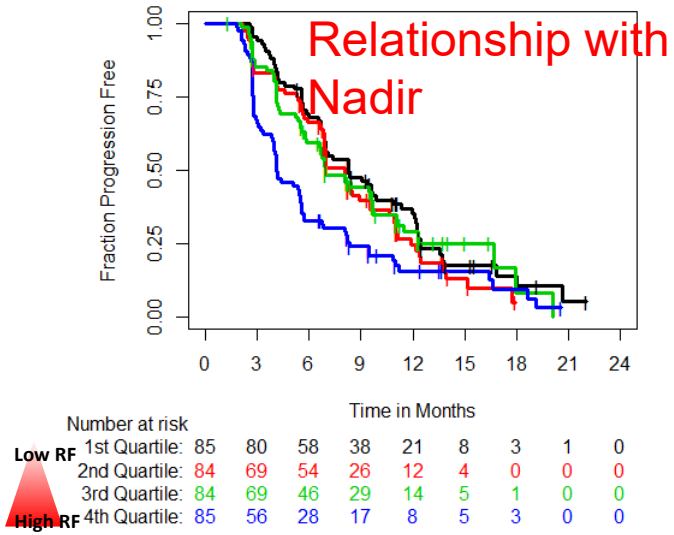
There is literature evidence that human tumours have significant cell death (D/L~0.9) whereas in animal models it is lower (D/L~0.5)

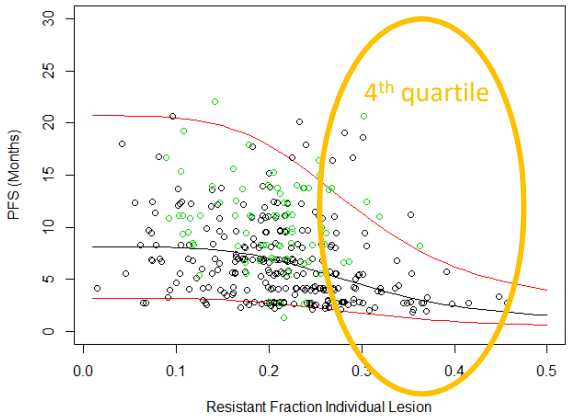
Consequences for resistance development in animal models vs clinic?

The linkage between resistance and PFS

Returning to EGFRi: Unclear relationship between tumour shrinkage and PFS

- Relationship between growing fraction and PFS exists – appears non-linear (KM-plots)
- Modelled relationship: acknowledge interval censored, data-descriptive approach, final model log-normal, used Emax model for functional relationship for covariate
  - Plot shows median, 2.5th and 97.5th PFS times as a function of growing fraction, black circles progressed patients, green circles patients right-censored





PFS is relative to nadir so no surprise not a strong relationship

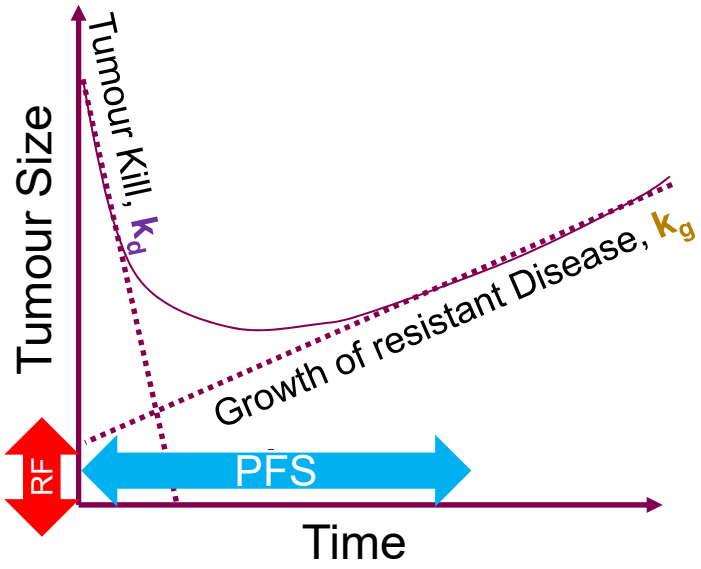
Can the poor PFS patients be spotted after first 2 visits?

A simple model explanation of why initial shrinkage and PFS are disconnected

$$Y(t) = Y(0)\left(RF.e^{k_g t} + (1 - RF).e^{-k_d t}\right)$$

Response kinetics defined by:

1. Baseline
2. Resistant fraction
3. Rate of shrinkage of drug sensitive fraction
4. Rate of regrowth of drug resistant fraction



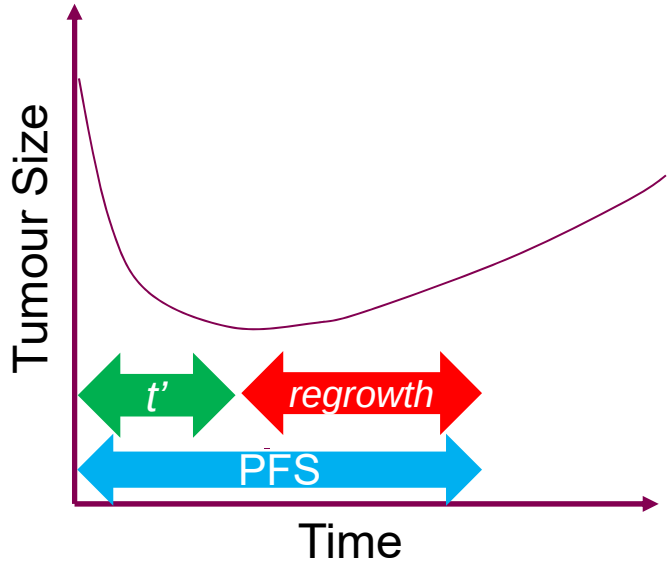
This simple model has appeared several times in the literature.

PFS is time to nadir (*t'*) + time to 20% increase

$$Y(t) = Y(0)\left(RF.e^{k_g t} + (1 - RF).e^{-k_d t}\right)$$

Response kinetics defined by:

1. Baseline
2. Resistant fraction
3. Rate of shrinkage of drug sensitive fraction
4. Rate of regrowth of drug resistant fraction



In the next few slides we will look at the relationship between these parameters

PFS is not always sensitive to resistant fraction

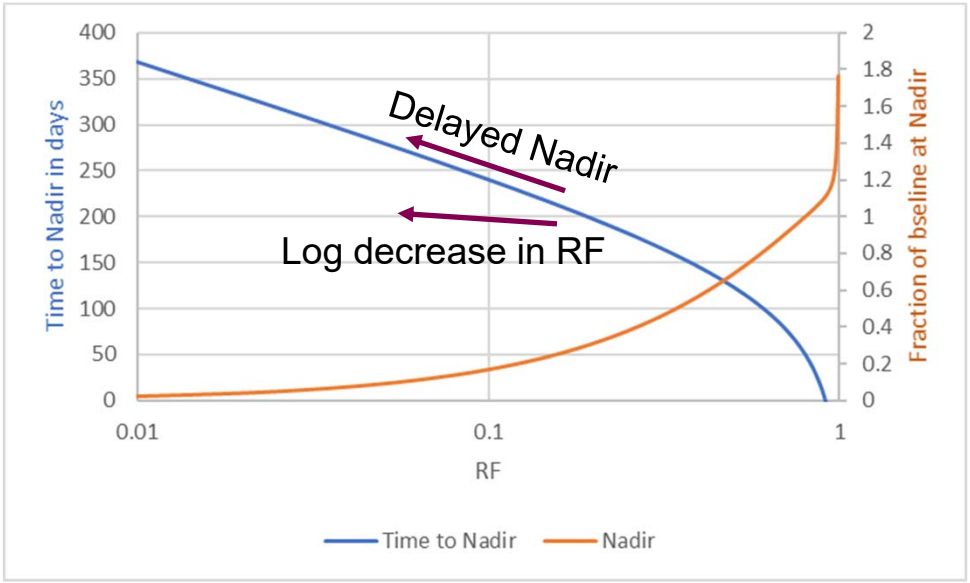
$$t' = \frac{1}{k_g + k_d} ln \left[\frac{k_d(1 - RF)}{k_g . RF} \right]$$

We can see RF needs to be reduced by order of magnitude to increase *t'*

$$RF = \frac{1}{1 + \frac{k_g}{k_d} e^{(k_g + k_d).t'}}$$

At *t'* the tumour will be this size

$$Y(0)(1 - RF)e^{-k_d t'}$$



Time to progression post nadir is defined by k_g

By nadir the tumour has been "purified" and now the resistant fraction is

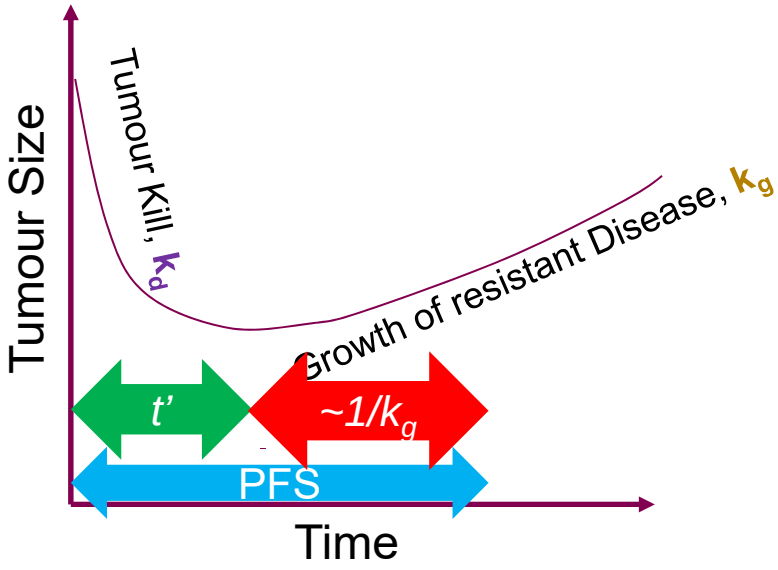
$$RF' = \frac{k_d}{k_g + k_d}$$

After this point the tumour the tumour will grow as:

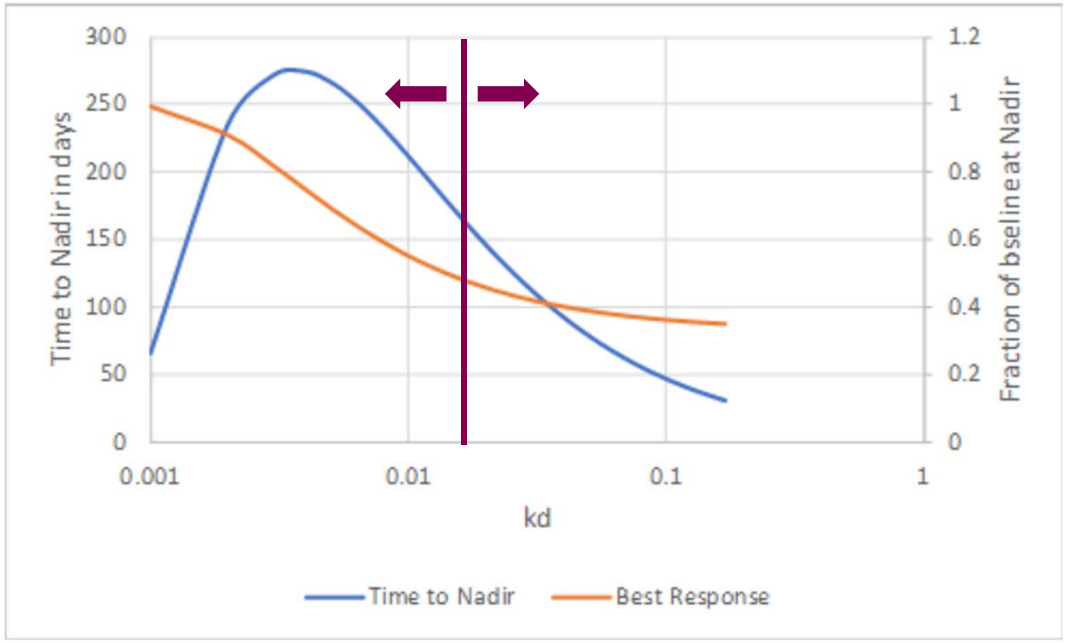
$$Y(t') = Y(0)(1 - RF)e^{-k_d t'}\left[\frac{k_d}{k_g}e^{k_g(t-t')} + e^{-k_d(t-t')}\right]$$

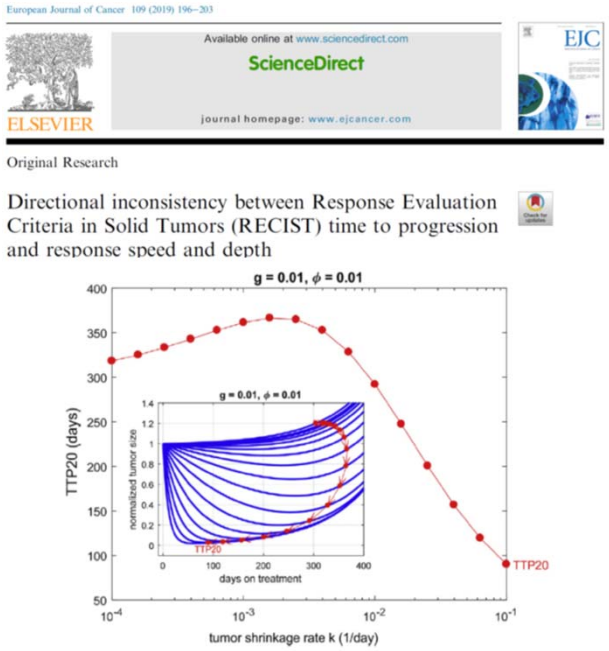
Progression at 20% increase from nadir

Progression is a function of 1/ k_g



Time to nadir and rate of tumour kill: Biphasic relationship





Reducing tumour kill rate could increase time to nadir without impacting best response significantly.
Observed via simulation by Millennium Pharmaceuticals Researchers

Linking Initial Response to OS

CLINICAL PHARMACOLOGY & THERAPEUTICS | VOLUME 86 NUMBER 2 | AUGUST 2009

FDA NSCLC model with different outgrowth (resistance) rates

$$TS_i(t) = BASE_i \cdot e^{-SR_i \cdot t} + PR_i \cdot t,$$

Treatment	M_BASE (cm)	M_SR (1/week)	M_PR (cm/week)
PCB	9.1 (0.33)	0.06 (0.004)	0.13 (0.02)
PC	8 (03)	0.038 (0.01)	0.14 (0.04)
DC	8.7 (0.31)	0.052 (0.01)	0.16 (0.02)
DCb	9.2 (0.38)	0.047 (0.005)	0.16 (0.02)
VC	8.5 (0.28)	0.063 (0.01)	0.17 (0.02)
DT	8.5 (0.82)	0.033 (0.01)	0.13 (0.02)
PT	7.4 (0.47)	0.023 (0.01)	0.25 (0.05)
PB[a] Placebo	8.6 (0.44)	0.0047 slow (0.001) 0.13 fast (0.004)	0.20 (0.02)
ET[a]	8.4 (0.32)	0.0045 slow (0.001) 0.11 fast (0.05)	0.058 (0.02)

DC, docetaxel and cisplatin; DCb, docetaxel and carboplatin; DT, docetaxel; ET, erlotinib; PB, placebo; PC, paclitaxel and carboplatin; PCB, paclitaxel, carboplatin, and bevacizumab; PT, pemetrexed; VC, vinorelbine and cisplatin.

- Tumour reduction at 8 weeks shown to be predictive of OS
- Prentice criteria: Many of these studies do not show OS effect differences are fully account for by tumour response. i.e. show treatment not a covariate
- Why do these treatments show different progression growth rates?

Key points:

PFS on target lesions as defined by RECIST 1.1 is time to nadir+time to 20% regrowth

PFS in a population will be increased by targeting more mutations because of an increased % objective response rate: Responders have PFS

PFS and OS in a patient will be increased by leaving slower growing disease: post nadir time ~$1/k_g$

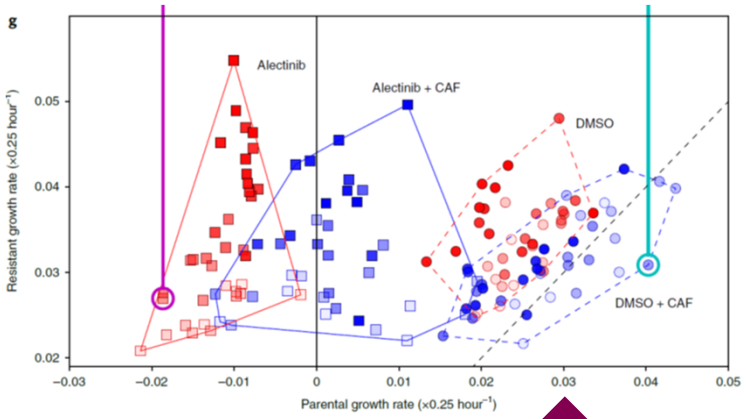
Resistance in nonclinical cancer models
Scope for understanding the clinical issues?

Competition assays: ALK inhibitor example

Nature Ecology and Evolution 3: 450. 2019

Fibroblasts and alectinib switch the evolutionary games played by non-small cell lung cancer

Artem Kaznatcheev[1,2*], Jeffrey Peacock[3], David Basanta[4], Andriy Marusyk[5*] and Jacob G. Scott[2,6*]

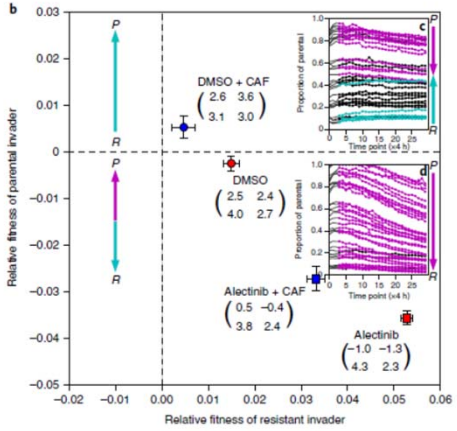


- Growth rate determined using fluorescent protein expression
- Density of drug sensitive parental cell line (opacity of point) alters the growth rate of resistant cells

$$\begin{array}{cc} & \begin{matrix} P & R \end{matrix} \\ \begin{matrix} P \\ R \end{matrix} & \begin{pmatrix} A & B \\ C & D \end{pmatrix} \end{array} \Rightarrow \begin{cases} \frac{d}{dt}N_P = N_P \overbrace{\left(A\frac{N_P}{N_T} + B\frac{N_R}{N_T}\right)}^{\hat{w}_P\text{: parental growth rate}} \\ \frac{d}{dt}N_R = N_R \underbrace{\left(C\frac{N_P}{N_T} + D\frac{N_R}{N_T}\right)}_{\hat{w}_R\text{: resistant growth rate}} \end{cases}$$

$$\Rightarrow \frac{dp}{dt} = p(1-p)\overbrace{\left(\underbrace{(B-D)}_{\substack{\text{relative fitness of} \\ \text{parental invader}}}(1-p) - \underbrace{(C-A)}_{\substack{\text{relative fitness of} \\ \text{resistant invader}}}p\right)}^{\text{gain function for } p}$$

where $N_T = N_P + N_R$ and $p = \frac{N_P}{N_T}$



Mathematical model where exponential growth rate is dependent on the presence of other clones.

Results suggest proliferation (fitness) of drug resistant cells is altered in the presence of drug sensitive cells

Competition assays in vitro and in vivo in vivo

LETTER Nature 520: 368. 2015

doi:10.1038/nature14336

Therapy–induced tumour secretomes promote resistance and tumour progression

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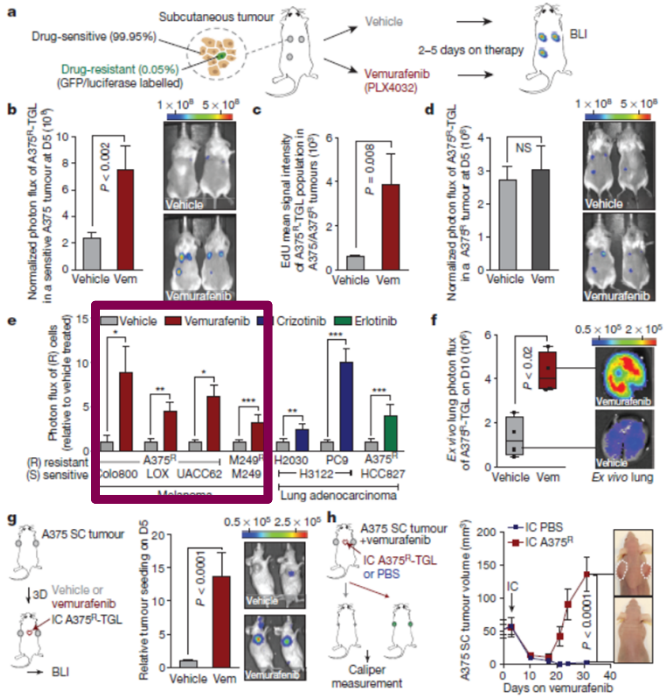
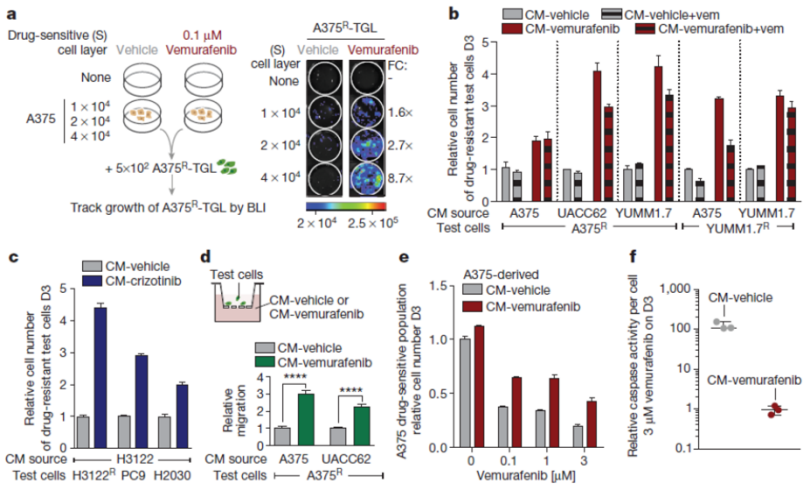


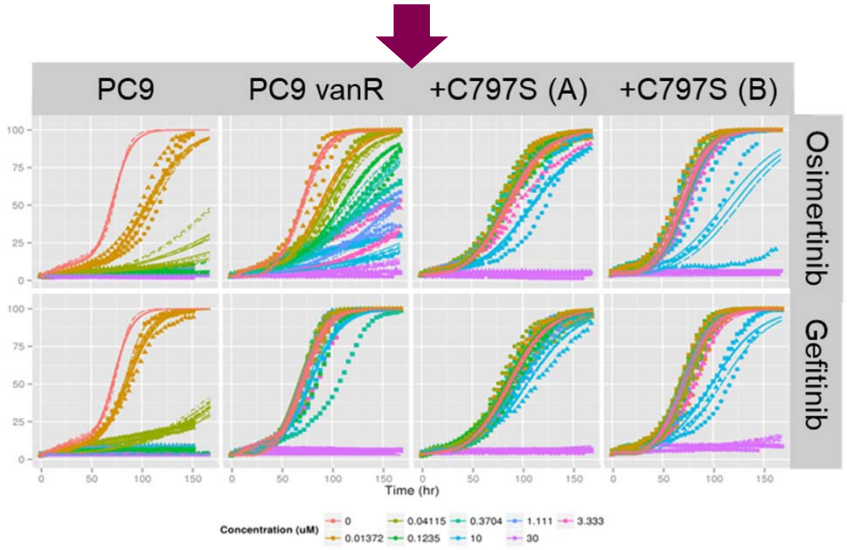


Similar interactions observed for EGFR, BRAF and ALK inhibitors

-Resistant cells grow more rapidly in the presence of Sensitive cells and drug

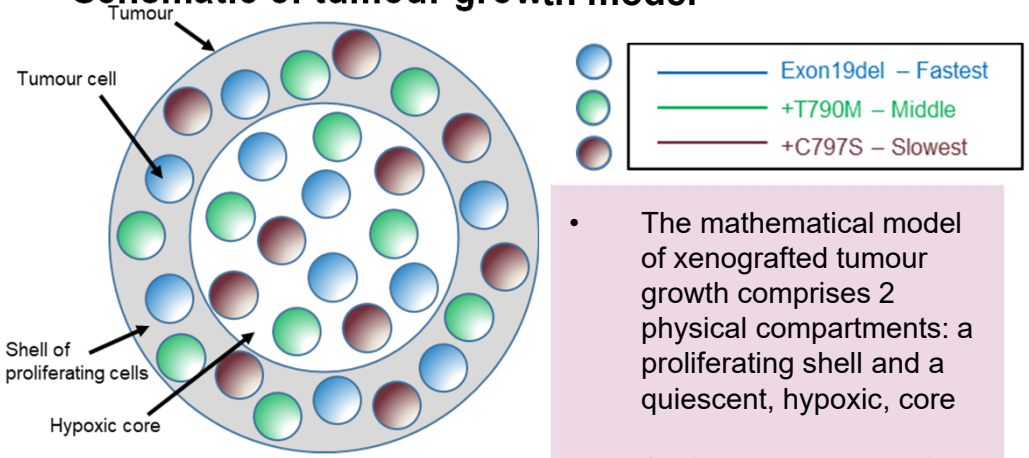
Model Application: In vitro profiling – In vivo extrapolation

In vitro time course of cell confluence with model fits super-imposed.



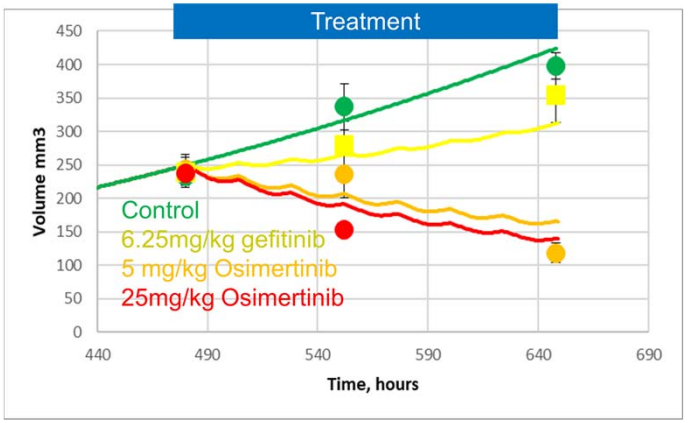
a) PC9 is sensitive to both drugs and grows most rapidly
b) PC9vanR is resistant to Gefitinib
c) The addition of C797S mutation renders the cells resistant to both drugs but also slow growth

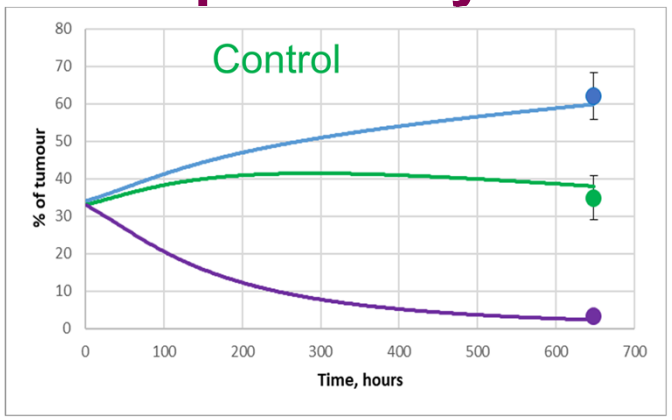
Schematic of tumour growth model



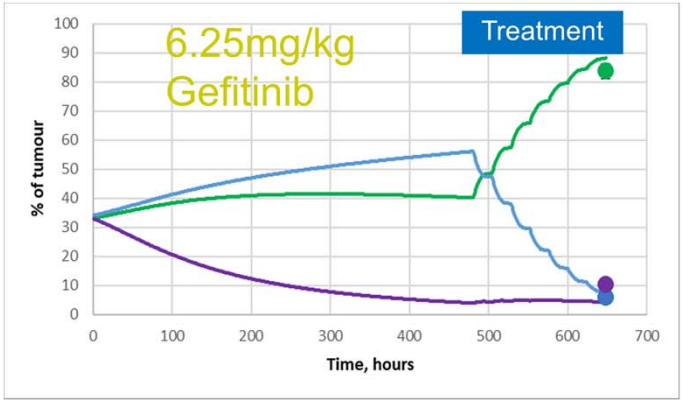
- The mathematical model of xenografted tumour growth comprises 2 physical compartments: a proliferating shell and a quiescent, hypoxic, core
- As the tumour grows the hypoxic region expands, thus the rate of growth reduces
- Intrinsic proliferation rates were set to those estimated from the in vitro experiments above

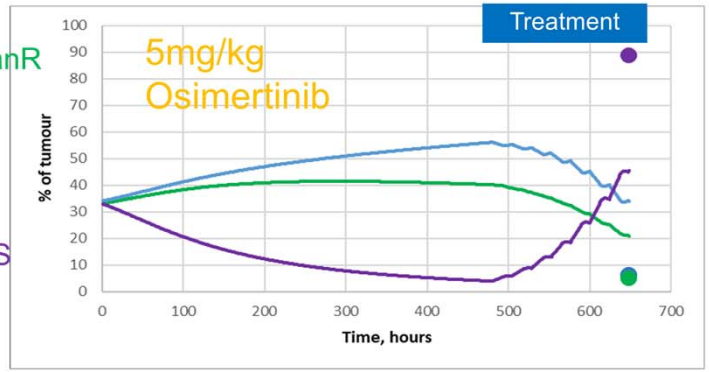
In vivo experiment: treatment dependent tumour growth kinetics and clonal expansion captured by model





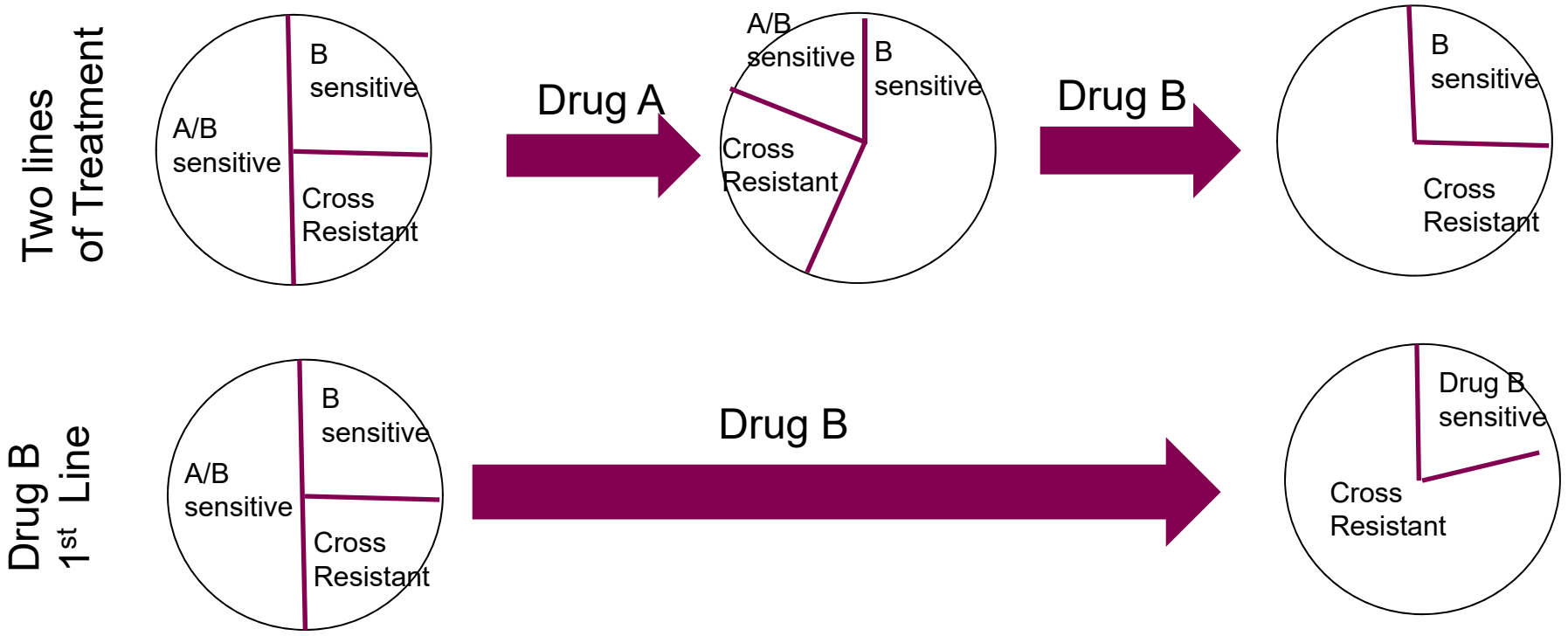
PC9s dominate untreated tumours due greatest proliferation as predicted by in vitro





Challenges for the future: Modelling multiple lines of therapy

Can we integrate clinical efficacy data from multiple lines of treatment to infer evolution in tumours?



Drug development starts in late line then moves to earlier patient populations

Such modelling would help decision making in which populations are appropriate

Conclusions

Understanding resistance kinetics will enable

- Optimisation of therapy
- Connection of early efficacy indicators to OS
- Predict the performance of treatments in earlier patient populations

Nonclinical assays and models exist to do this as well as Clinical data analysis

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Confidentiality Notice