

Identification, reconstruction and analysis of disease-related signaling networks from RNA-seq data

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Institute for Systems Genomics

JAX Big Genomic Data Skills Training for Graduate Professors

Outline of Presentation

Brief Introduction to Systems Biology

Pipeline for Network Reconstruction

Step I: Optimal Functional Module of Genes

- DEGs
- First degree connected component (OFTEN)
- Second degree connected component (SOC)

Step II: Transcription factors enriched on Functional Module of Genes

Step III: Identification of Master Regulators and Network Reconstruction

Step IV: Network Analysis

- Combinations of Interventions (OCSANA)

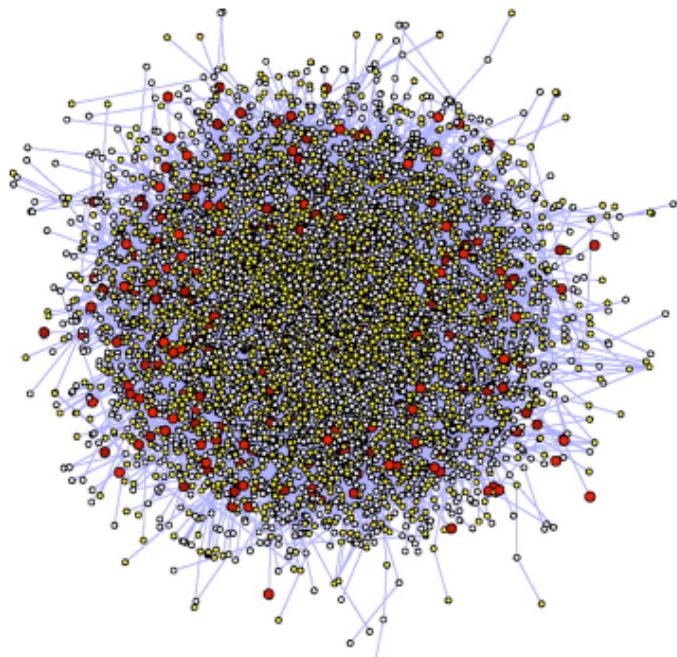
Other examples on ongoing research

- MyD88 phagosomal signals in Lyme disease
- CD8 T Cell Activation and Migration in Vivo
- Prediction of interventions in TNBC through mathematical network control

Systems are entities composed of well-defined components. When integrated, the components act together as to form a functioning whole with dynamical behaviors and responses to the environment.

Characteristics

- Non-linear behavior
- Feedback
- Self organization
- Robustness and lack of central control
- Put picture of complex systems



Systems Biology

- ◆ A branch of science that seeks to integrate different levels of information to understand how biological systems function.

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- ◆ L. Hood: “Systems biology defines and analyses the interrelationships of all of the elements in a functioning system in order to understand how the system works.”

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 - ◆ L. Hood: “Systems biology defines and analyses the interrelationships of all of the elements in a functioning system in order to understand how the system works.”
 - ◆ It is not the number and properties of system elements but their relations!

Networks and the Core Concepts of Systems Biology

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(i) Complexity emerges at all levels of the hierarchy of life

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- (iii) The whole is more than the sum of the parts.

Networks and the Core Concepts of Systems Biology

- (i) Complexity emerges at all levels of the hierarchy of life
- (ii) System properties emerge from interactions of components
- (iii) The whole is more than the sum of the parts.
- (iv) Applied mathematics provides approaches to modeling biological systems.

Systems Levels

Ecosystems

Organs

Tissues

Cells

Pathways

Proteins/genes

...

Organ and tissue networks

Inter-cellular networks

Intra-cellular networks

Signaling Networks

Metabolic Networks

Protein interaction networks

Protein kinase-substrate networks

Gene Regulatory Networks

Gene - miRNA Regulatory Networks

Chromatin interaction networks

Other networks:

Disease networks

Drug-target interaction networks

...,etc.

Systems Biology

- ◆ Is it a new area of study? NO and YES

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- ◆ Systems theory and theoretical biology are old

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- ◆ Systems theory and theoretical biology are old
- ◆ Experimental and computational possibilities are new

von Bertalanffy, 1901-1972

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\$15.95

GENERAL SYSTEM THEORY

Gathered here are Ludwig von Bertalanffy's writings on general system theory, selected and edited to show the evolution of systems theory and to present its applications to problem solving. An attempt to formulate common laws that apply to virtually every scientific field, this conceptual approach has had a profound impact on such widely diverse disciplines as biology, economics, psychology, and demography.

A German-Canadian biologist and philosopher, Ludwig von Bertalanffy (1901–1972) was the creator and chief exponent of general system theory. He is the author of ten books including *Robots, Men, and Minds* and *Modern Theories of Development*, both which have been published in several languages.

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ISBN 0-8076-0636-7, pb, \$7.95

The Relevance of General Systems Theory

ISBN 0-8076-0659-6, hb, \$8.95

Hierarchy Theory

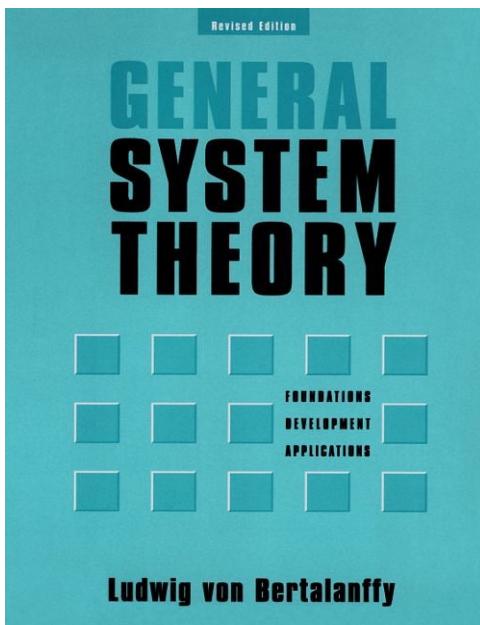
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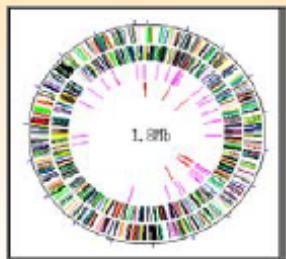
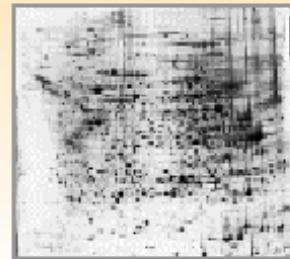
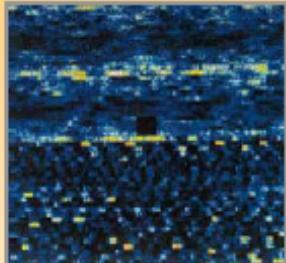
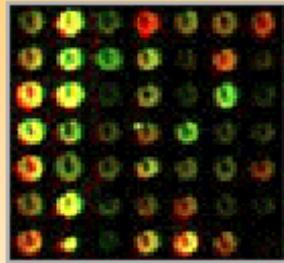


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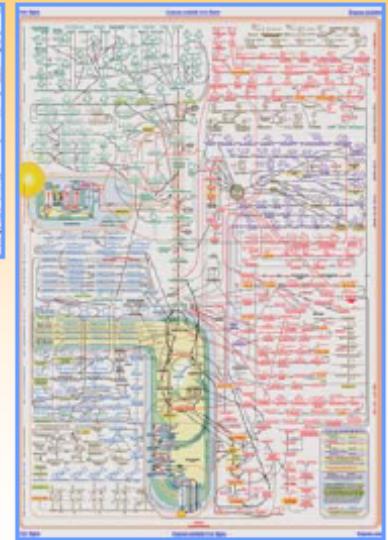
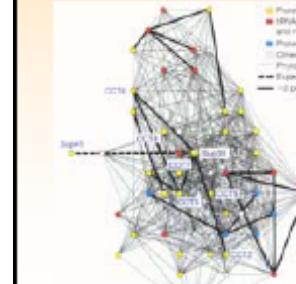
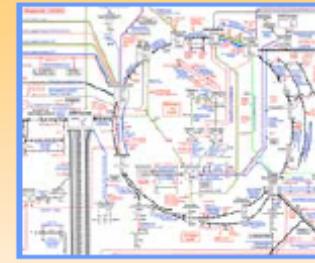
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Omics-revolution shifts paradigm to large systems

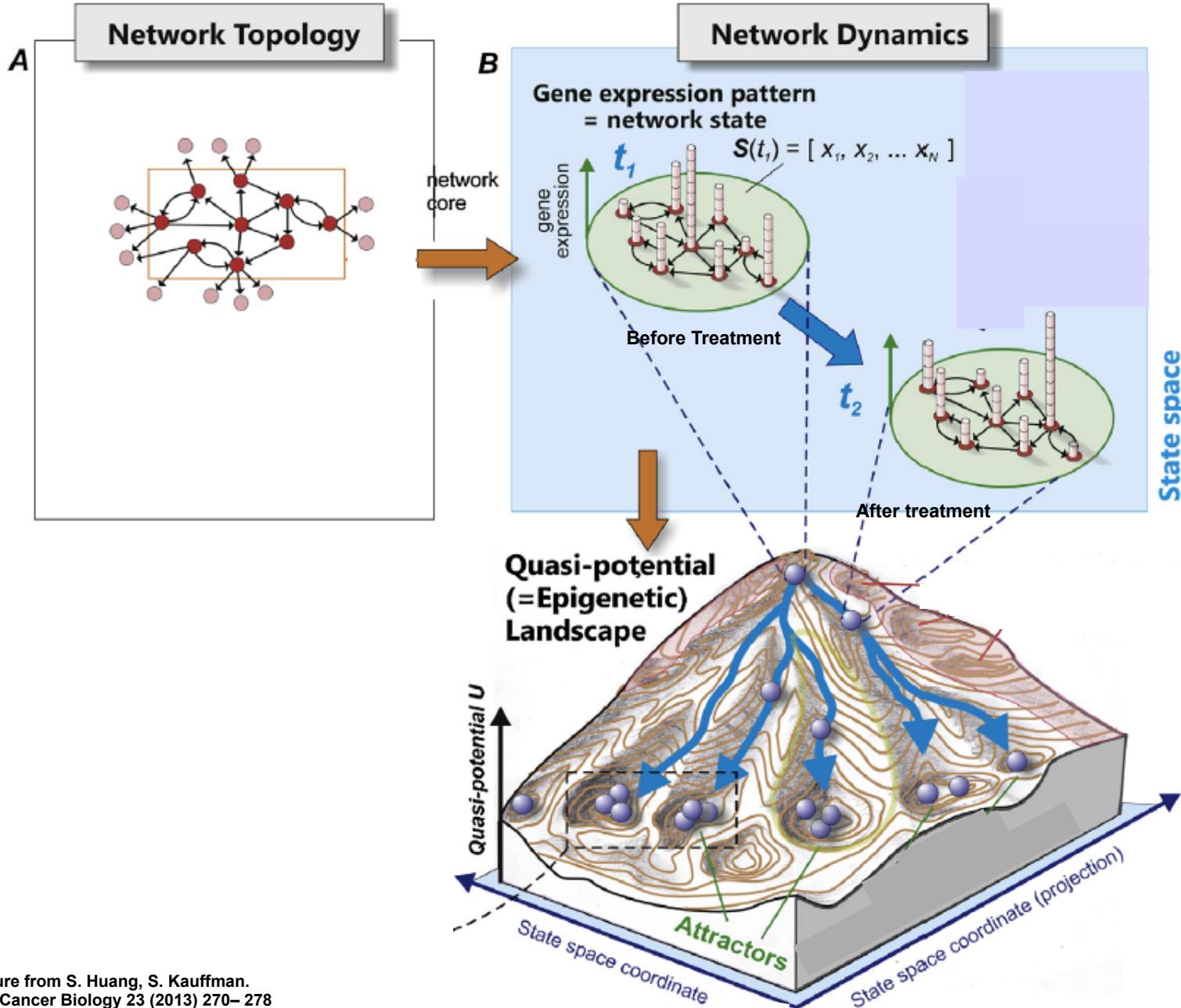
High Throughput Data

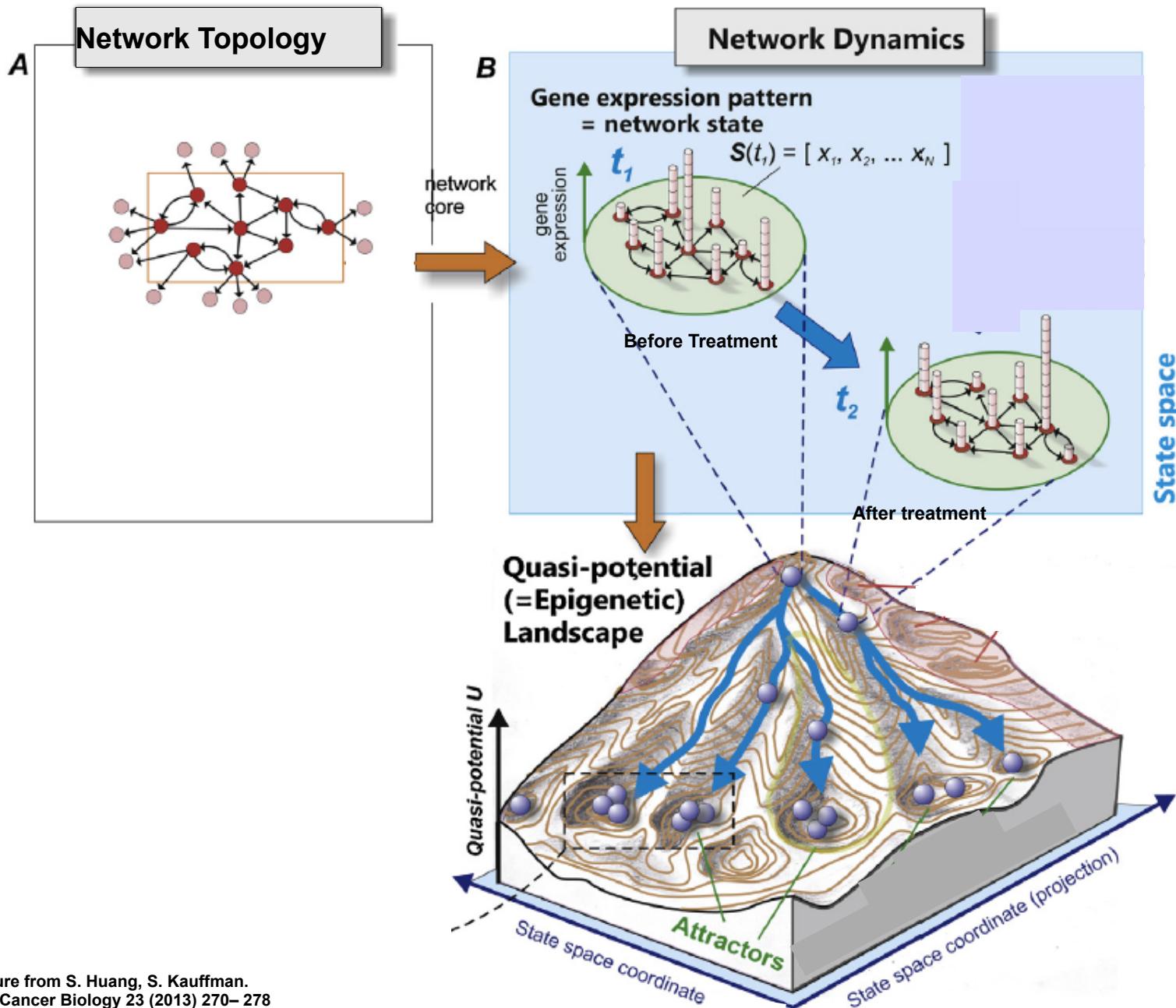


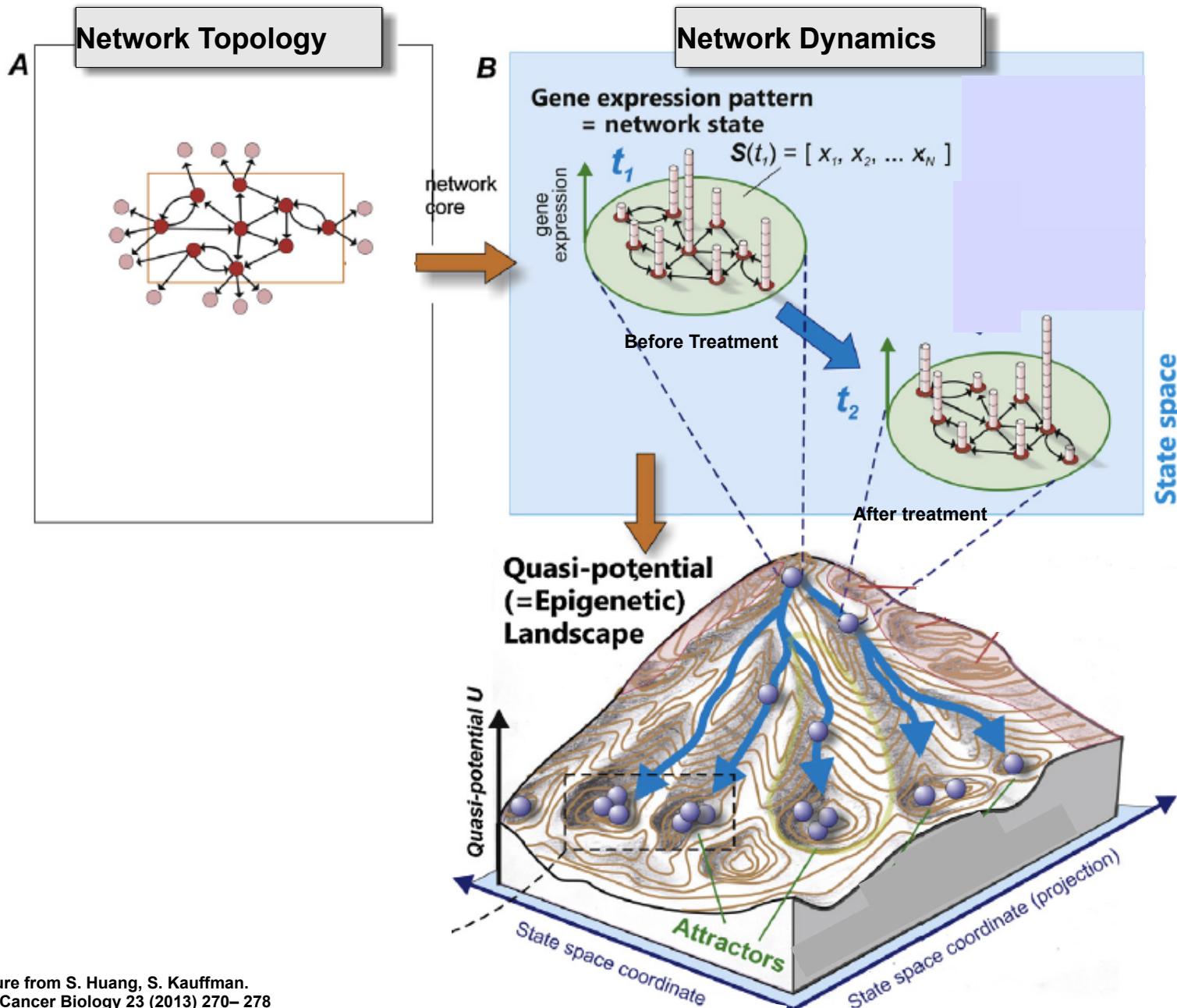
Cellular Complexity

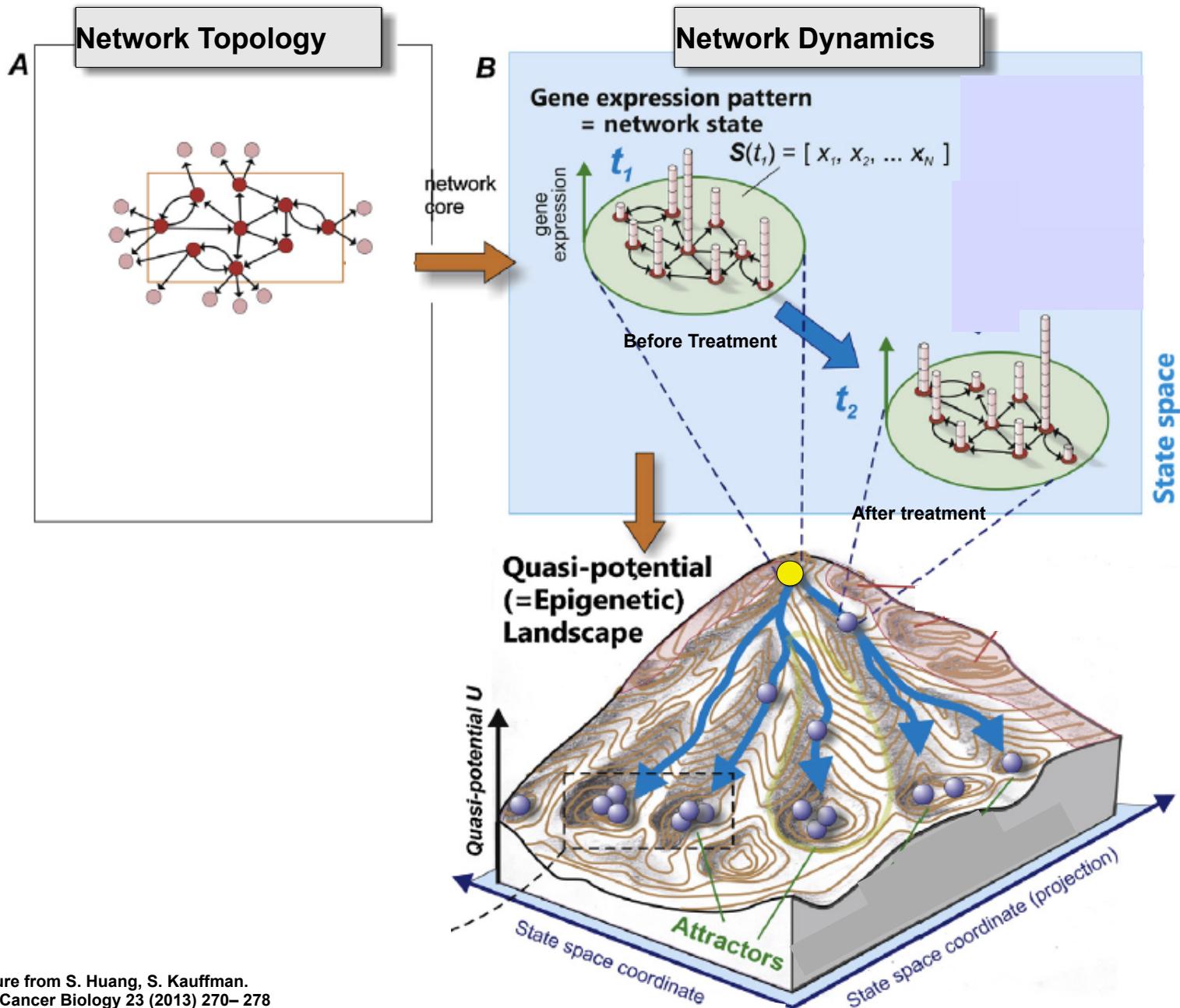


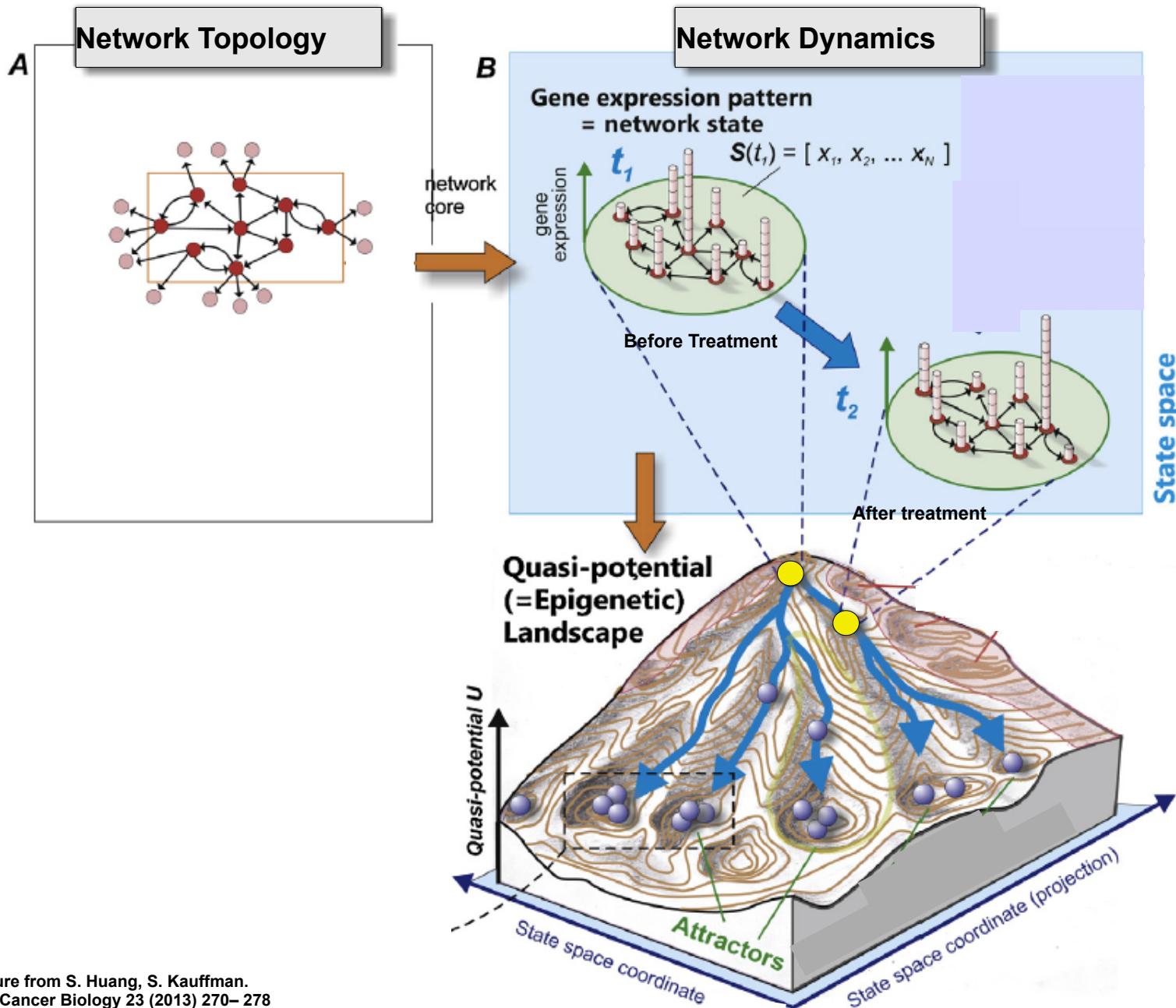
- Integrative bioinformatics
- (Network) modeling
- (Dynamic) modeling

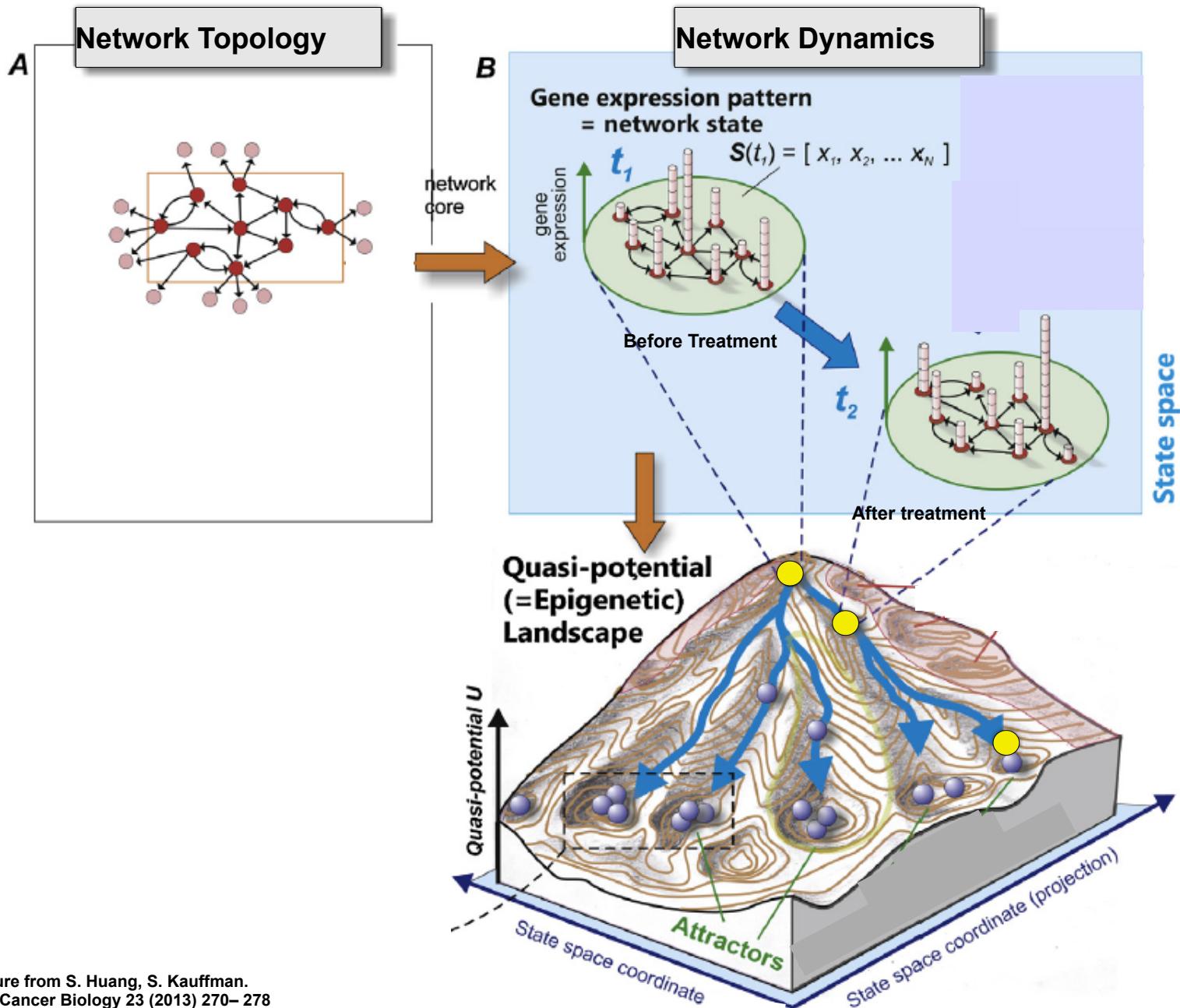


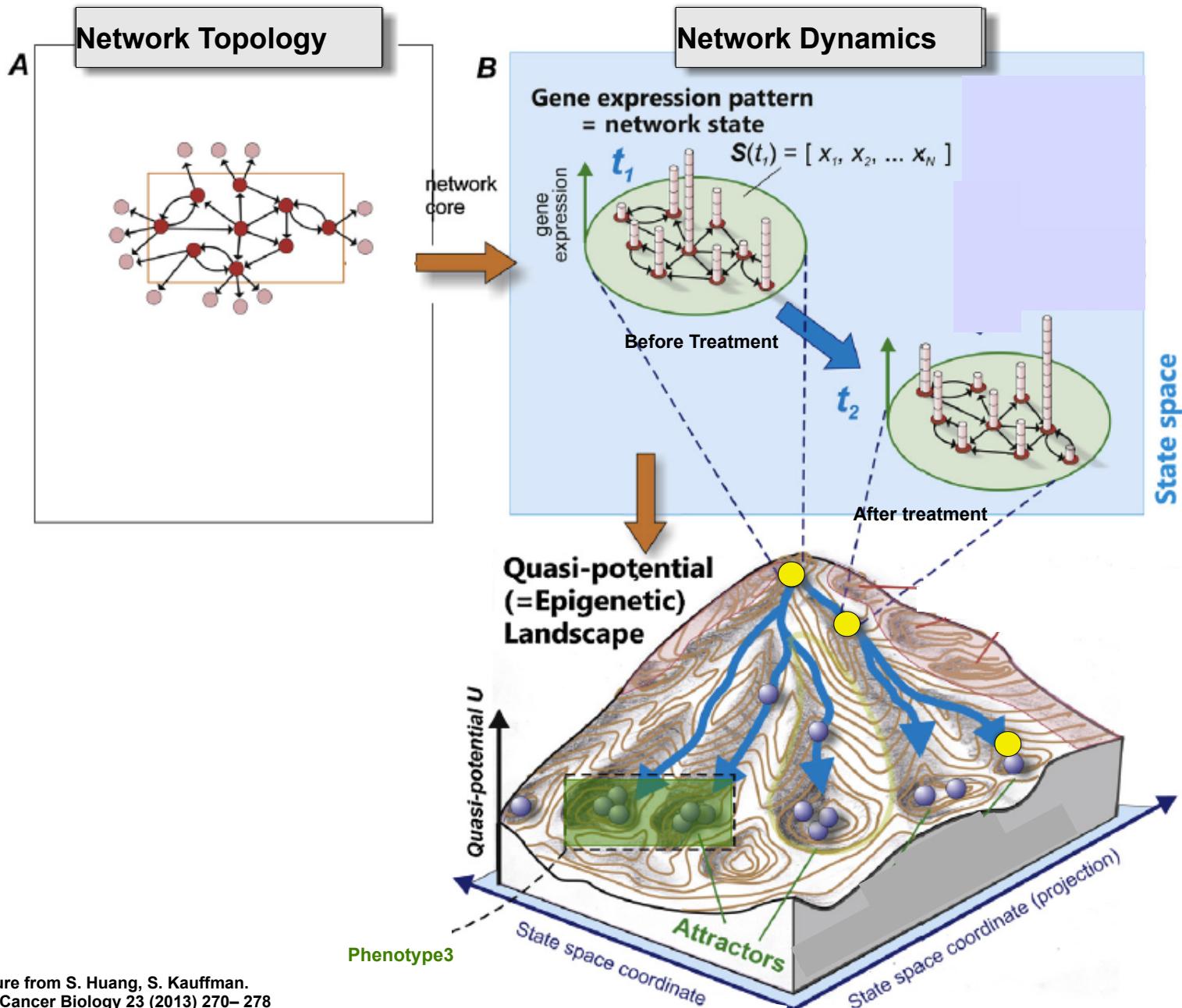


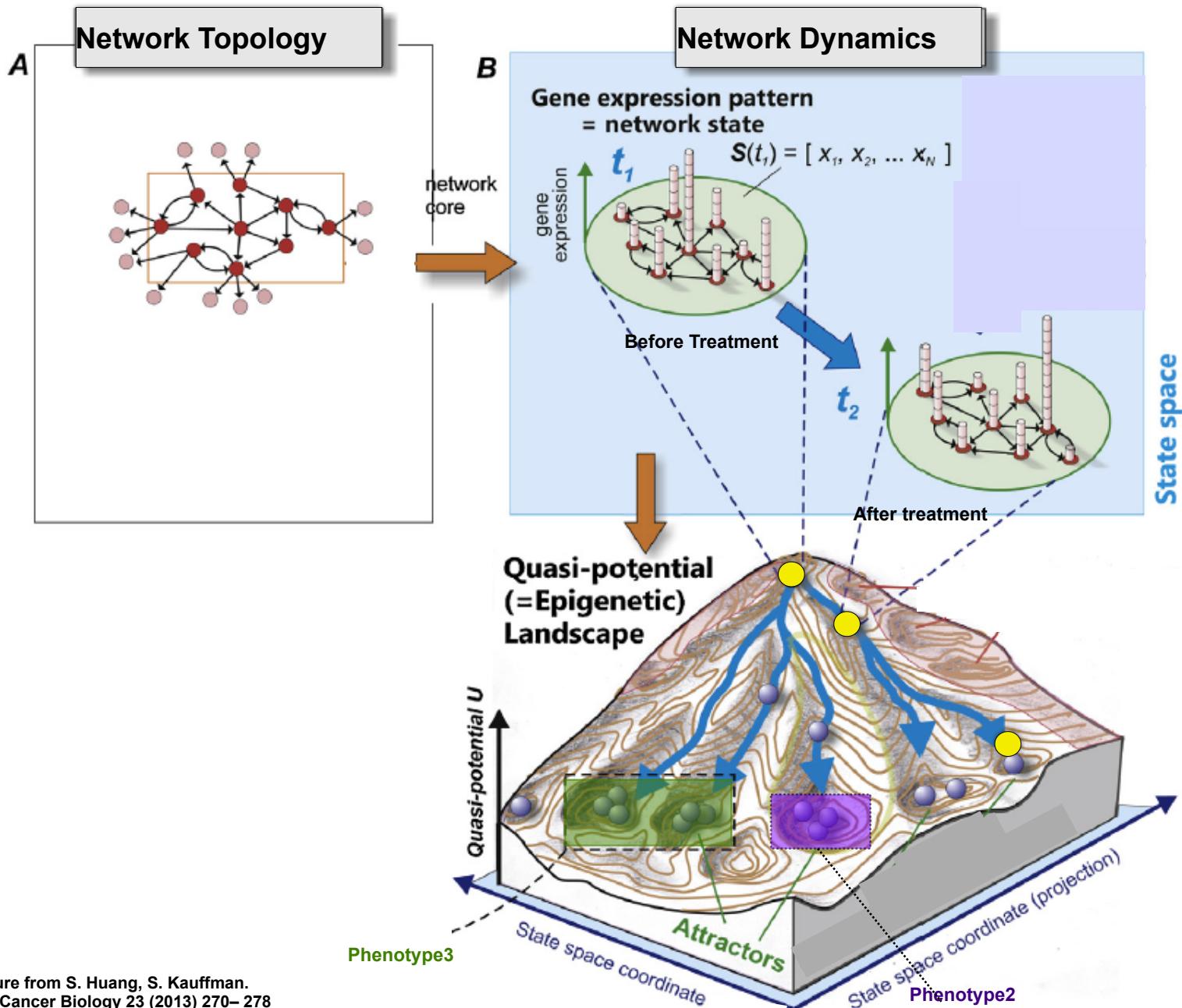


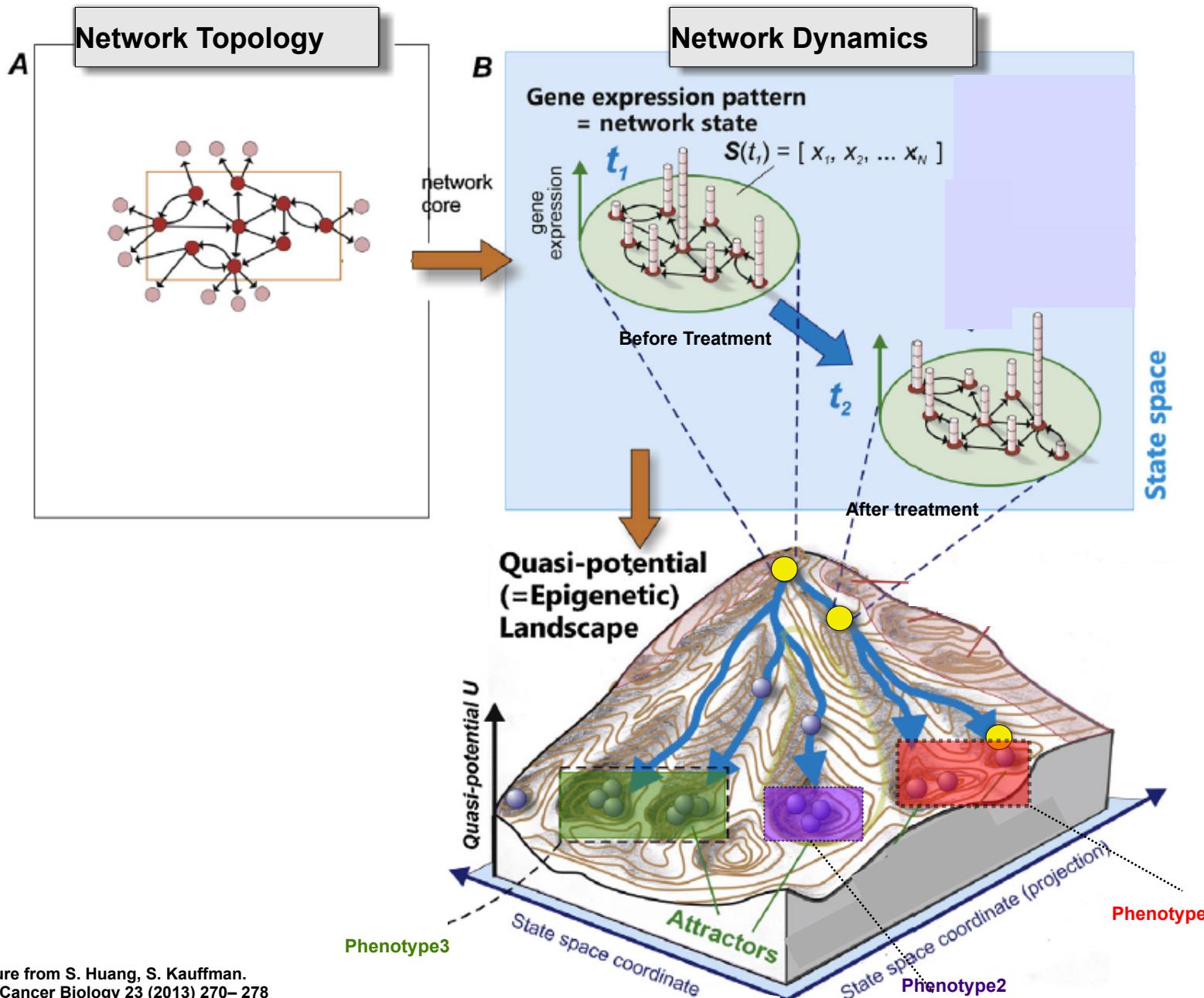












Identification, reconstruction and analysis of disease-related signaling networks from RNA-seq data

Motivation Example: HER2-positive Breast Cancer

- HER2 (also known as ErbB2 or Her2/neu) stands for Human Epidermal Growth Factor Receptor 2.

- Each normal breast cell contains copies of the HER2 gene which encodes the HER2 protein (also called **HER2 receptor**).

- HER2 is a member of the **HER receptor tyrosine kinase family**, which includes three other members: Epidermal growth factor receptor (EGFR or HER1), HER3 and HER4.

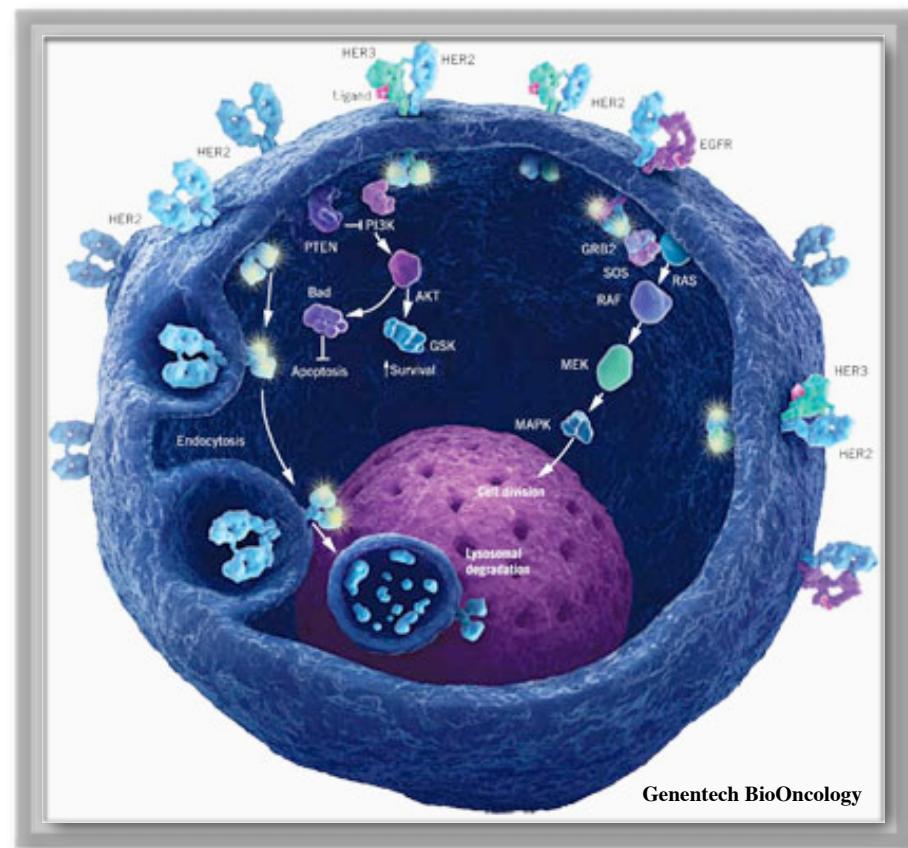
- HER2, the preferred heterodimerization partner of the other HER receptors, does not have a ligand and is **activated** by

- **overexpression** and **homodimerization**, or
- **ligand-mediated stimulation of another HER receptor by heterodimerization**.

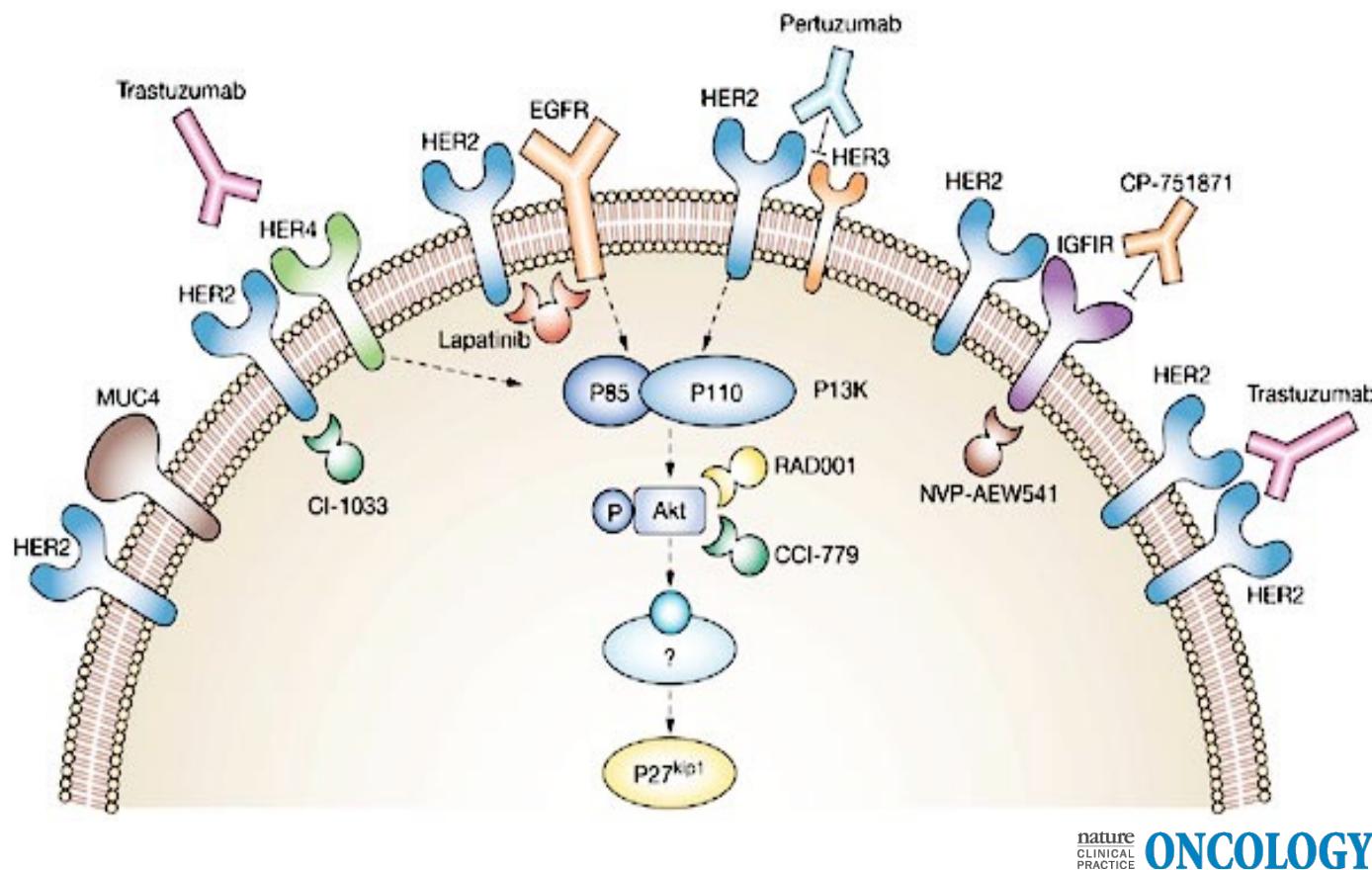
- In normal cells, HER2 protein acts as a receptor for human Epidermal Grow Factor (EGF). When EGF attaches to Her2 receptor, it stimulates the cell to **divide and grow**.

- In **HER2+ breast cancer**, the cancer cells have an abnormally high number of HER2 genes per cell (gene amplification) thus a higher number of HER2 protein on the surface of these cancer cells. This is called HER2 protein over-expression (HER2+).

- **Approximately 25% of breast cancer patients have tumors that are HER2+ . This abnormality in HER2 production can occur in other types of cancer.**



Approved Targeted-Therapies for Her2+ Breast Cancer



- These targeted therapies have shown significant clinical benefit.
- However, a large percentage of patients with advanced HER2+ BC eventually relapse after treatment, suggesting that **tumors acquire or intrinsically possess mechanisms for escape from HER2 inhibition**.

Challenges and Unmet Needs in HER2+ Breast Cancer

- **Redundancy** and **cross-talk** between intracellular signaling pathways are thought to facilitate the development of resistance in most breast cancer patients [1]
- Resistance may be mediated through an **altered interaction between the receptor and antibody**
- **Tumor heterogeneity** [5]
- The use of some of these targeted therapies may also be limited by the development of drug intolerance.

To overcome these issues, several strategies are proposed such as the identification of additional targets [2], enhancement the effector functions of MAbs [3] and combination therapies [4].

[1] Cauglin et al. Breast Cancer Res Treat, 124 (1) (2010), pp. 1–11

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With the use of high-throughput data, we propose a Systems Biology to identify a comprehensive signal transduction network representative for Her2+ Human Breast Cancer and propose combinations of therapeutic interventions to overcome some of these resistance mechanisms.

Signal Transduction Network Analysis for the Identification of Combinations of Therapeutic Combinations

The pipeline of network analysis consists of 4 main steps:

4. Construction of **combinations of interventions** with OCSANA and identification of optimal therapeutic interventions.



3. **Identification of a Core Signal Transduction Network of Dysregulated Pathways in HER2+ BC.** Assembling of a **comprehensive network** for the set of functionally enriched genes, their transcription factor and master regulators.



2. Identification of **transcription factors** and **master regulators** responsible for the expression patterns observed in the functionally enriched gene set identified on step 1.



1. **Protein-protein interaction** (PPI) network analysis for the identification of Functionally Enriched Gene Set in HER2+ BC.

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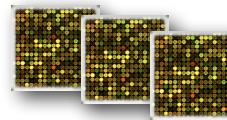


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Stage I
Functionally
enriched set of
genes



BiNoM

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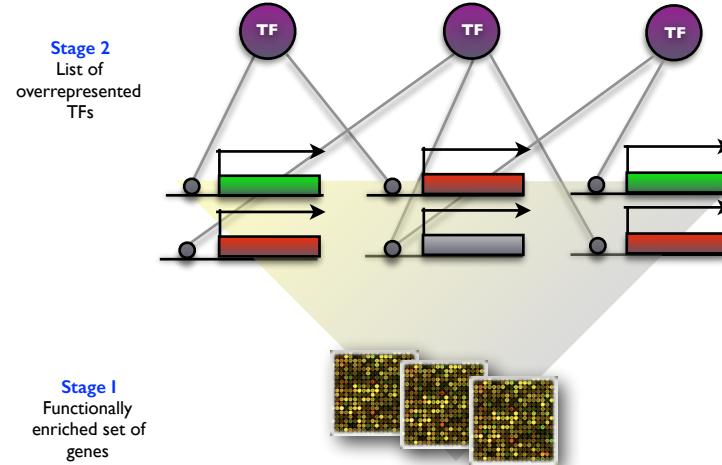
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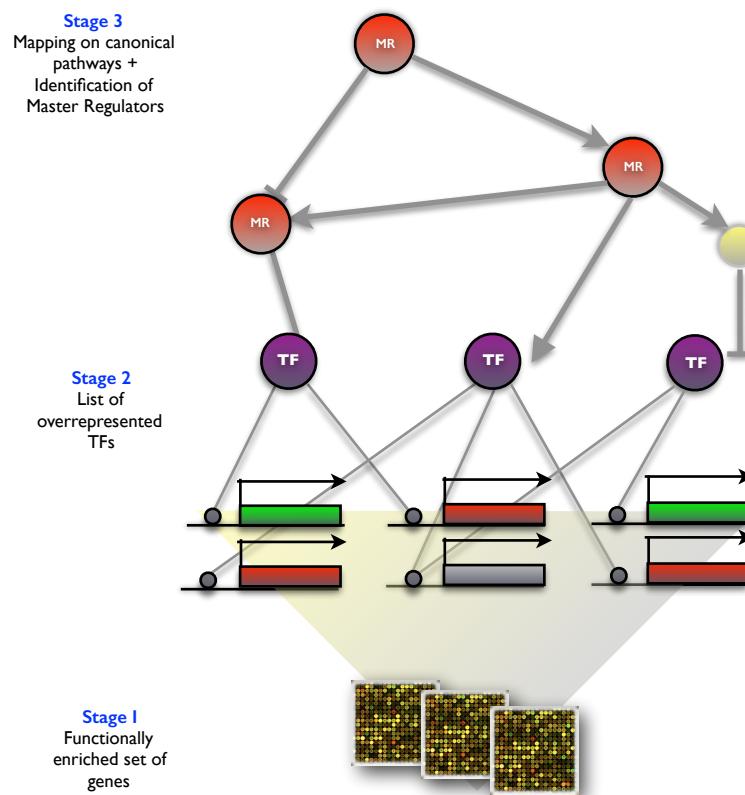
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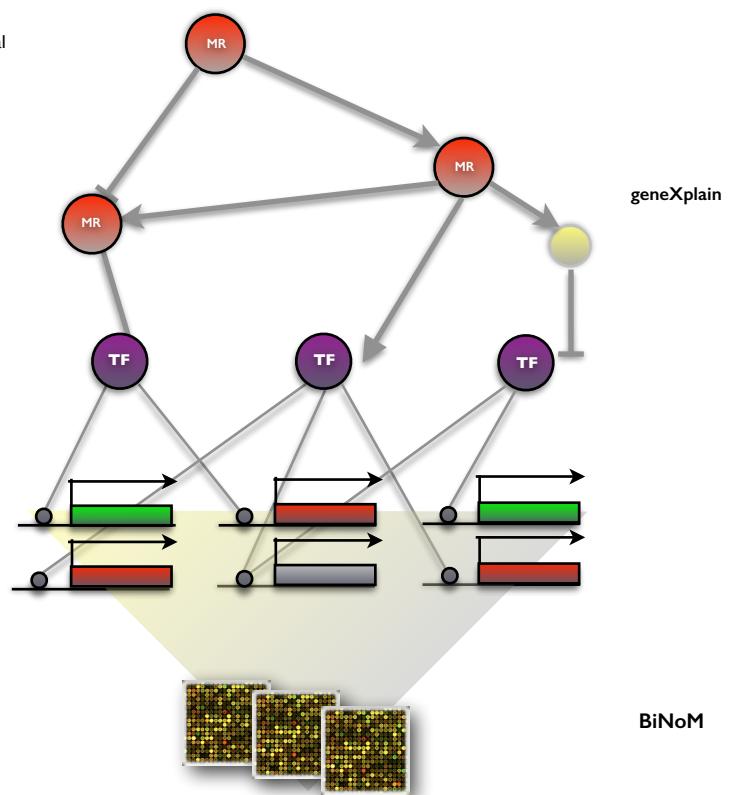


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Stage 3
Mapping on canonical pathways +
Identification of Master Regulators

Stage 2
List of
overrepresented
TFs

Stage 1
Functionally
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genes



BiNoM

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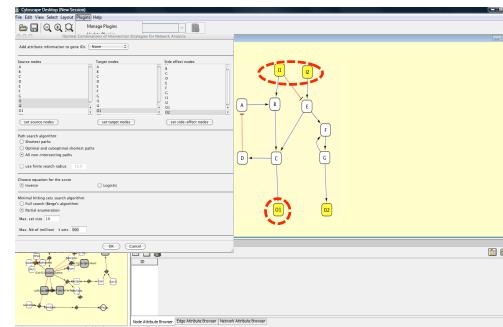


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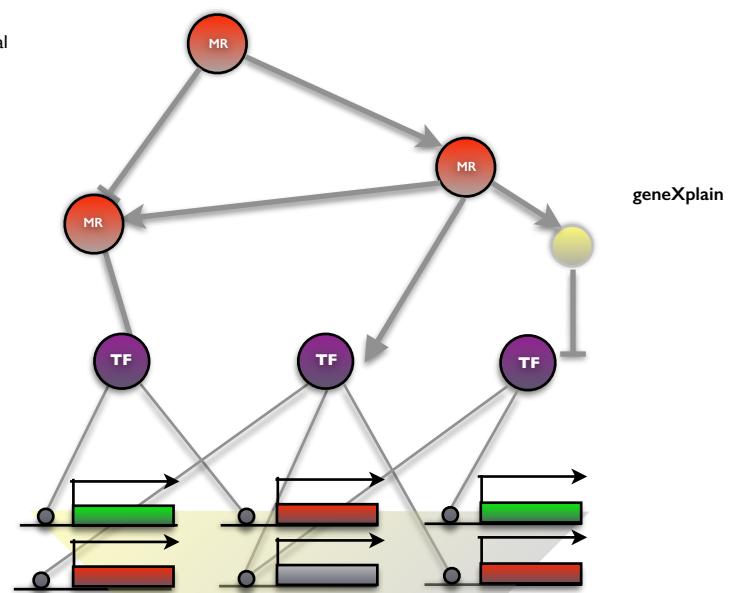
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Stage 4
Identification of Optimal Combinations of Interventions from Network Analysis



OCSANA

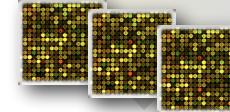
Stage 3
Mapping on canonical pathways + Identification of Master Regulators



geneXplain

Stage 2
List of overrepresented TFs

Stage 1
Functionally enriched set of genes

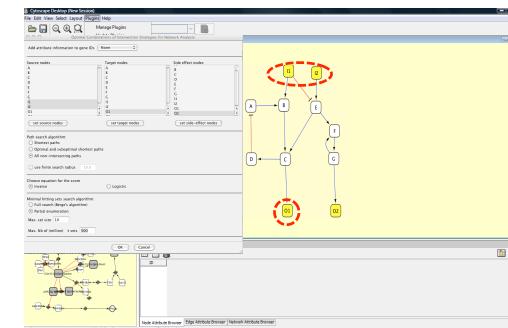


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Signal Transduction Network Analysis for the Identification of Combinations of Therapeutic Combinations

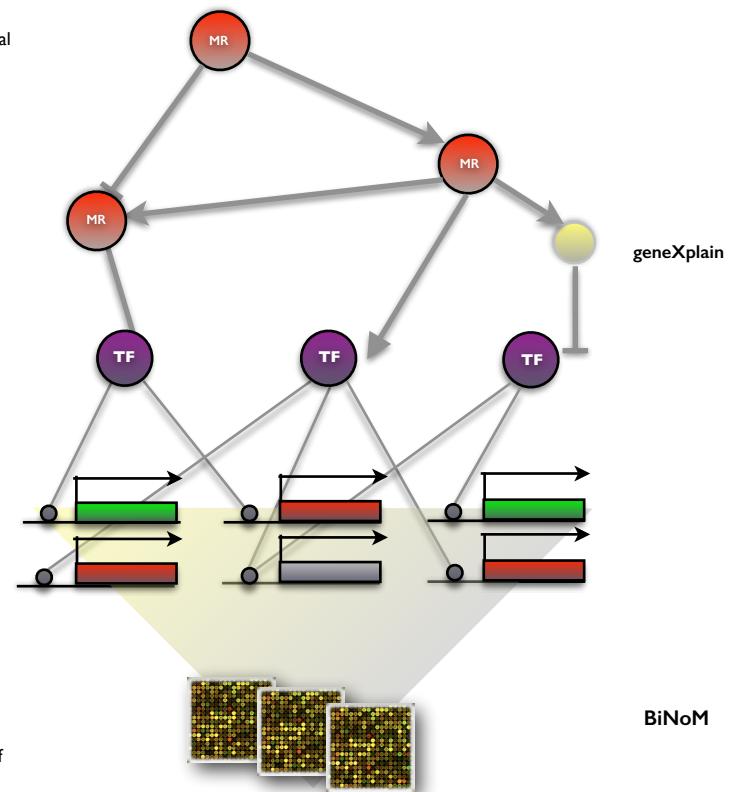
Internal Control: Verify that HER2 is a top master regulator for HER2+ Breast Cancer

Stage 4
Identification of
Optimal
Combinations of
Interventions from
Network Analysis



OCSANA

Stage 3
Mapping on canonical
pathways +
Identification of
Master Regulators



Stage 1
Functionally
enriched set of
genes

BiNoM

Stage I : Identification of Functionally Enriched Module of Genes

From our data we are able to generate a ranked list of genes based on their differentially expression. We want to

- 1) Detect the **optimal number of genes that will act as a disease biomarker**
- 2) Detect the **functional “core”** at the top of the ranked list of genes

Considering biological properties of the genes and their functional relationships.

We considered the work introduced in

Kairov U, Karpenyuk T, Ramanculov E, Zinovyev A. Network analysis of genes lists for finding reproducible prognostic breast cancer gene signatures. *Bioinformation* 2012; **8**(16).

to generate three possible Enriched Networks for HER2+ breast cancer as a seed to our pipeline.

Stage I : Identification of Functionally Enriched Networks

How are Functional Modules Identified?

Input:

- 1) HPRD database of PPI (9856 proteins and 49504 interactions)
- 2) Top ranked DEGs between Her2 and Normal samples

Output: Functional Module of proteins

The genes from the DEGs list were identified in HRPD and direct protein-protein interactions connecting such DEGs were extracted.

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Two main functional modules can be extracted:

1. Optimal Functionally Enriched Network (OFEN): Selected from the largest connected component of DEGs (Seed Nodes)

Connectivity of proteins inside the extracted component was compared to the connectivity of the same genes in the global PPI network (to estimate specificity of the connectivity degree).

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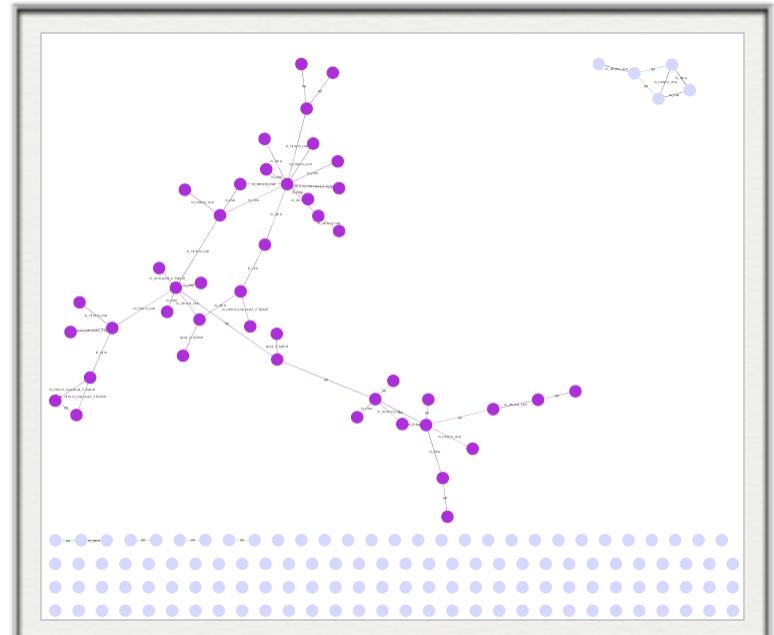
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OFten Module for HER2+ Breast Cancer vs Normal Tissue Sample

300 DEGs between HER2+ BC and Normal Samples
44 genes are connected by PPI in the largest connected component

Stage I : Identification of Functionally Enriched Networks

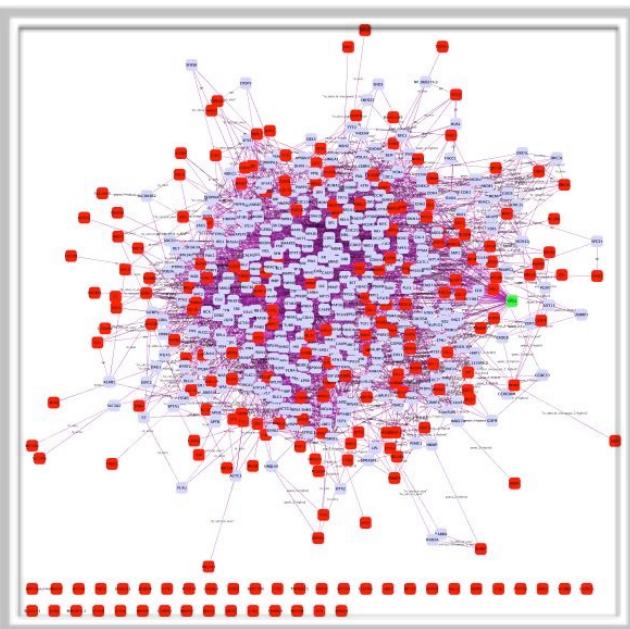
How are Functional Modules Identified? (Continuation)

2. Second Order Connectivity (SOC) Modules: Seed Nodes together with nodes that are not differentially expressed but play an important role in connecting seed nodes in the network (Connector Nodes)

Stage I : Identification of Functionally Enriched Networks

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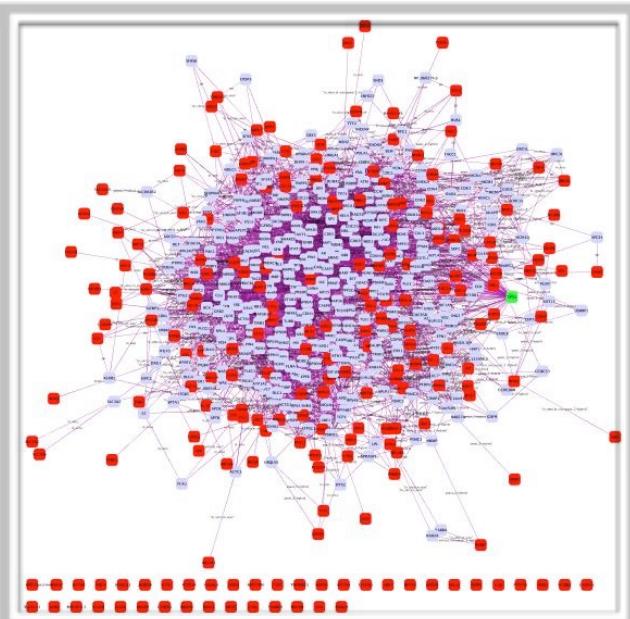
SOC Module for Her2 vs Normal Tissue Sample

300 DEGs between HER2+ and Normal Samples
773 genes a large connected component

Stage I : Identification of Functionally Enriched Networks

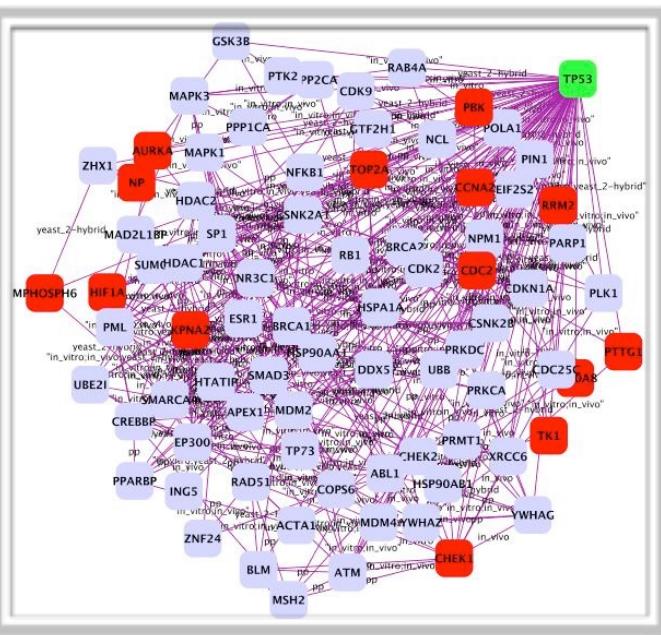
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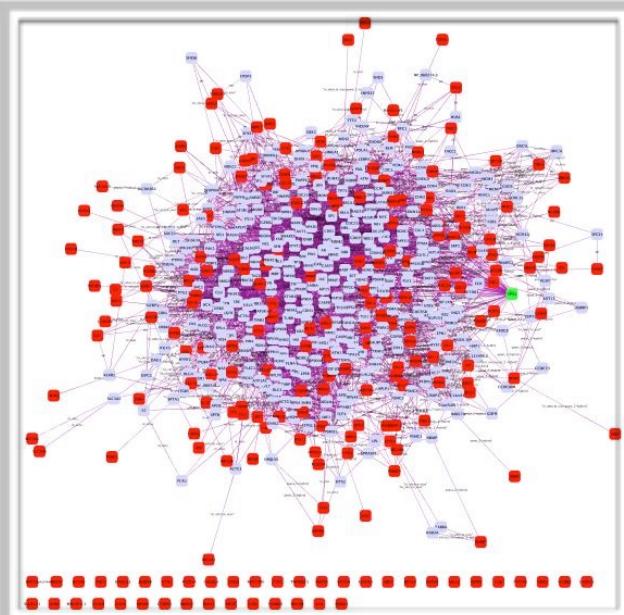
Example TP53 Connector Node

In green we have Connector Node TP53. In red are the DEGs that are connected through TP53.

Stage I : Identification of Functionally Enriched Networks

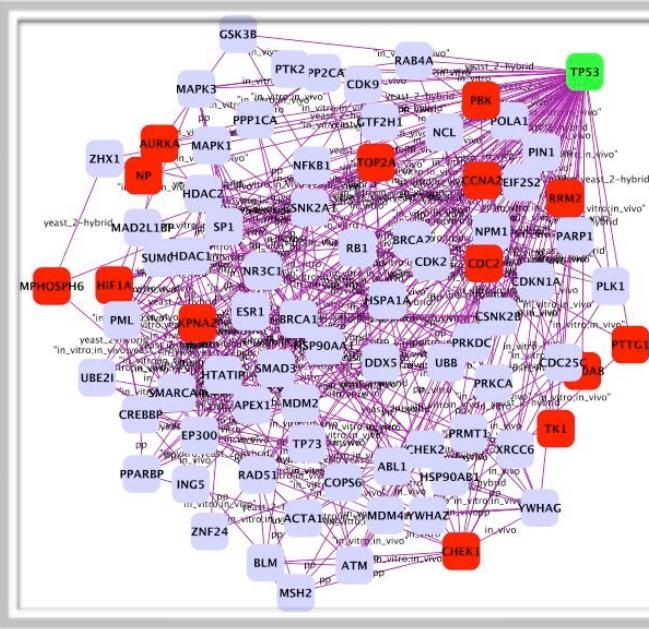
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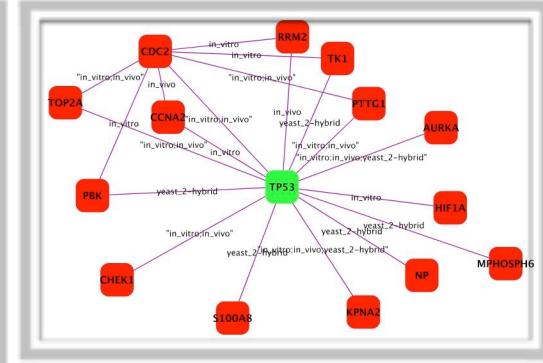


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Example TP53 Connector Node
In green we have Connector Node TP53. In red are the DEGs that are connected through TP53.



Example TP53 Connector Node

For a better appreciation of the relevance of connector, we show here the isolated TP53 subnetwork together with the DEGs connected.

Stages II and III: Identification of Transcription Factors and Master Regulators in for Her2+ Human Breast Cancer

File View Data Analyze Network search result Help

No running process
Ready: FA (+ 35 more)
[Advanced]

Tree Search Select >

HER2 x

FOC_300DEGs:Sheet1 [44,21,79,41] x

F-match MyNewProfile_HER2_Optimizedh0.0

+ Os 1mod 1gr 6-10p [0.802] x

S81 [104,71,212,137] x

S82 (S81&S56 (Her2_mean > 4)) [74, x]

S83 (S81\ResultDifferentialExpressionB x)

S84_TFsMATCH_FOC300 [92,67,187]

Proteome BKL Disease View 0.05n

S86_AllTFs300CMA&MATCH [95,7]

Transpath molecule classification

Up Dis3 FDR KN [408] x

Up Dis4 FDR KN [287] x

Up Dis5 FDR KN [280] x

Up Dis6 FDR KN [276] x

Up Dis7 FDR KN [278] x

Up Dis8 FDR KN [269] x

Up Dis9 FDR KN [263] x

Up Dist10 FDR KN [284] x

GO annotation, BKL manual curation 0.05ma

Molecular Function 0.05max 2min on 'FOC_3

ProcessBiological 0.05max 2min on 'FOC_30

Proteome BKL Disease View 0.05max 2min o

[+] Network keynodes: Up Dis6 FDR KN

Filter (clear | filter bar): Z-Score > 1, FDR < 0.05 (107 of 276 rows) Rows per page: 500

Export: Plain text | XLS | RTF

Mark: All (107) | None | Invert

| | Molecule name | Molecule classification | #Hits in network | Hits list | Distance | #Non-relevant reachable node | |
|----------|---------------|---|------------------|---|----------|------------------------------|--|
| Filter » | (none) | (none) | (none) | (none) | (none) | (none) | |
| □ (*) | ErbB2 | enzymes; transferases EC 2; transferases EC 2.7; phosphotransferases EC 2.7.1; protein kinases; protein tyrosine kinases; RTK; EGFR; EGFR(h); ErbB2(h) enzymes; transferases EC 2; transferases EC 2.7; phosphotransferases EC 2.7.1; protein kinases; protein tyrosine kinases; RTK; EGFR; ErbB2; ErbB2(h) membrane-transducing components; receptors; RTK; EGFR; EGFR(h); ErbB2(h) (main classification) membrane-transducing components; receptors; RTK; EGFR; ErbB2; ErbB2(h) (main classification) proto-oncogene; ErbB2; ErbB2(h) | 61 | ATF-1, ATF-4, B-Myb, BCL-6, c-Ets-1, c-Ets-2, c-Fos, c-Jun, c-Myb-isoform1, CAR, CDP-isoform1, CREB, Delta40p53, deltaCREB, DeltaNp63alpha, DeltaNp73alpha, E2F-1, E2F-2, E2F-3a, E2F-5, Elk1-isoform1, HSF1-L, IRF-3, IRF-7A, LEF-1B, LEF1-isoform1, LEF1-isoform3, MEF-2C, MEF-2C/delta8, MEF2A-isoform1, Net, NR1B1-isoform1, NR1B2-isoform2, p53-isoform1, p53beta, p63alpha, p63gamma, p73alpha, p73beta, p73delta, p73gamma, pRB, RXR-alpha, RXR-beta, SAP-1a, SAP-1b, SREBP-1a, SREBP-1c, SREBP-2, STAT1alpha, ... | 4 | 411 | |
| | | | | ATF-1, ATF-4, BCL-6, c-Ets-1, c-Ets-2, c-Fos, c-Jun, c-Myb-isoform1, CAR, CDP-isoform1, CREB, Delta40p53, deltaCREB, DeltaNp63alpha, DeltaNp73alpha, E2F-1, E2F-2, E2F-3a, E2F-5, Elk1-isoform1, HSF1-L, IRF-3, IRF-7A, LEF-1B, LEF1-isoform1, LEF1-isoform3, MEF-2C, MEF-2C/delta8, MEF2A-isoform1, Net, NR1B1-isoform1, NR1B2-isoform2, p53-isoform1, p53beta, p63alpha, p63gamma, p73alpha, p73beta, p73delta, p73gamma, pRB, RXR-alpha, RXR-beta, SAP-1a, SAP-1b, SREBP-1a, SREBP-1c, SREBP-2, STAT1alpha, ... | | | |

Stages II and III: Identification of Transcription Factors and Master Regulators in for Her2+ Human Breast Cancer

- We sort the upstream regulators that we have identified, according to their z-scores
- Using HER2 (ERBB2) as an internal control, we compare the different methods for selection of functional modules by observing the ranking position assigned to HER2, according to z-score
- Since HER2 is a receptor, we perform as well a comparative study focusing specifically on all the **receptor master regulators** to identify another possible molecular players in Her2+ breast cancer and to generate hypothesis on their combined therapeutic effect.

Below is the table with the comparative results. Smaller ranking position reflect a better method to identify functional modules:

| Method for the Identification of Functional Module | Ranking Position of HER2 Comparison with all upstream regulators | Ranking Position of HER2 Comparison Among <u>Receptor</u> Upstream Regulators |
|--|--|---|
| First Order Connectivity Module from PPI Network | 7 | 2 |
| Second Order Connectivity Module from PPI Network | 72 | 12 |
| Highest-t-test-ranked DEGs | 71 | 28 |

Stages II and III: Identification of Transcription Factors and Master Regulators in for Her2+ Human Breast Cancer

- ♦ We sort the master regulators according to their significance scores and the number of TFs that they regulate
- ♦ **Using HER2 (ERBB2) as an internal control, we compare the different methods for selection of functionally enriched sets of genes by observing the ranking position assigned to HER2, according to their significance score**
- ♦ Based on the ranking of HER2 in the list of Master Regulators (MRs), we selected the OFTEN set of genes at distance 4
 - ⦿ HER2 top MR according to ExPlain score (Master regulator of largest coverage of TFs downstream in the shortest possible distance)
 - ⦿ HER2 covers more than 60% of TFs downstream coverage
 - ⦿ List of MRs including other elements of the ErbB family

Stage III: Assembling a Comprehensive Signaling Pathway Network for HER2+ BC

Now that we have identified a list of master regulators we proceed to assemble a comprehensive signal transduction network for HER2+.

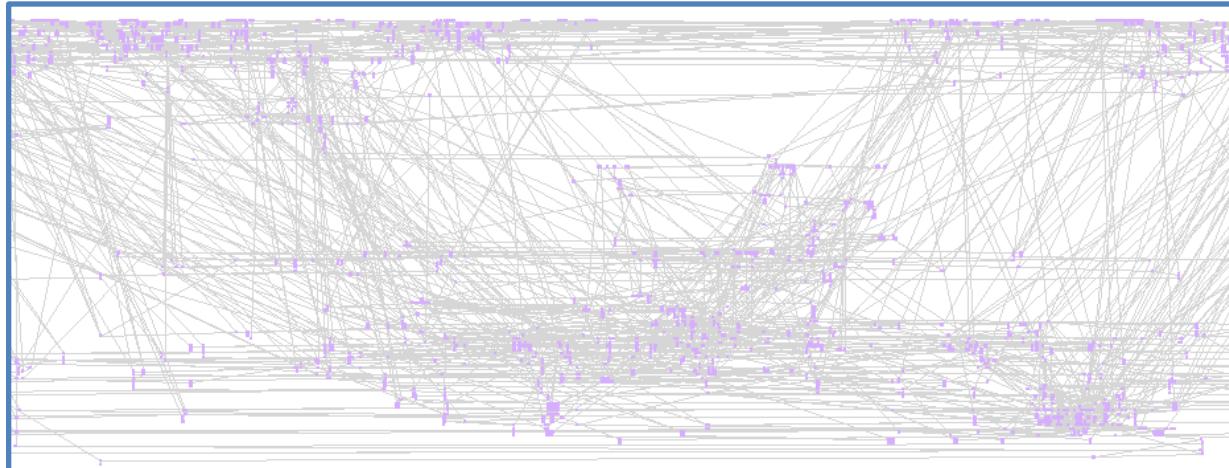
The Core Signaling Network for HER2+ BC: From ExPlain we retrieve the signaling network that contains the OFTEN seed genes, the identified TFs, intermediate molecules and the identified MRs. This network contains **791 nodes** and **2978 edges**.

To introduce all known redundancy and cross-talk in this signaling network, we consider:

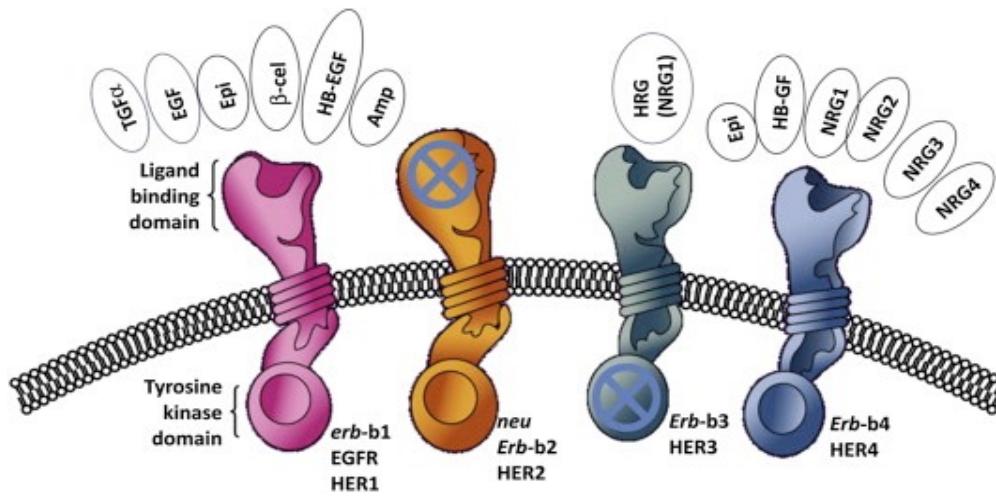
Canonical Pathways: Using TRANSPATH we retrieve all the canonical pathways known that join all the aforementioned players (a total of 71 canonical pathways). This network contains **942 nodes** and **1168 edges**.

In BiNoM we proceed to merge these two networks to one comprehensive map. This **Comprehensive Network** consists of **1495 genes** and **3841 edges**.

NOTICE: In each one of the aforementioned networks we verify that only those nodes corresponding to genes are **expressed in our HER2+ BC tissues**.

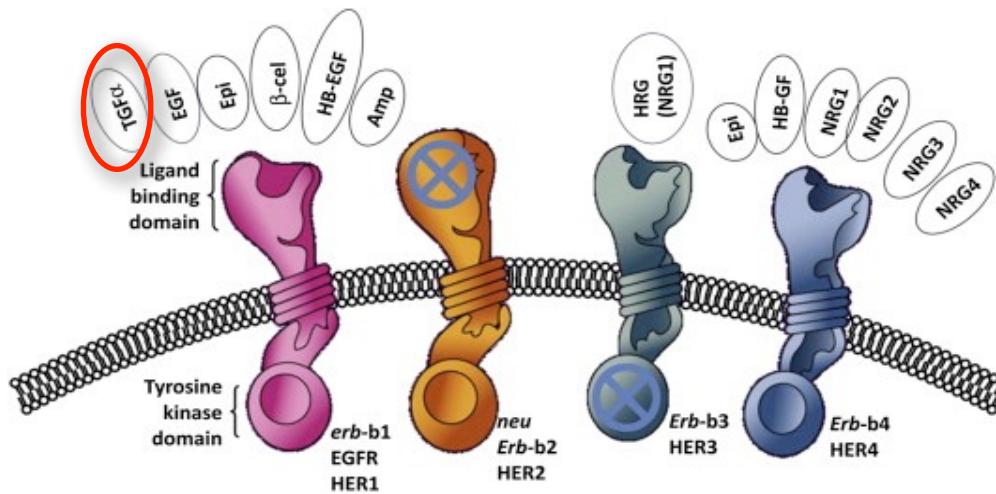


Family of HER receptors and associated activating ligands.



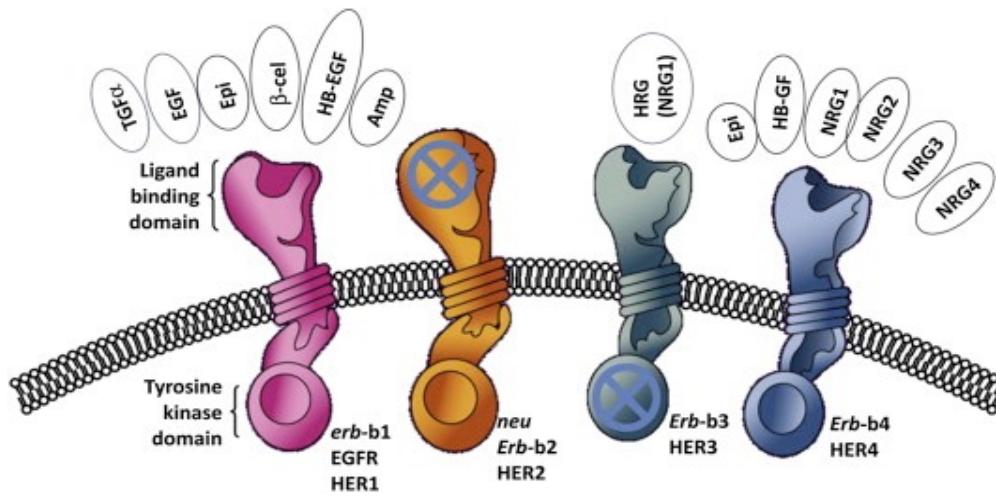
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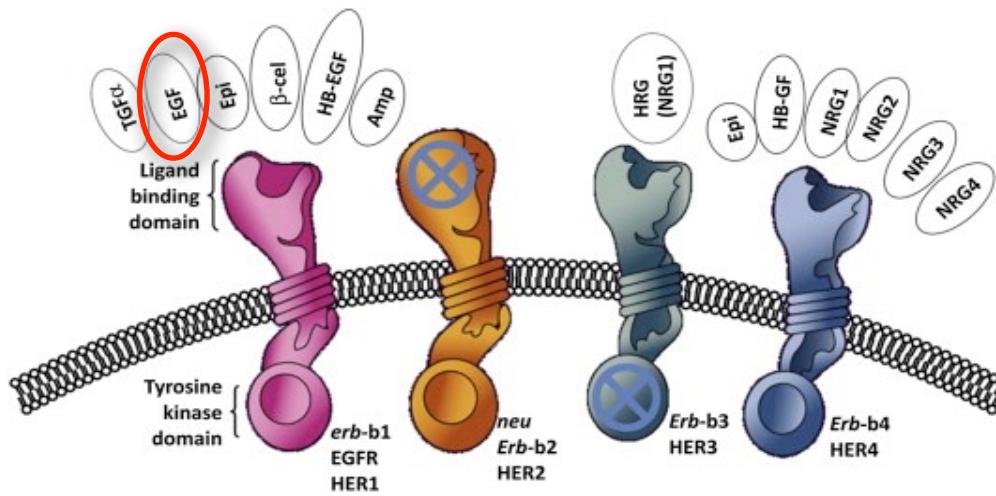
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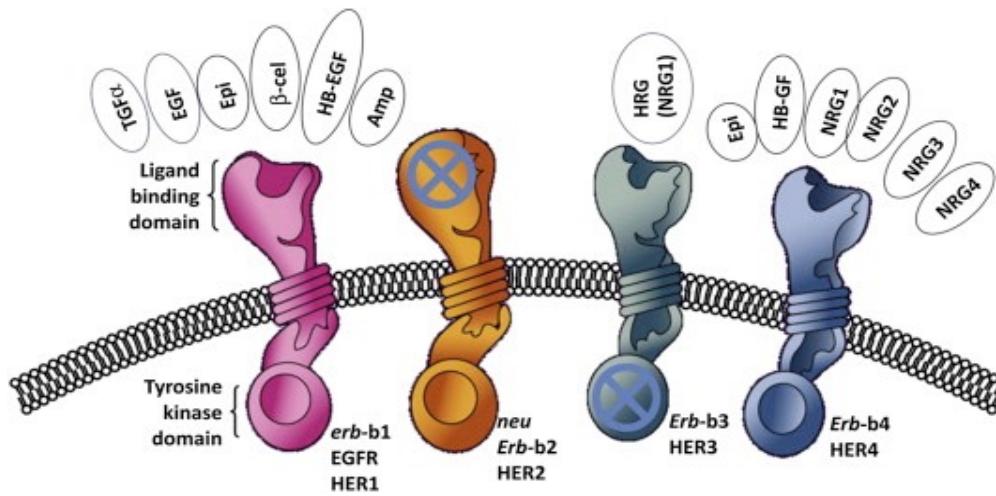
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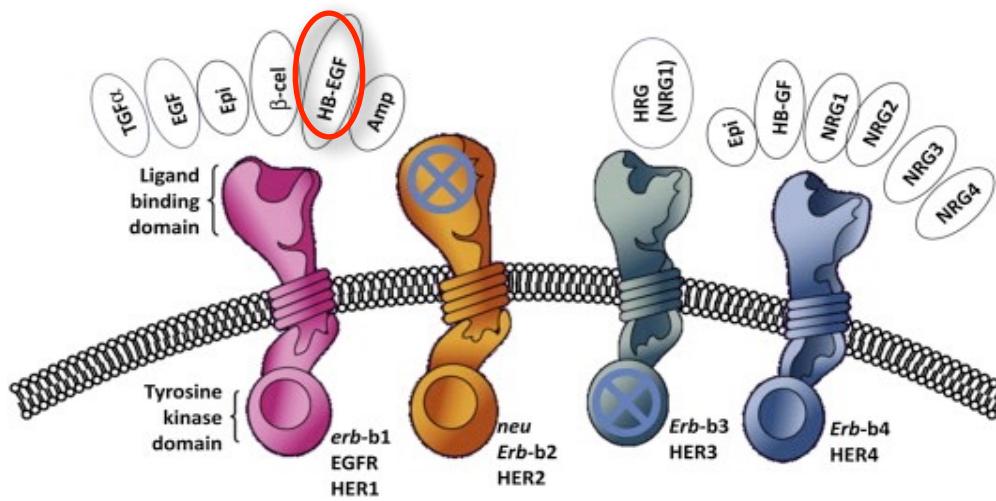
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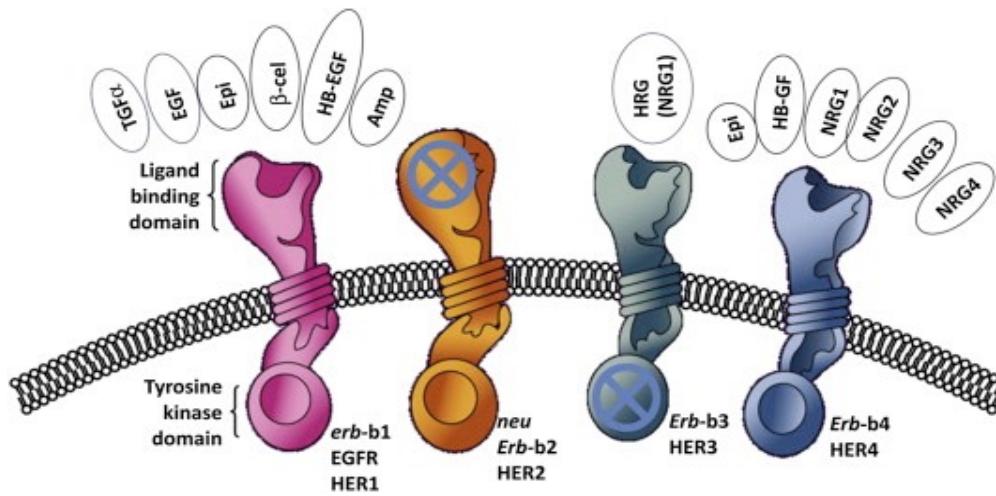
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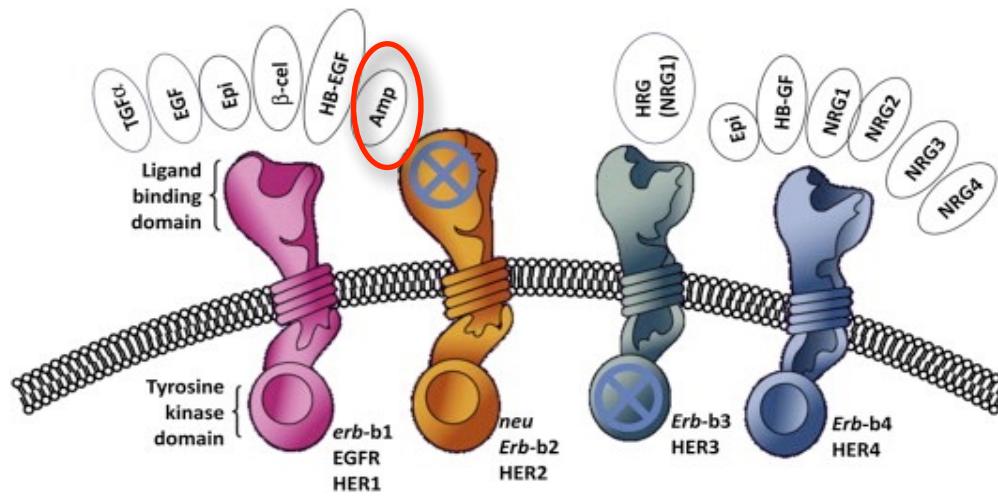
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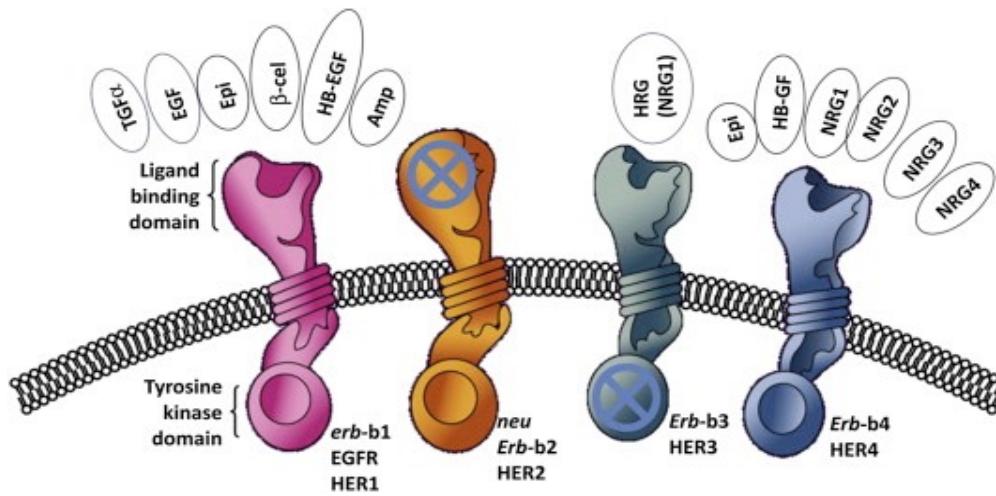
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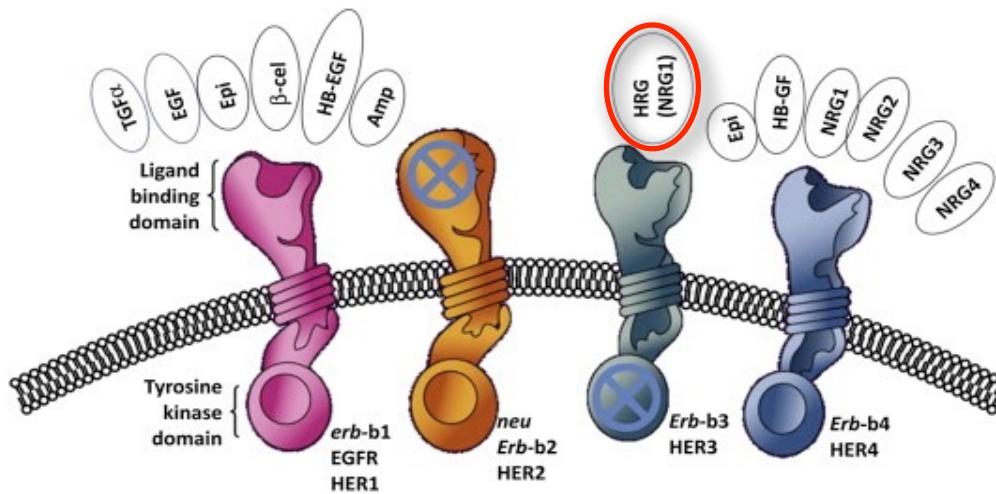
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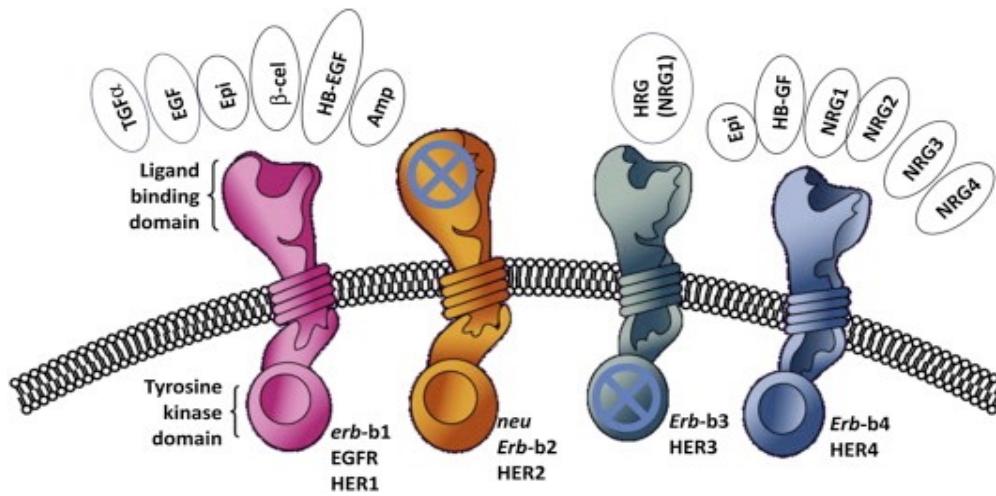
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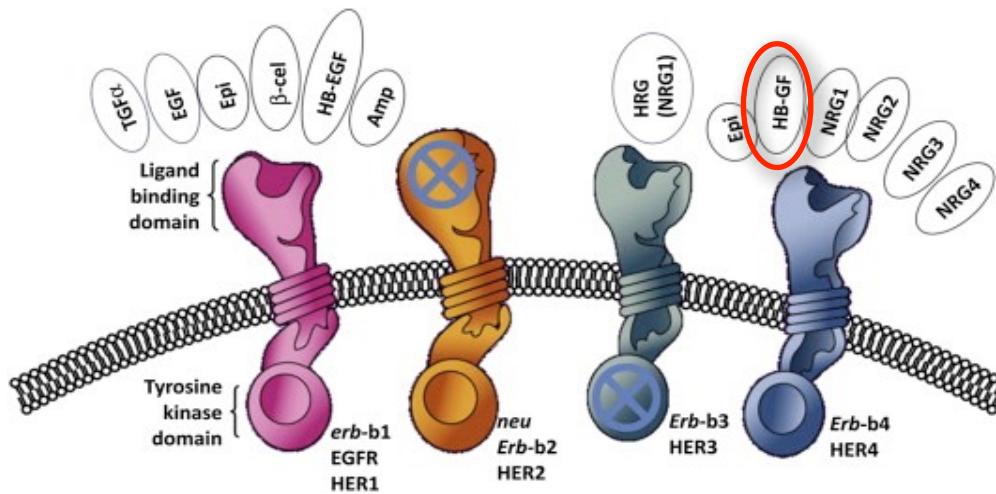
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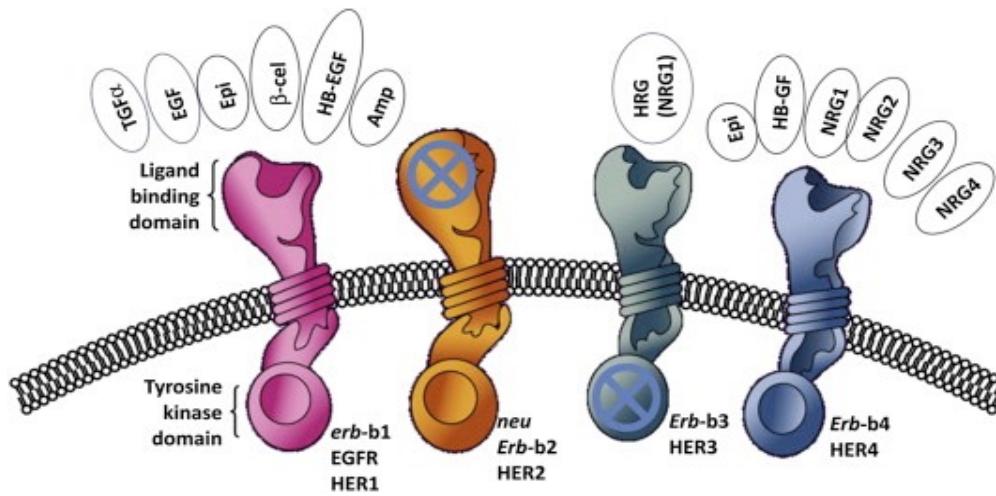
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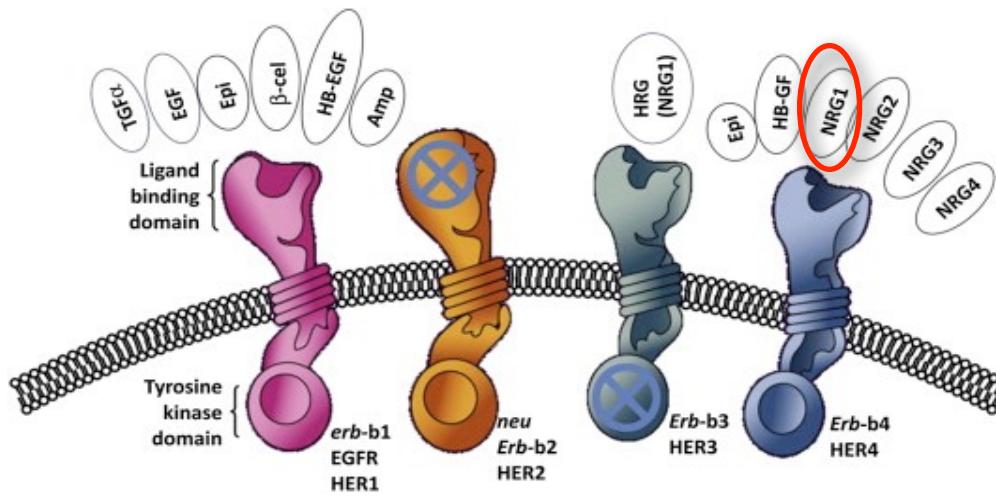
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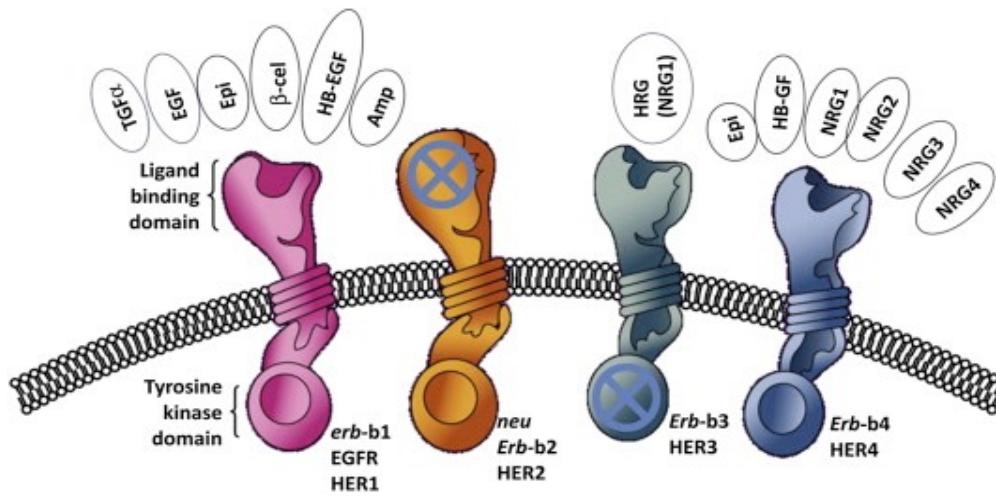
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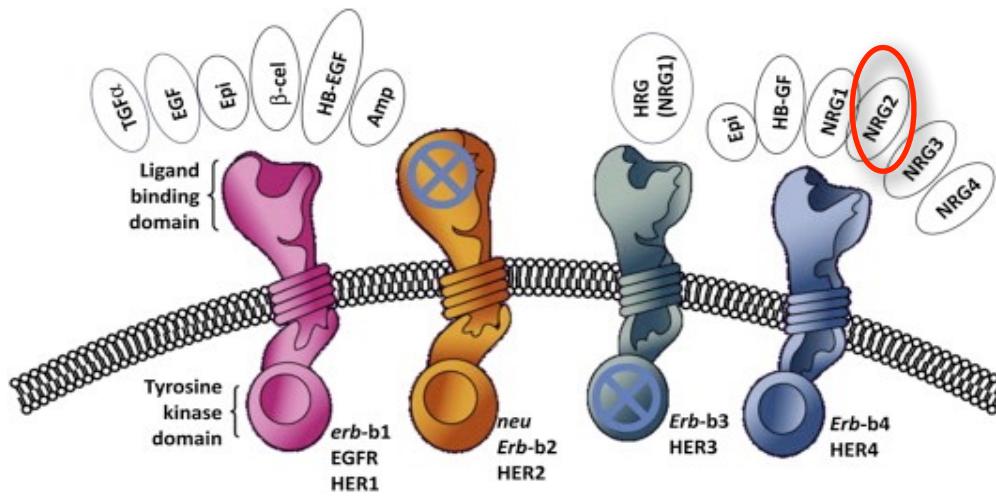
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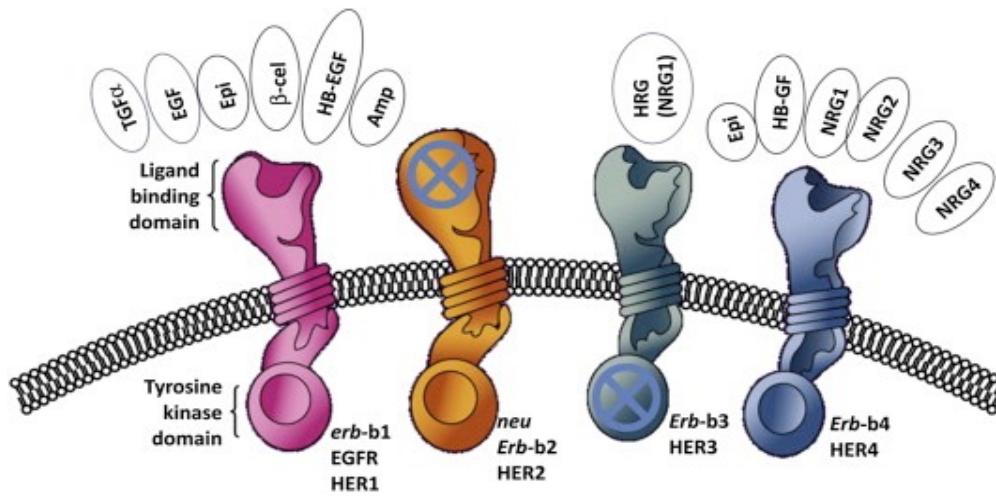
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Identification of Transcription Factors and Master Regulators in for Her2+ Human Breast Cancer

First top ranked upstream regulator (receptors-related list):

FCGR2B

- Fc fragment of IgG, low affinity IIb, receptor (CD32)
- Involved in phagocytosis
- Negatively regulates phagocytosis
- **Extracellular effect (immune response)**

Second top ranked upstream regulator (receptors-related list):

HER2

- Internal control
- HER2+ breast cancer marker

+



Mechanism:

Active phagocytosis is essential for successful anti-HER2 treatment and fast tumor clearance

Therapeutic duo proposals (theoretical prediction):

HER2 and FCGR2B

Identification of Transcription Factors and Master Regulators in for Her2+ Human Breast Cancer

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References Supporting Our Theoretical Prediction:

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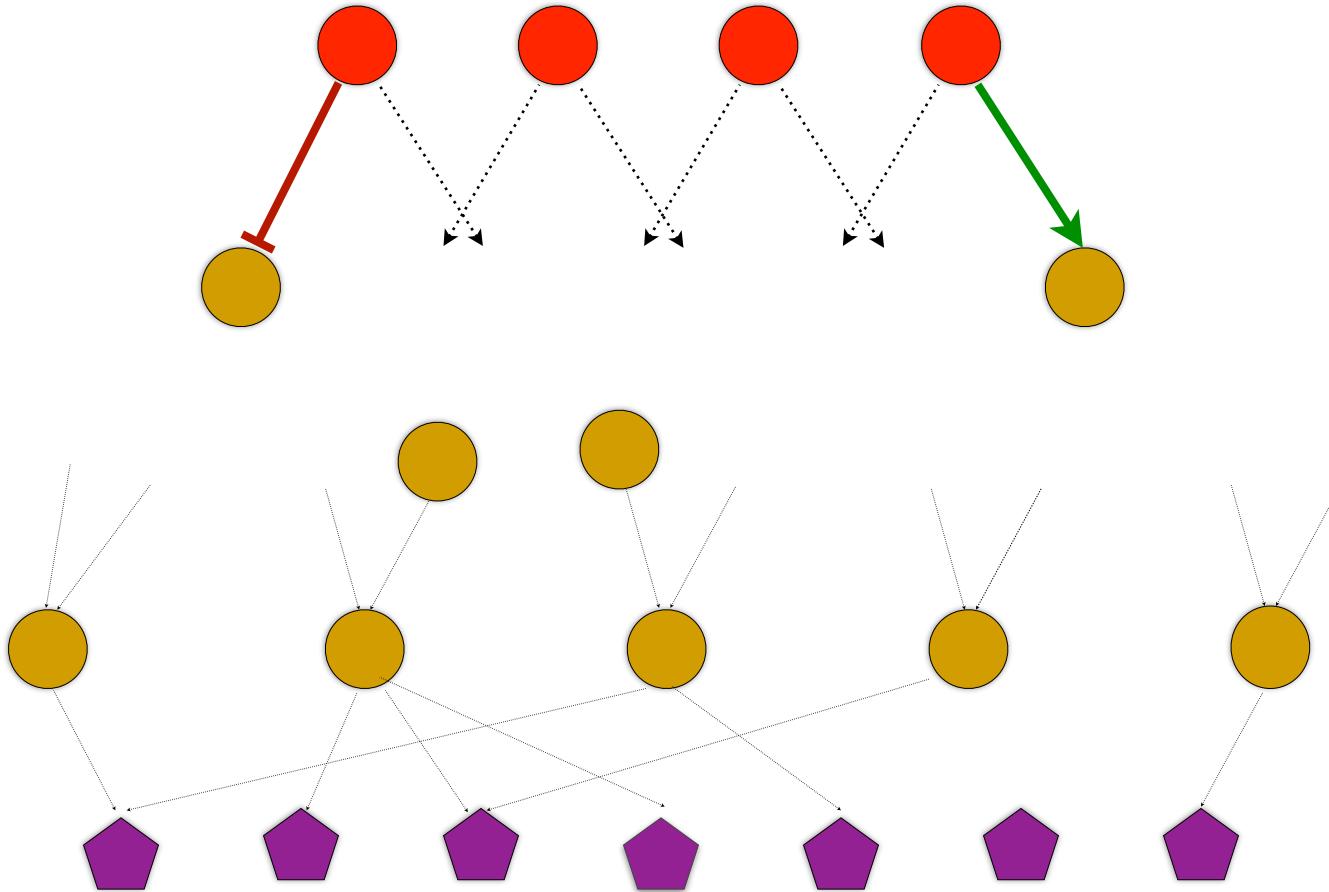
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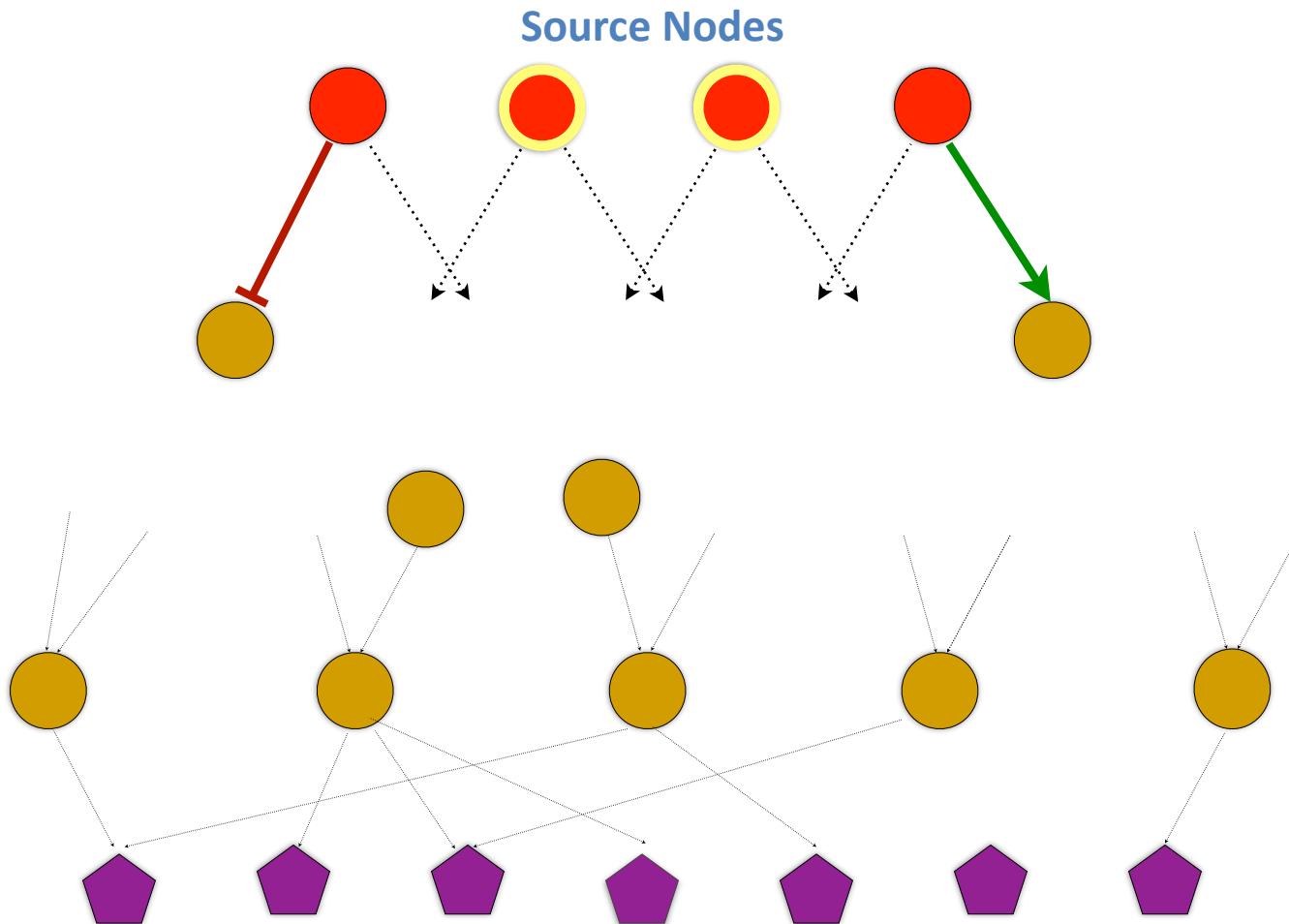
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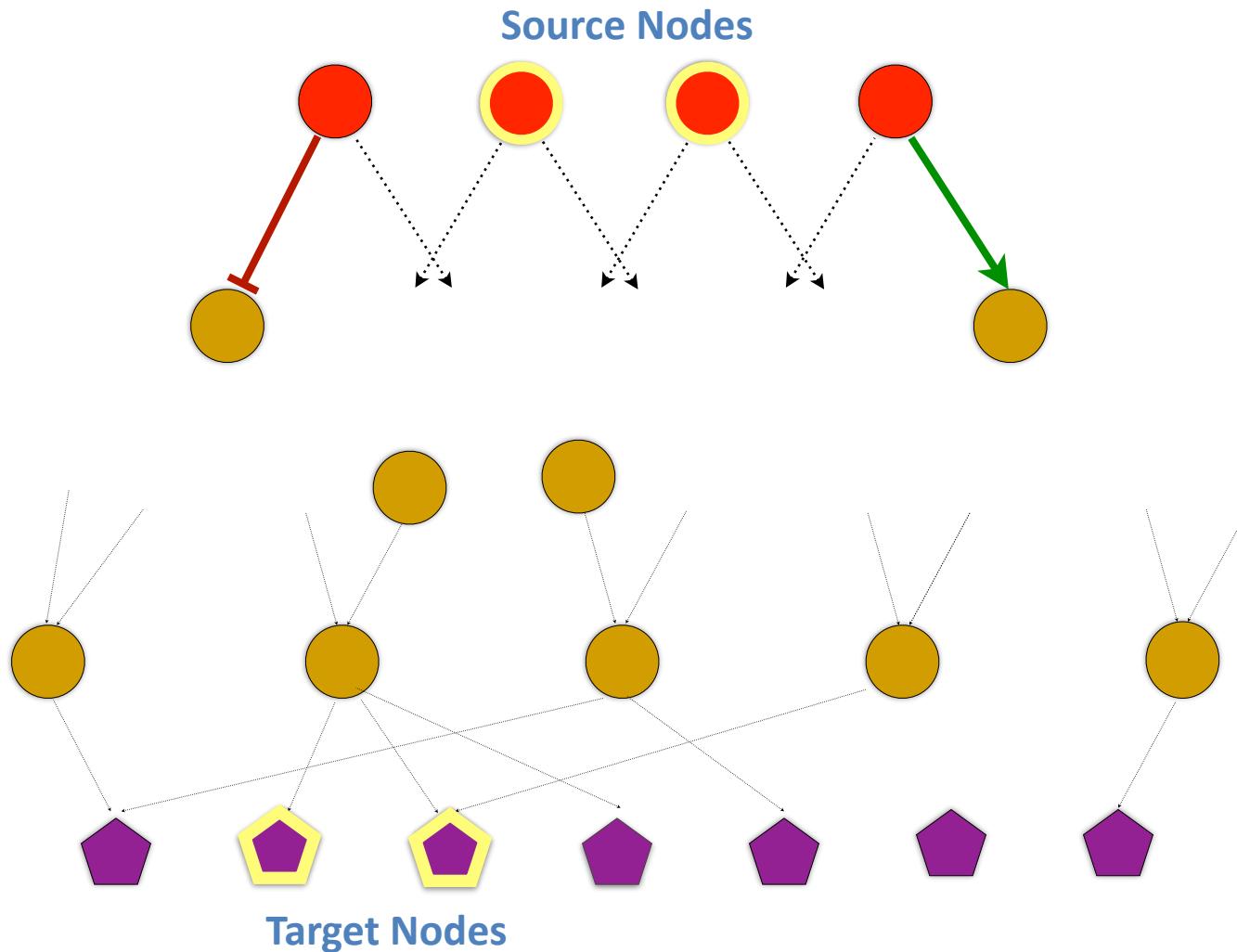
Optimal Combinations of Interventions



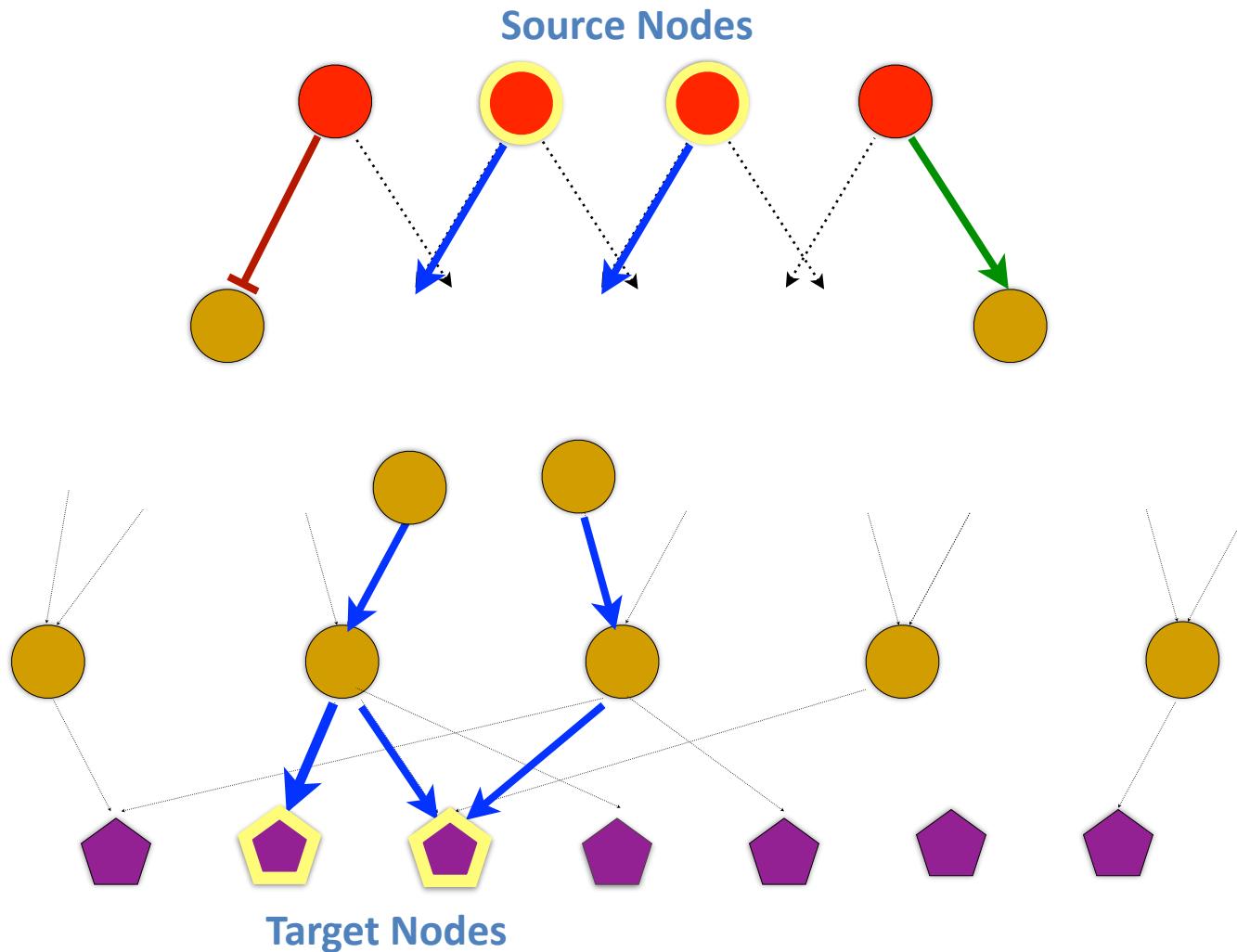
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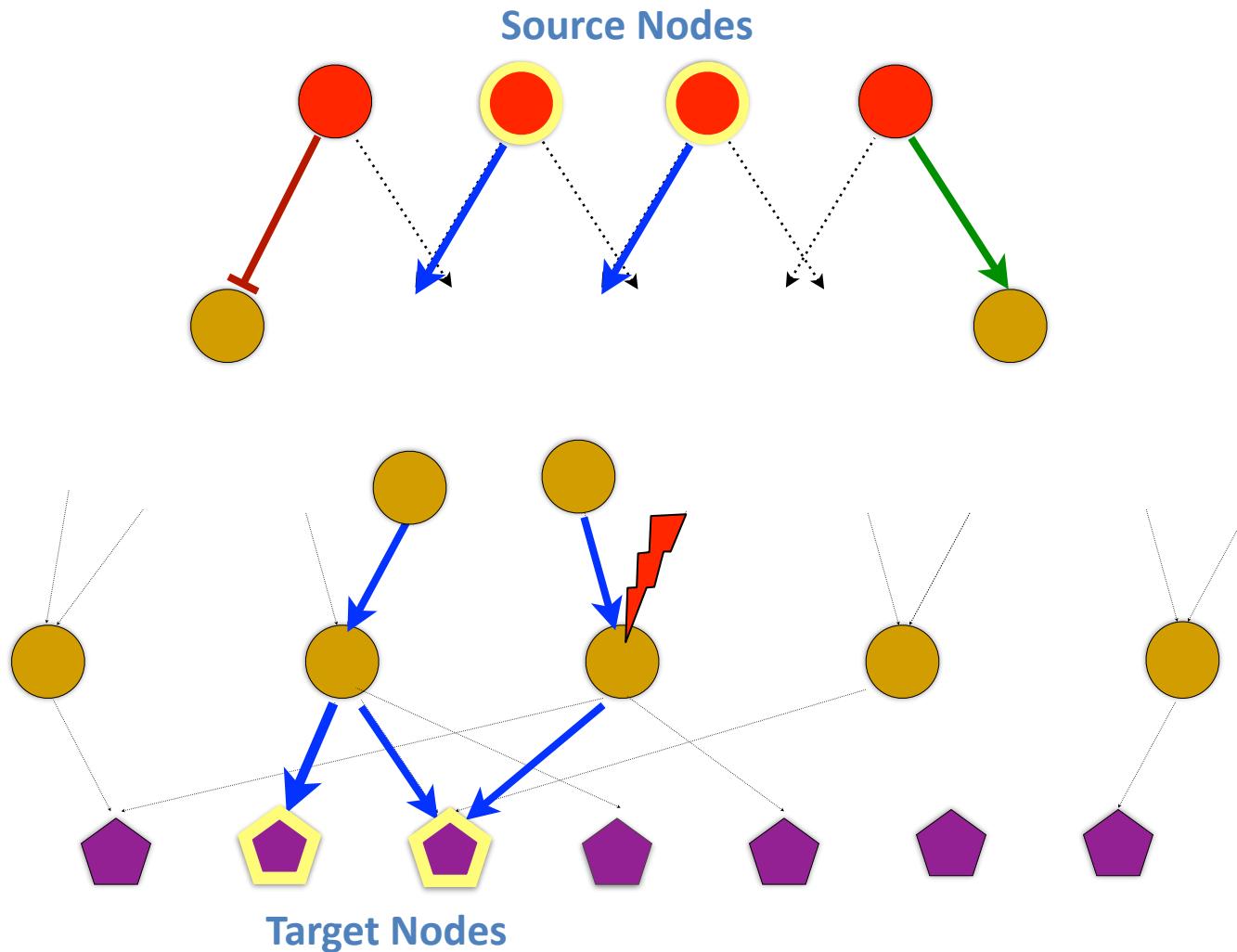
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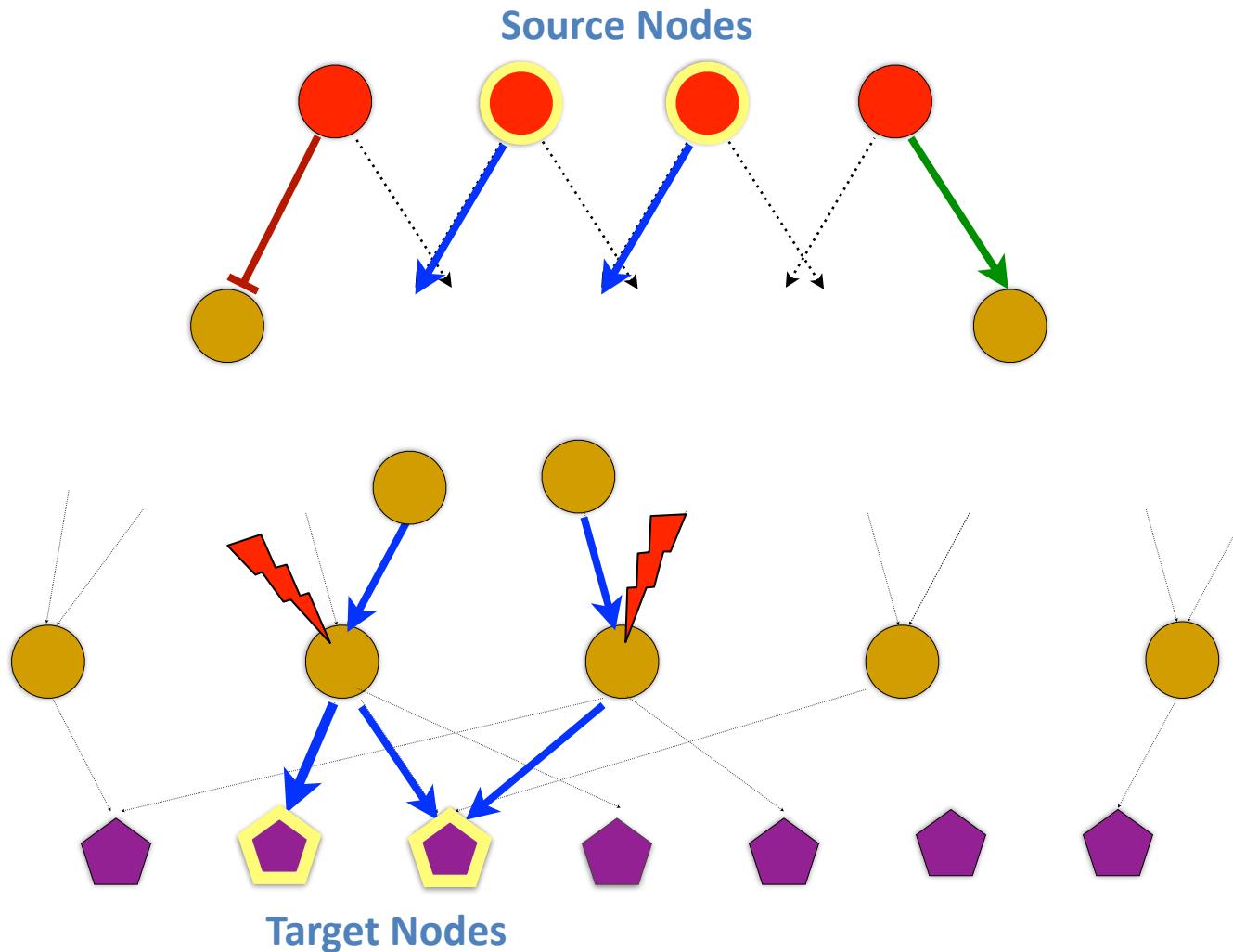
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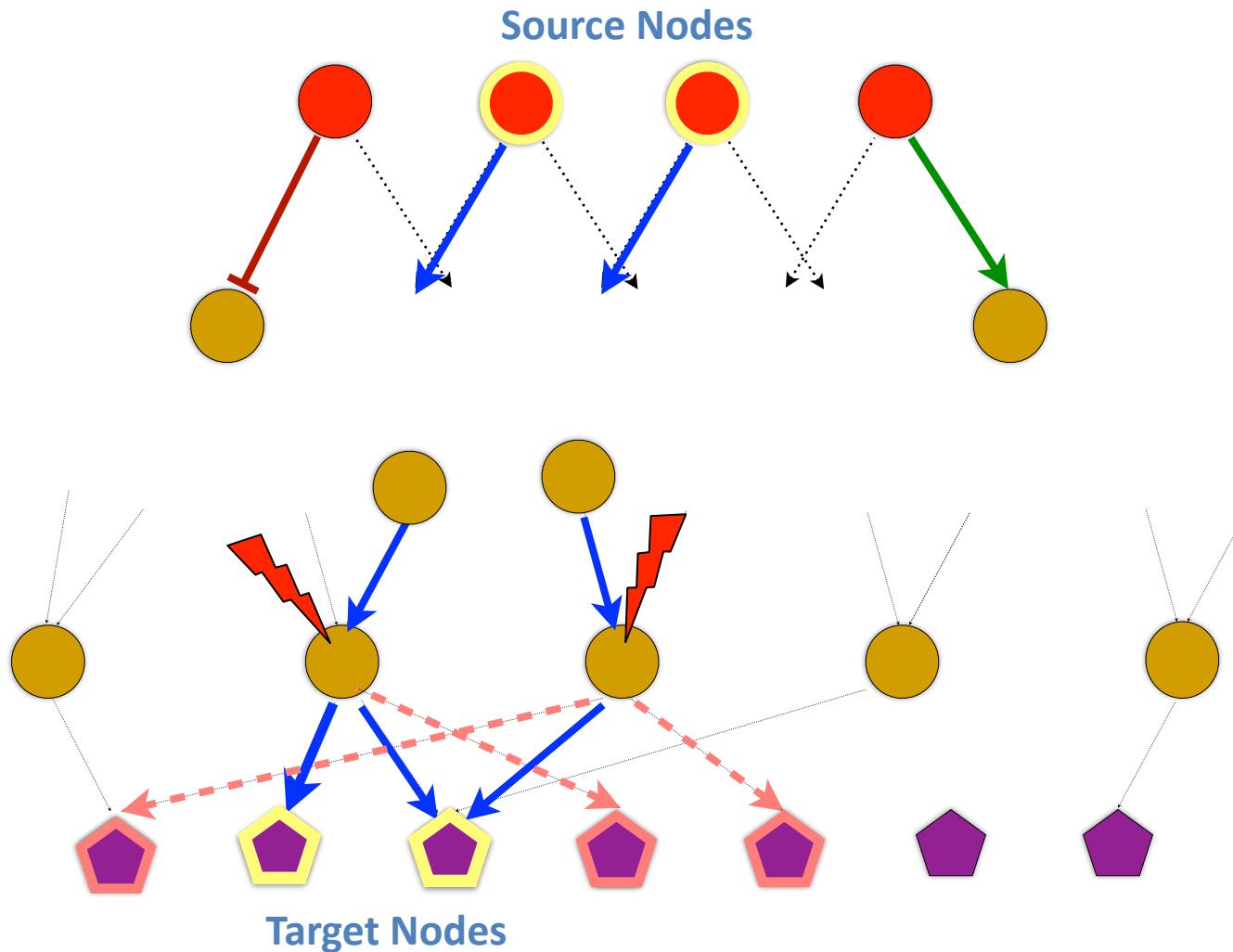
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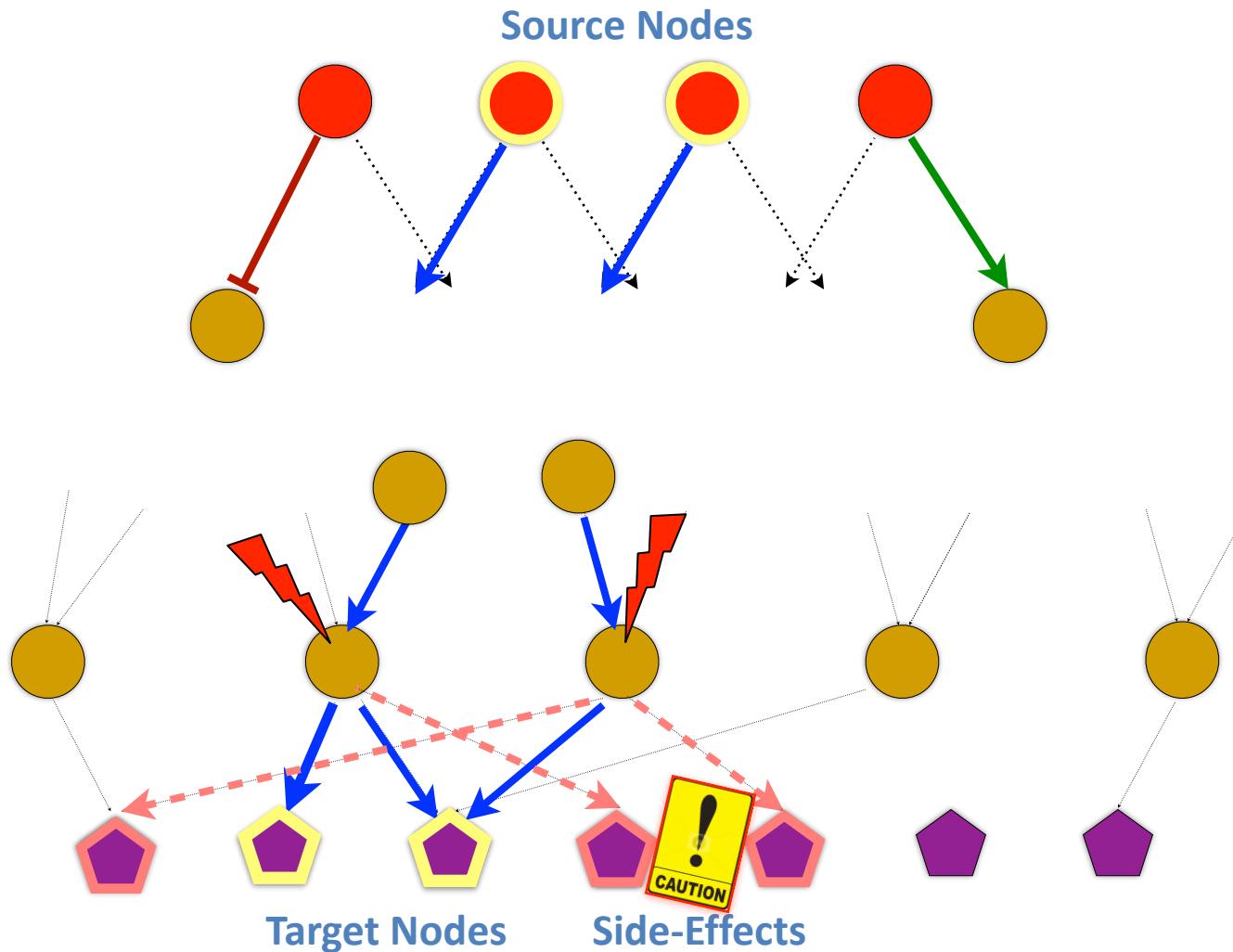
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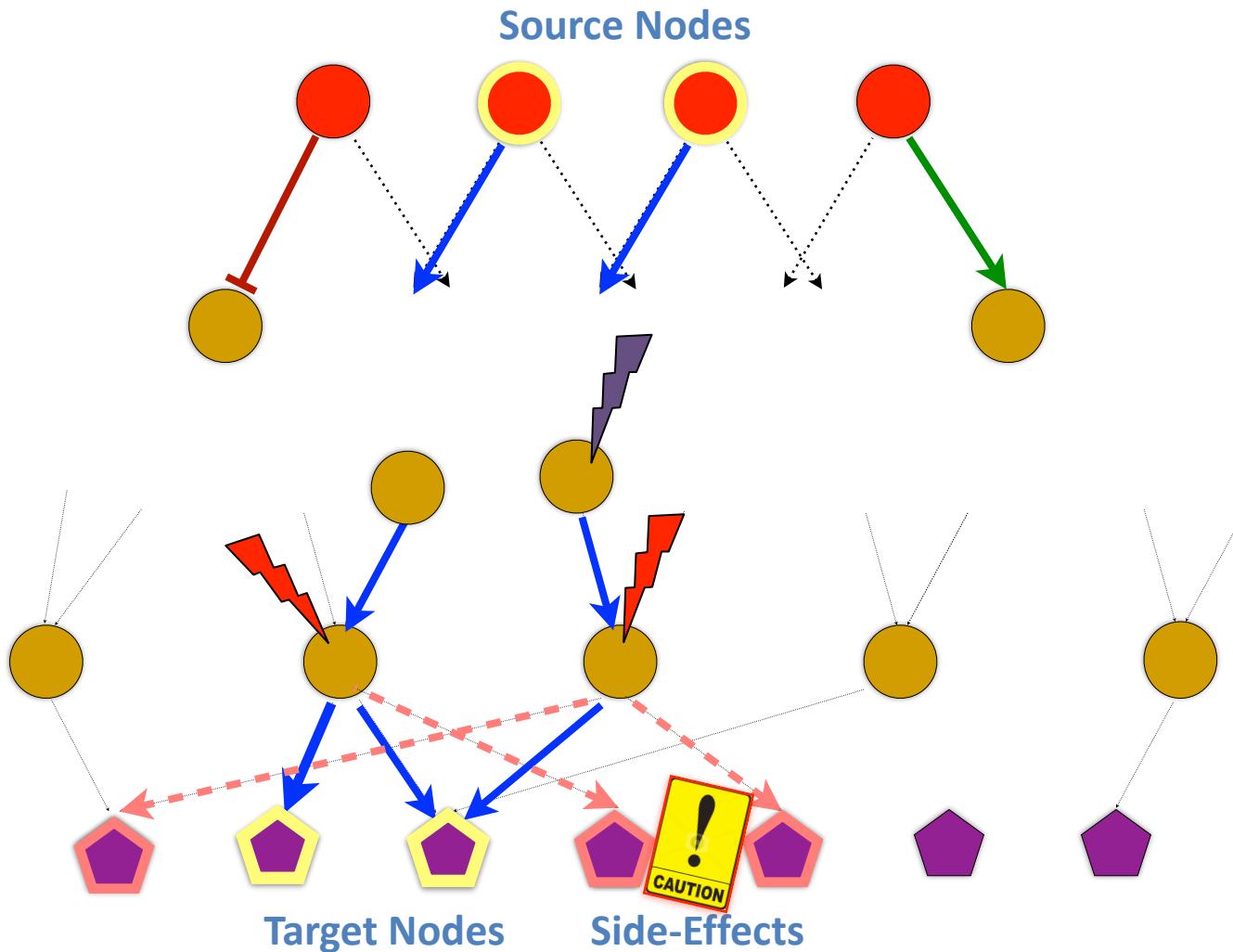
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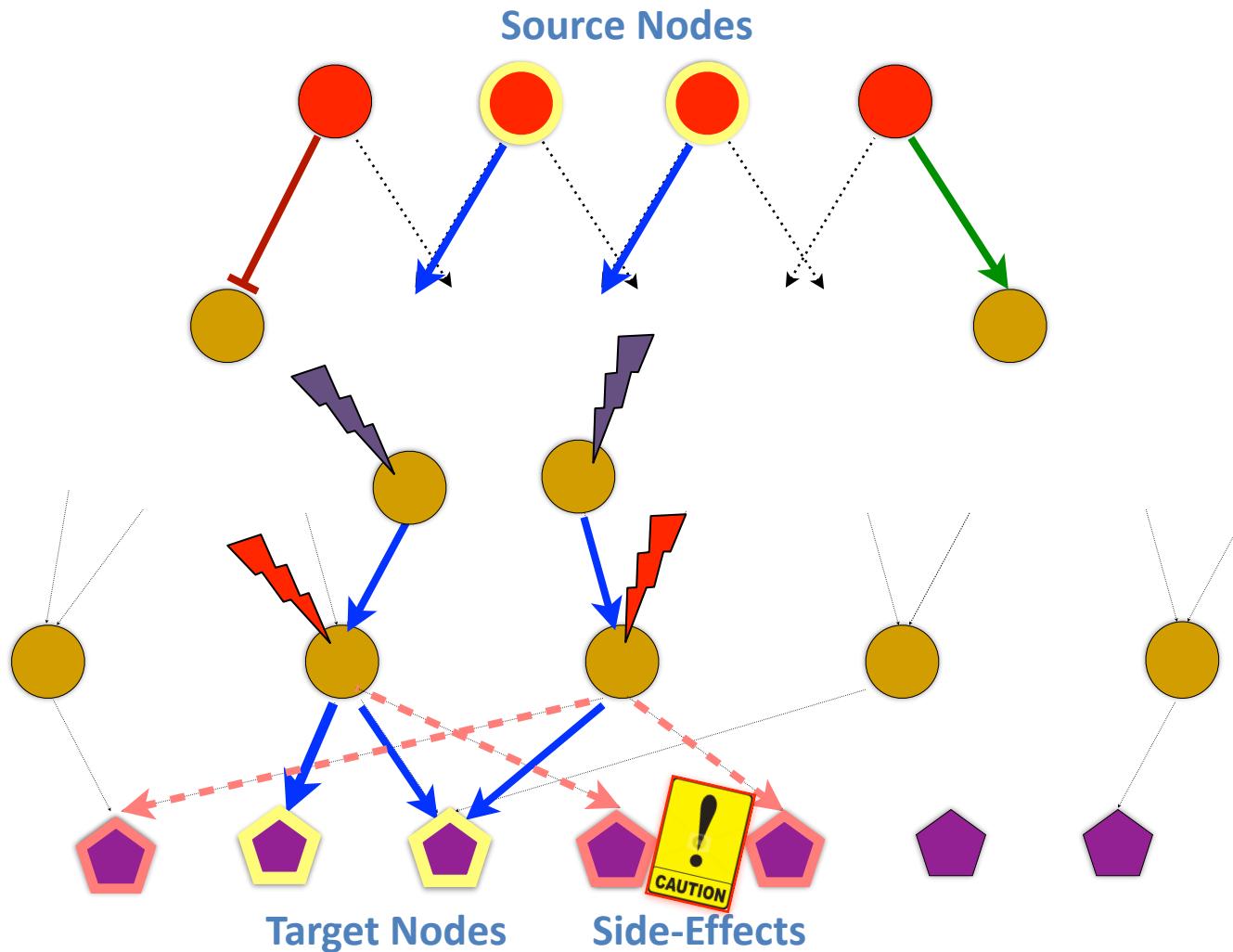
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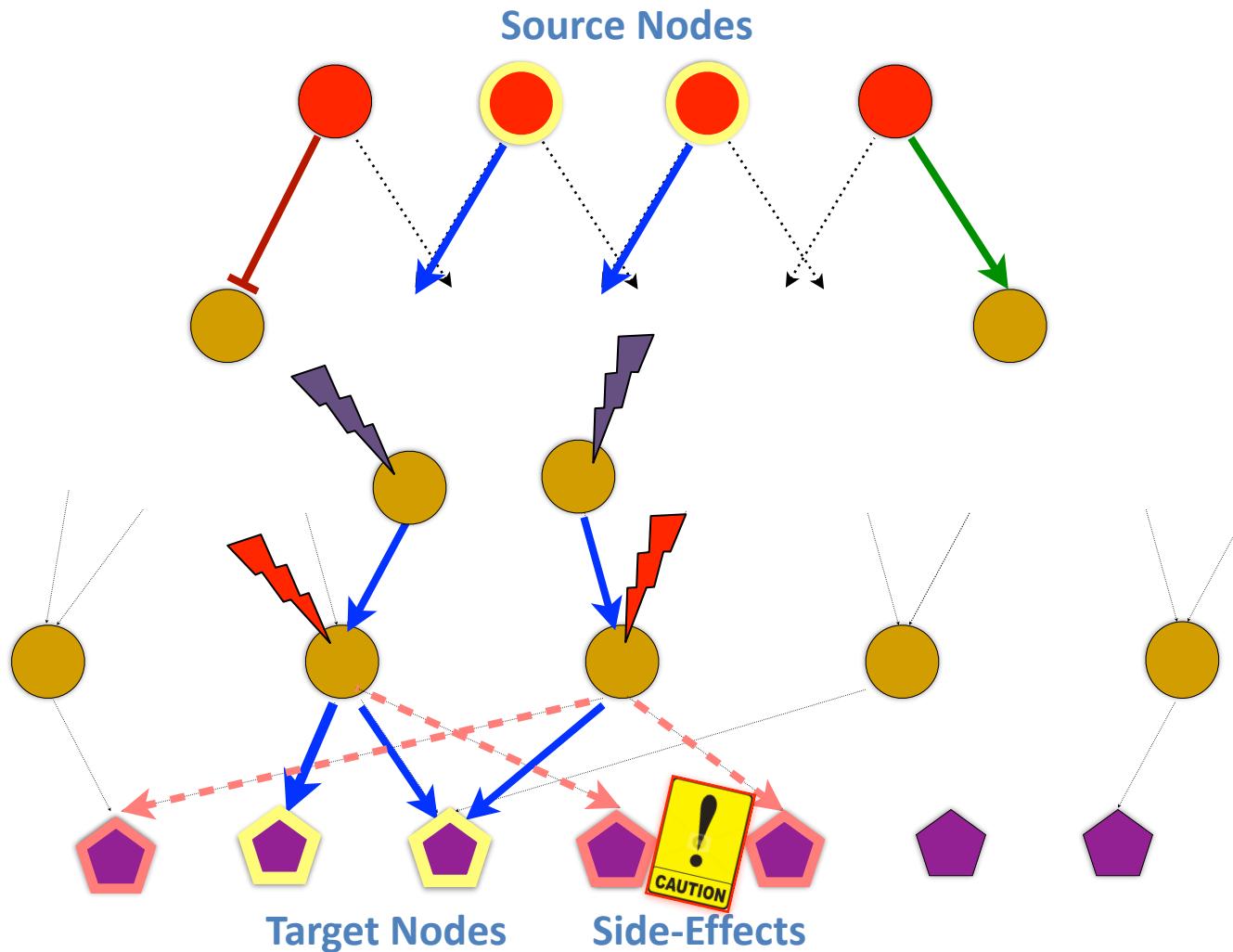
Optimal Combinations of Interventions



Optimal Combinations of Interventions



Optimal Combinations of Interventions



- Which Combination of Interventions (CI) is more optimal? **Prioritization of CIs**



OCSANA: Optimal Combinations of Interventions from Network Analysis

INTRODUCTION

Targeted therapies interfering specifically one protein activity, are promising strategies in the treatment of diseases like cancer. However, accumulated empirical experience has shown that targeting multiple proteins in signaling networks involved in the disease, is often necessary. Thus one important problem in biomedical research is the design and prioritization of optimal combinations of interventions to repress a pathological behavior, while minimizing side-effects.

OCSANA (Optimal Combinations of Interventions from Network Analysis) is a new software designed to identify and prioritize optimal and minimal, combinations of interventions to disrupt the paths between source nodes and target nodes. When specified by the user, OCSANA seeks to additionally minimize the side-effects that a combination of interventions can cause on specified off-target nodes. With the crucial ability to cope with very large networks, OCSANA includes exact and selective enumeration approaches for the combinatorial interventions' problem.

More information can be found at:

[Vera-Licona P, Bonnet E, Barillot E, Zinovyev A. \(2013\) OCSANA: Optimal Combinations of Interventions from Network Analysis. Bioinformatics, 29 \(12\): 1571-1573.](#)

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OCSANA: optimal combinations of interventions from network analysis

Paola Vera-Licona^{1,2,3,*}, Eric Bonnet^{1,2,3}, Emmanuel Barillot^{1,2,3} and Andrei Zinovyev^{1,2,3}

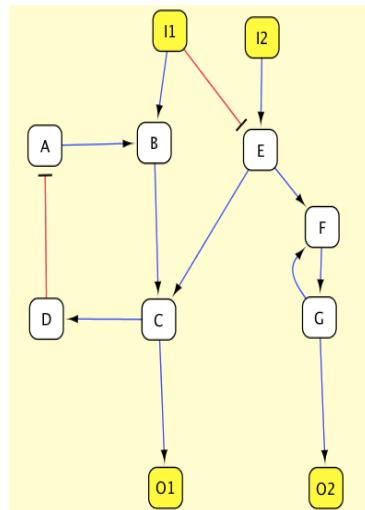
* To whom correspondence should be addressed.

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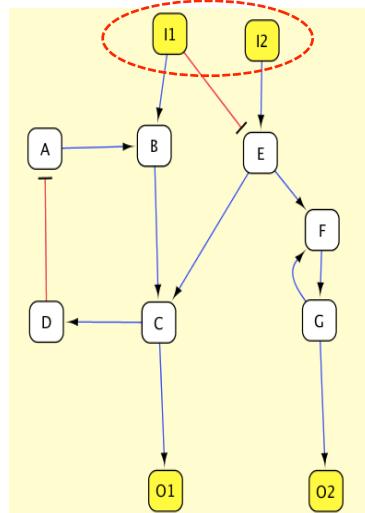
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Bioinformatics (2013) 29 (12): 1571-1573.
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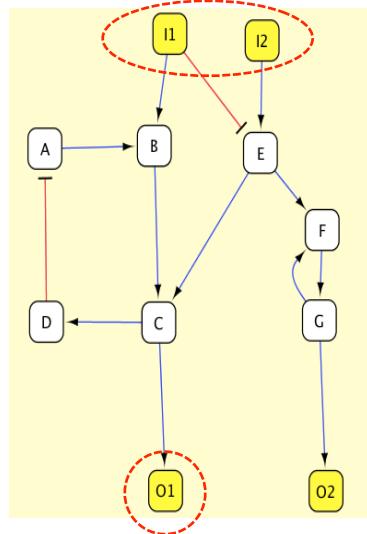
Stage IV: Identification of Optimal Combinations of Interventions from Network Analysis with OCSANA



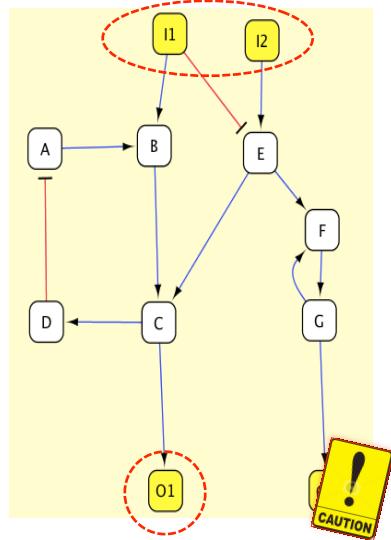
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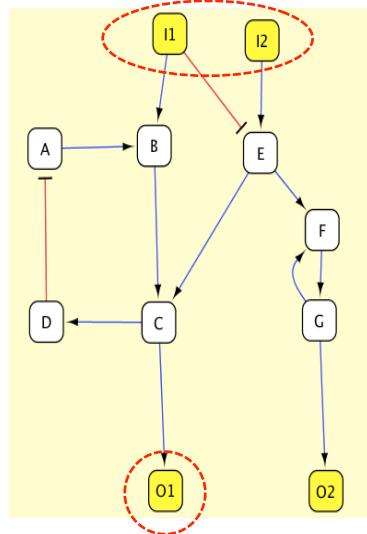
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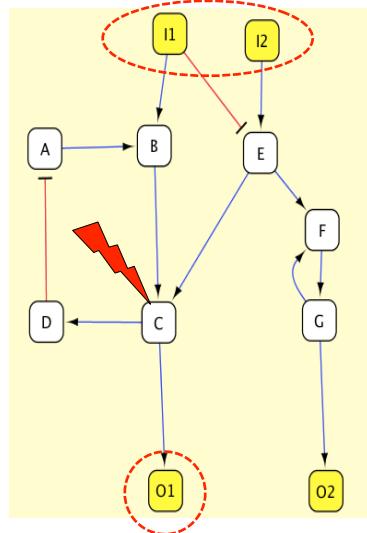
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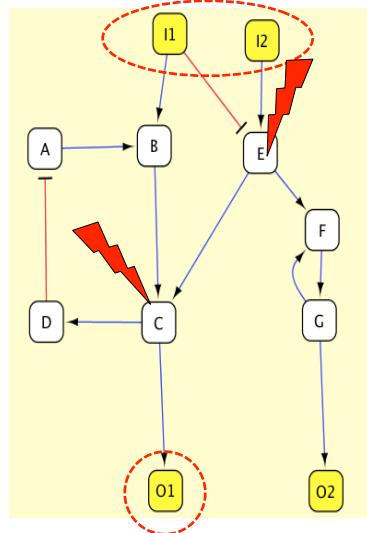
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Stage IV: Identification of Optimal Combinations of Interventions from Network Analysis with OCSANA

Optimal intervention set report

--- Optimal Combinations of Interventions Report ---

OPTIONS

Source nodes: I1 I2
 Target nodes: O1
 Side effect nodes: O2

Path search algorithm: All non-intersecting paths
 Finite search radius for All non-intersecting paths: inf

CI algorithm selected: Approximation solution
 Max. Nb of million of combinations to test: 50

RESULTS

Modifications to the network: 0 undefined effect edges were converted to activation effect edges out of 13

Found 3 elementary paths and 5 elementary nodes

I1->B->C->O1
 I1->E->C->O1
 I2->E->C->O1

Search for CI size 3
 Total nb of possible combinations: 4
 Tested nb of combinations: 4

Search for CI size 4
 Total nb of possible combinations: 1
 Tested nb of combinations: 1

Total timing for the search: 0 sec.

Found 4 optimal CIs.

| Optimal CI | Size | OCSANA score | PIQUANT score *Set score | SideEffects score |
|------------|------|--------------|--------------------------|-------------------|
| [C] | 1 | 3 | 3 | 0 |
| [E , B] | 2 | 0.833 | 1.5 | 0.333 |
| [I1 , I2] | 2 | -0.417 | 0.333 | 0.5 |
| [E , I1] | 2 | -0.167 | 1 | 0.583 |

Stage IV: Identification of Optimal Combinations of Interventions from Network Analysis with OCSANA

Cytoscape Desktop (New Session)

File Edit View Select Layout Plugins Help

Control Panel

Network

M-Phase2-scaled2.xml

Optimal Combinations of Intervention Strategies for Network Analysis

Add attribute information to gene IDs: **None**

Source nodes: A, B, C, D, E, F, G, I1, I2, O1

Target nodes: A, B, C, D, E, F, G, I1, I2, O1, O2

Side effect nodes: B, C, D, E, F, G, I1, I2, O1, O2

set source nodes **set target nodes** **set side-effect nodes**

Path search algorithm:

- Shortest paths
- Optimal and suboptimal shortest paths
- All non-intersecting paths

use finite search radius: 10.0

Choose equation for the score

- Inverse
- Logistic

Minimal hitting sets search algorithm:

- Full search (Berge's algorithm)
- Partial enumeration

Max. set size: 10

Max. Nb of (million) it sets: 500

OK Cancel

Phase2-scaled2.xml

Optimal intervention set report

--- Optimal Combinations of Interventions Report ---

OPTIONS

Source nodes: I1 I2
Target nodes: O1
Side effect nodes: O2

Path search algorithm: All non-intersecting paths
Finite search radius for All non-intersecting paths: inf

CI algorithm selected: Approximation solution
Max. Nb of million of combinations to test: 50

RESULTS

Modifications to the network: 0 undefined effect edges were converted to activation effect edges out of 13

Found 3 elementary paths and 5 elementary nodes

I1->B->C->O1
I1->E->C->O1
I2->E->C->O1

Search for CI size 3
Total nb of possible combinations: 4
Tested nb of combinations: 4

Search for CI size 4
Total nb of possible combinations: 1
Tested nb of combinations: 1

Total timing for the search: 0 sec.

Found 4 optimal CIs.

| Optimal CI | Size | OCSANA score PIQUANT score *Set score | SideEffects score |
|------------|------|--|-------------------|
| [C] | 1 | 3 | 0 |
| [E , B] | 2 | 0.833 | 1.5 |
| [I1 , I2] | 2 | -0.417 | 0.333 |
| [E , I1] | 2 | -0.167 | 1 |
| | | | 0.583 |

Attribute Browser

Stage IV: Identification of Optimal Combinations of Interventions from Network Analysis with OCSANA

Stage IV: Identification of Optimal Combinations of Interventions from Network Analysis with OCSANA

1) Does ErbB2 has access to all the OFTEN set of genes that are highly dysregulated in HER2?

YES. Thus it is natural to consider it as THE guilty gene causing the disease.

Found **3657 elementary paths and 208 elementary nodes if we consider ANSIP of length at most 10.**

Stage IV: Identification of Optimal Combinations of Interventions from Network Analysis with OCSANA

1) Does ErbB2 has access to all the OFTEN set of genes that are highly dysregulated in HER2?

YES. Thus it is natural to consider it as THE guilty gene causing the disease.

Found 3657 elementary paths and 208 elementary nodes if we consider ANSIP of length at most 10.

2) Considering only ErbB2 as **THE guilty gene** (thus considering it as the only one source node), is it possible to find CIs such that ALL the pathways to OFTEN dysregulated genes in HER2+ BC?

YES.

Found 17 optimal CIs. The only CI of size 1 is ErbB2 itself and the other CIs require to be of size at least 3.

However we know that this view of the problem might be reduced as resistance to treatment targeting ErbB2 alone, might occur.

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3) Considering all the MRs as source nodes, is it possible to find CIs < 6 such that ALL the pathways to OFTEN dysregulated genes in HER2+ BC?

Found 380942 elementary paths and 588 elementary nodes to be blocked simultaneously.

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NO!

4) We simulated an scenario where both drugs, **Trastuzumab** and **Lapatinib** are combined. We aim to reveal complementary intervention strategies to ensure all the pathways are intervened.

We applied OCSANA algorithm to our network top 3 master regulators.

Found 36 CIs of size at least three. Here is and example with one of the most prevalent interventions suggested:

| Intervention Set | Size | Score |
|-------------------|------|---------|
| [SRC, PIP3, ERK2] | 3 | 6654.37 |

Feedback upregulation of HER3 (ErbB3) expression and activity attenuates antitumor effect of PI3K inhibitors

Anindita Chakrabarty^a, Violeta Sánchez^a, María G. Kuba^b, Cammie Rinehart^a, and Carlos L. Arteaga^{a,c,d,1}

Departments of ^aMedicine, ^bPathology, and ^cCancer Biology and ^dBreast Cancer Research Program, Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, TN 37232

Edited by Peter K. Vogt, The Scripps Research Institute, La Jolla, CA, and approved February 4, 2011 (received for review December 2, 2010)

We examined the effects of an inhibitor of PI3K, XL147, against clinical development; it exhibits an IC₅₀ against WT and mutant human breast cancer cell lines with constitutive PI3K activation. p110α of approximately 40 nM (12).

Upon inhibition of PI3K, the cell can maintain some level of PIP3 through partial restoration of HER3 phosphorylation which may limit the net inhibitory effect of the PI3K inhibitor thus suggesting that additional blockage of PIP3 is indeed necessary (for example via the antagonist PTEN).

Additionally in [Abramson et al. CCR 2011;17(5)] is suggested, as with one of our predicted combinations that, to inhibit the HER2 network and its output PI3K/Akt another rational therapeutic combination is trastuzumab or lapatinib plus a HER3 or an AKT inhibitor.

Literature Validation (SRC)

Nat Med. 2011 Apr;17(4):461-9. doi: 10.1038/nm.2309. Epub 2011 Mar 13.

Combating trastuzumab resistance by targeting SRC, a common node downstream of multiple resistance pathways.

Zhang S, Huang WC, Li P, Guo H, Poh SB, Brady SW, Xiong Y, Tseng LM, Li SH, Ding Z, Sahin AA, Esteva FJ, Hortobagyi GN, Yu D.

Department of Molecular and Cellular Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA.

Abstract

Trastuzumab is a successful rationally designed ERBB2-targeted therapy. However, about half of individuals with ERBB2-overexpressing breast cancer do not respond to trastuzumab-based therapies, owing to various resistance mechanisms. Clinically applicable regimens for overcoming trastuzumab resistance of different mechanisms are not yet available. We show that the nonreceptor tyrosine kinase c-SRC (SRC) is a key modulator of trastuzumab response and a common node downstream of multiple trastuzumab resistance pathways. We find that SRC is activated in both acquired and de novo trastuzumab-resistant cells and uncover a novel mechanism of SRC regulation involving dephosphorylation by PTEN. Increased SRC activation conferred considerable trastuzumab resistance in breast cancer cells and correlated with trastuzumab resistance in patients. Targeting SRC in combination with trastuzumab sensitized multiple lines of trastuzumab-resistant cells to trastuzumab and eliminated trastuzumab-resistant tumors *in vivo*, suggesting the potential clinical application of this strategy to overcome trastuzumab resistance.

In this work the authors showed how SRC, a non-membrane tyrosine kinase, is a common signaling node in trastuzumab resistance caused by different mechanisms in HER2-positive breast cancers. A SRC inhibitor restored trastuzumab sensitivity *in vitro* and in mouse tumor models, suggesting a new way to tackle drug resistance in breast tumors.

Dual mTORC1/2 and HER2 Blockade Results in Antitumor Activity in Preclinical Models of Breast Cancer Resistant to Anti-HER2 Therapy

Celina García-García¹, Yasir H. Ibrahim¹, Violeta Serra¹, María Teresa Calvo¹, Marta Guzmán¹, Judit Grueso¹, Claudia Aura², José Pérez¹, Katti Jessen³, Yi Liu³, Christian Rommel³, Josep Tabernero¹, José Baselga^{4,5}, and Maurizio Scaltriti^{4,5}

Abstract

Purpose: The PI3K/Akt/mTOR pathway is an attractive target in HER2-positive breast cancer that is refractory to anti-HER2 therapy. The hypothesis is that the suppression of this pathway results in sensitization to anti-HER2 agents. However, this combinatorial strategy has not been comprehensively tested in models of trastuzumab and lapatinib resistance.

Experimental Design: We analyzed *in vitro* cell viability and induction of apoptosis in five different cell lines resistant to trastuzumab and lapatinib. Inhibition of HER2/HER3 phosphorylation, PI3K/Akt/mTOR, and extracellular signal-regulated kinase (ERK) signaling pathways was evaluated by Western blotting. Tumor growth inhibition after treatment with lapatinib, INK-128, or the combination of both agents was evaluated in three different animal models: two cell-based xenograft models refractory to both trastuzumab and lapatinib and a xenograft derived from a patient who relapsed on trastuzumab-based therapy.

Results: The addition of lapatinib to INK-128 prevented both HER2 and HER3 phosphorylation induced by INK-128, resulting in inhibition of both PI3K/Akt/mTOR and ERK pathways. This dual blockade produced synergistic induction of cell death in five different HER2-positive cell lines resistant to trastuzumab and lapatinib. *In vivo*, both cell line-based and patient-derived xenografts showed exquisite sensitivity to the antitumor activity of the combination of lapatinib and INK-128, which resulted in durable tumor shrinkage and exhibited no signs of toxicity in these models.

Conclusions: The simultaneous blockade of both PI3K/Akt/mTOR and ERK pathways obtained by combining lapatinib with INK-128 acts synergistically in inducing cell death and tumor regression in breast cancer models refractory to anti-HER2 therapy. *Clin Cancer Res*; 1–10. ©2012 AACR.

Other Examples on Ongoing Research

- **MyD88 phagosomal signals in Lyme disease**

Dr. Juan Salazar, Pediatrics Department at UConn Health & Connecticut Children's Hospital

- **CD8 T Cell Activation and Migration in Vivo**

Dr. Kamal Khanna, Department of Microbiology at NYU

- **Prediction of interventions in TNBC through mathematical network control**

Dr. Ed Liu, JAX-GM

Dr. Reinhard Laubenbacher, Center for Quantitative Medicine at UConn Health & JAX-GM

Conclusions

In this talk we have introduced a Systems Biology analysis pipeline that combines 3 different software packages to build three layers of our disease-related network.

Through a comparative study, we emphasized the importance of selecting the set of differential expressed genes upon which we build the upstream layer of the network.

We introduce OCSANA for the systematic finding and prioritization of optimal combinations of interventions.

Reconstruction and analysis of signaling networks can be applied to other problems aside discovery of therapeutic interventions. One such example was illustrated with the study of the role of MyD88 in lyme disease.