Using EGFR signaling networks to guide therapeutic strategies

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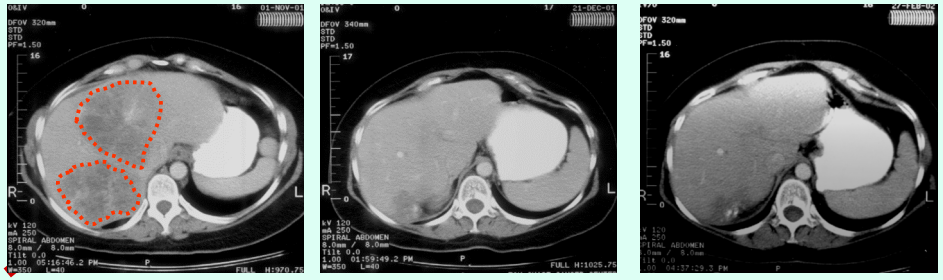
Problem: the limited efficacy of therapies directed at well-validated cancer targets

Some targeted drugs work well, but only in a minority of patients:

Some work initially, but rapidly lose efficacy

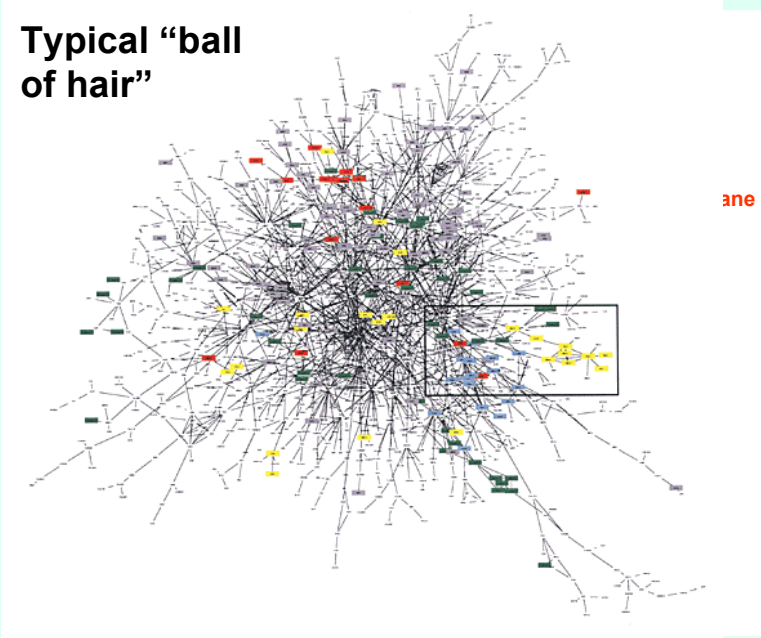
Best case: one patient, EGFR-targeted therapy



Before treatment:
metastases in liver

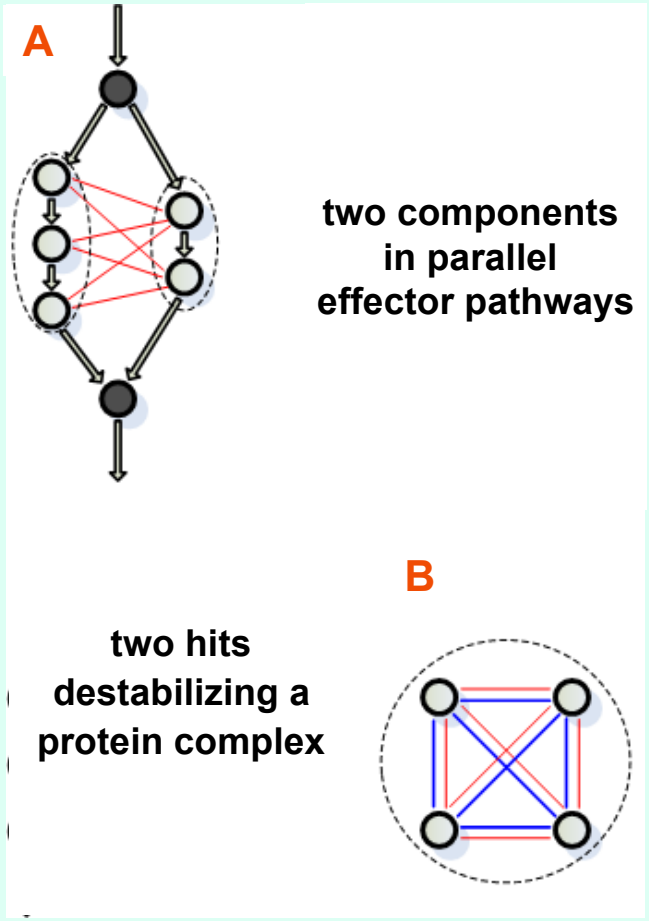
6 weeks:
Metastases regressed

8 months:
No regrowth



<u>Likely explanation</u>:
the availability of multiple possible signaling detours

Hypothesis/Concept: escape pathways will cluster near incapacitated targets



Ma et al, 2008

Synthetic lethality =
a binary event leading to network failure

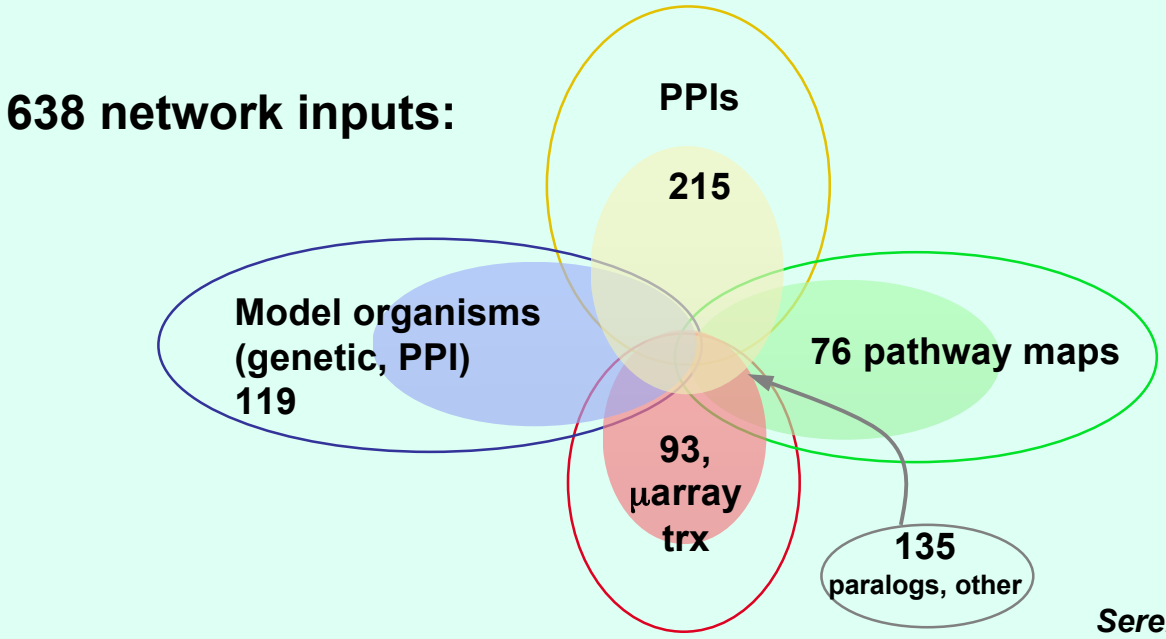
"Large-scale mapping of genetic interactions among nonessential yeast genes showed that **synthetic interactions are highly biased toward genes that have related functions**". Tong et al, Science 303, 808–813, 2004.

"Perhaps the most notable property of the essential genetic network (based on synthetic lethal analysis) is its density…our results indicate that **essential genes are highly connected hubs on the genetic interaction network**". Daverwala et al, Nat Genet 37:1147, 2005

"Thus, **genetic profiles of members of PPI pairs tend to correlate** better, not only to their interaction partners within the same species, but also to the orthologs of their interaction partner in an evolutionarily distant organism". Roguev et al Science 322:405, 2008

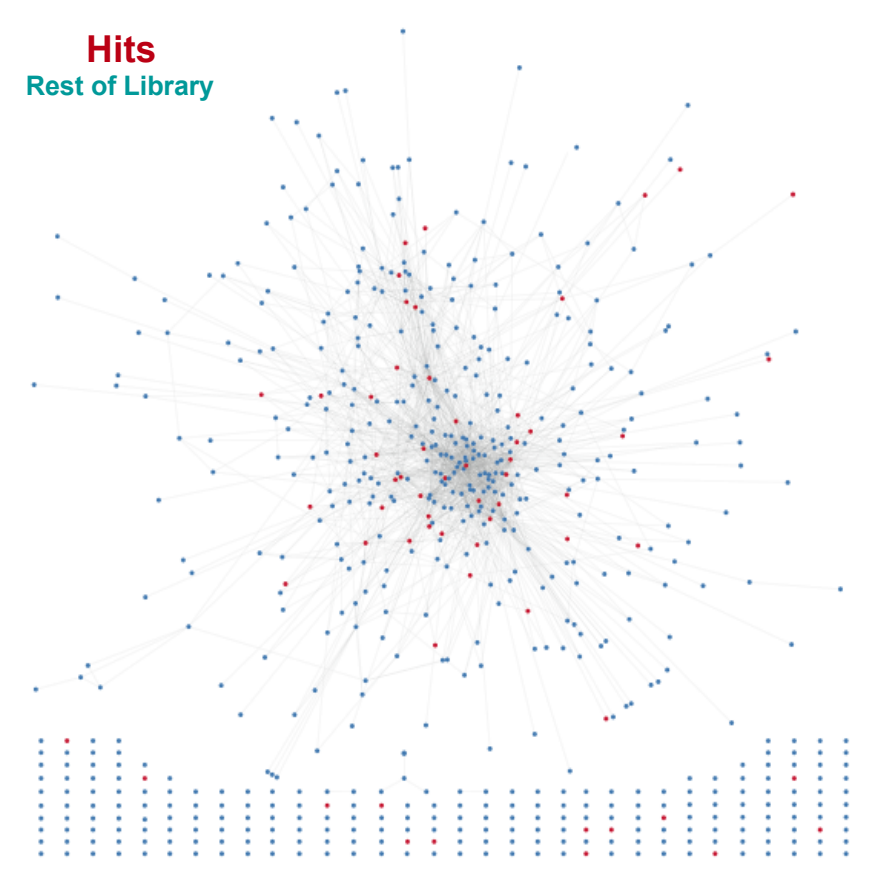
Approach: develop and screen siRNA library enriched around EGFR

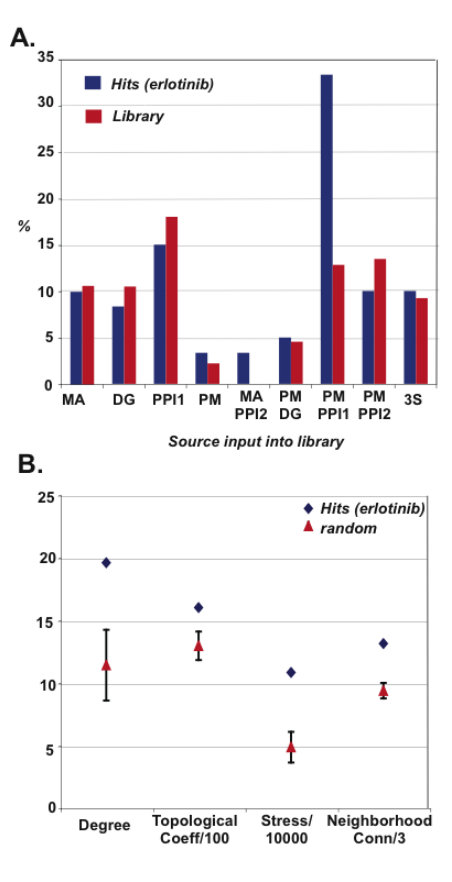
Key reference: Astsaturov et al, *Science Signaling* 3(140) ra:67, 2010



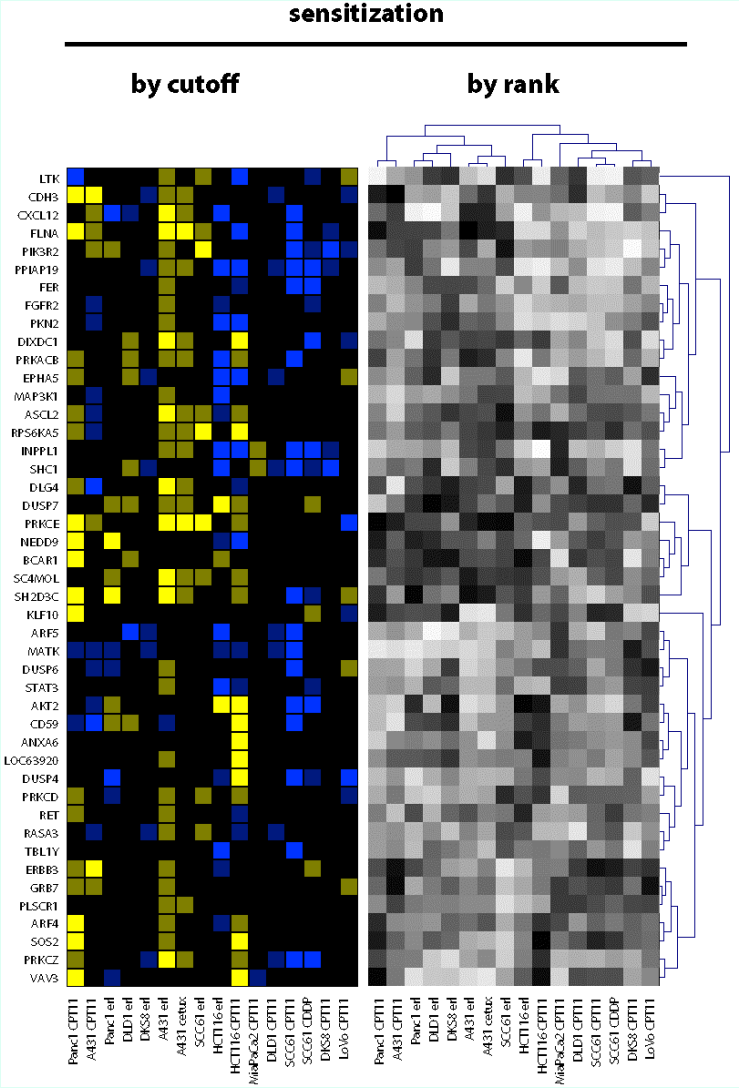
Serebriiskii

Results: hits concentrate among first order PPIs of EGFR “pathway”



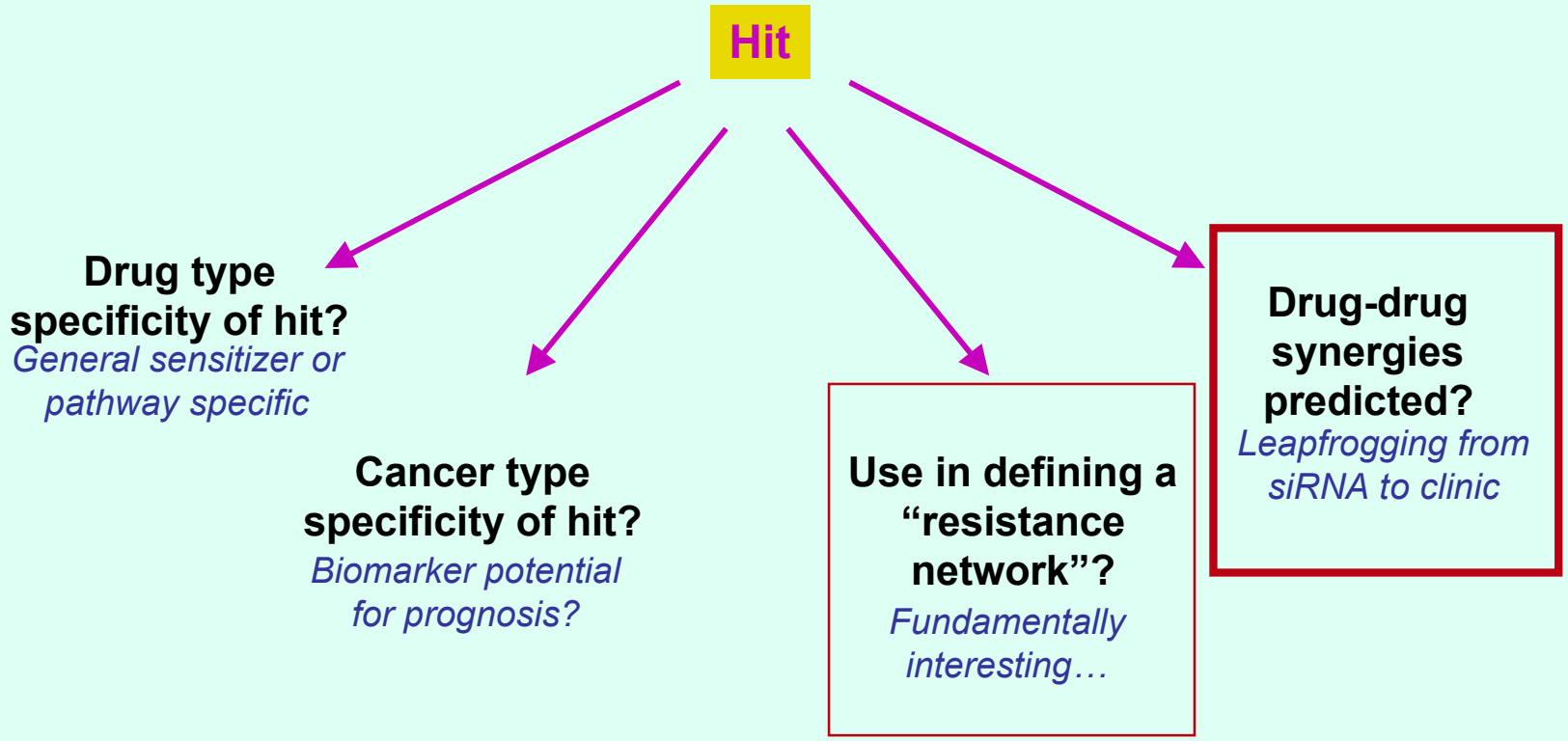


Hit activity profiles

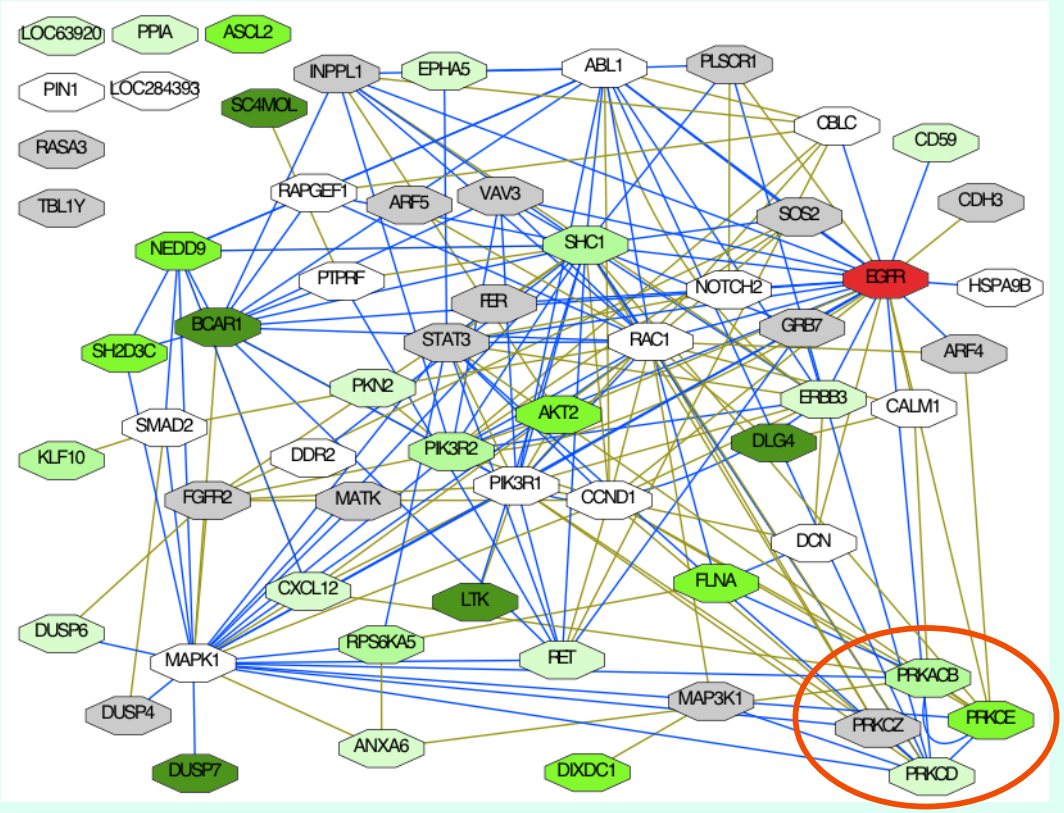


--Some specific for EGFR, some general for other inhibitors
--Some intrinsically reduce viability, others do not
--Some active in multiple cell lines, others limited to one or two lines
--Some selectively induce apoptosis in a drug-dependent manner

Utility of results - possible directions



Hit network, 1st pass follow-up: combinations with drugs directly targeting hits

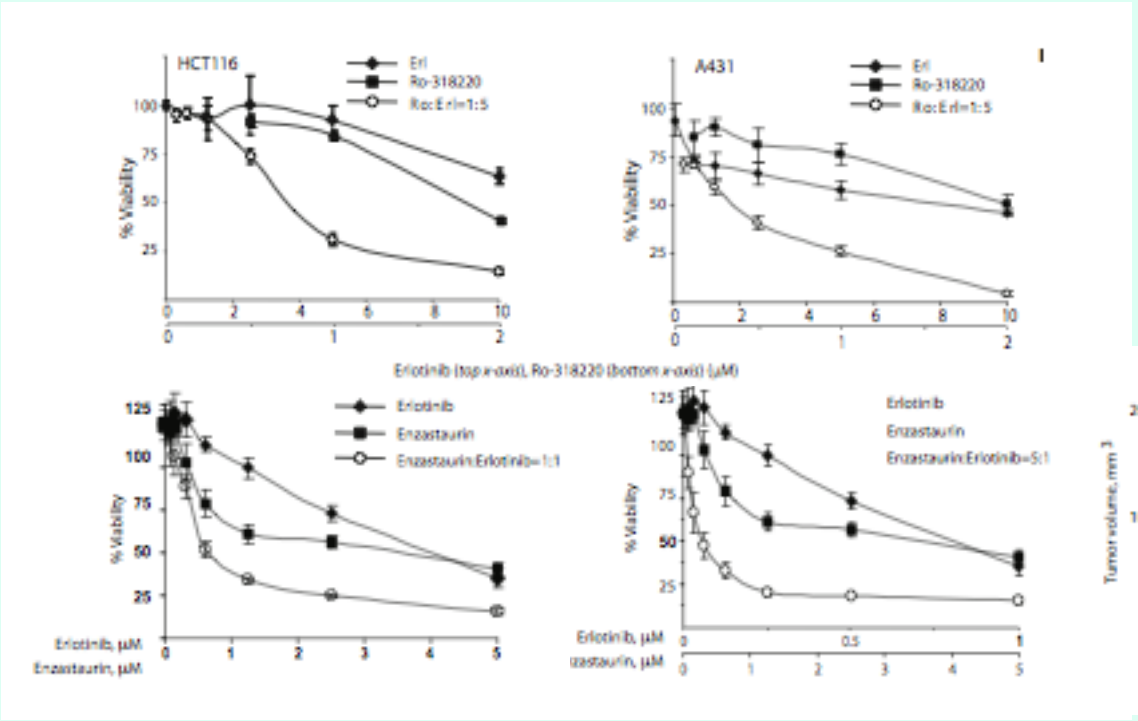


In vitro and in vivo confirmation of efficacy: PKC inhibitors with erlotinib

HCT116/proliferation

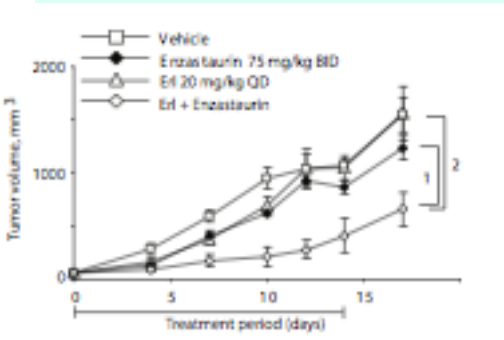
A431/proliferation

With Rottlerin (Ro-318220)

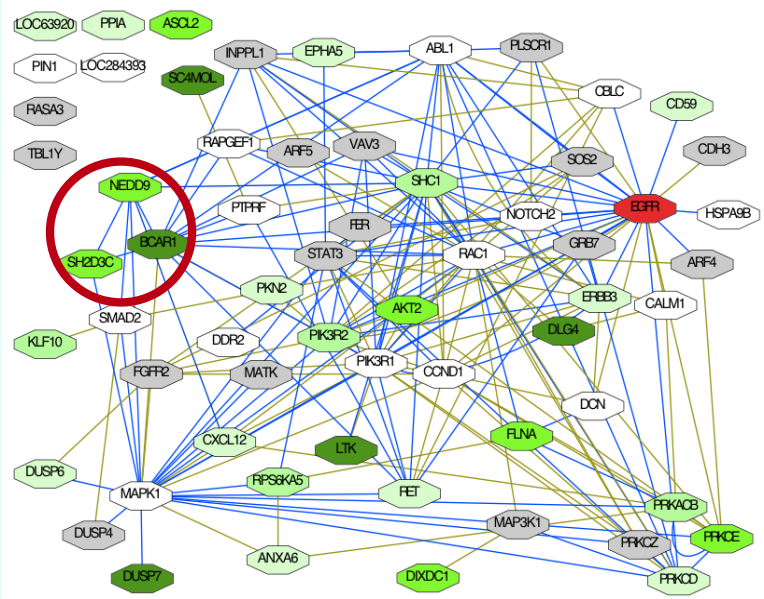


With Enzastaurin

xenograft



Hit Network, 2nd pass follow-up: Focus on BCAR1-SH2D3C-NEDD9 cluster to hypothesize new drug combinations



Relevant biology:

Scaffolding proteins with numerous partners

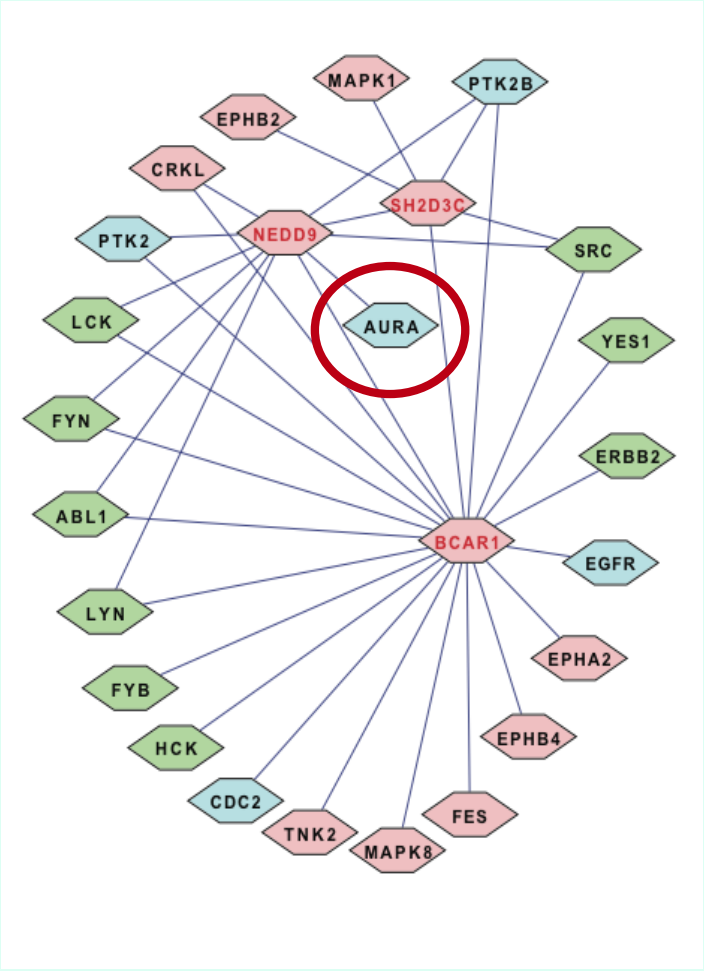
Central intermediates in integrin survival and invasion signaling (FAK, SRC)

NEDD9 and BCAR1 both strongly promote growth of mammary tumors in HER2/neu mouse models

Interactions with other proteins closer to canonical EGFR-signaling network (SHC…)

BUT: *not catalytic, not obviously druggable*

Extrapolate to Aurora-A



Hypothesis

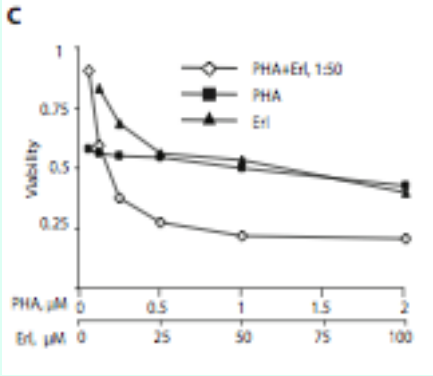
catalytic targets closely linked to this cluster are well positioned for synthetic lethality

Aurora-A; Mitotic kinase, plus

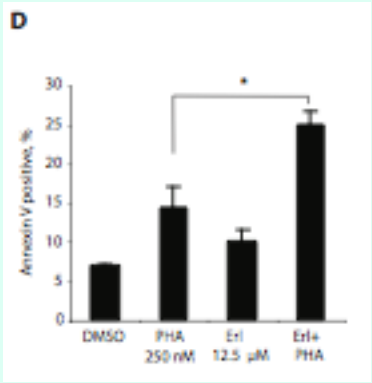
-resorption of cilia
-binding (regulation) of RalA
-centrosomal maturation
-microtubule organization and dynamics in interphase cells
-rapid environmental response to calcium

Synergy, EGFR-Aurora-A inhibition

Reduction of cell growth in vitro



Induction of apoptosis

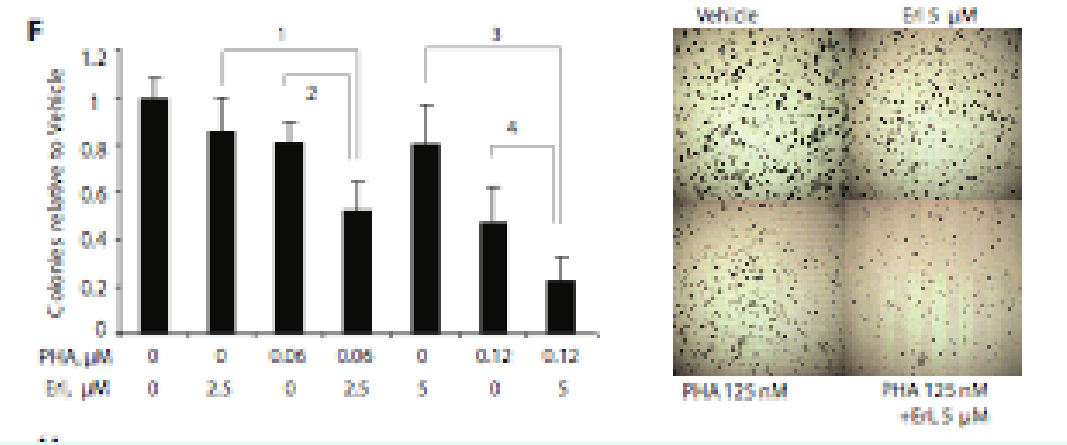


Chou-Talalay analysis: multiple combinations of EGFR and AurA inhibitors result in synergy

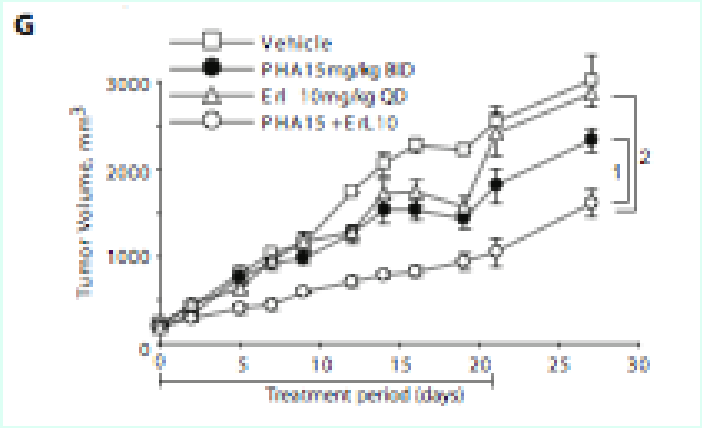
Cell line	Inhibitors		Molar Ratio	Coefficient of Interaction (average ± std. dev.) ED50	ED75	ED90
HCT116	erlotinib	PHA-680632	1:50	0.80 ± 0.04	0.28 ± 0.18	0.14 ± 0.13
			1:100	0.58 ± 0.16	0.31 ± 0.12	0.21 ± 0.06
	cetuximab	PHA-680632	1:16	0.46 ± 0.27	0.62 ± 0.05	1.07 ± 0.78
			1:33	0.31 ± 0.10	0.45 ± 0.06	0.73 ± 0.39
	erlotinib	C1368	1:25	0.64 ± 0.12	0.71 ± 0.17	0.89 ± 0.56
	cetuximab	C1368	1:8	0.42 ± 0.18	0.31 ± 0.10	0.24 ± 0.06
A431	erlotinib	PHA-680632	1:5	0.46 ± 0.11	0.37 ± 0.17	0.34 ± 0.23

Continued, anchorage-independent growth and in vivo

Reduced growth in soft agar

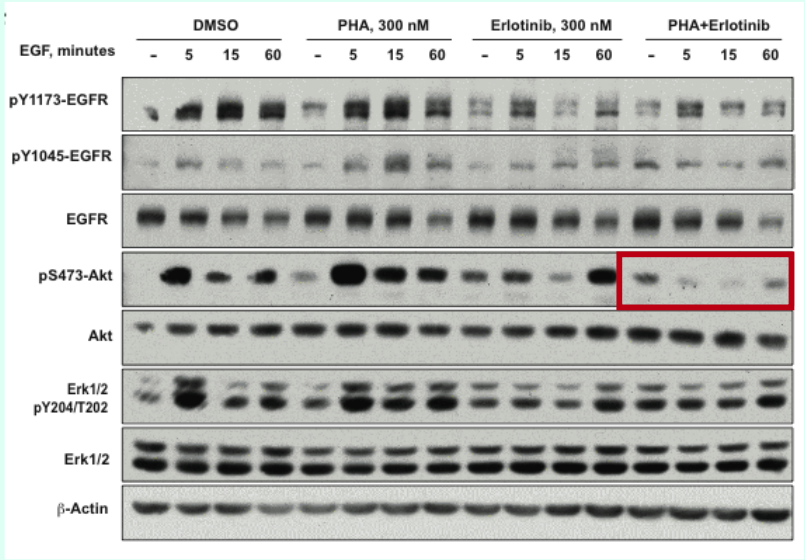


Xenograft activity

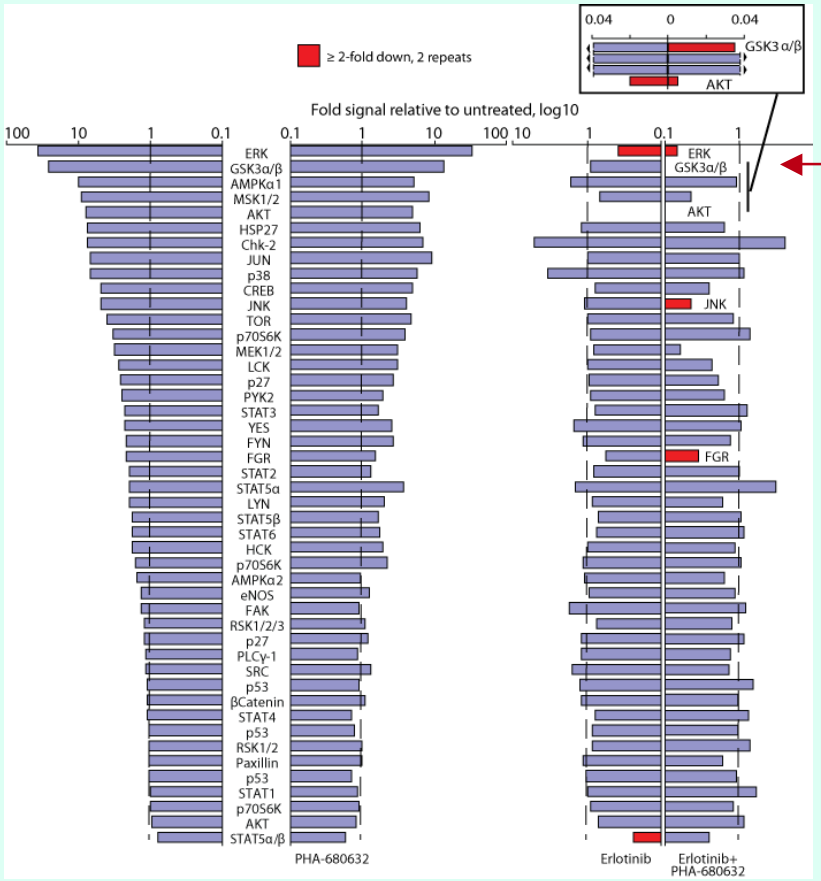


Dual EGFR-Aurora-A inhibition limits AKT activity

Dual inhibition of AurA and EGFR blocks AKT activation



Under EGF-responsive conditions, dual inhibition of Aurora-A and EGFR depresses activation of canonical effector kinases, and..

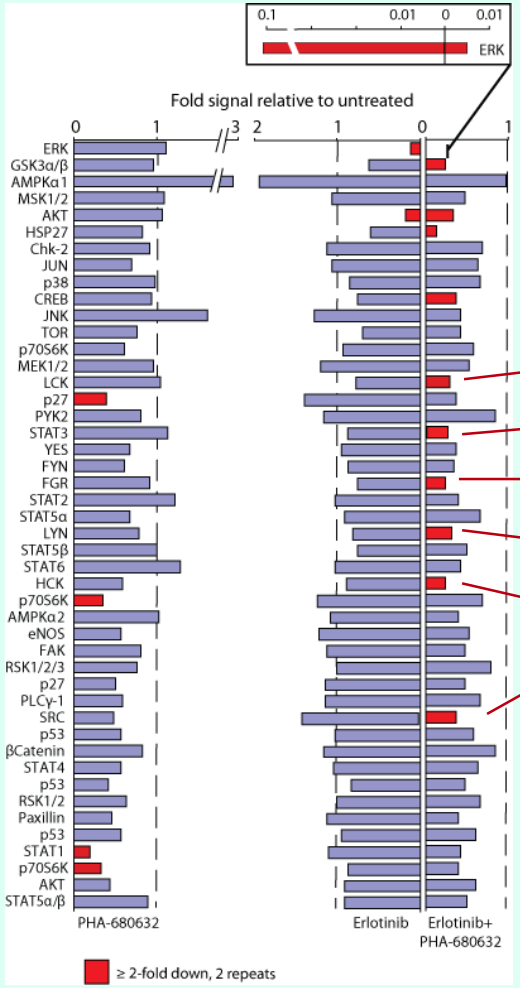


GSK3β:

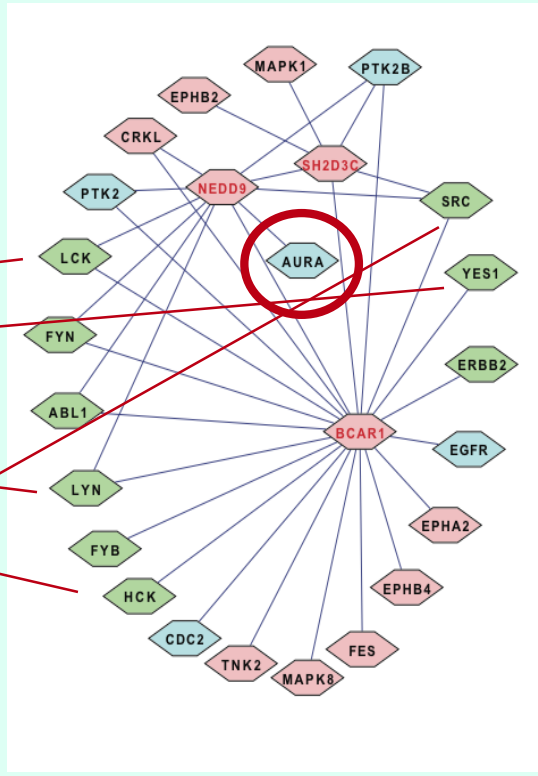
AuroraA partner, controls β-catenin signaling…

significance?

Under full serum conditions, dual inhibition of Aurora-A and EGFR inhibits cluster of Src family kinases



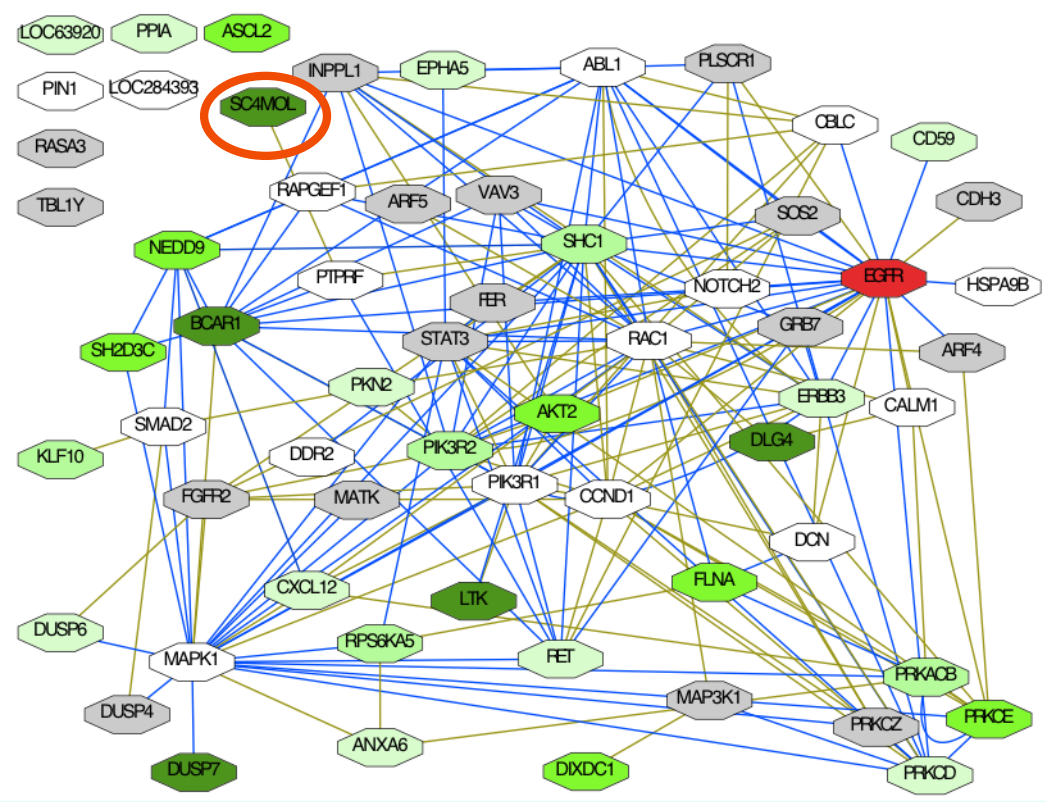
(SRC)

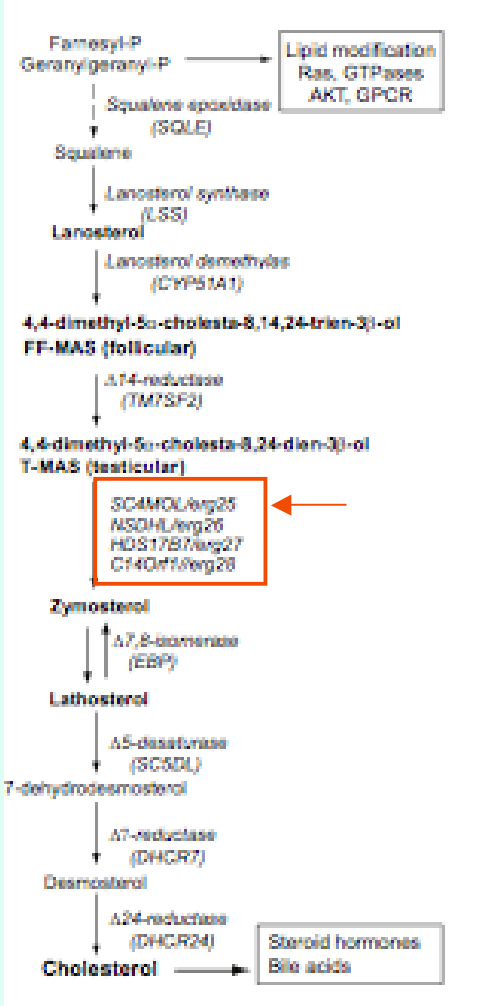


Already evidence that dual targeting of Src and EGFR is beneficial: Epidermal growth factor receptor cooperates with Src family kinases in acquired resistance to cetuximab. Wheeler et al, Cancer Biol Ther 2009, etc…

We are exploring effect of dual inhibition of Src and Aurora-A

Hit network, 3rd pass investigation: Exploring new biology around SC4MOL





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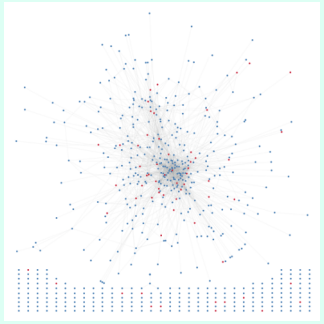
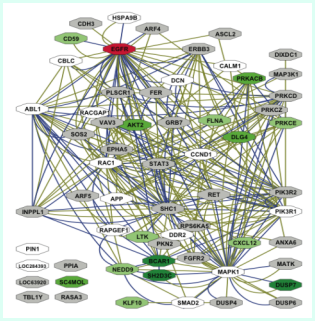
Summary

Network



Sub-network structure



Re-expanded towards drug targets

Biomarkers

3 Way Synergy

Biology

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