Informatics-guided screening approaches to improve cancer therapy

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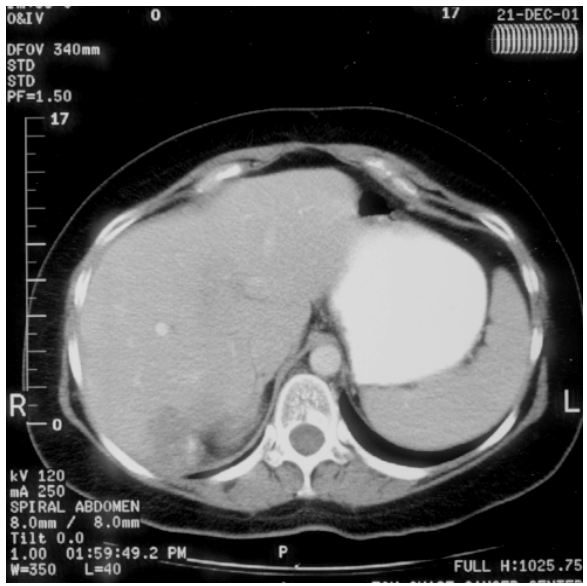
The clinical problem: which patients will respond to which therapies?

Best case: one patient, protein-targeted therapy



Before treatment:
metastases in liver

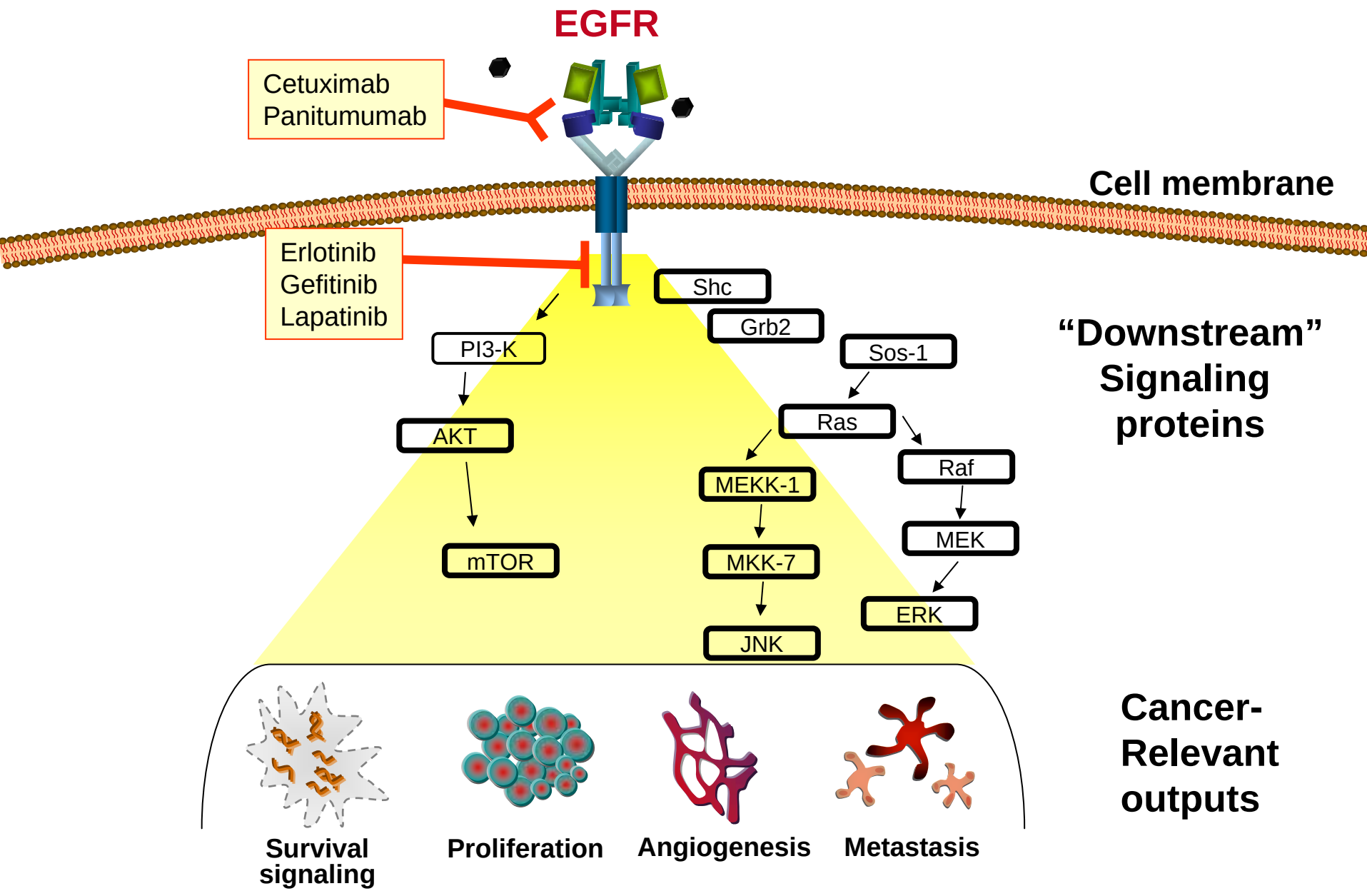
6 weeks:
Metastases regressed

8 months:
No regrowth

Why do many (apparently) similar tumors fail to respond to this drug?

How can we actively improve response rates?

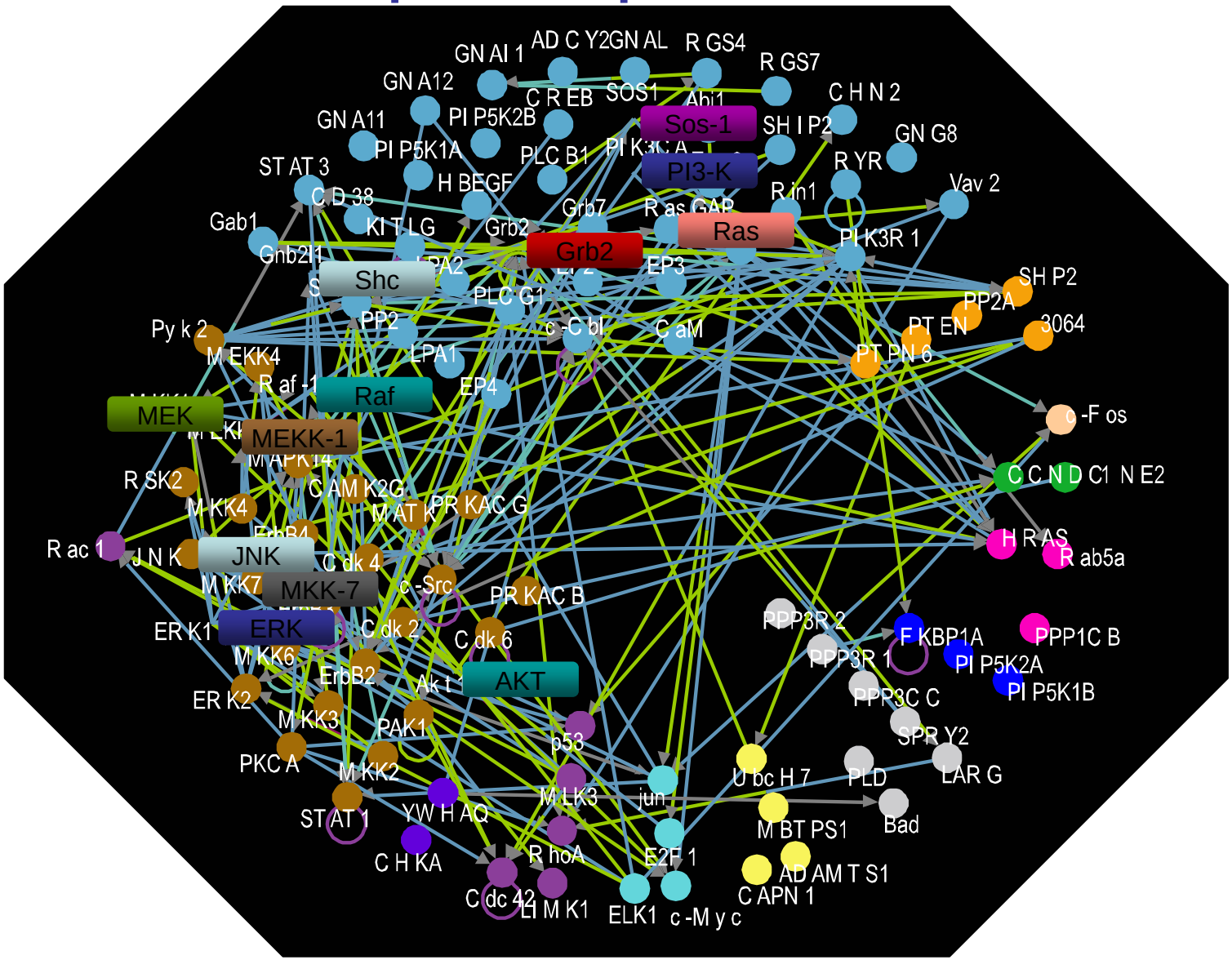
EGFR: a central and heavily targeted pathway



"Downstream" Signaling proteins

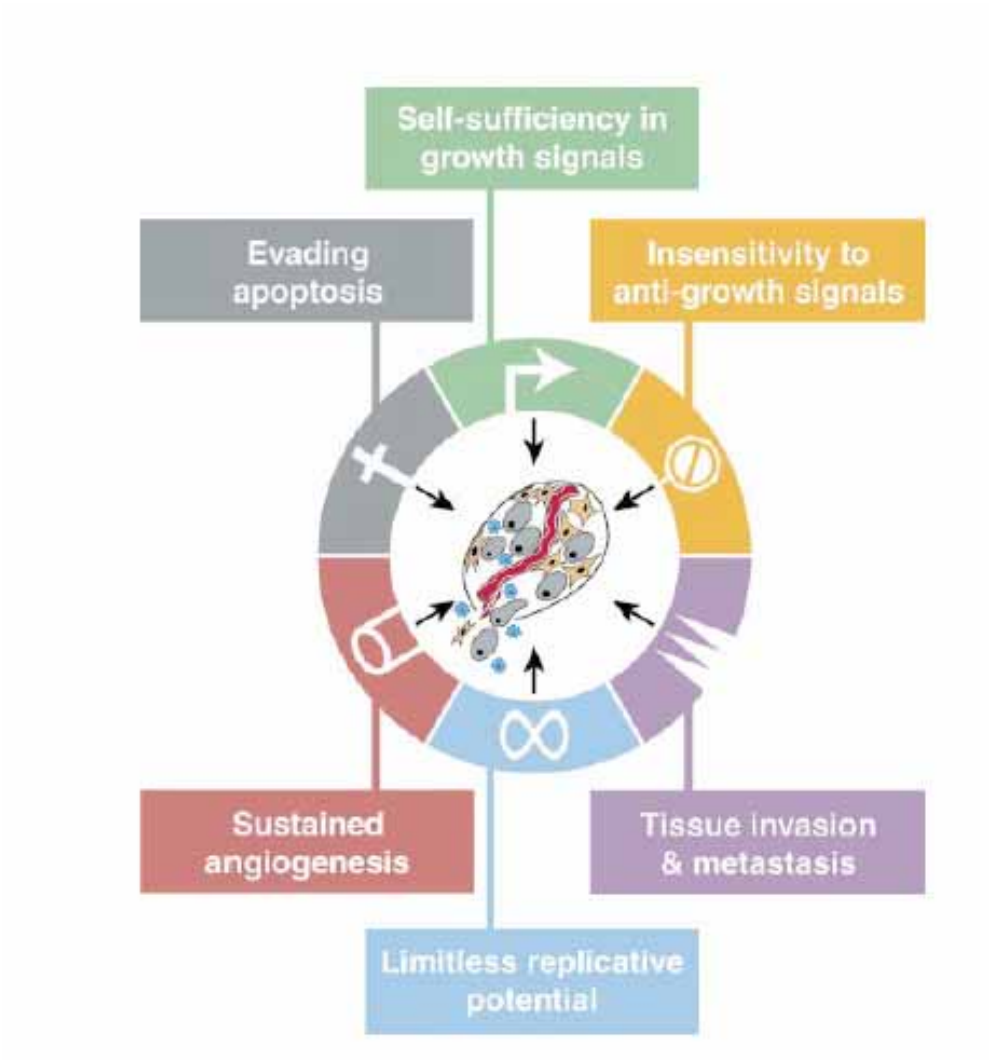
Cancer-Relevant outputs

In fact, the EGFR-centered signaling network is quite complicated…



Hypothesis: network robustness provides rescue routes: *an analogy*

Cancer is extremely complicated:
Drug combination strategies are inevitable



Hanahan & Weinberg, Cell, 2000

Our project

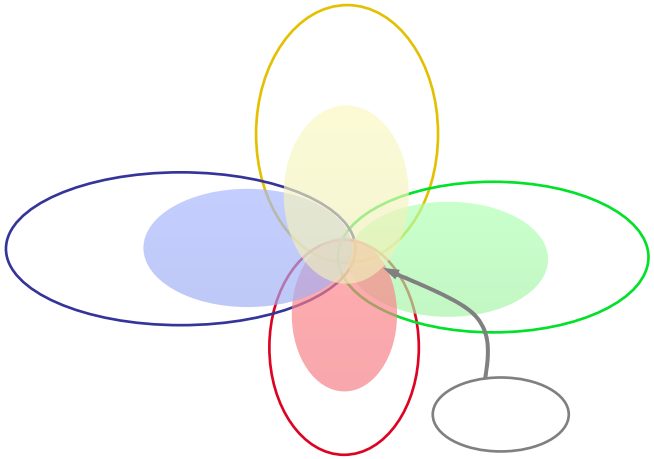
Use bioinformatics tools to build a physical/functional network around high-value targets, based on the idea that resistance factors will cluster near the primary target

Build an siRNA library to probe the network for sensitization to drugs of interest

Analyze the hits to

- **understand resistance network structure**
- **identify biomarkers for treatment susceptible/resistant**
- **identify new proteins to target**
- **design new clinical synergies using existing, approved drugs**

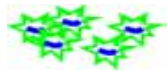
Building and screening an EGFR-centered network



High Confidence or Overlap

Source	Total	
Experts: STKE, Biocarta, Systems-biology.org, NetPath, Protein Lounge	277	76
PPIs: BOND, Biogrid, EMBL IntAct, HPRD, KEGG, Prolinks	1439 (1st and 2nd rank)	215
Microarray: GEO (NIH)	348	93
Model Organisms: Michigan Proteome Consortium, fly -> human orthologs	105; 65 have 1 or more orthologs	119
Paralogous clusters for key genes		125
Selected based on literature		~10

= 638 siRNAs



Reverse transfection siRNA-lipid complex

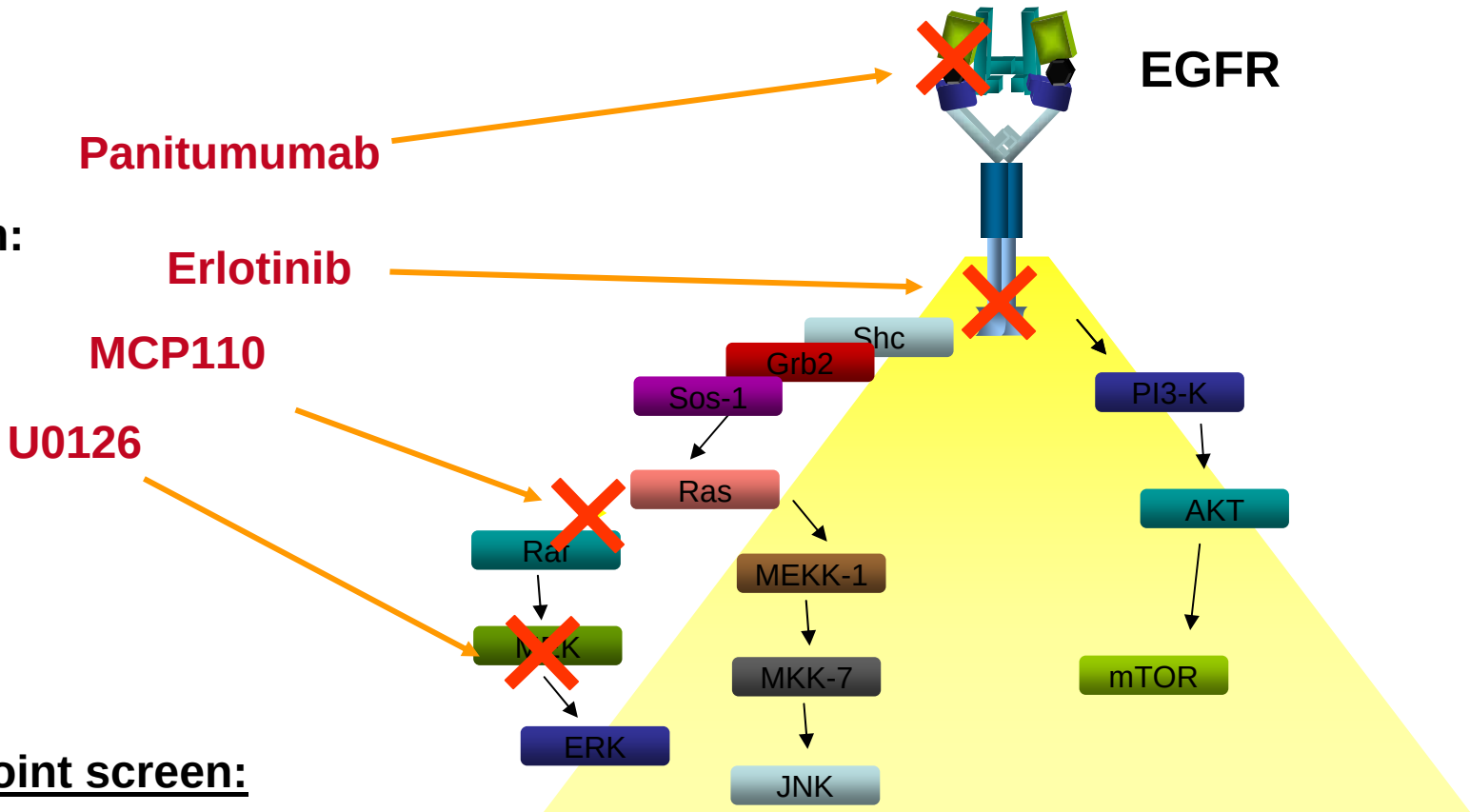
+/- Drug

Incubation

Screens Performed

Cell lines: A431 (sensitive)
HCT116 (K-Ras^{mut}, resistant)

Screen with:

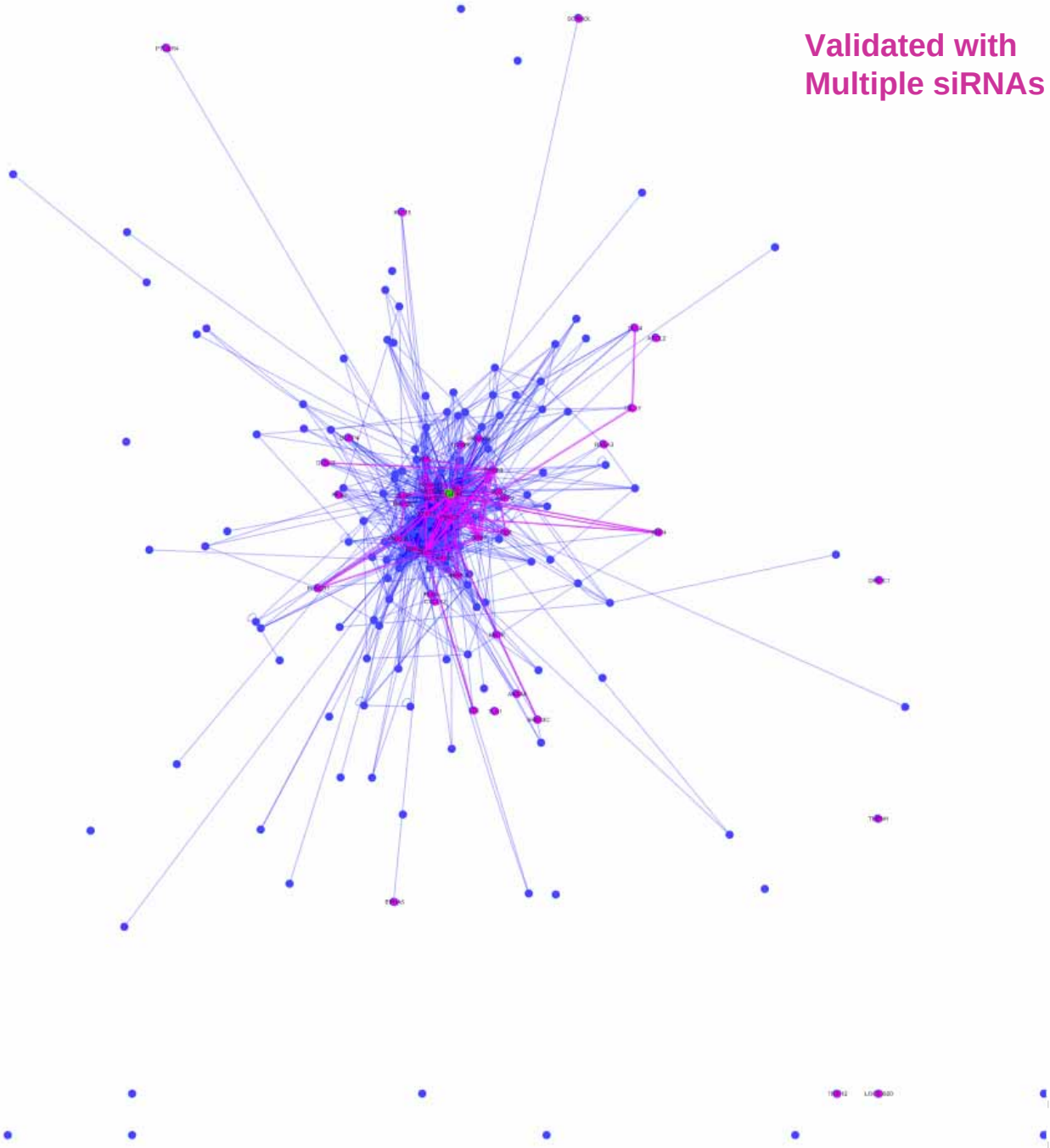


Counterpoint screen:

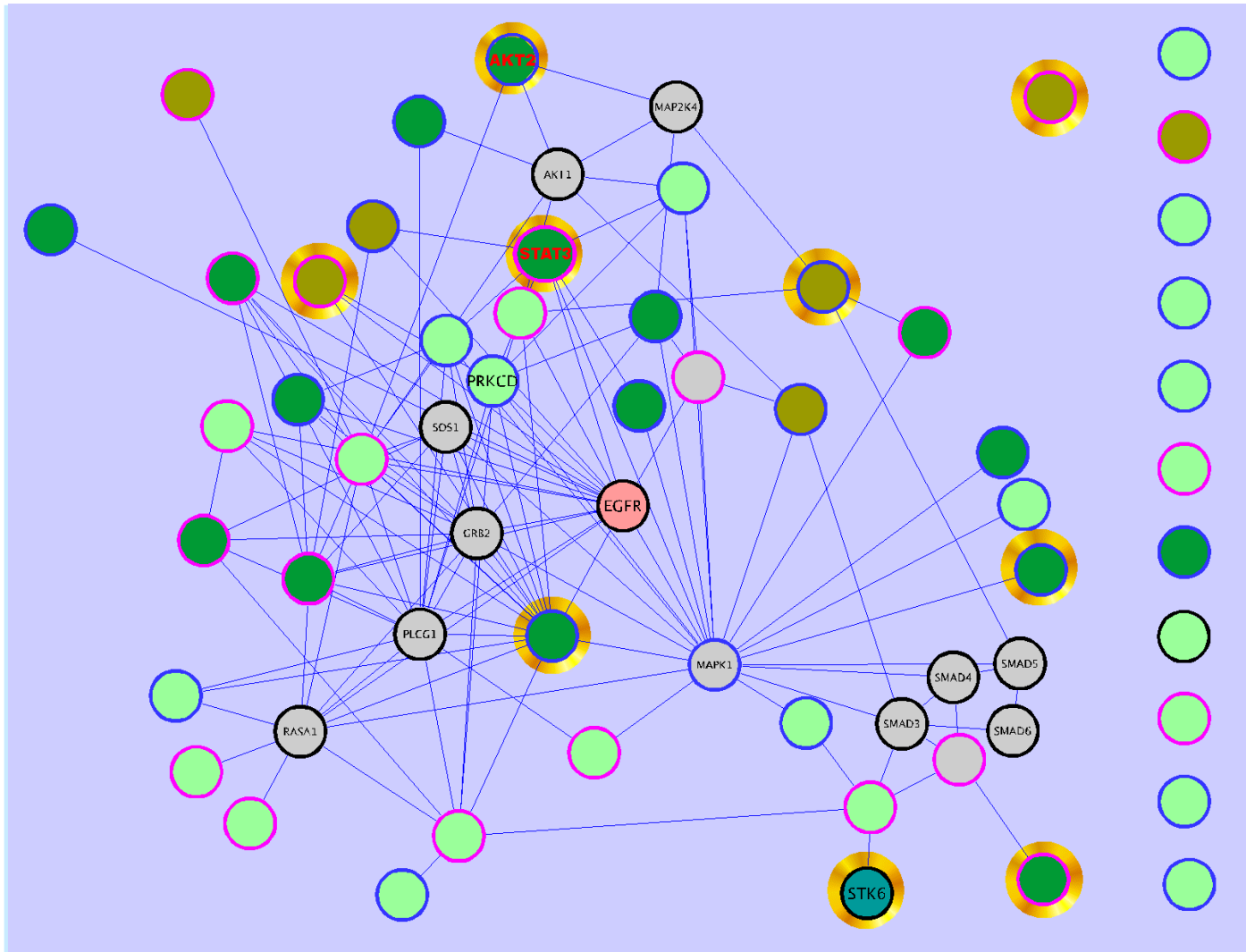
Topo I inhibitor CPT11

Hits

Validated with
Multiple siRNAs



Hit properties (subset)



A431 confirmed hits
lower expression levels

HCT116 only
confirmed hits

HCT116 & A431
common hits

erlotinib & CPT11

erlotinib

red labels, drug available; golden haloes, selected for combinatorial experiments

What next?
in progress

For the Top 40

Run panel across set of different cell lines with defined lesions

Goal: define most commonly active

->nominate biomarkers for resistance, targeting

Test for super-sensitizing pair-wise combinations of siRNAs

Goal: establish ways to completely shut down rescue routes

Test for additional drugs, radiation

Goal: EGFR/Ras specific, or general apoptosis sensitizer?

Leapfrog to drug synergies

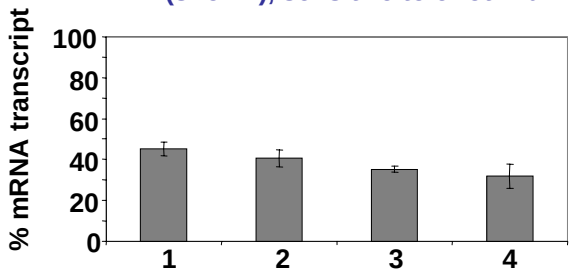
Goal: into clinic as quickly as possible

Establish mode of action of the hits

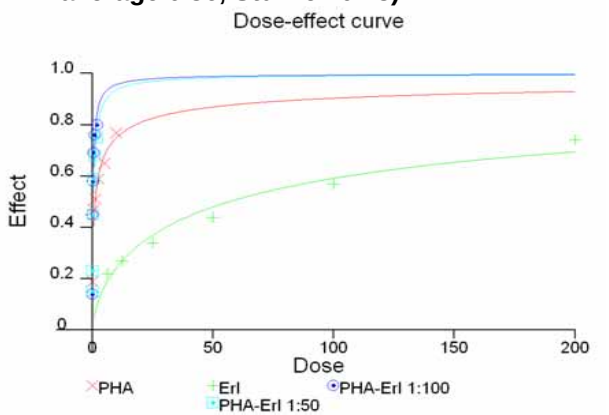
Goal: it's just interesting

EGFR and Aurora-A/STK6: from siRNA-drug to drug-drug

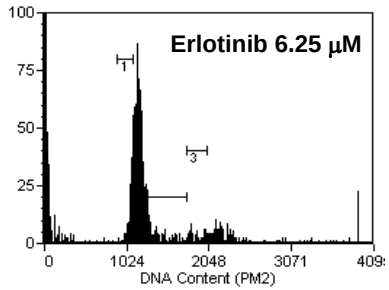
4 independent siRNAs deplete Aurora kinase A mRNA (shown), sensitize to erlotinib

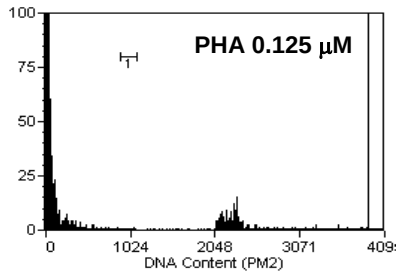


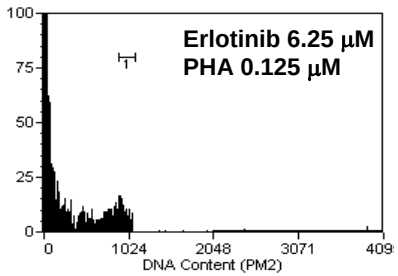
Chou-Tallalay analysis showing strong synergy of PHA 680632 with Erlotinib used in ratios of 1:50 and 1:100 (CI average 0.36, Std Dev.0.13)



Combination induces efficient apoptosis







-Results validated with separate, Aurora-A-specific drug

-Preliminary data suggests synergy with cetuximab

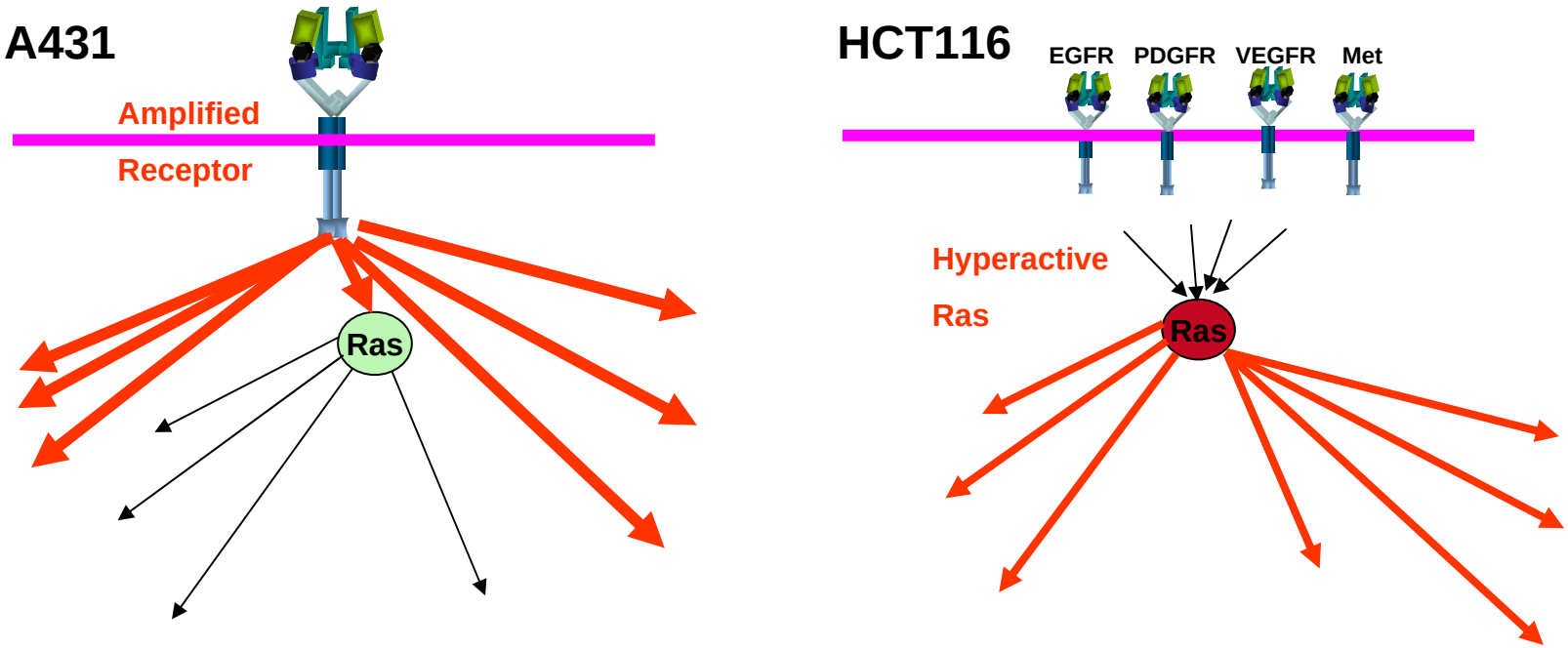
-Testing multiple cell lines

-Preparing for xenograft (summer)

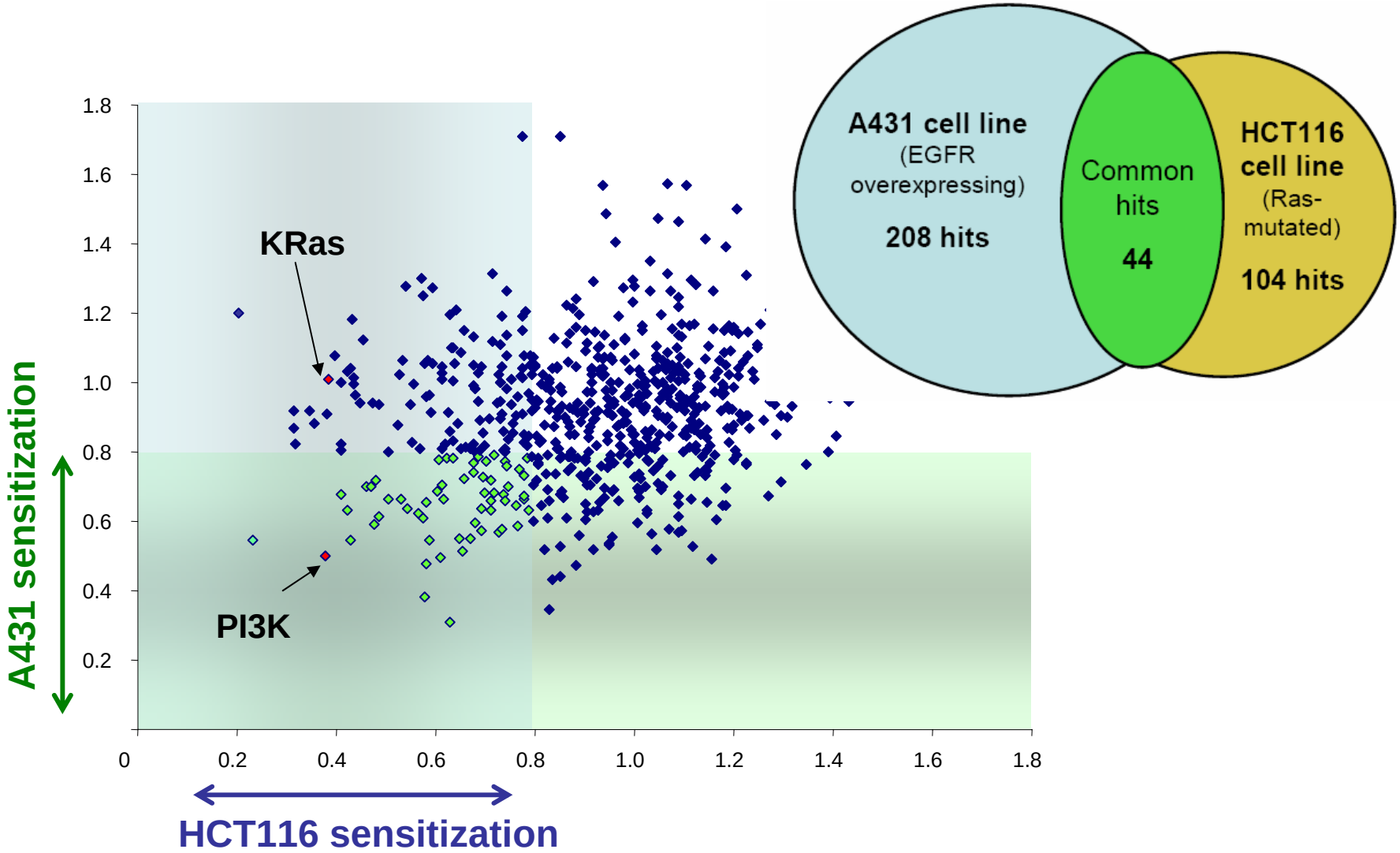
-Preparing for phase I trial (fall/winter) with FCCC colleagues

Other drugs poised to test

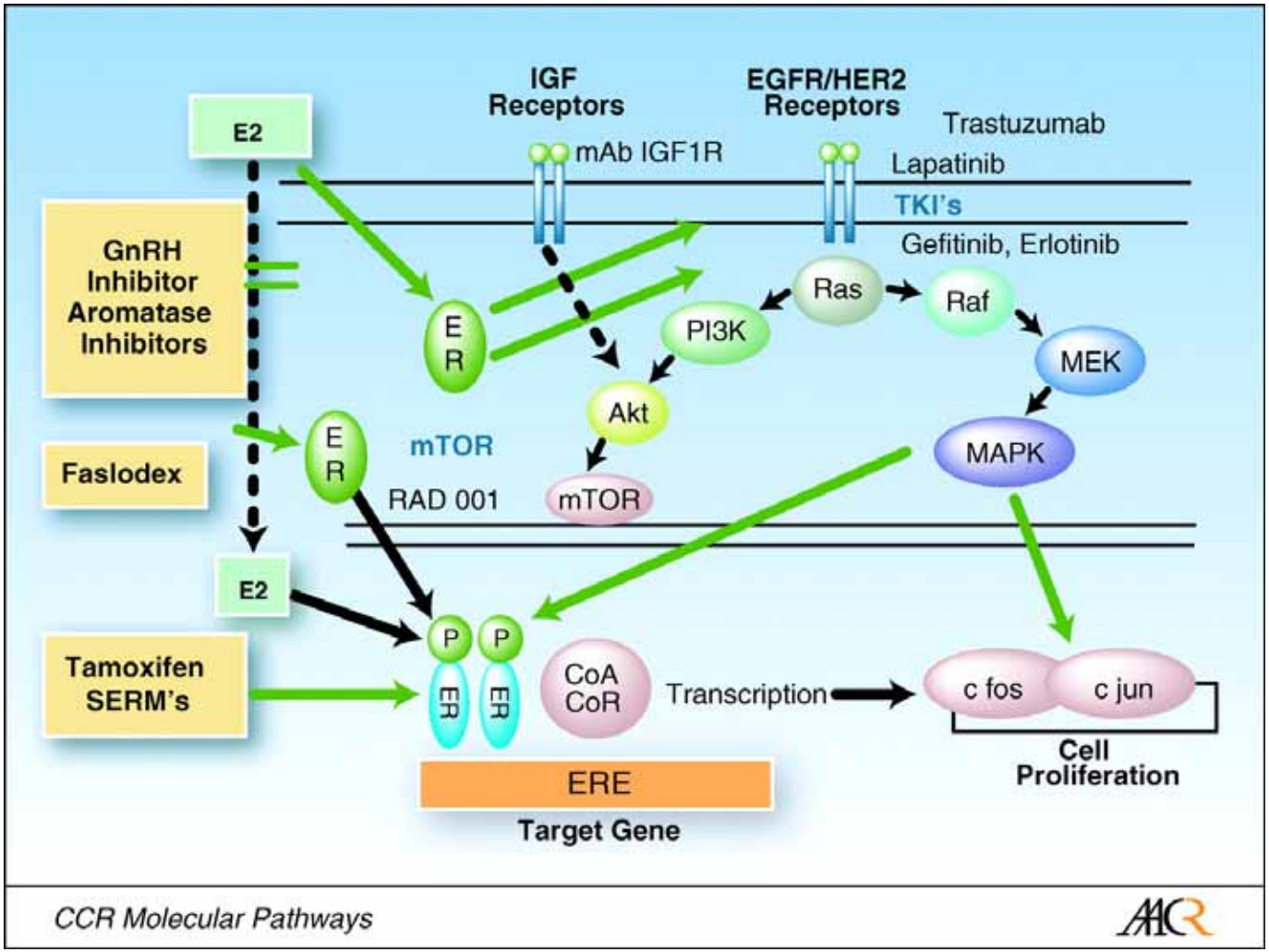
Further applicability: illuminating resistance networks specific to defined oncogenic lesions



Screening hits segregate by line



Extending the approach: estrogen signaling, cross-talk with EGFR

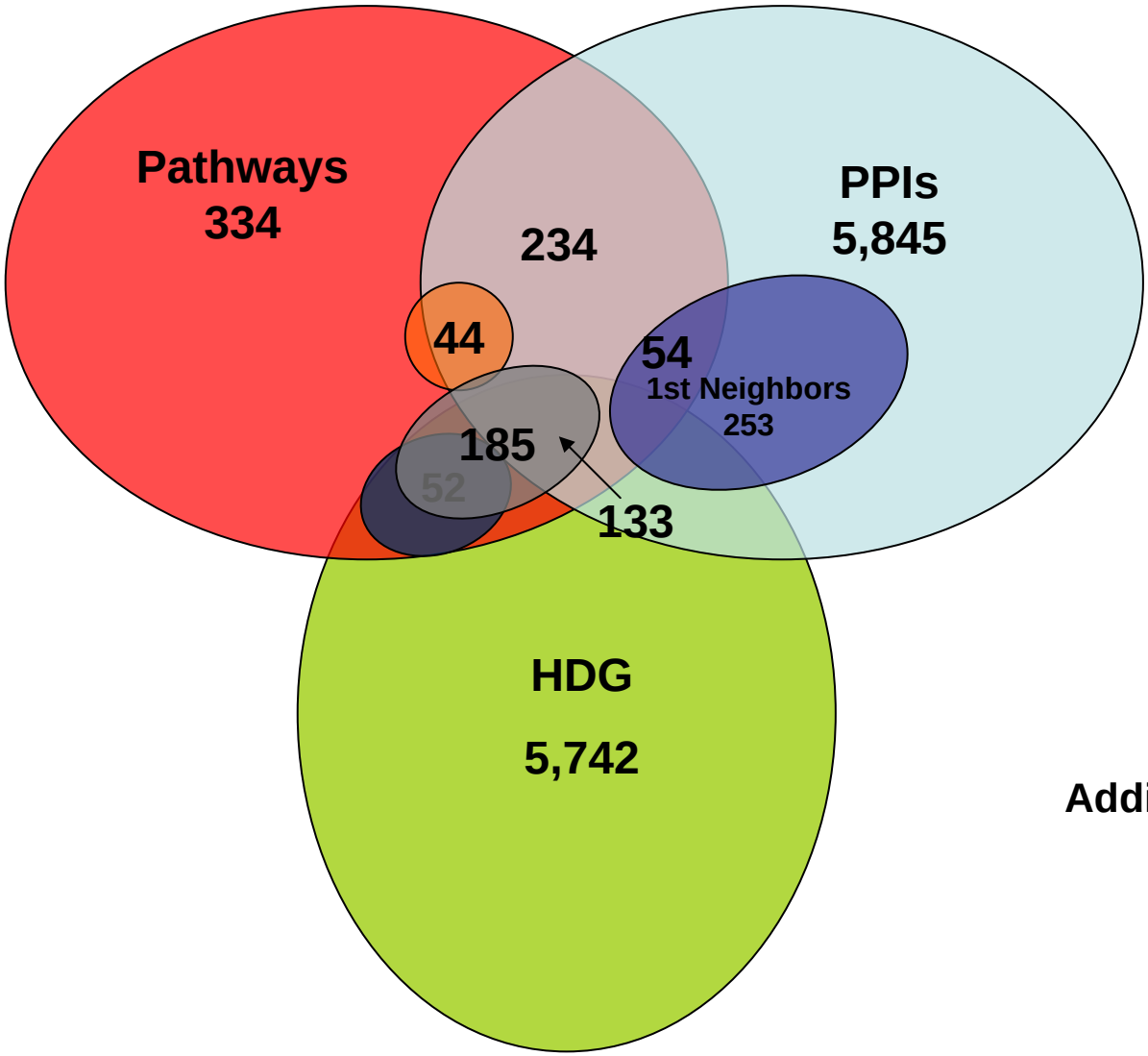


Seeds

ESR1 (ERα)
ESR2 (ERβ)
ESRRA
ESRRG
ARO (Cyp19A1)

Moy, B. et al. Clin Cancer Res 2006;12:4790-4793

In progress: estrogen pathways/ network construction



Estrogen Receptor Network:

- first neighbors 253
- pathway and PPIs 234
- core Pathway 44
- Druggable targets 52
- Unique nodes 486

Adding: microarray-based, paralog sets large complexes

Acknowledgments

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