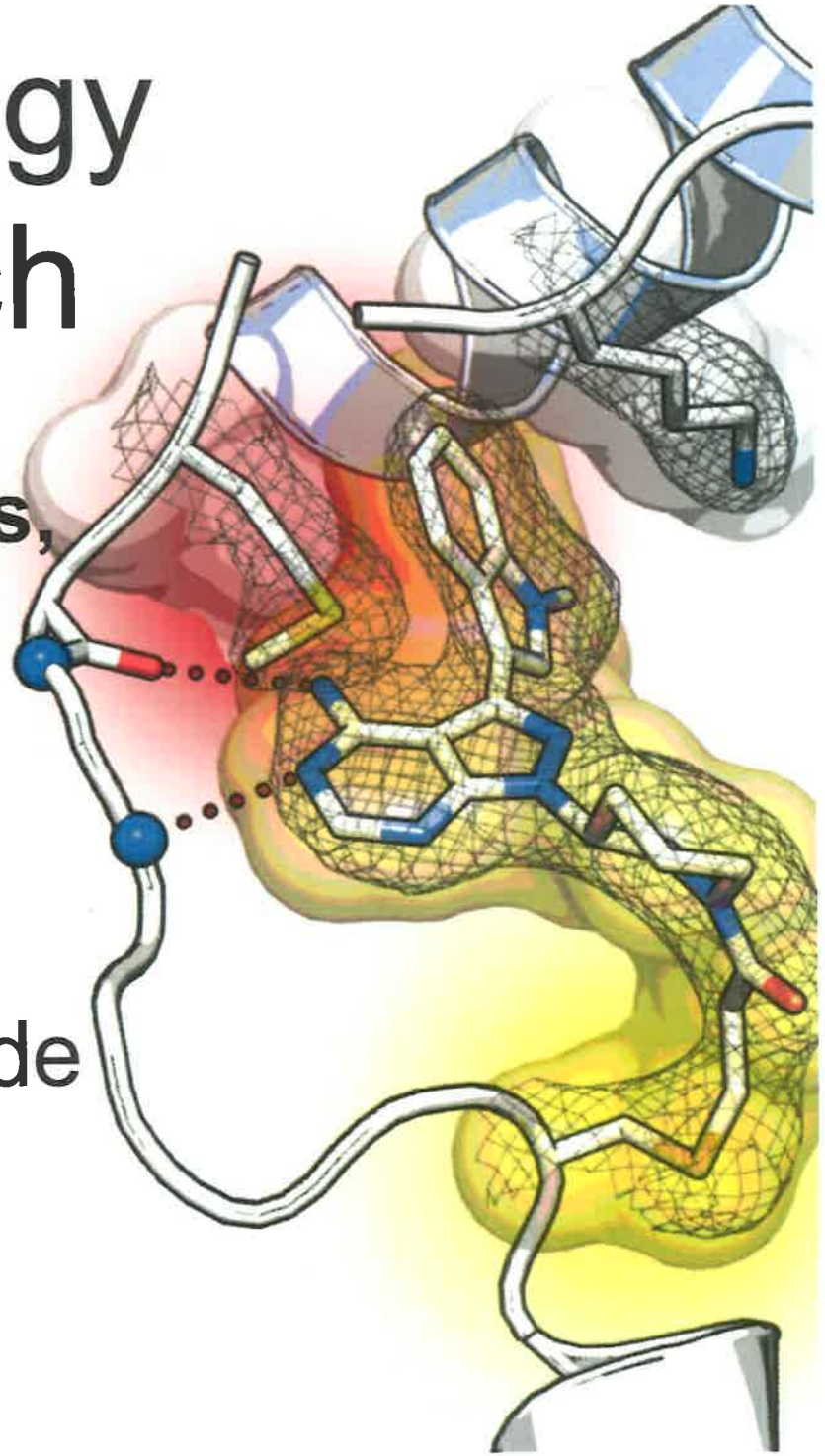
Chemical Oncology for Drug Research

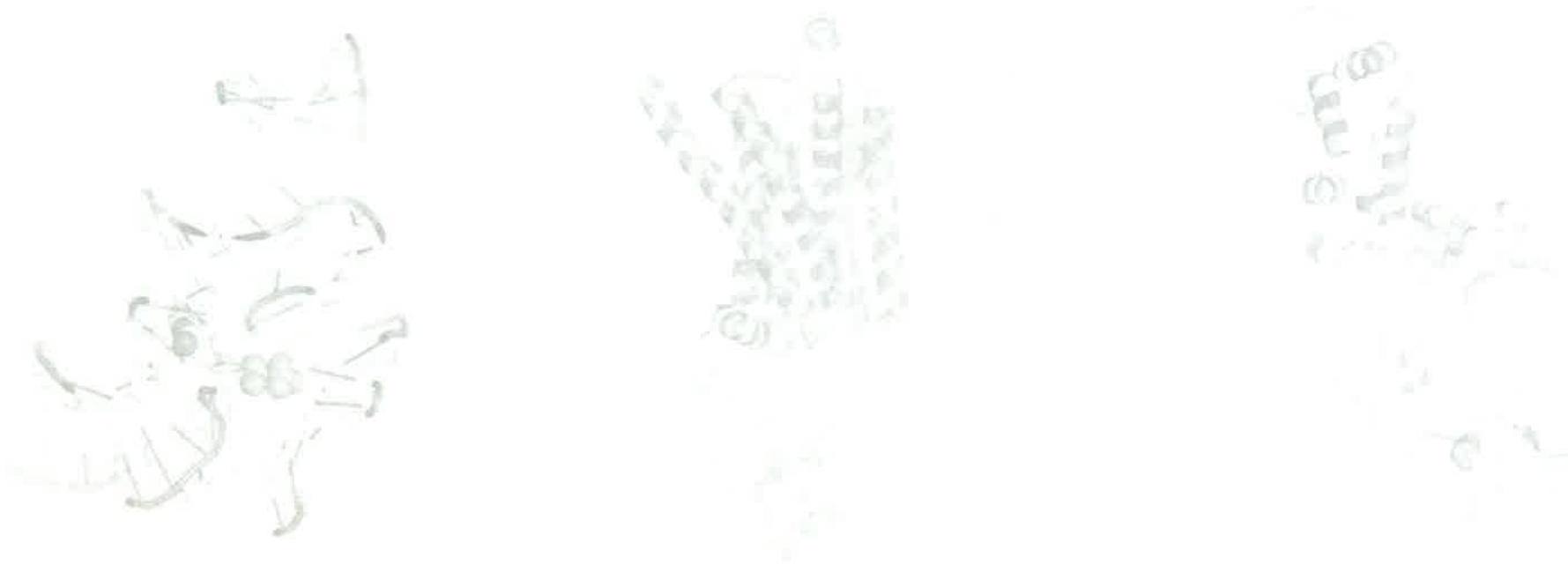
converging cancer genetics, structural biology and medicinal chemistry



daniel.rauh@tu-dortmund.de

ÖPhG-SAPhW Tagung, Innsbruck, 2017

Small molecules



Basic research: Protein kinases, drug resistance, transcription factors, …

Translation: Basic academic research into pharmaceutical application

Chemical Oncology

converging cancer genetics, structural biology and medicinal chemistry …

Roman Thomas, Köln

Martin Sos, Köln

Reinhard Büttner, Köln

Jürgen Wolf, Köln

Christian Reinhardt, Köln

Sebastian Bauer, Essen

Martin Schuler, Essen

Jens Siveke, Essen

Stephan Hahn, Bochum

Jan Hengstler, Dortmund

NOVARTIS

Bundesministerium für Bildung und Forschung

janssen

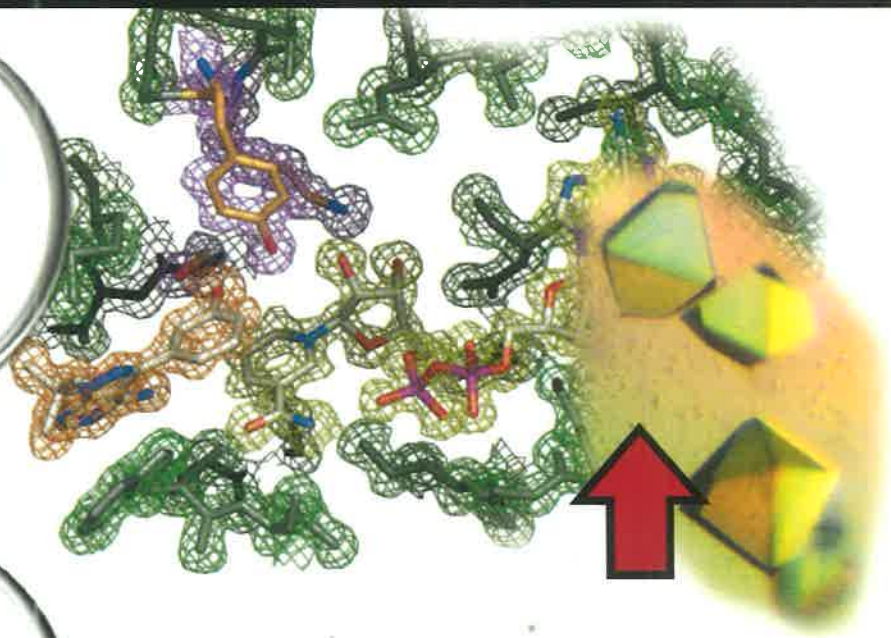
technische universität dortmund

TAROS

Deutsche Forschungsgemeinschaft

DFG

Small Molecules for Dissecting Protein Function



structural biology

p38α Thr Gatekeeper

Hydrophobic Sub-Pocket

C-Helix Glu

Hinge Region

Phe Sp… Pock…

Allosteric site

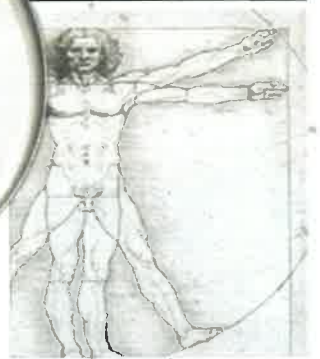
ATP site

Rauh Lab

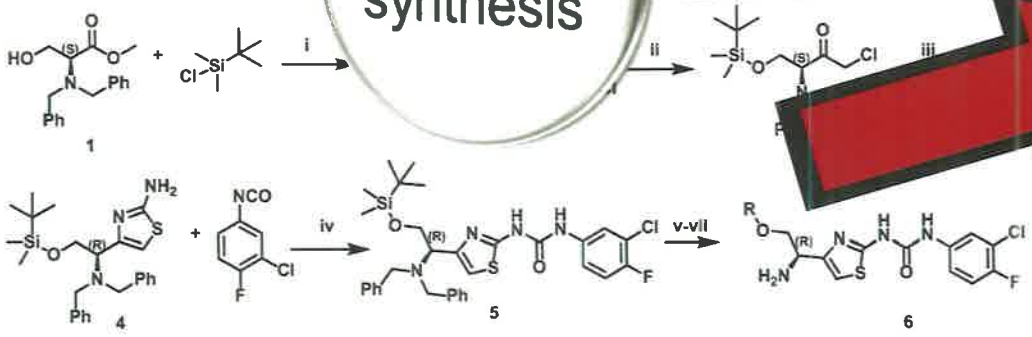
organic synthesis

biology + pharma-cology

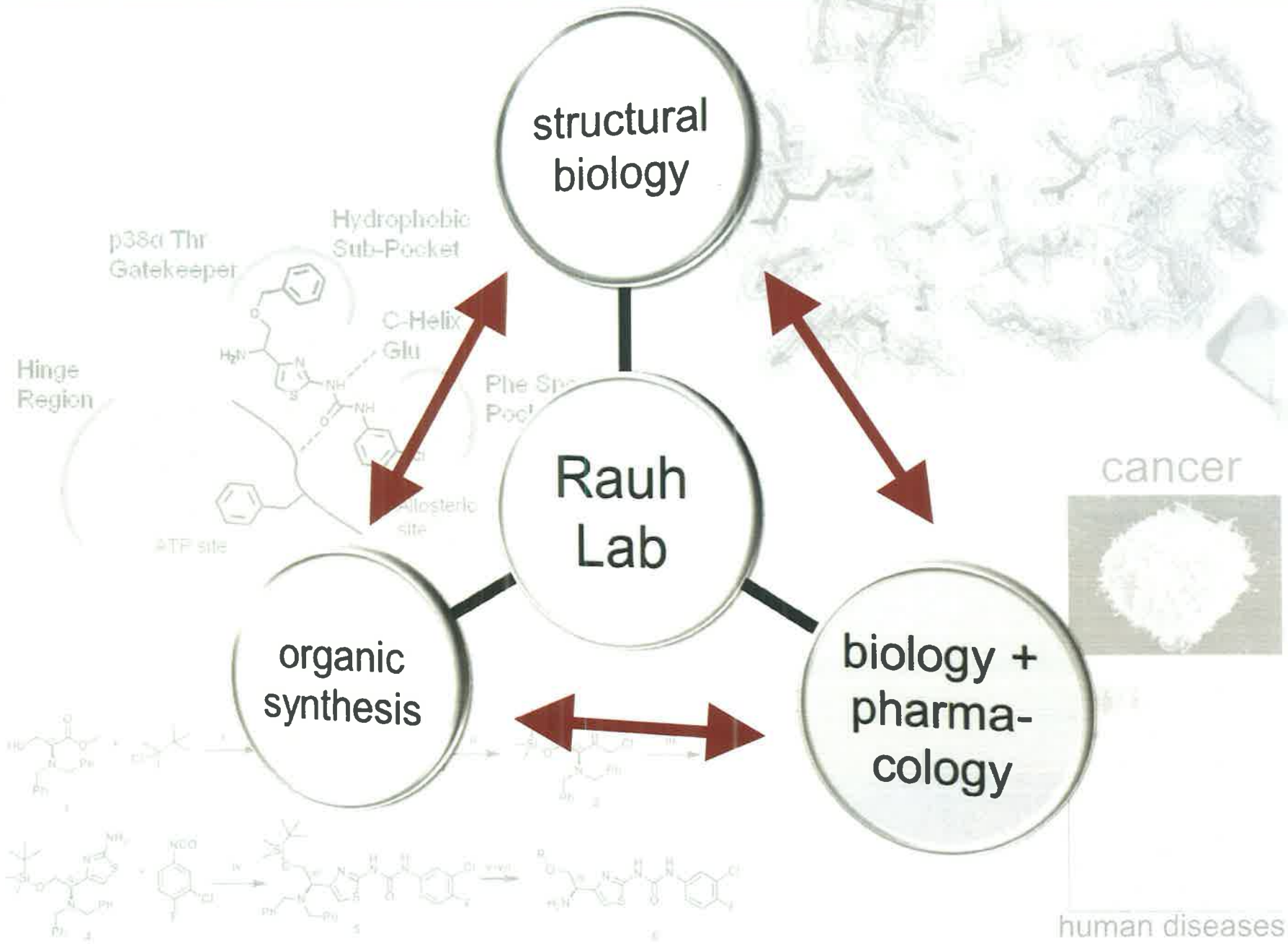
cancer



human diseases

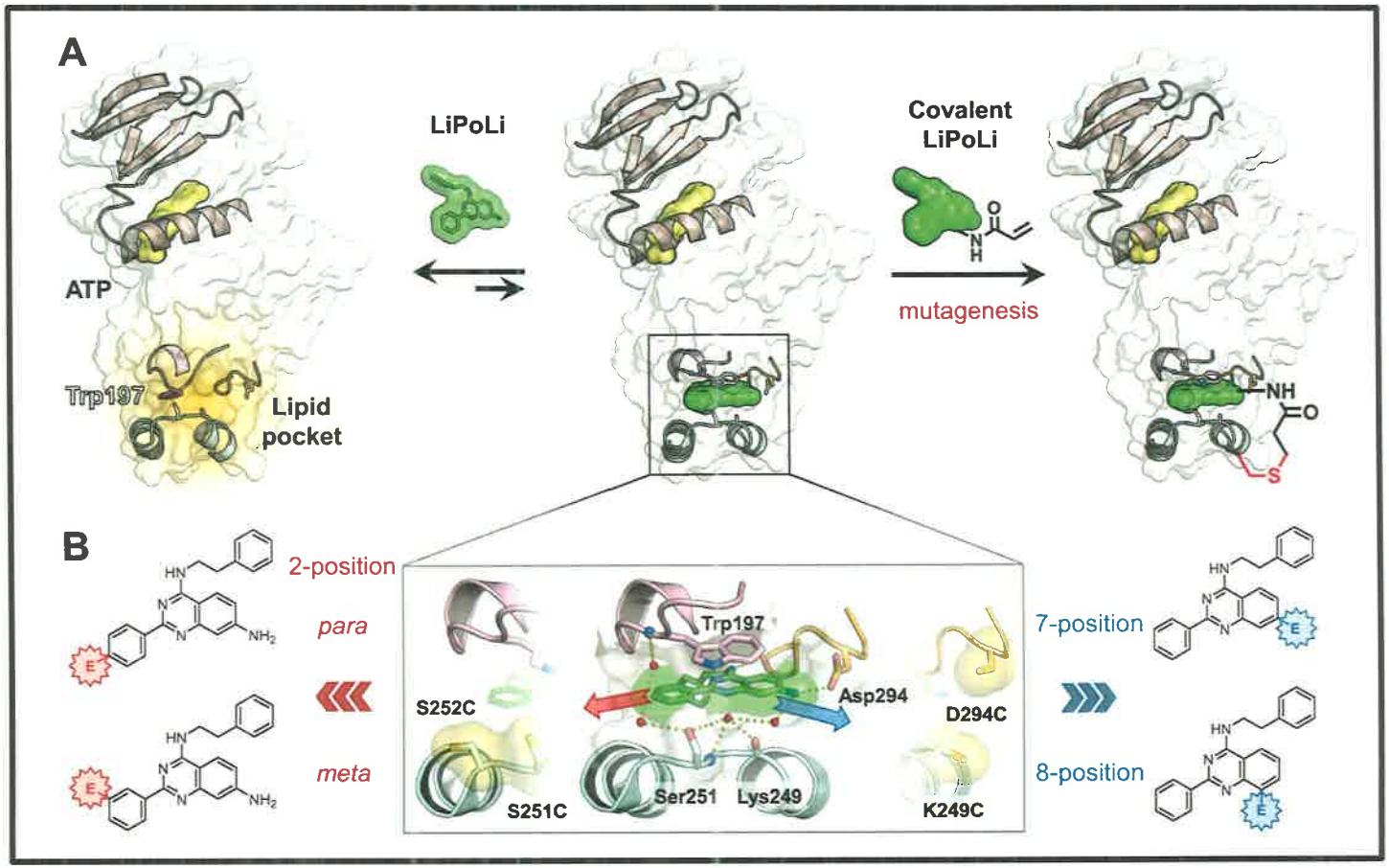


Small Molecules for Dissecting Protein Function



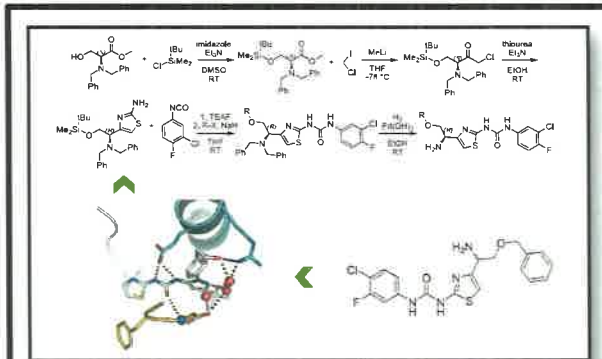
Small Molecules for Dissecting Protein Function

Screening for and development of probes



Mike Bührmann, Bianca Wiedemann, Julia Hardick, *et al.*, *unpublished*

Small Molecules for Dissecting Protein Function



compound design and synthesis

- **35.000** diversity-based compounds
- selected by various filters e.g. PAINS

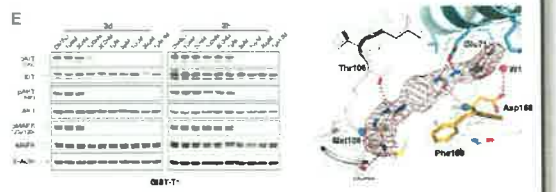
2.000 proprietary compounds

compound library

- fast and efficient compound transfer
- full barcode compatible
- **384-** and **1536**-well compatible

contact free compound transfer

- **hit follow-up**
- **binding studies (SPR, ITC, X-ray)**



hit validation, functional studies

R A S P E L D

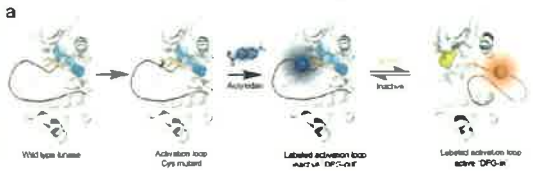
high-quality compound library,

freedom to operate

robust, straightforward setup

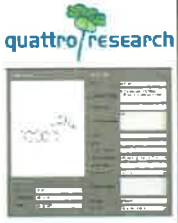
meaningful assays, cost-efficient

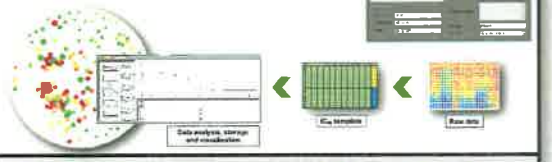
- **biochemical** and **cellular screens**



assay formats and screens

- **HTS data analysis**
- efficient comp. management





data clustering and analysis

- **multi-label system**
- fast HTS reader
- 96-, 384-, 1536-well plates

HTS multilabel reader

- **bulk filling and transfer** of buffers, substrates, enzymes, cells

parallelized dispensing

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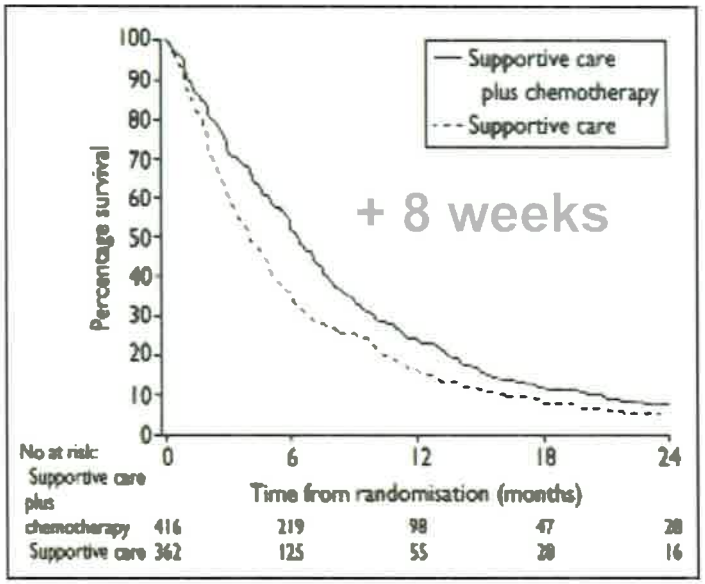
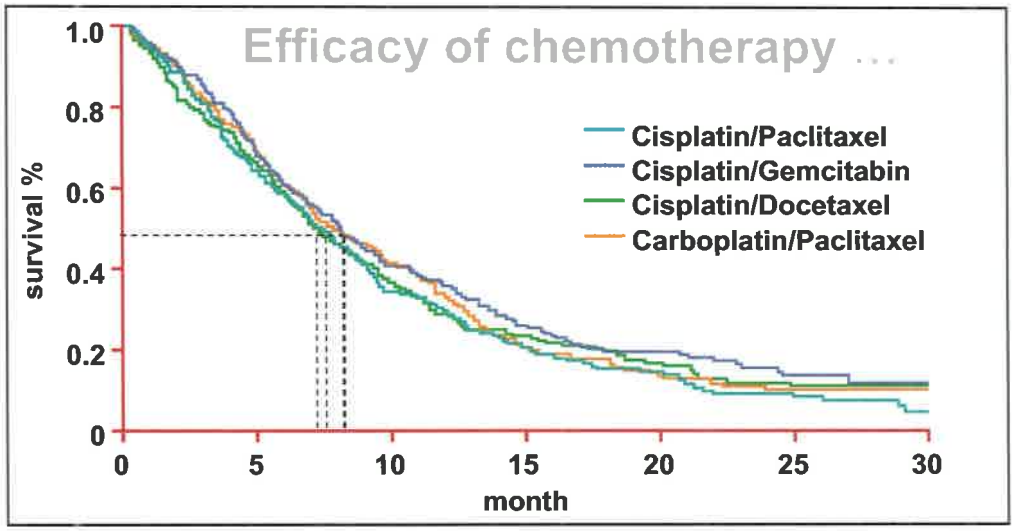
Progress in the treatment of lung cancer?





NSCLC Collaborative Group & MRC UK (Cullen **1997**)

J. Schiller, *N Engl J Med.* **2002**, *346*, 92-8.

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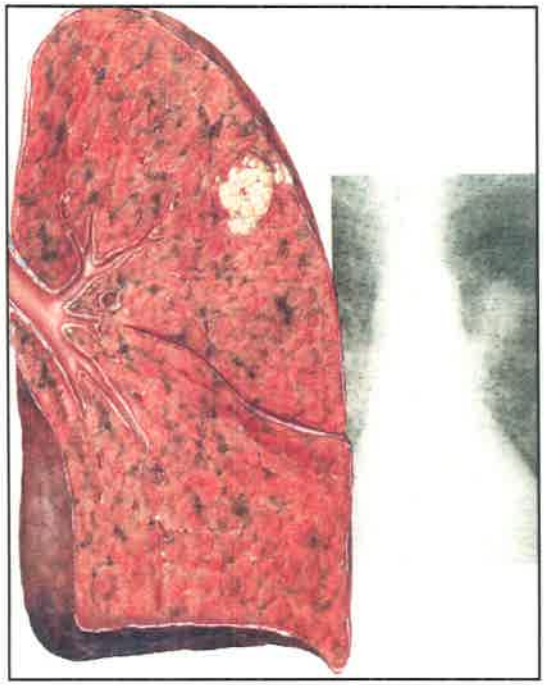
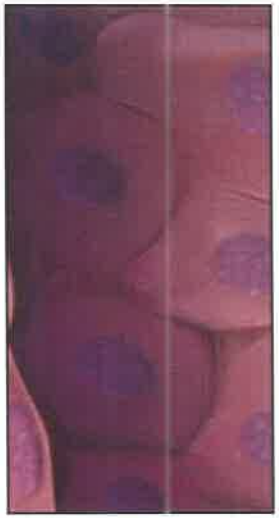
Understanding tumor biology – molecular biology revolution

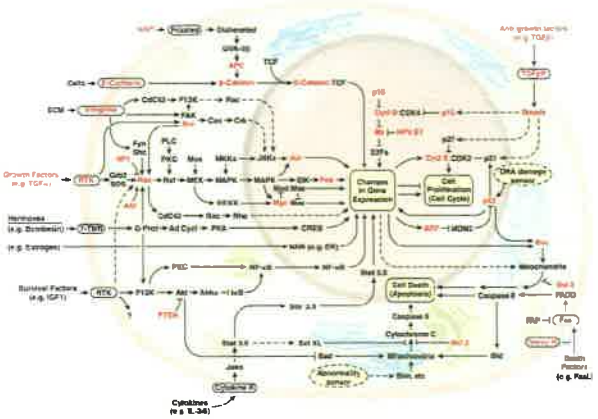
organ



tissue



cell

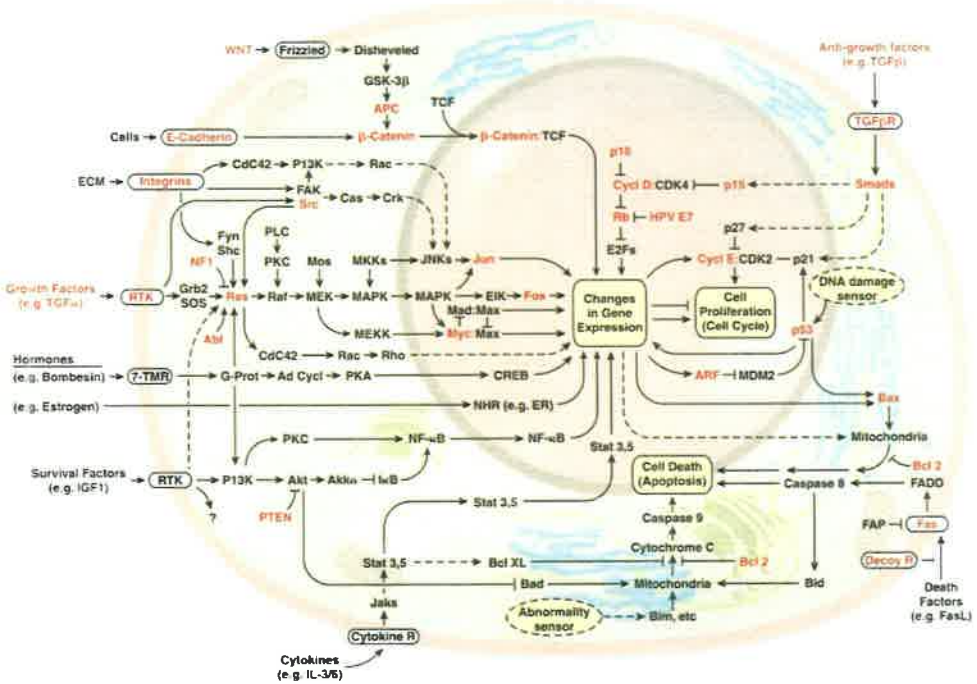


Precision medicine

Cancer is a disease of the genome!

Last decade: revolutionary epoch of cancer science & cancer medicine

- Molecular, atomic **understanding** of cancer
- **Knowledge** about genes; why cancer; how it manifests and spreads …



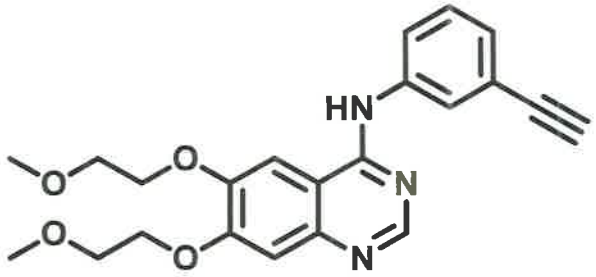
R.A. Weinberg, et al., *Cell* **2000**, *100*
"The Hallmarks of Cancer"; R.A. Weinberg, et al., *Cell* **2011**, *144,* "Hallmarks of Cancer: The Next Generation"

Precision medicine

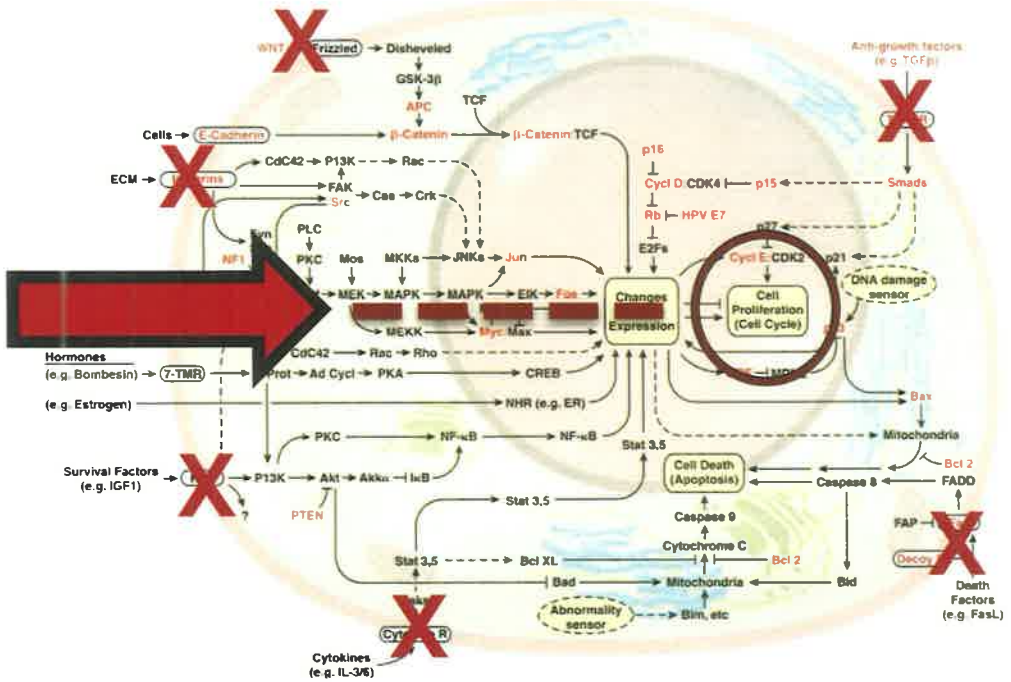
Cancer is a disease of the genome!

Precision medicine

- Seeing the “dragon”, its weaknesses
- attacking the weaknesses



Erlotinib, approved by the FDA 2005



R.A. Weinberg, et al., *Cell* **2000**, *100*
“The Hallmarks of Cancer”; R.A. Weinberg, et al., *Cell* **2011**, *144,* “Hallmarks of Cancer: The Next Generation”

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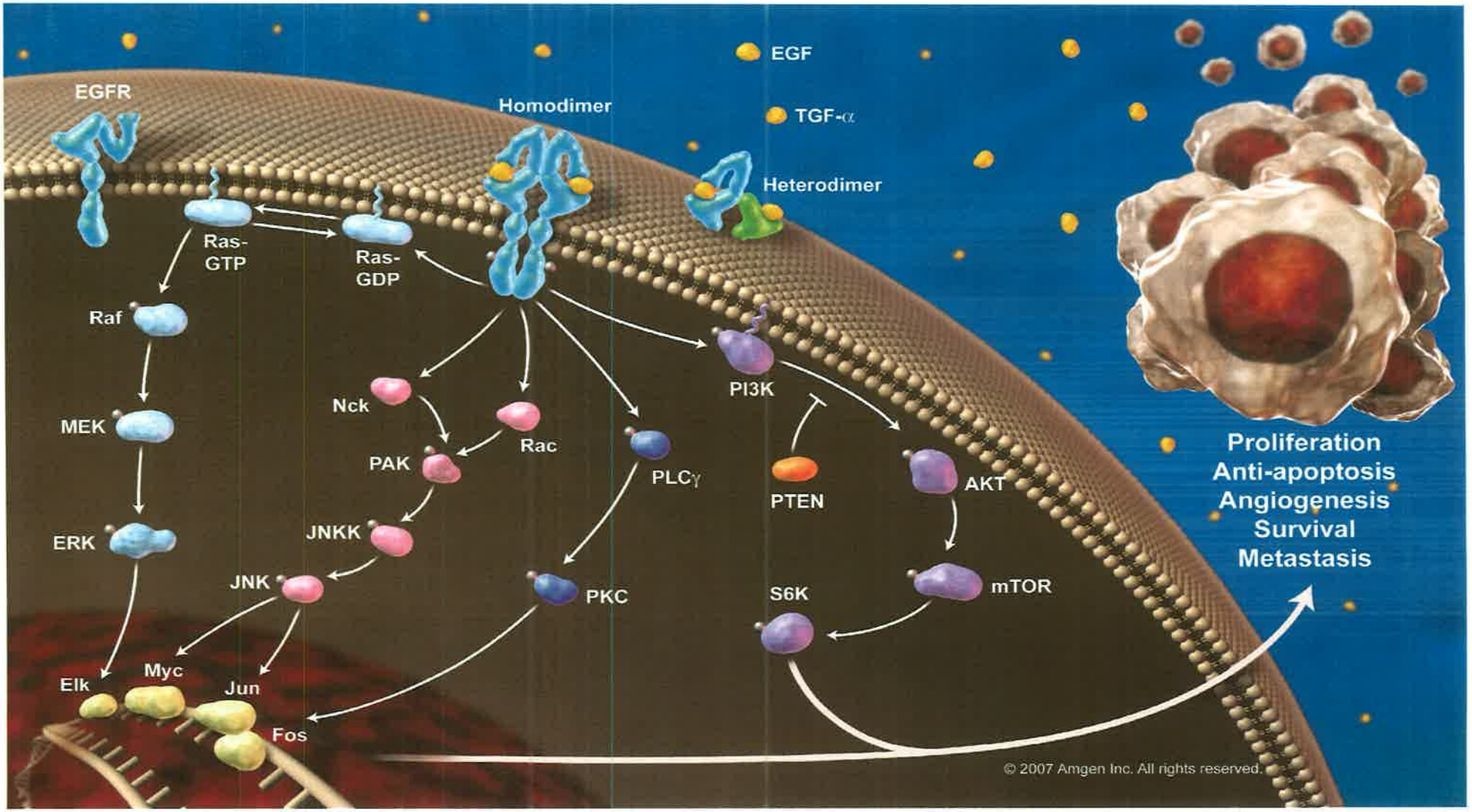
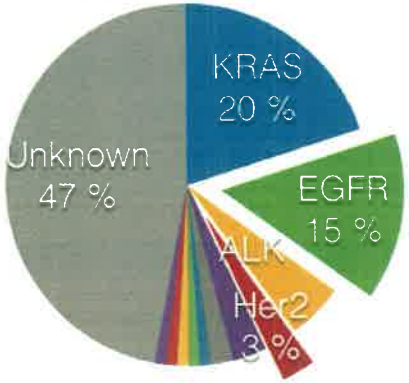
EGFR: molecular signaling switch of tumor cells

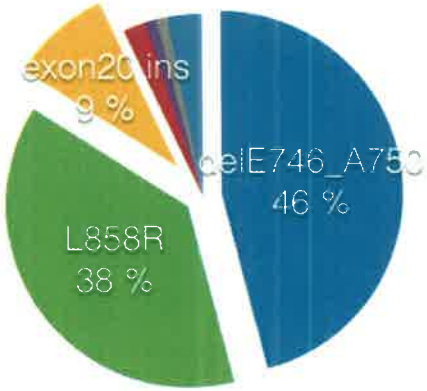


Her mutations in lung cancer

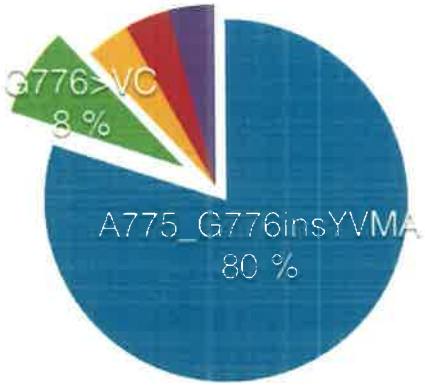
driver mutations in NSCLC[1]



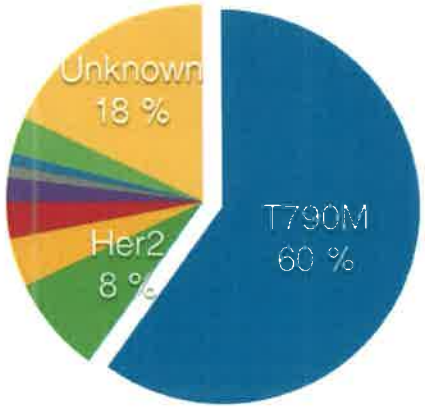
EGFR mutations[2]



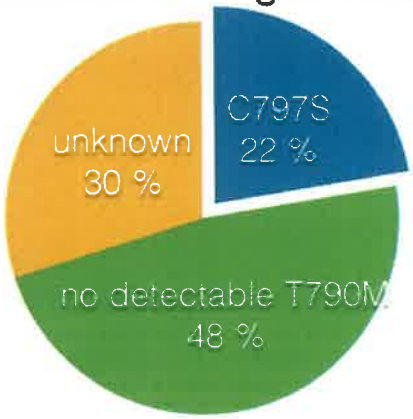
Her2 mutations[5]



resistance to 1st gen TKIs[3]



resistance to 3rd gen TKIs[4]

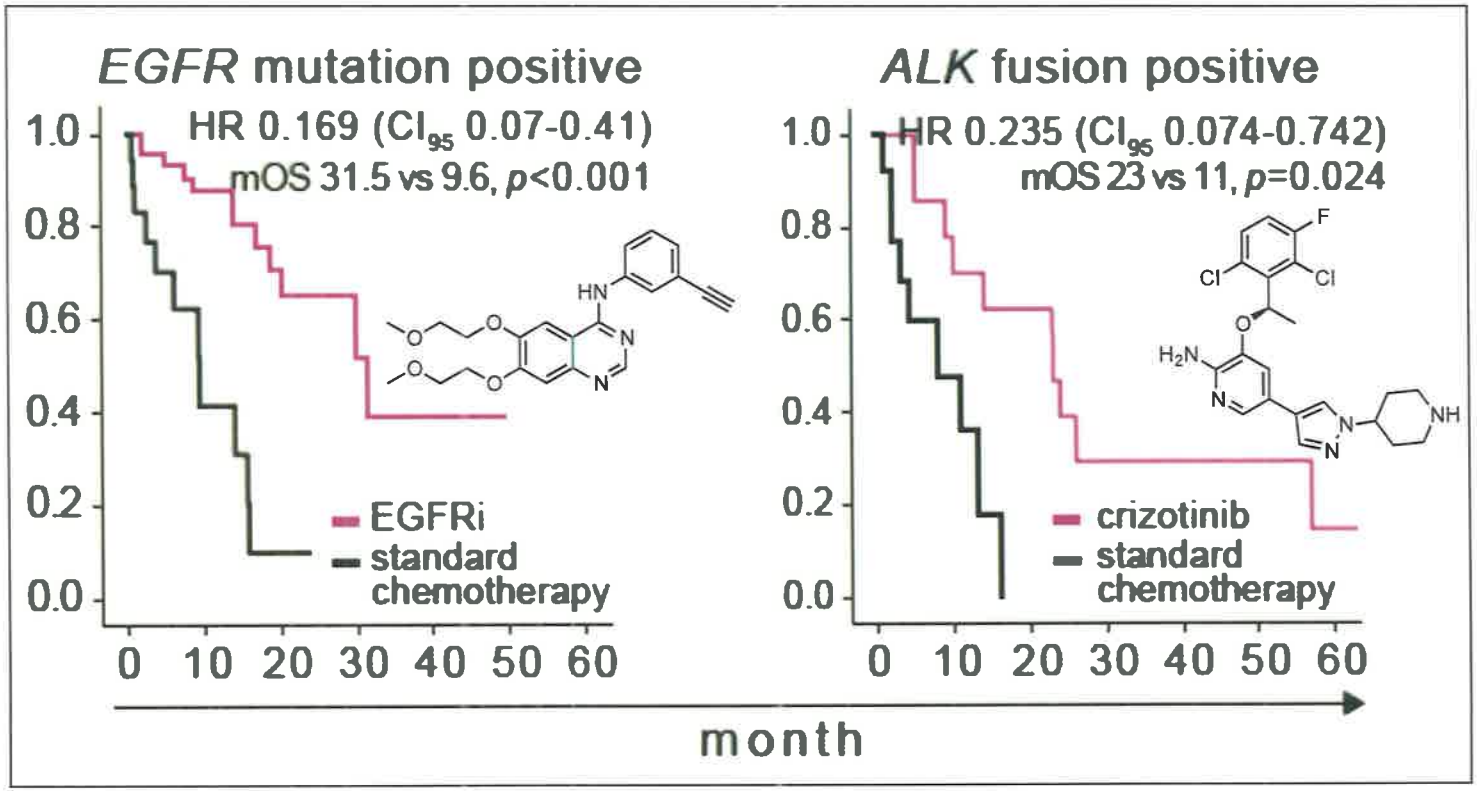


with Roman Thomas, Martin Sos,
Reinhard Büttner, Jürgen Wolf
Carsten Schultz-Fademrecht, Lead
Discovery Center GmbH

[1] M.E. Arcila, et al., *Clinical cancer research* **2012**, *18*, 4910-4918.
[2] W. Pao, et al., *Nature medicine* **2012**, *18*, 349-351.
[3] H.A. Yu, et al., *Clin Cancer Res* **2013**, *19*, 8, 2240-2247.
[4] G. R. Oxnard, et al., *in 16th World Conference on Lung Cancer* (*Denver, Colorado,* **2015**).
[5] M.E. Arcila, et al., *Mol Cancer Ther* **2013**, *12*, 2, 220-229.

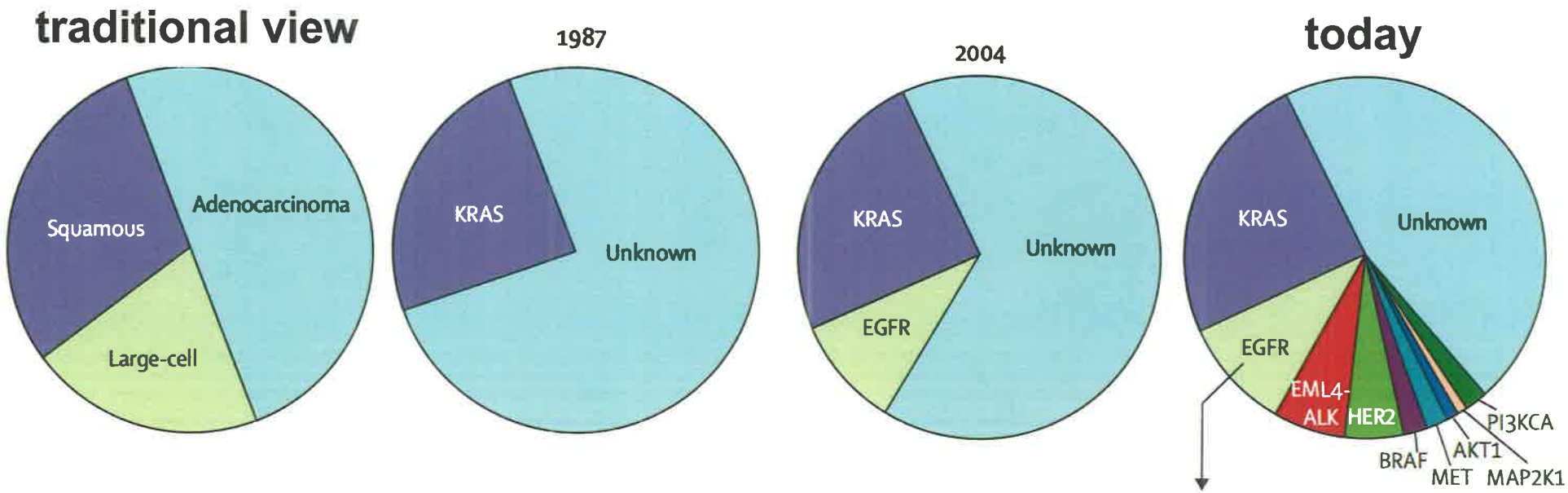
Lung cancer serves as a paradigm

Improved survival of EGFR^{mut} and ALK^{fusion} patients with precision medicine as compared to chemotherapy



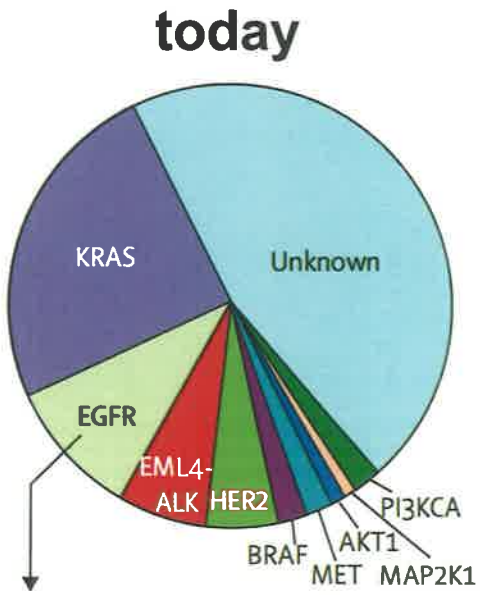
Curtesy Reinhard Büttner, Köln, The Clinical Lung Cancer Genome Project and Network Genomic Medicine, Sci Transl Med 2013

Challenges



Predictive response (diagnostics + therapy)!

Challenges

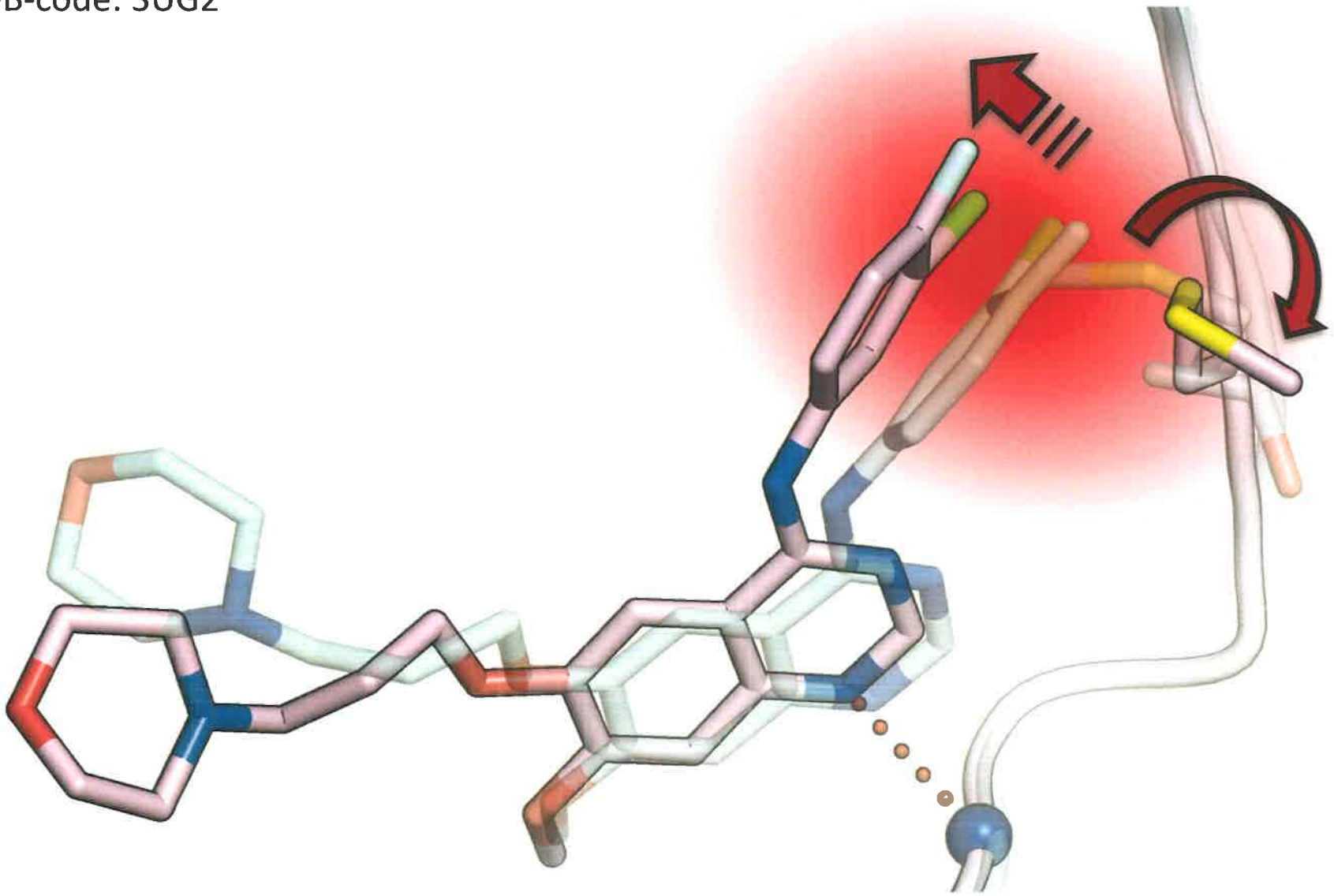


therapy)!

03/2017, only 24 drugs …

emergence of acquired drug resistance …

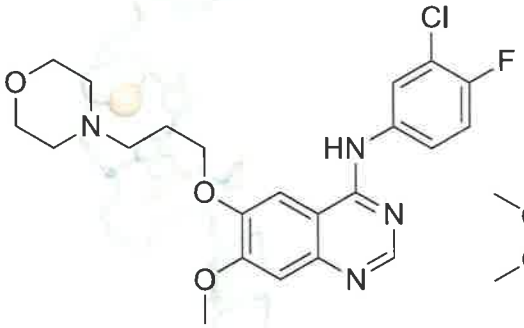
Gefitinib in complex with EGFR-T790M
Yoshikawa et al., Oncogene 2012
PDB-code: 3UG2



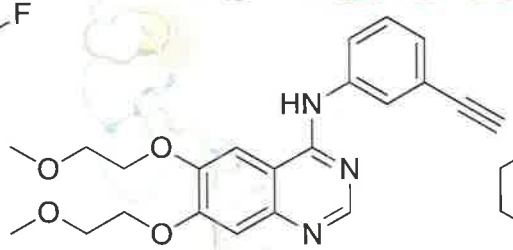
a structural view …

drug resistance in EGFR

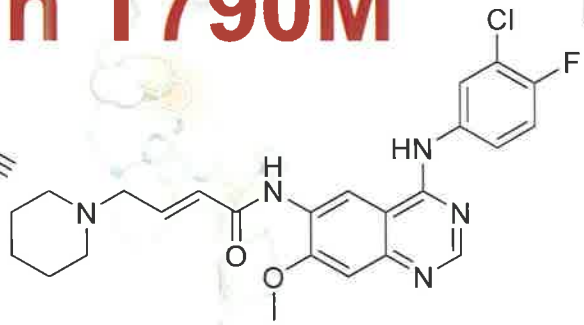
classic 4-aminoquinazolines are prone to fail in T790M



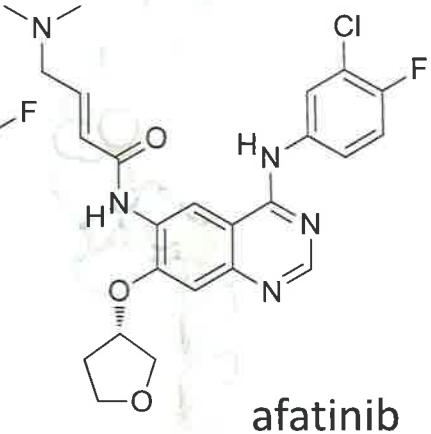
gefitinib



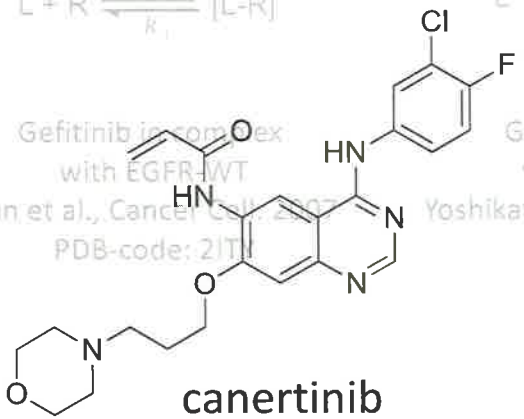
erlotinib



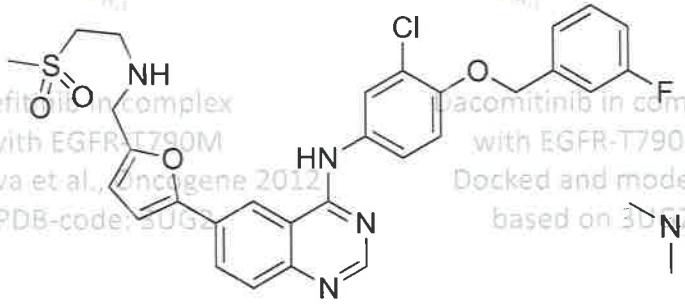
dacomitinib



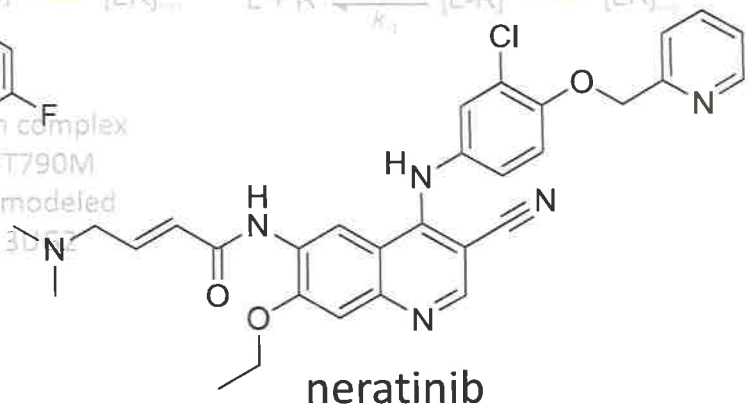
afatinib



canertinib



lapatinib



neratinib

J. Engel, et al., *J. Med. Chem.* **2015**, *58*, 6844.
D. Basu, et al., *BMC*. **2015**, *23*, 5075.
M. L. Sos, et al., *Cancer Res* **2010**, *70*, 868.
V. G. Pawar, et al., *J Med Chem* **2010**, *53*, 2892.
A. Richters, et al., *ACS Chem Biol* **2015**, *10*, 289.
J. Heuckmann, et al., *J Clinical Oncol.* **2012**, *30*, 3417.
S. Klüter, et al., *ChemBioChem* **2010**, *11*, 2557.
A. Michalczyk, et al., *Bioorg Med Chems.* **2010**, *16*, 3482.

drug resistance in EGFR

classic 4-aminoquinazolines are prone to fail in T790M

develop more potent TKIs

avoid the gatekeeper!

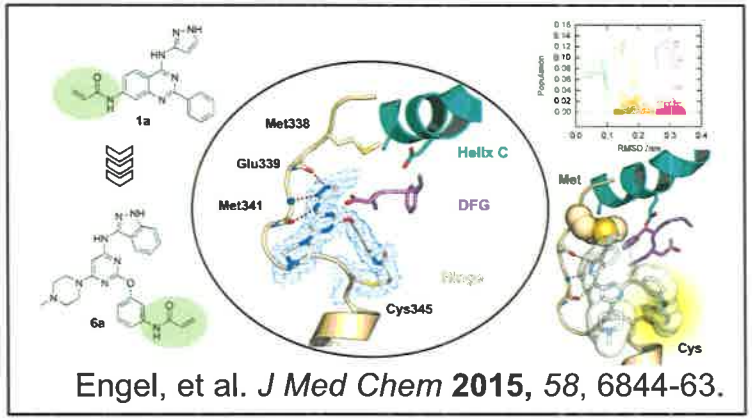
do not hit EGFR-WT

be specific for T790M

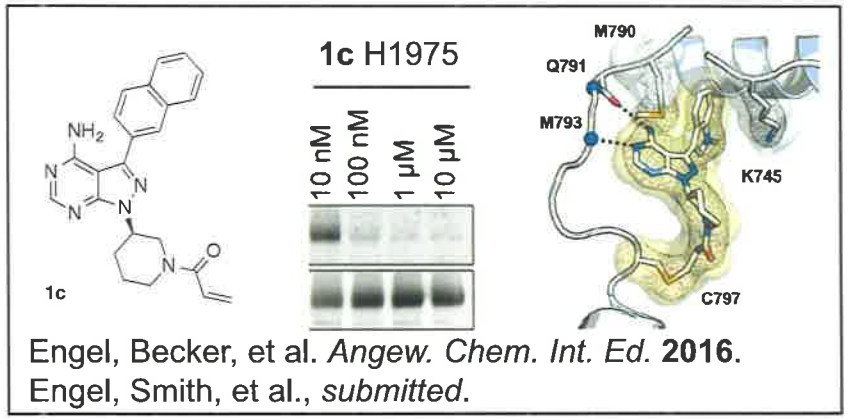
drug-target residence time, be covalent!

D. Basu, et al., *BMC*. **2013**, *23*, 5075.
M. L. Sos, et al., *Cancer Res* **2010**, *70*, 868.
V. G. Pawar, et al., *J Med Chem* **2010**, *53*, 2892.
J. Heuckmann, et al., *J Clinical Oncol.* **2012**, *30*, 3417.
S. Klüter, et al., *ChemBioChem* **2010**, *11*, 2557.
A. Michalczyk, et al., *Bioorg Med Chems.* **2010**, *16*, 3482.

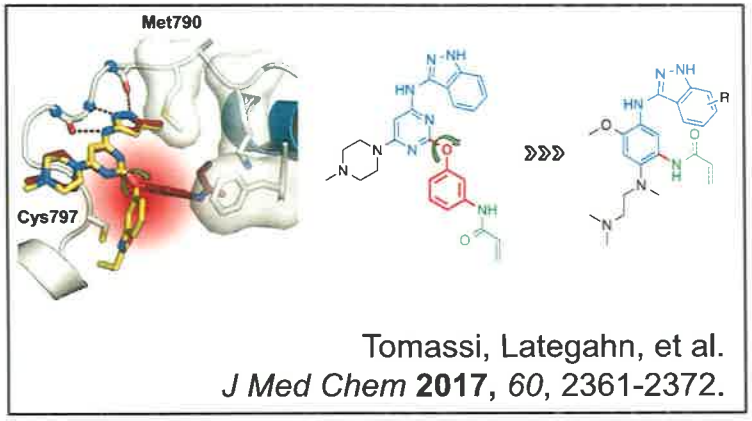
Hope and Disappointment: Covalent Inhibitors to Overcome Drug Resistance in Non-Small Cell Lung Cancer



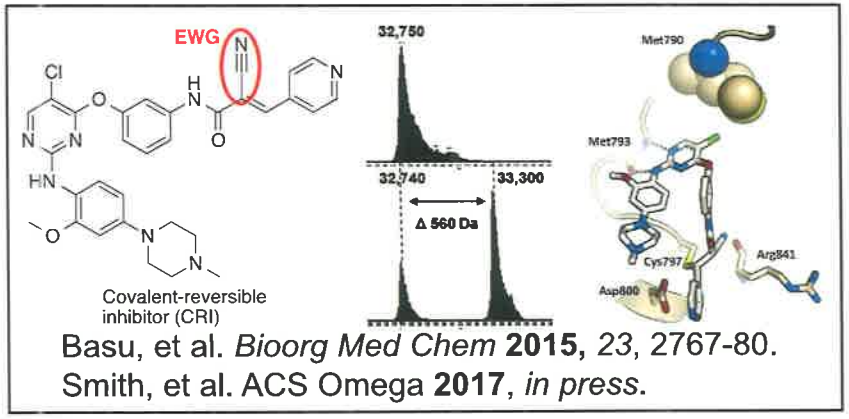
Engel, et al. *J Med Chem* **2015,** *58*, 6844-63.



Engel, Becker, et al. *Angew. Chem. Int. Ed.* **2016.**
Engel, Smith, et al., *submitted*.



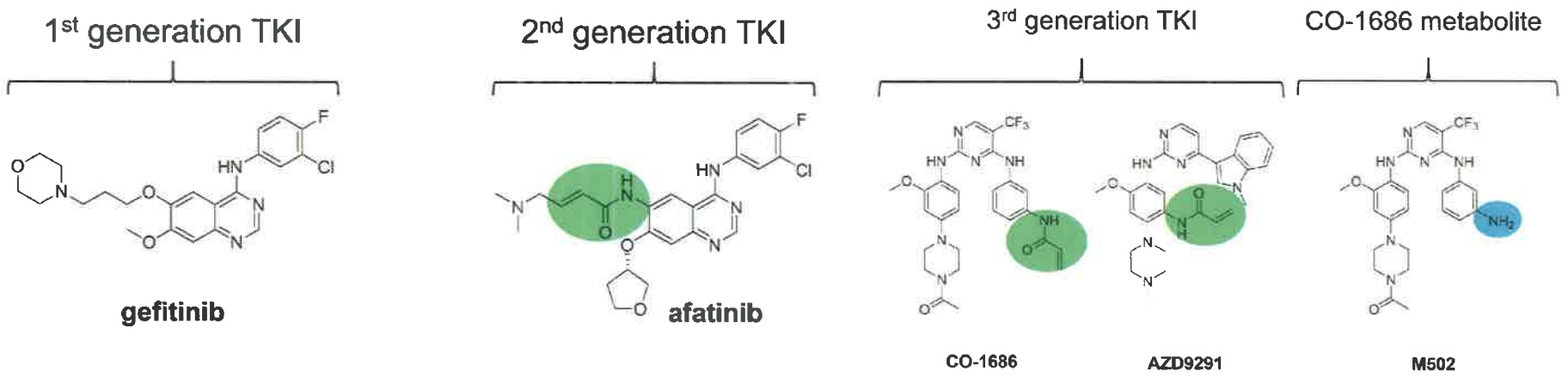
Tomassi, Lategahn, et al.
J Med Chem **2017,** *60*, 2361-2372.

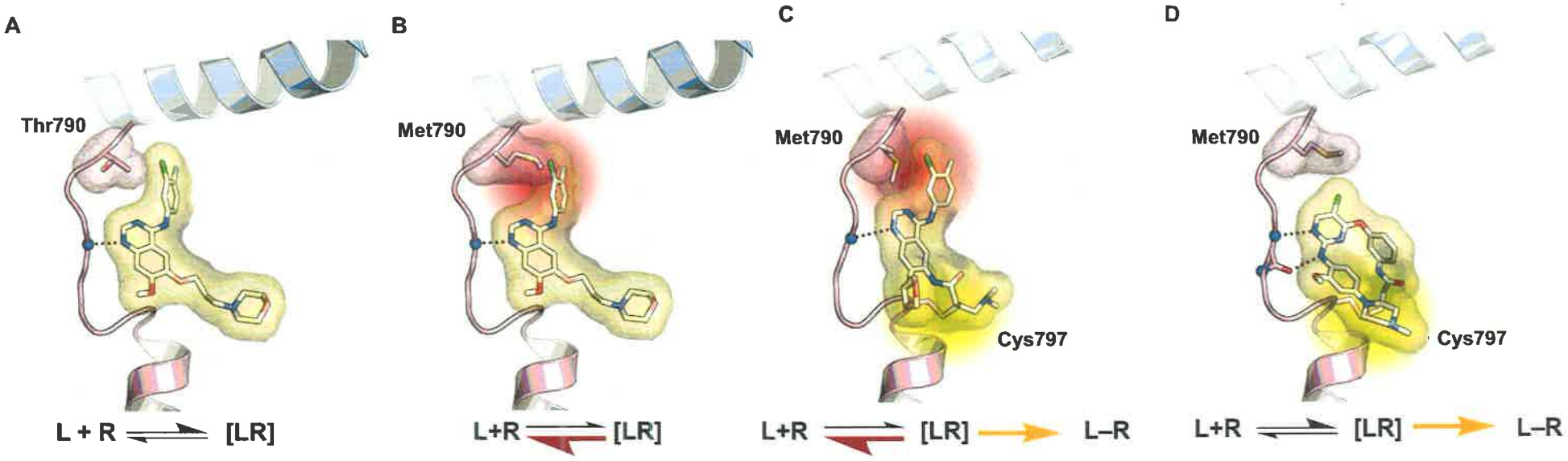


Basu, et al. *Bioorg Med Chem* **2015,** *23*, 2767-80.
Smith, et al. ACS Omega **2017**, *in press*.

Engel, Lategahn et al., *ACS Med Chem Lett,* **2016**, *7(1):2-5.*

Hope and Disappointment: Covalent Inhibitors to Overcome Drug Resistance in Non-Small Cell Lung Cancer





Engel, Lategahn et al., *ACS Med Chem Lett,* **2016**, *7(1):2-5.*

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 APRIL 30, 2015 VOL. 372 NO. 18

AZD9291 in EGFR Inhibitor–Resistant Non–Small-Cell Lung Cancer

"… administered AZD9291 at doses of 20 to 240 mg once daily … no dose-limiting toxic effects occurred at the doses evaluated."

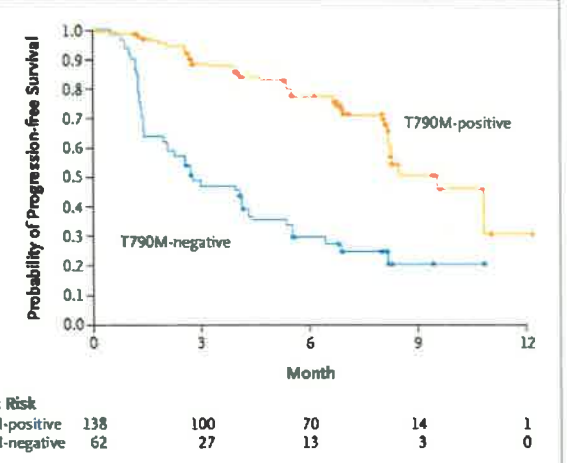
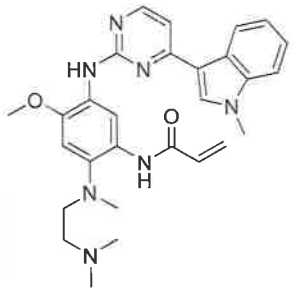


Figure 3. Progression-free Survival According to Status with Respect to *EGFR* T790M.

Shown are Kaplan–Meier estimates of progression-free survival among patients with advanced non–small-cell lung cancer who received AZD9291 at doses of 20 to 240 mg daily. In the subgroup of patients with T790M-positive disease (yellow), the median progression-free survival was 9.6 months. In the subgroup of patients with T790M-negative disease (blue), the median progression-free survival was 2.8 months. Shaded areas indicate 95% confidence intervals, and dots censored observations.

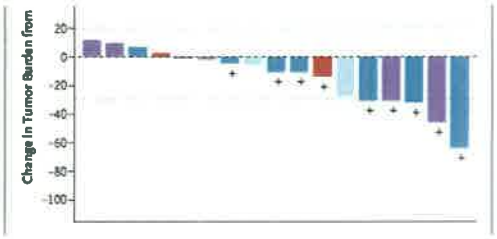


Osimertinib

ORIGINAL ARTICLE

Rociletinib in *EGFR*-Mutated Non–Small-Cell Lung Cancer

"… patients to be enrolled received the free-base … (150 mg once daily to 900 mg twice daily) ... remaining patients received the hydrogen bromide salt (HBr) form (500 mg twice daily to 1000 mg twice daily). A maximum tolerated dose (the highest dose associated with a rate of dose-limiting toxic effects of less than 33%) was not identified."



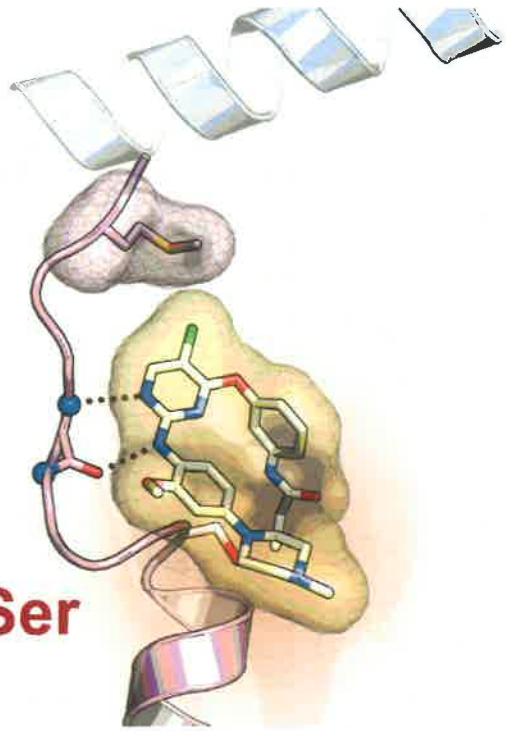
nature medicine

Acquired *EGFR* C797S mutation

The allelic content of the C797S mutation acquired upon treatment with third generation EGFR inhibitors impacts sensitivity to subsequent treatment strategies

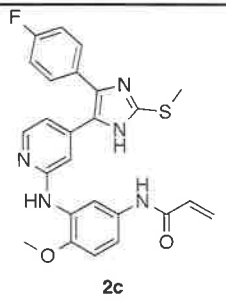
Need for 4th generation TKIs?

(EGFR) tyrosine kinase inhibitor (TKI) AZD9291. We first performed next-generation sequencing of cfDNA from seven subjects and detected an acquired *EGFR* C797S mutation in one; expression of this mutant *EGFR* construct in a cell line rendered it resistant to AZD9291. We then performed droplet digital PCR on serial cfDNA specimens collected from 15 AZD9291-treated subjects. All were positive for the T790M mutation before treatment, but upon developing AZD9291 resistance three molecular subtypes emerged: six cases acquired the C797S mutation, five cases maintained the T790M mutation but did not acquire the C797S mutation and four cases lost the T790M mutation despite the presence of the underlying *EGFR* activating mutation. Our findings provide insight into the diversity of mechanisms through which tumors acquire resistance to AZD9291 and highlight the need for therapies that are able to overcome resistance mediated by the *EGFR* C797S mutation.



published online 4 May 2015;
doi:10.1038/nm.3854

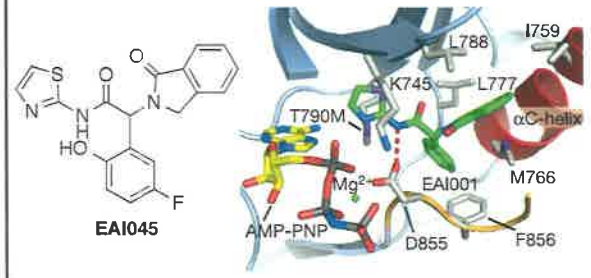
Need for 4th generation TKIs!

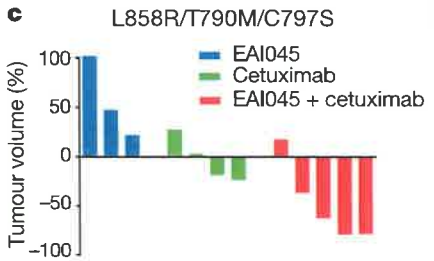


	IC_{50} [nM] against EGFR L858R/T790M/C797S
1a	21
1b	336
2c	90
2d	167
Staurosporine	1
WZ4002	7294

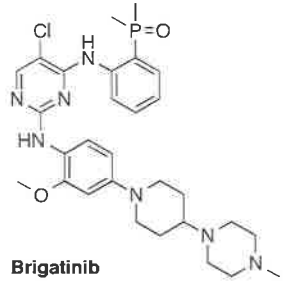
2c

Günther, et al. *Angew. Chem. Int. Ed.* **2016**, *55*, 1-6.

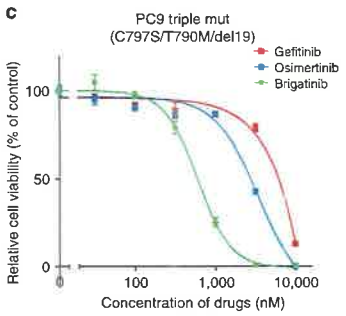




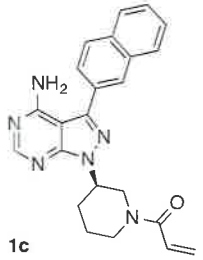
Jia, et al. *Nature* **2016,** *534*, 129-32.



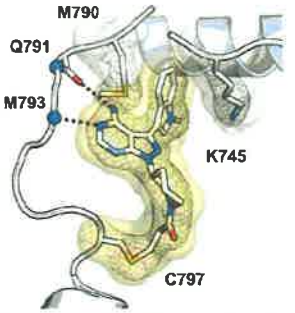
Brigatinib



Uchibori, et al. *Nat Commun* **2017,** *8*, 14768.



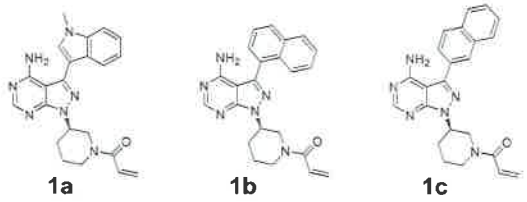
1c



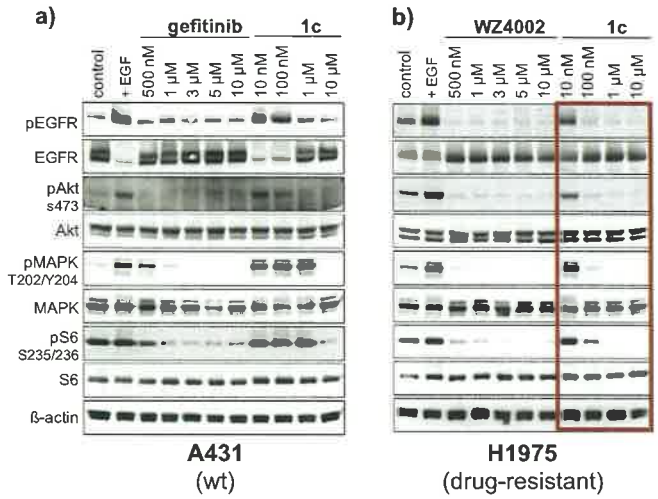
Engel, Becker, et al.
Angew. Chem. Int. Ed. **2016**, *55*, 10909-12.

in vitro Evaluation

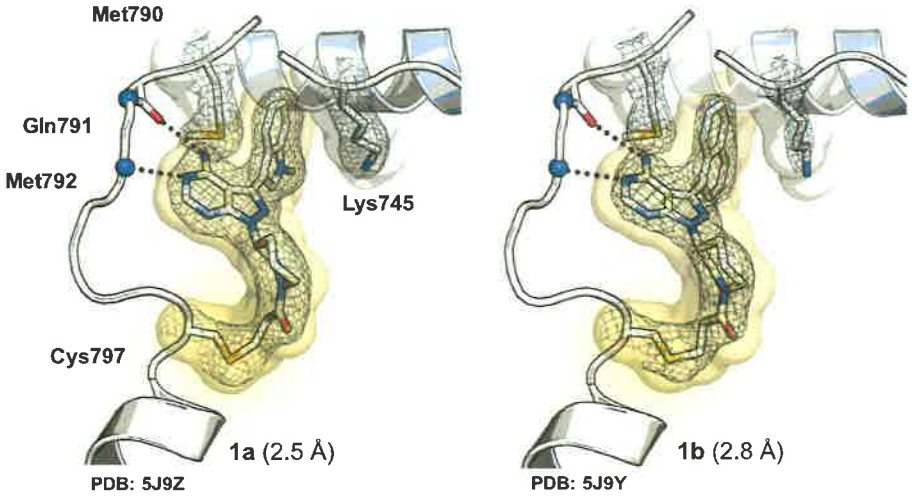
	GI_{50} EGFR [μM]		IC_{50} EGFR [μM]		
	A431 (wt)	H1975 (L858R/T790M)	wt	L858R	L858R/T790M
1a	8.9	0.21	0.035	0.004	0.003
1b	24	0.49	0.058	0.001	0.002
1c	3.6	0.14	0.016	0.001	<0.001



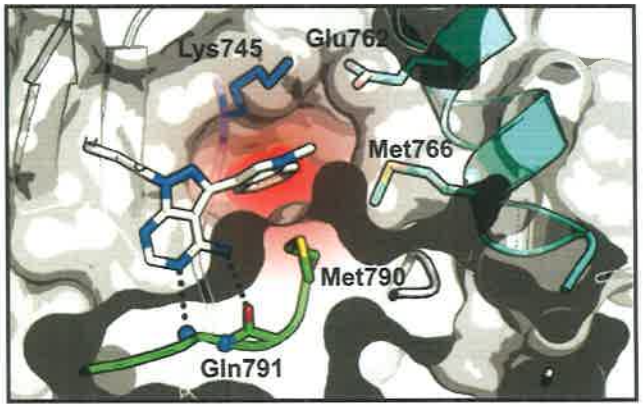
highly potent & mutant-selective in drug-resistant H1975 cells



dose-dependent effect on EGFR phosphorylation and its downstream signaling



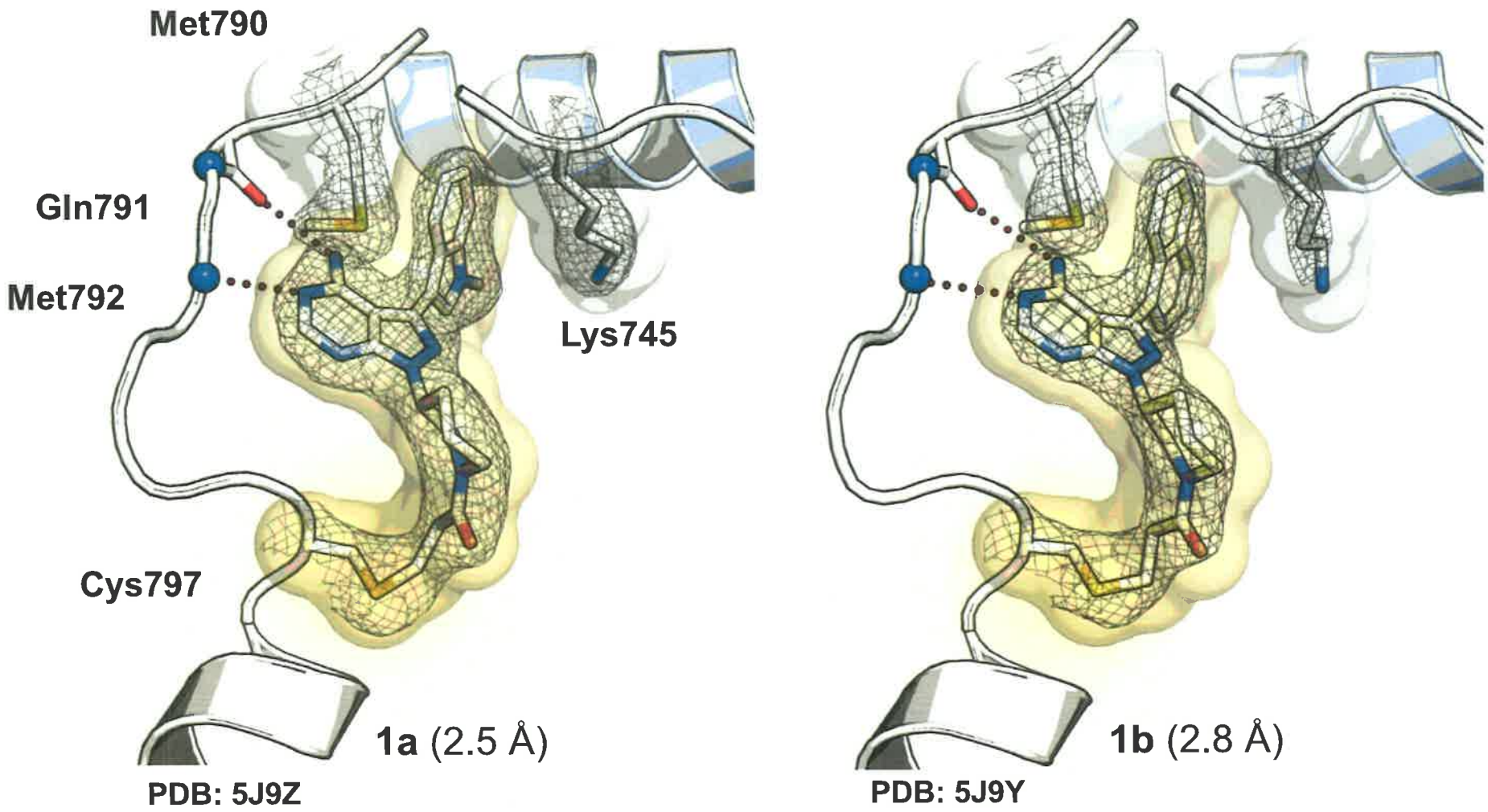
covalent bond formation with Cys797 lipophilic interaction (Met790&Lys745)



covalent bond formation with Cys797 lipophilic interaction (Met790&Lys745)

Engel, Becker et al., *Angew Chem Int Ed Engl* **2016**, *55*, 10909-12.
Tomassi, Lategahn et al., *J Med Chem*, **2017**, *60*, 2361-2372.

Complex Crystal Structure in EGFR-T790M



covalent bond formation with Cys797
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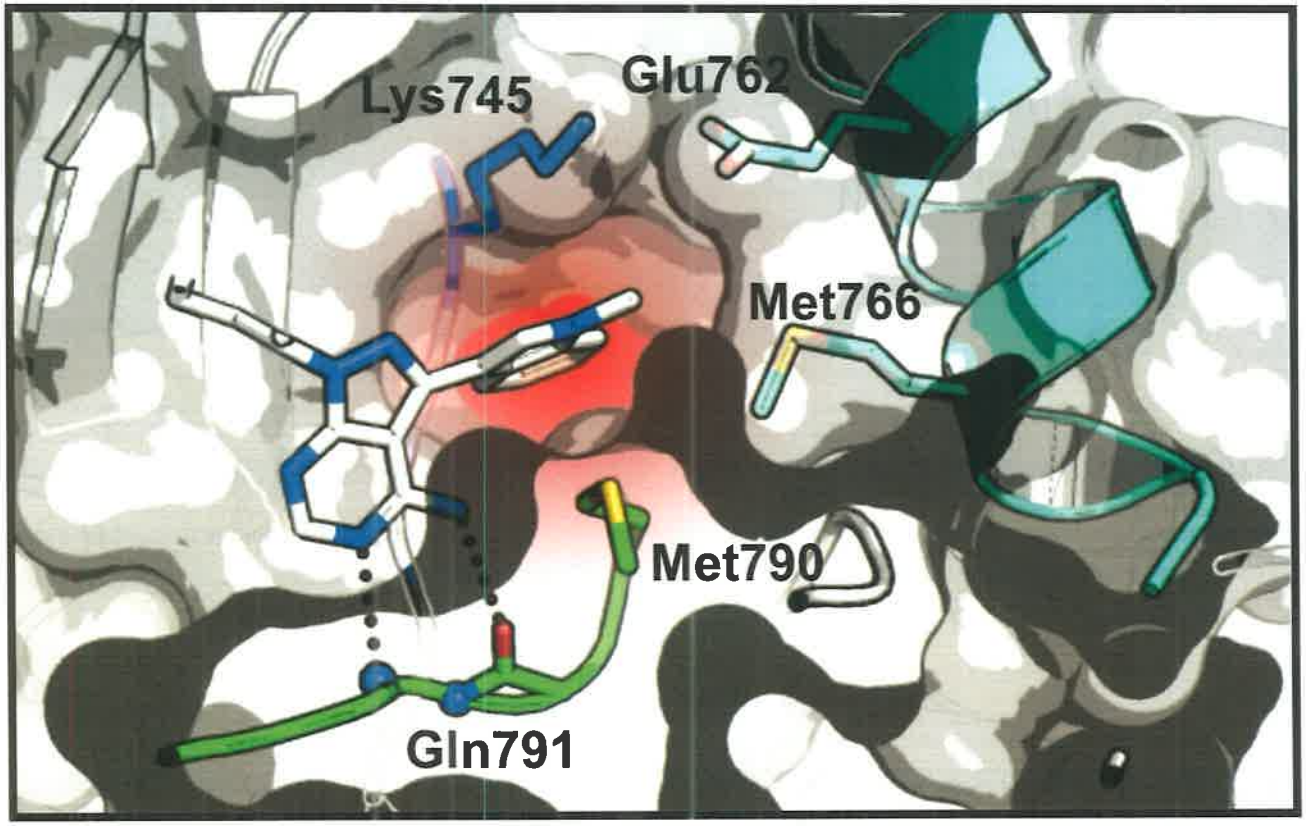
Engel, Becker et al., *Angew Chem Int Ed Engl* **2016**, *55*, 10909-12.

Complex Crystal Structure in EGFR-T790M



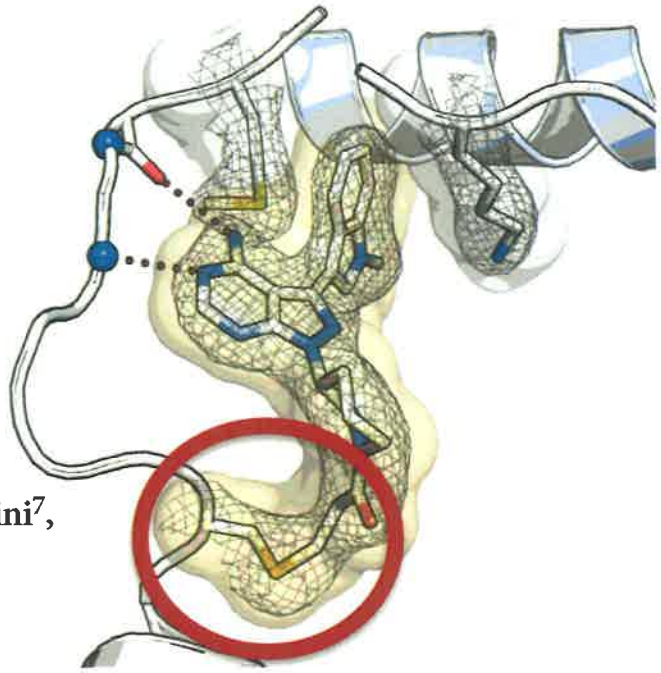
covalent bond formation with Cys797
lipophilic interaction (Met790&Lys745)

Engel, Becker et al., *Angew Chem Int Ed Engl* **2016**, *55*, 10909-12.

Acquired Drug Resistance – C797S Mutation

Acquired *EGFR* C797S mutation mediates resistance to AZD9291 in non–small cell lung cancer harboring *EGFR* T790M

Kenneth S Thress[1], Cloud P Paweletz[2,3], Enriqueta Felip[4,5], Byoung Chul Cho[6], Daniel Stetson[1], Brian Dougherty[1], Zhongwu Lai[1], Aleksandra Markovets[1], Ana Vivancos[4], Yanan Kuang[2,3], Dalia Ercan[2], Sarah E Matthews[2], Mireille Cantarini[7], J Carl Barrett[1], Pasi A Jänne[2,3] & Geoffrey R Oxnard[2]



can we target <u>both drug-resistant mutants</u>

(T790M & T790M/C797S)

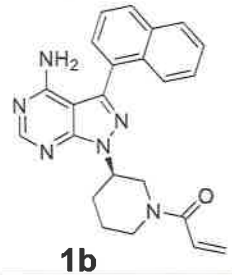
with <u>one and the same drug</u>?

Thress et al., *Nat Med* **2015**, *21*, 560-62.

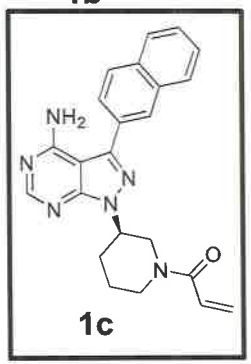
Niederst et al., *Clin Cancer Res* **2015**, *17*, 3924-33.

Acquired Drug Resistance – C797S Mutation

1a



1b



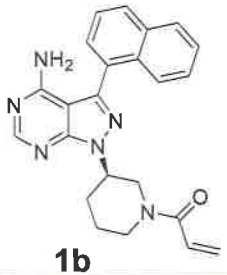
1c

Compound	EGFR	IC_{50} [nM]
1a	L858R/T790M	2.5
	L858R/T790M/C797S	838
1b	L858R/T790M	1.9
	L858R/T790M/C797S	7900
1c	L858R/T790M	<1
	L858R/T790M/C797S	88
AZD9291	L858R/T790M	<1
	L858R/T790M/C797S	77

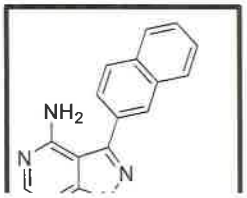
Engel, Becker et al., *Angew Chem Int Ed Engl* **2016**, *55*, 10909-12.

Acquired Drug Resistance – C797S Mutation

1a



1b



Compound	EGFR	IC_{50} [nM]	K_i [nM]	k_{inact} [min^{-1}]
1a	L858R/T790M	2.5	16	0.29
	L858R/T790M/C797S	838	1008	-
1b	L858R/T790M	1.9	58	0.31
	L858R/T790M/C797S	7900	1068	-
1c	L858R/T790M	<1	1.5	0.17
	L858R/T790M/C797S	88	49	-
AZD9291	L858R/T790M	<1	1.5	0.33
	L858R/T790M/C797S	77	25	-

high affinity towards gatekeeper (Met790)

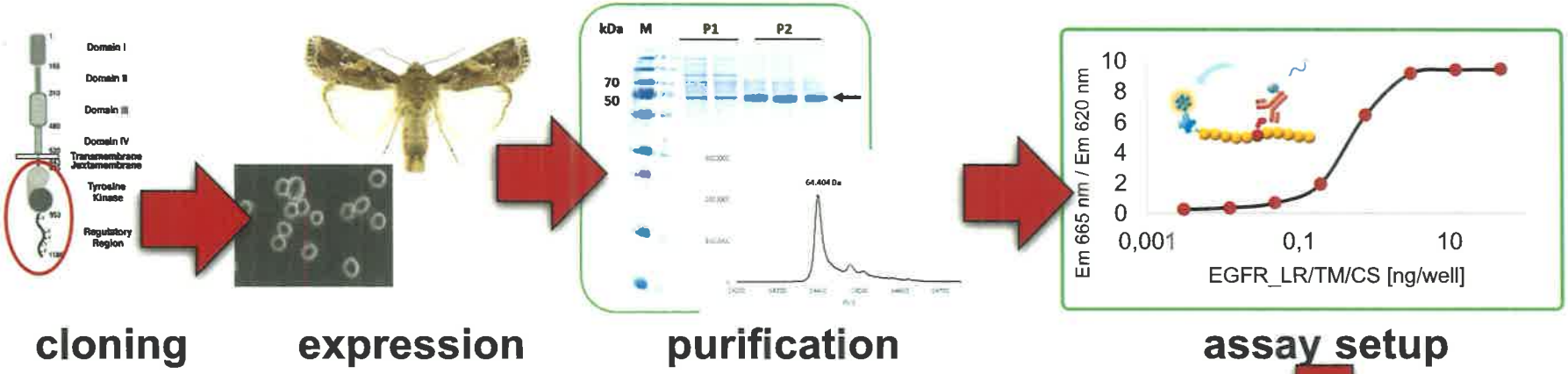
is key for

targeting T790M/C797S resistance

Engel, Becker et al., *Angew Chem Int Ed Engl* **2016**, *55*, 10909-12.

Acquired C797S mutation - screening for novel chemical entities

biochemical screen



cloning **expression** **purification** **assay setup**

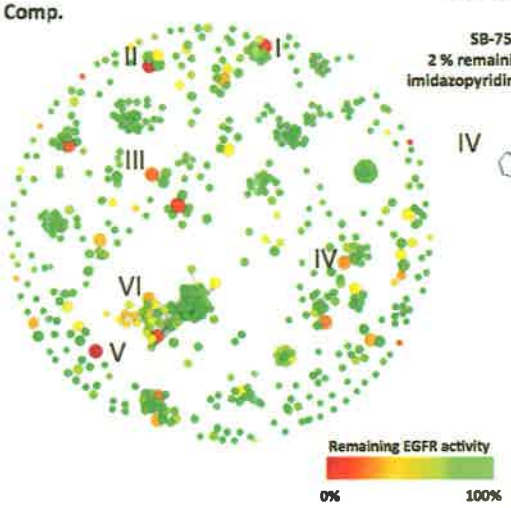
Screen of GSK and Roche Library

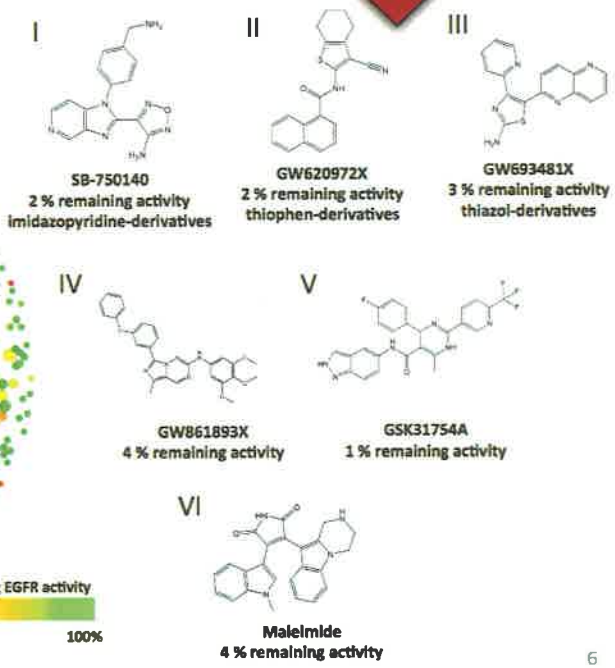
conditions:

1 µM compound; 0.08 nM EGFR LRTMCS;

30 min pre-incubation; triplicate

600 Comp.

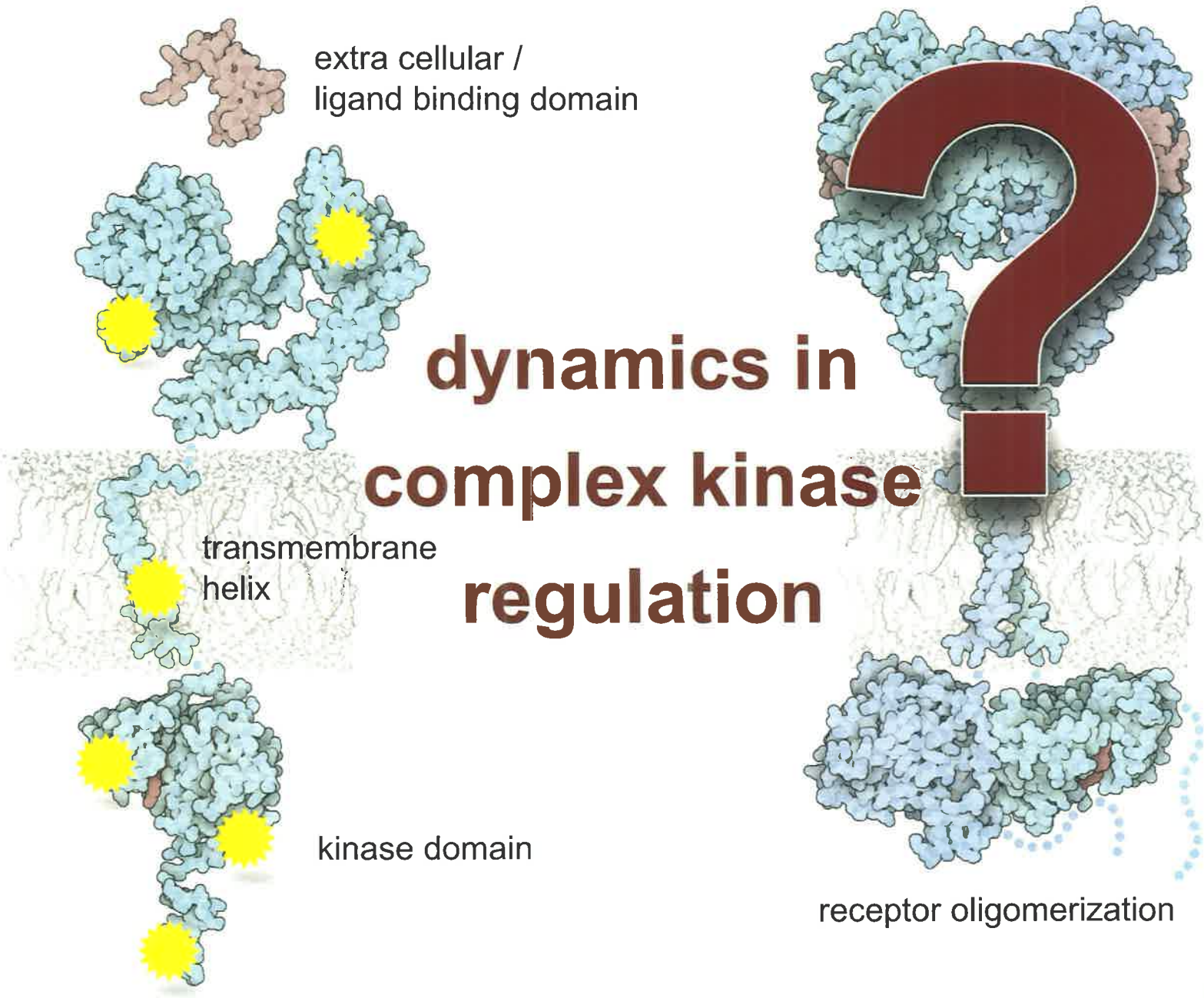




Knapp, S. *et al.*, *Nat. Chem. Biol.*, 2013, 9, 3-6.

Marina Keul, Hannah L. Tumbrink, Jonas Lategahn

Complex regulation of EGFR

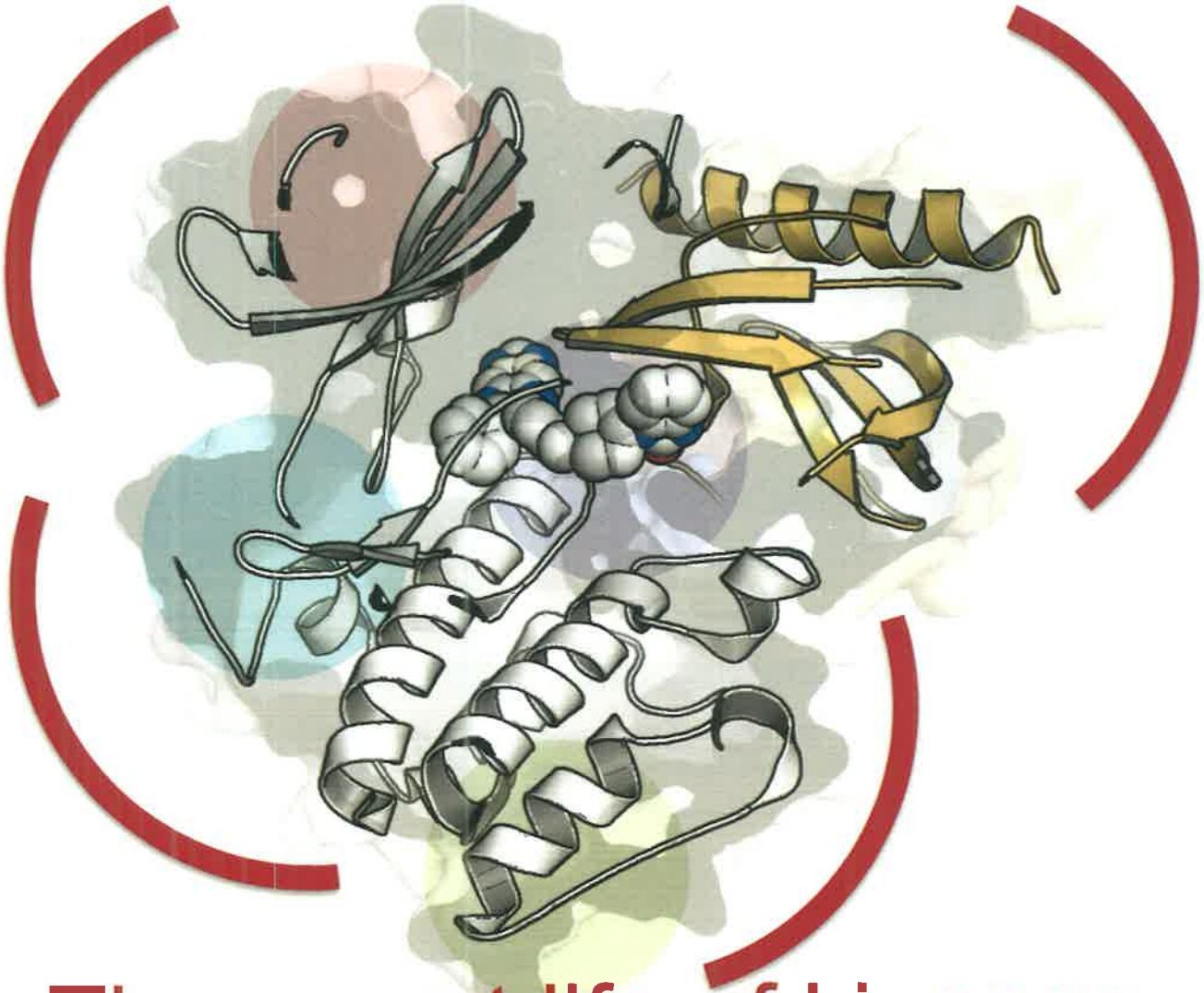


dynamics in

complex kinase

regulation

www.rcsb.org



The secret life of kinases beyond catalysis

Rauch, J. ... Walter Kolch, *Cell Commun Signal.* **2011**