Network dynamics driving cancer metastasis: from design principles to therapeutic approaches

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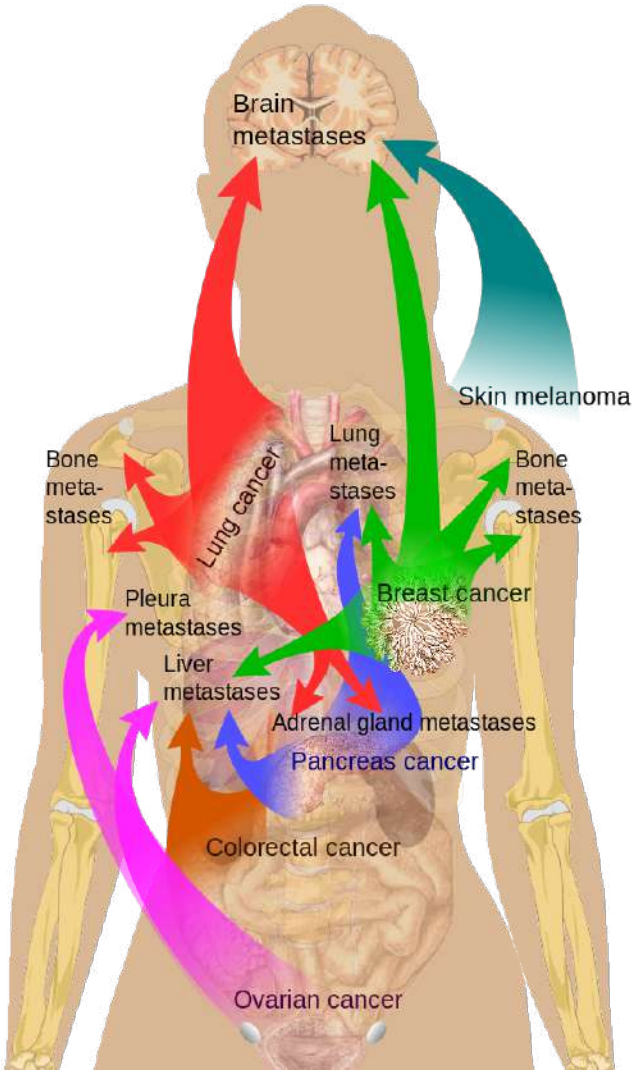
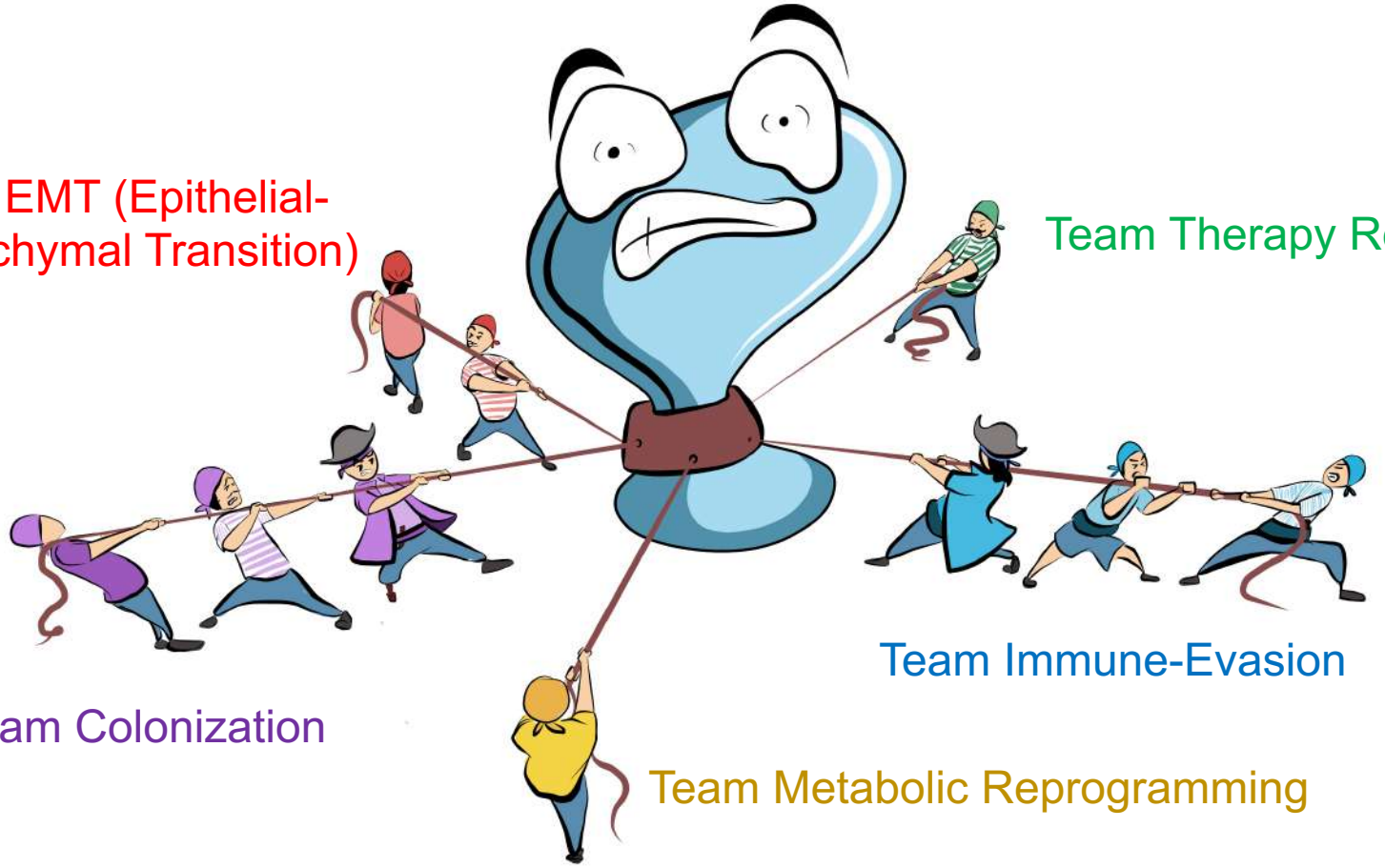
Metastasis : the cause of 90% of all cancer deaths



Metastasis has extremely high attrition (> 99.9%) rates.

What traits cells need to successfully metastasize?

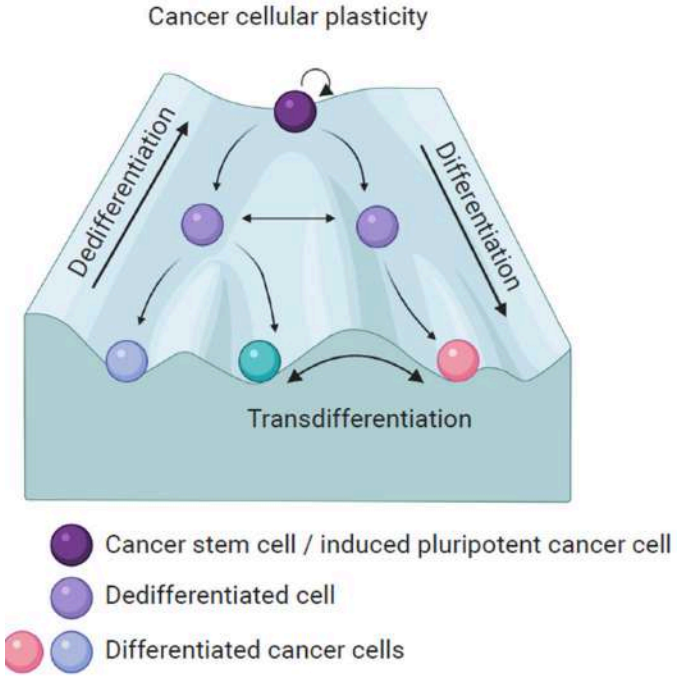


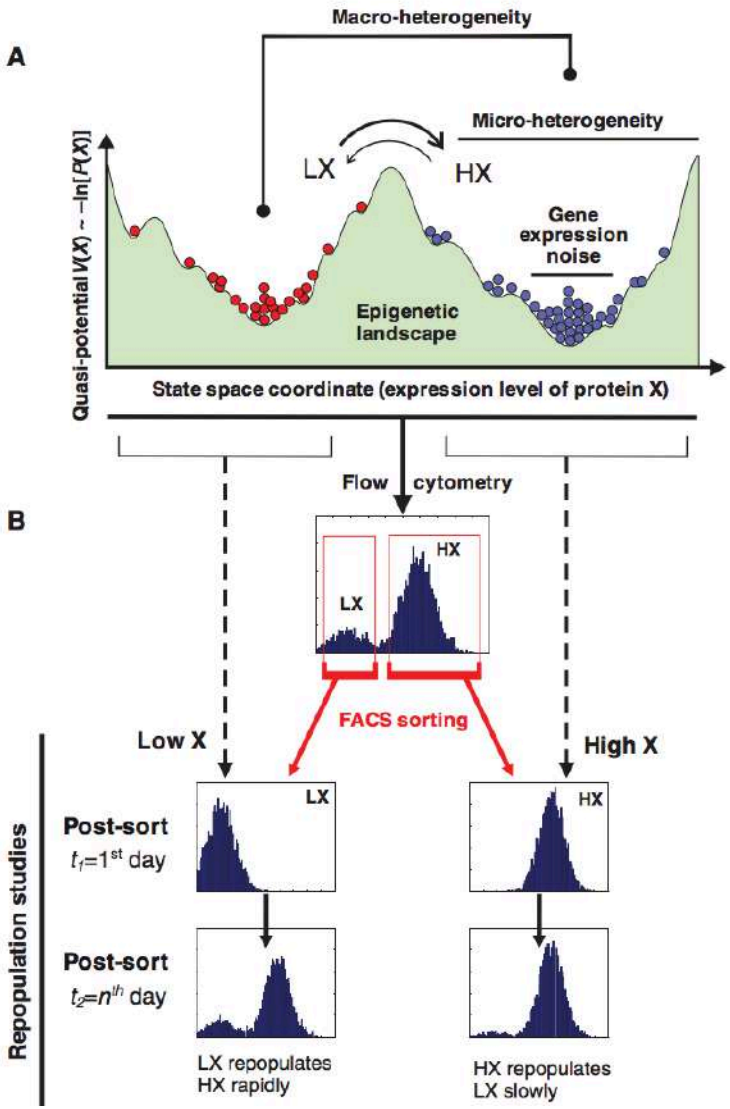
We need **a dynamic and systems-level understanding** of the process to identify how cells alter these multiple traits together

Credits: Atchuta Srinivas Duddu

Phenotypic plasticity and non-genetic heterogeneity

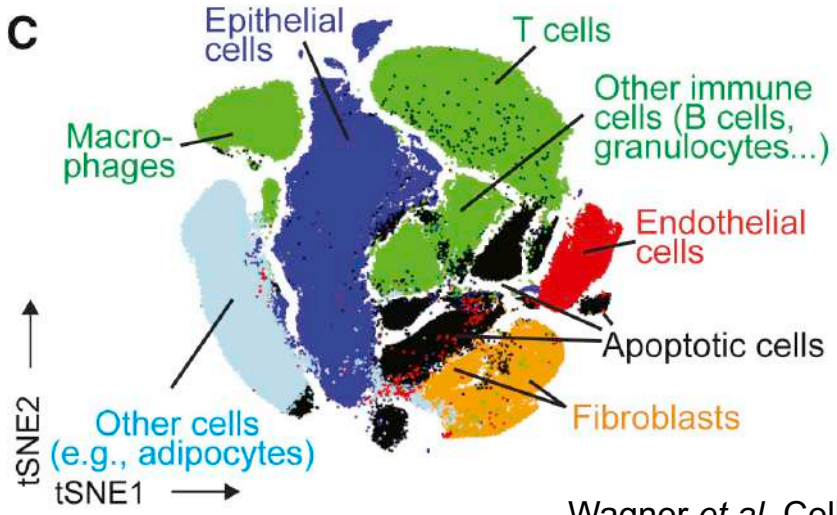
Cellular/Phenotypic plasticity: Ability of cells to switch their phenotype/behavior reversibly in response to environmental conditions





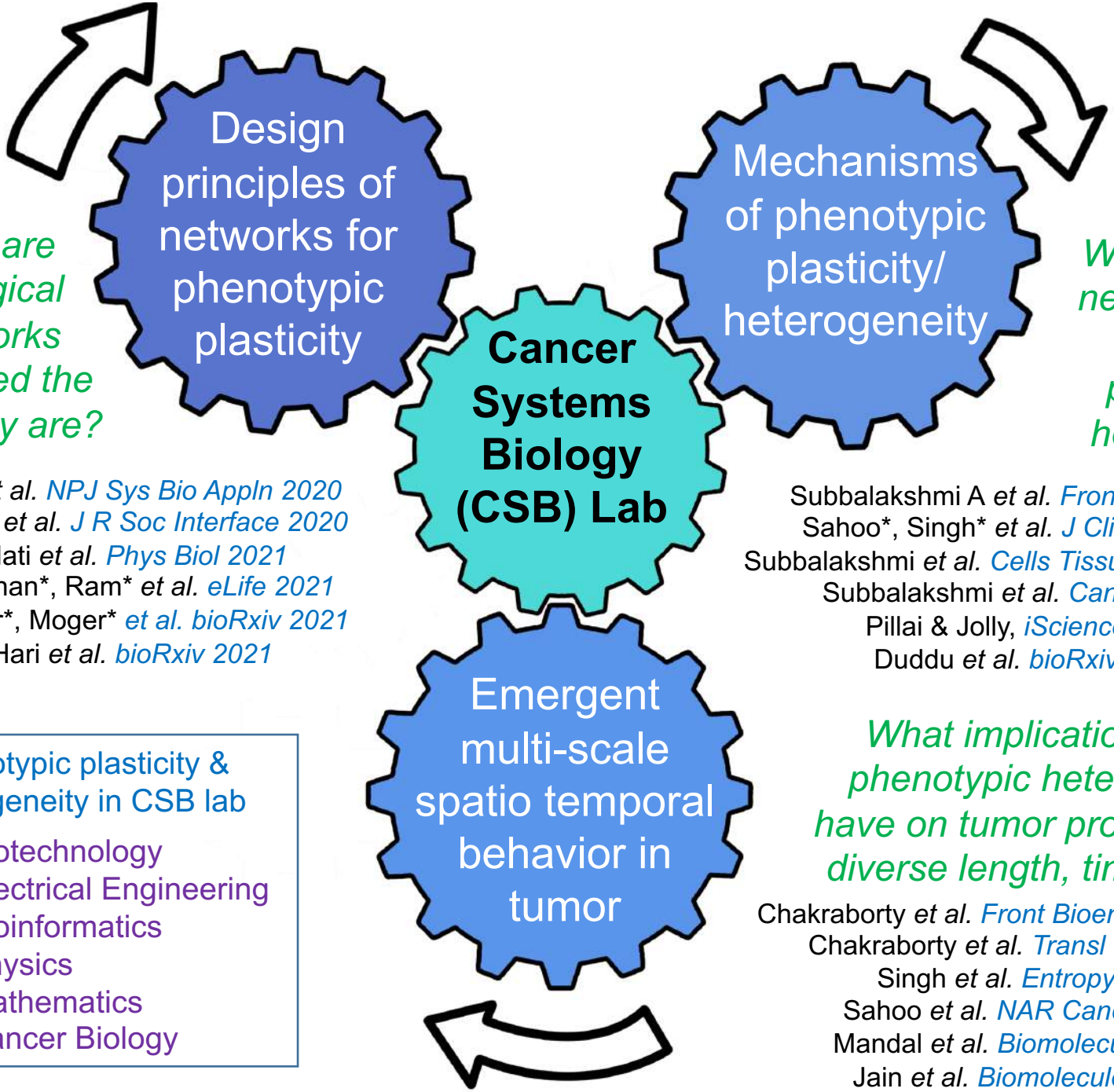
Huang *et al.* Development 2009
Granados *et al.* Int J Mol Sci 2020

Open questions about plasticity & heterogeneity in cancer



Wagner *et al.* Cell 2019

- How many states can cancer cells exist in?
- How do they switch among these states?
- How do they coordinate behavior among these different axes?
- Can we suggest ways to control plasticity and heterogeneity in a dynamic evolving system?



Why are biological networks designed the way they are?

Hari *et al.* *NPJ Sys Bio Appln 2020*
Duddu *et al.* *J R Soc Interface 2020*
Hati *et al.* *Phys Biol 2021*
Chauhan*, Ram* *et al.* *eLife 2021*
Hebbar*, Moger* *et al. bioRxiv 2021*
Hari *et al.* *bioRxiv 2021*

What regulatory networks enable phenotypic plasticity and heterogeneity?

Subbalakshmi A *et al.* *Front Oncol 2020*
Sahoo*, Singh* *et al.* *J Clin Med 2020*
Subbalakshmi *et al.* *Cells Tissues Organs 2021*
Subbalakshmi *et al.* *Cancers 2021*
Pillai & Jolly, *iScience 2021*
Duddu *et al.* *bioRxiv 2021*

Phenotypic plasticity & heterogeneity in CSB lab

∫
Biotechnology
Electrical Engineering
Bioinformatics
Physics
Mathematics
Cancer Biology

What implications does phenotypic heterogeneity have on tumor progression at diverse length, time scales?

Chakraborty *et al.* *Front Bioeng Biotech 2020*
Chakraborty *et al.* *Transl Oncol 2021*
Singh *et al.* *Entropy 2021*
Sahoo *et al.* *NAR Cancer 2021*
Mandal *et al.* *Biomolecules 2022*
Jain *et al.* *Biomolecules 2022*

Acknowledgements

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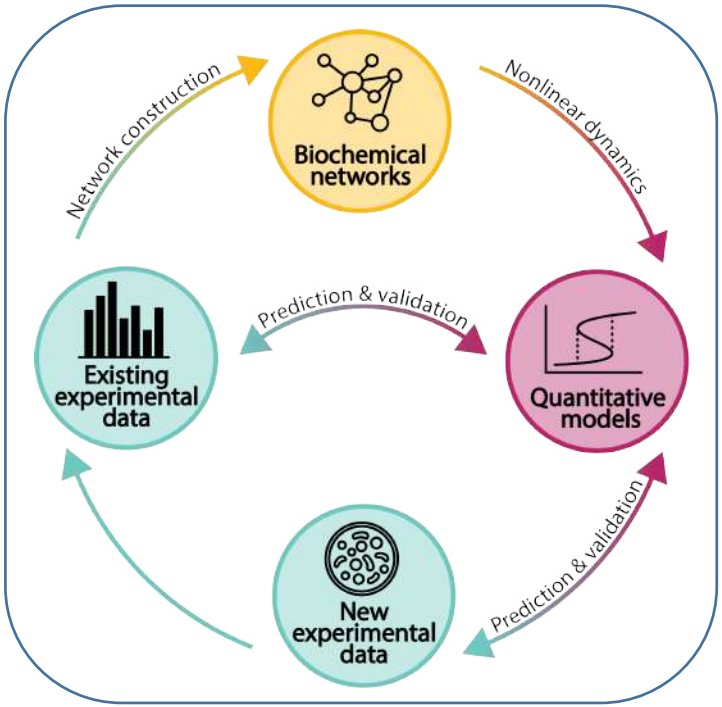


∫ Oncologists
Clinicians
Mathematicians
Physicists
Chemists
Engineers

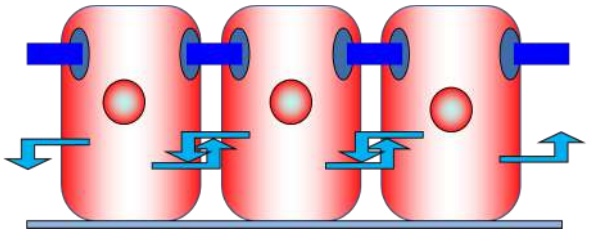
Herbert Levine
Jose N Onuchic
Shubham Tripathi
Wen Jia
Federico Bocci

Kishore Hari
Sarthak Sahoo
Maalavika Pillai
Lakshya Chauhan
Gubbala Udayram
Paras Jain
Atchuta S Duddu
Divyoj Singh
Susmita Mandal
Subbalakshmi A R
Srinath Muralidharan

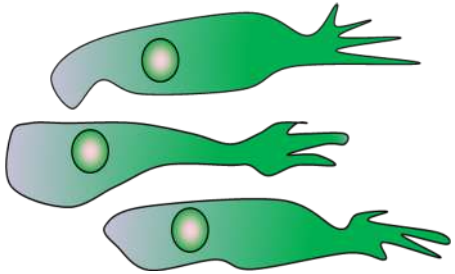
Partha Sharthi Dutta
Sudipta Sinha
Sukanta Sarkar

Caterina La Porta
Stefano Zapperi
Franc Font-Clos

EMT/MET: The engine of metastasis

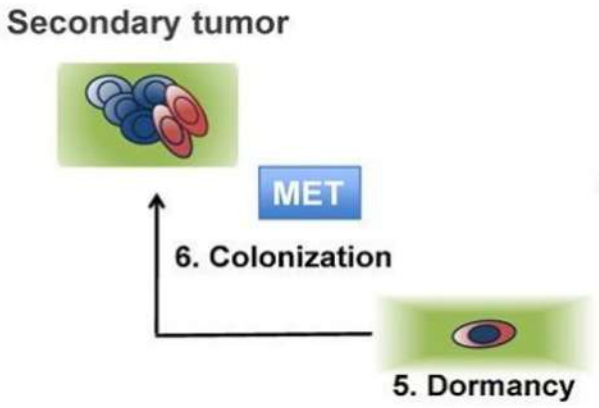


Adhere to neighbors
Do NOT migrate or invade
Epithelial (E)

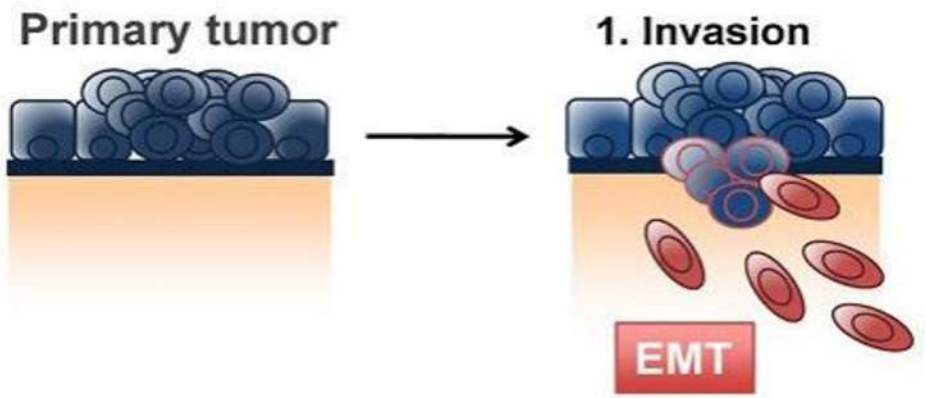


Do NOT adhere to neighbors
Migrate and invade
Mesenchymal (M)

Mesenchymal-to-Epithelial Transition (MET)

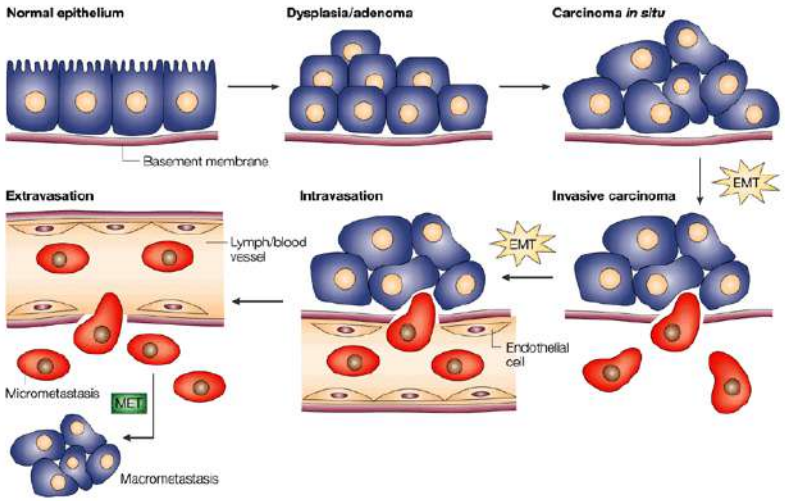


Epithelial-to-Mesenchymal Transition (EMT)

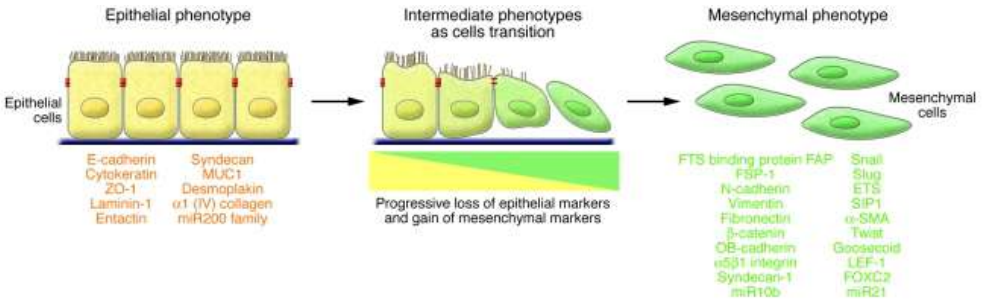


Scheel & Weinberg, Semin Cancer Bio 2012

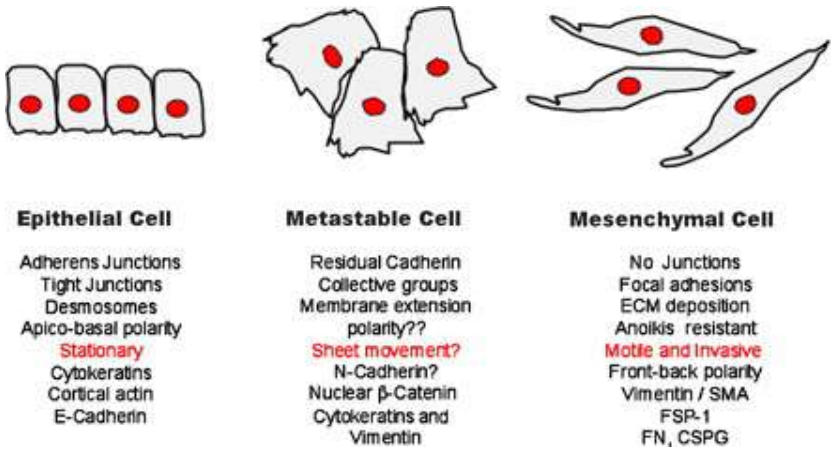
Role of EMT in cancer metastasis (2002 – 2012)



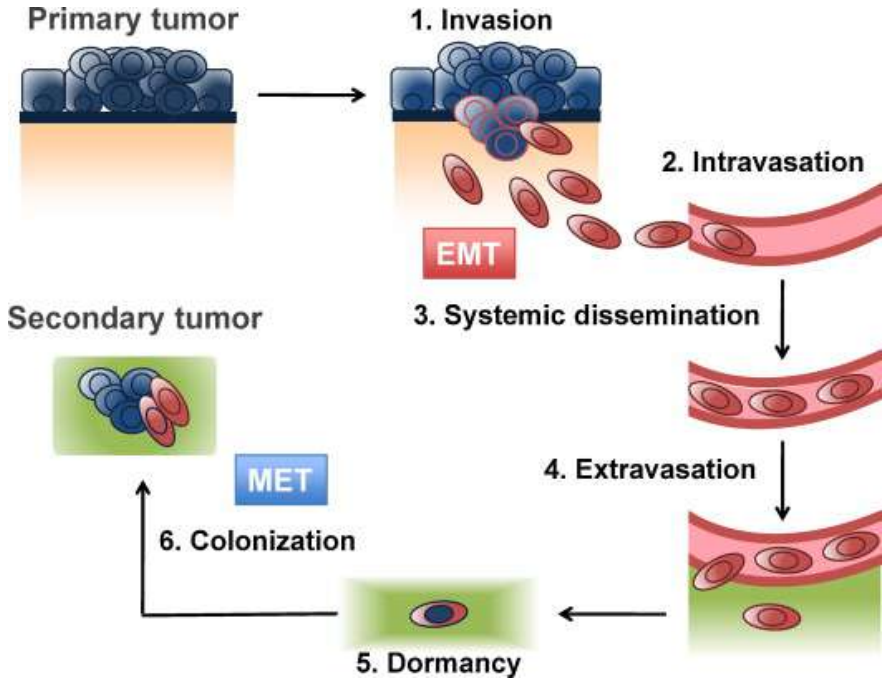
Thiery JP, Nat Rev Cancer 2002



Kalluri & Weinberg, J Clin Invest 2009

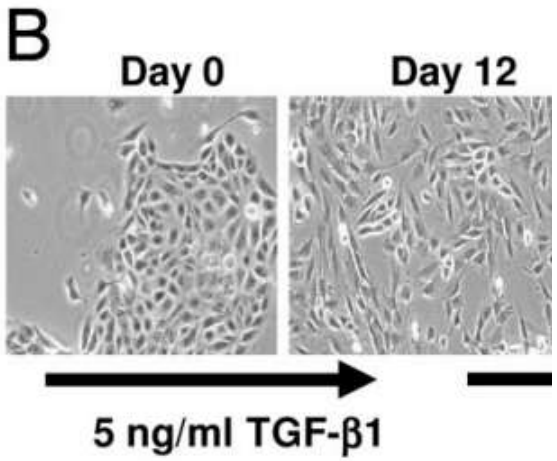


Lee *et al.* J Cell Biol 2006

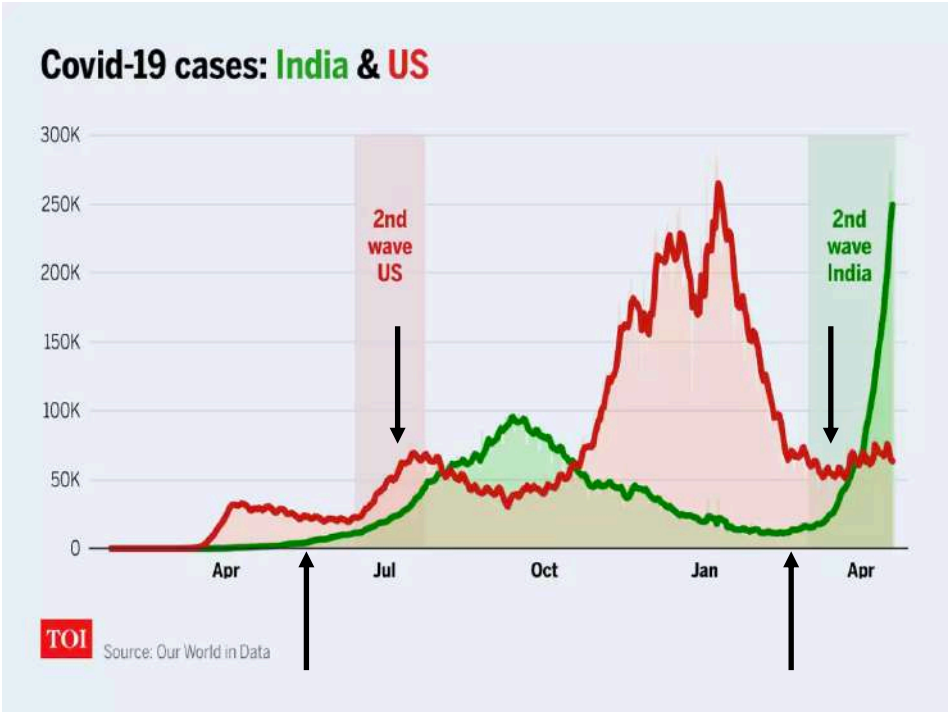


Scheel & Weinberg, Semin Cancer Biol 2012

Is EMT/MET a binary process?

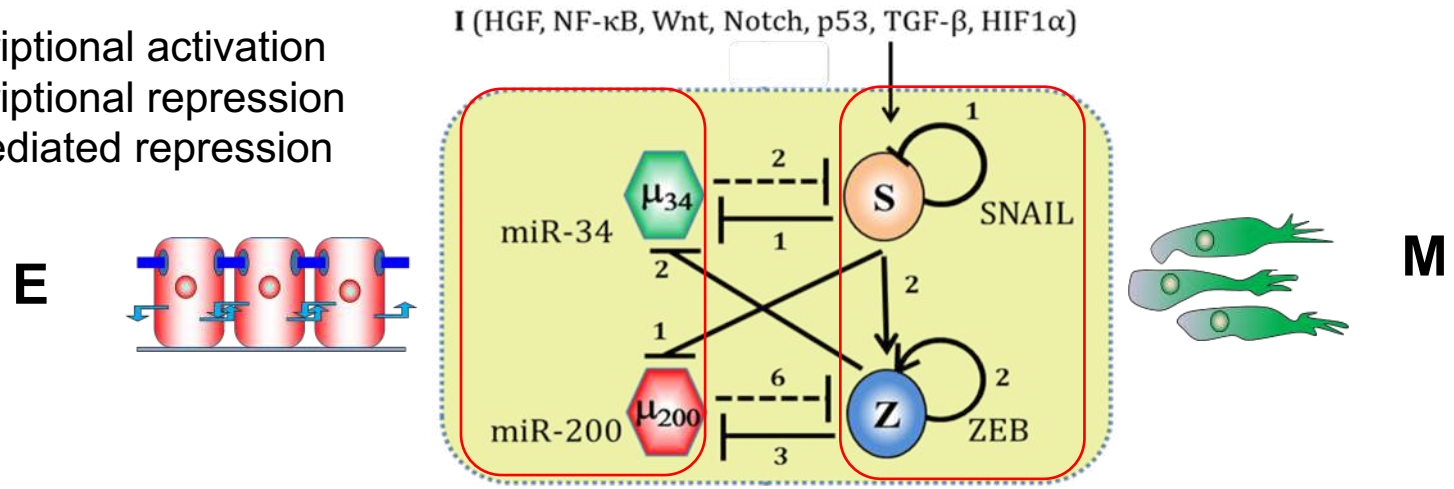


Mani *et al.*
PNAS 2007



Network that controls EMT/MET

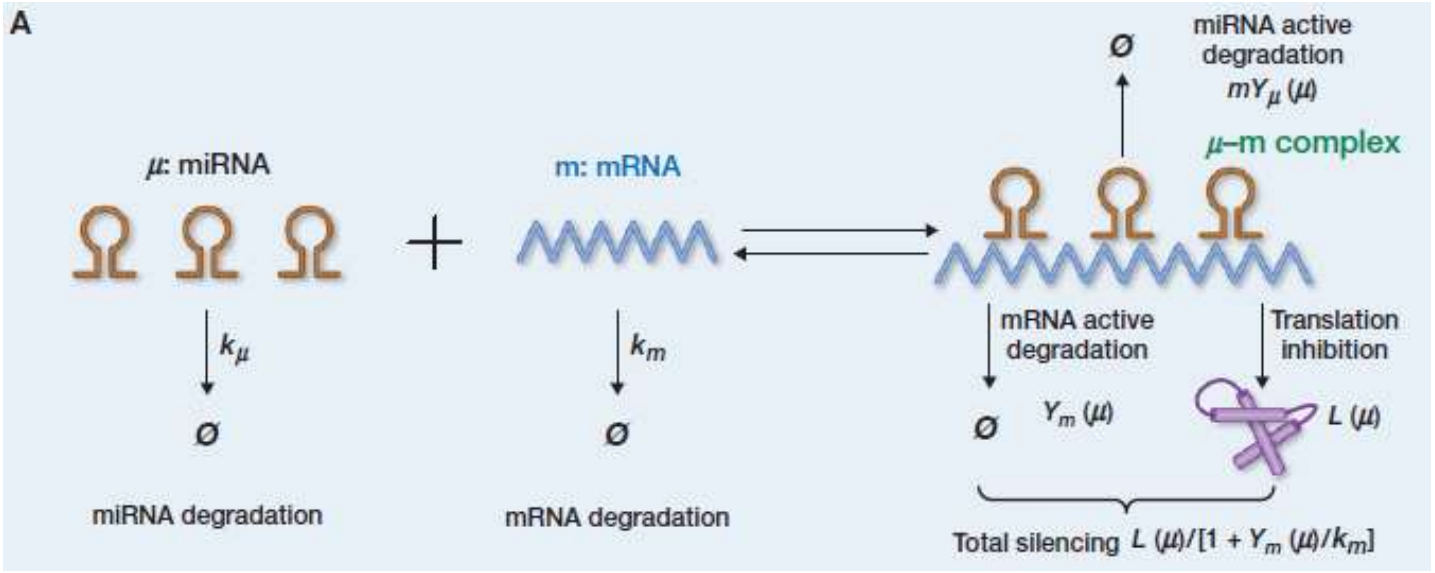
Transcriptional activation
Transcriptional repression
miR-mediated repression



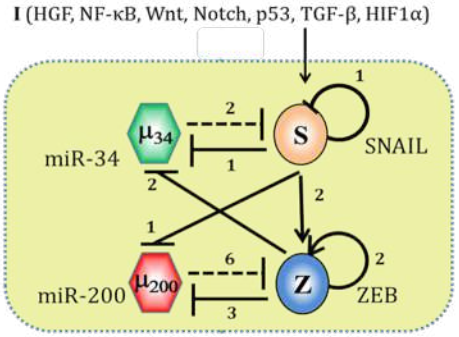
- Each arrow/bar indicates a quantitative input-output relationship.
- Such models have been extensively built for simpler microorganisms.
- Can we decode the emergent properties of these nonlinear interactions?

Lu*, Jolly* *et al.* PNAS 2013

Mathematical model formulation



Lu*, Jolly* *et al.* PNAS 2013



Production

Degradation

miR regulation

TF regulation

$$\frac{d\mu_{200}}{dt} = g_{\mu_{200}} H^S(Z,\lambda_{Z,\mu_{200}}) H^S(S,\lambda_{Z,\mu_{200}}) - m_Z Y_\mu(\mu_{200}) - k_{\mu_{200}}\mu_{200}$$ miR-200

$$\frac{dm_Z}{dt} = g_{m_Z} H^S(Z,\lambda_{Z,m_Z}) H^S(S,\lambda_{S,m_Z}) - m_Z Y_m(\mu_{200}) - k_{m_Z} m_Z$$ ZEB mRNA

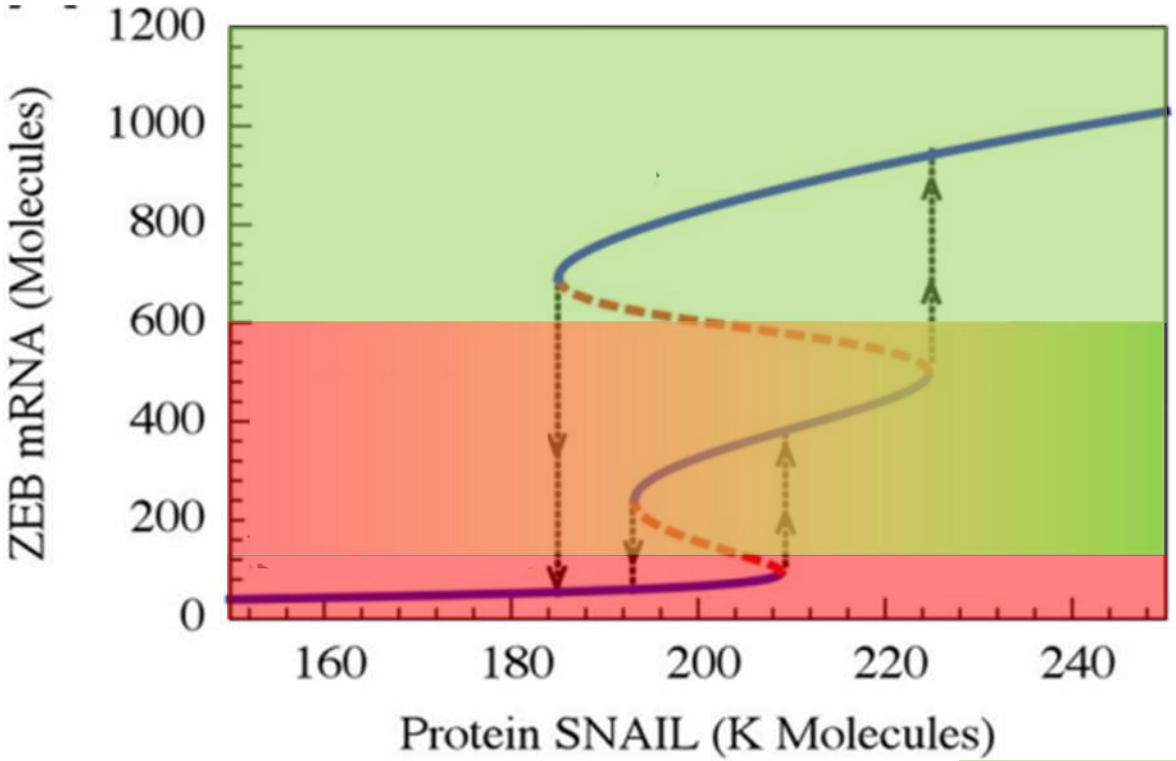
$$\frac{dZ}{dt} = g_Z m_Z L(\mu_{200}) - k_Z Z$$ ZEB

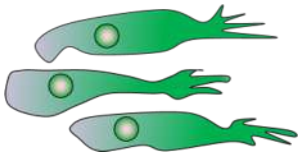
$$\frac{d\mu_{34}}{dt} = g_{\mu_{34}} H^S(Z,\lambda_{Z,\mu_{34}}) H^S(S,\lambda_{Z,\mu_{340}}) - m_S Y_\mu(\mu_{34}) - k_{\mu_{34}}\mu_{34}$$ miR-34

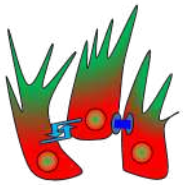
$$\frac{dm_S}{dt} = g_{m_S} H^S(S,\lambda_{S,m_S}) H^S(I,\lambda_{I,m_S}) - m_S Y_m(\mu_{34}) - k_{m_S} m_S$$ SNAIL mRNA

$$\frac{dS}{dt} = g_S m_S L(\mu_{34}) - k_S S$$ SNAIL

Model prediction: EMT is NOT binary



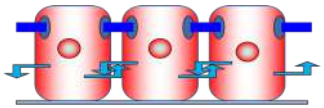


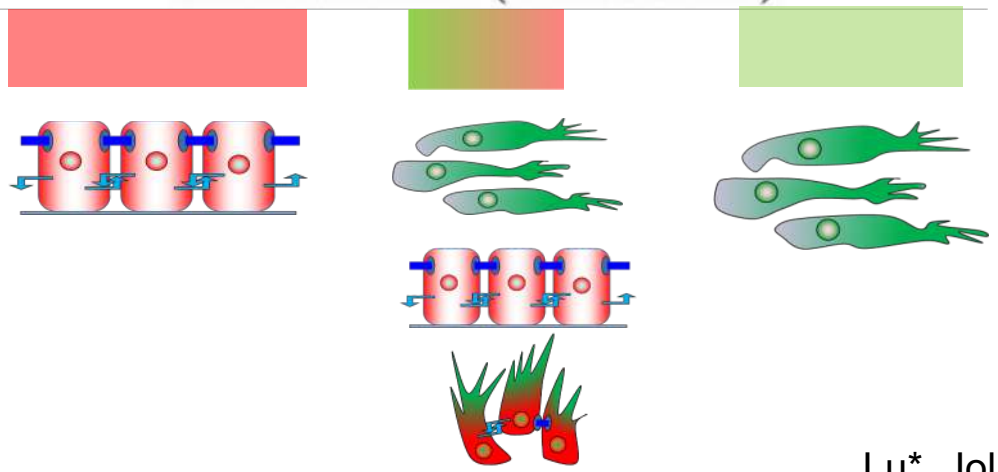


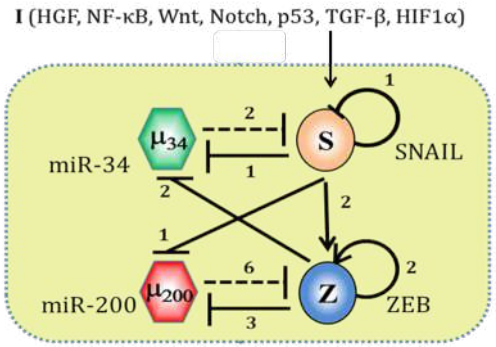
Hybrid E/M

Adhere AND

migrate collectively

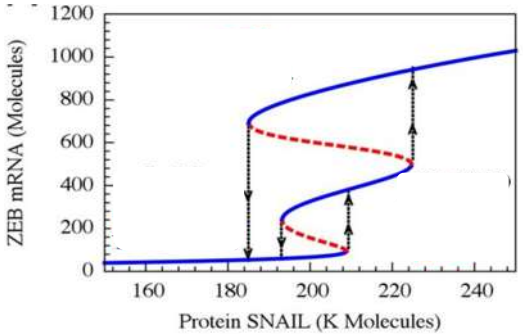






Lu*, Jolly* *et al.* PNAS 2013

Mathematical modeling for EMT dynamics

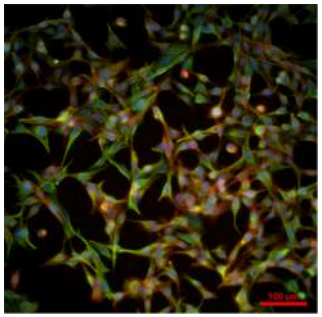


Predictions from mathematical model:

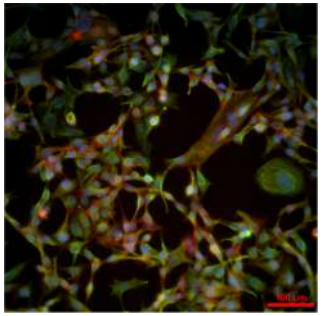
1. Cells can stably exist in hybrid E/M state
2. Isogenic cells can exist in different EMT states
3. Cells can 'spontaneously' switch their states

Lu*, Jolly* *et al.* PNAS 2013

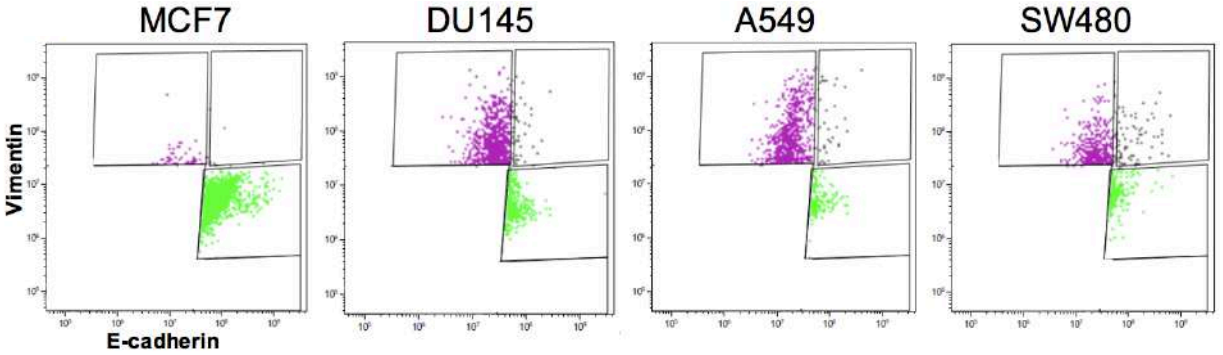
Experimental validation:



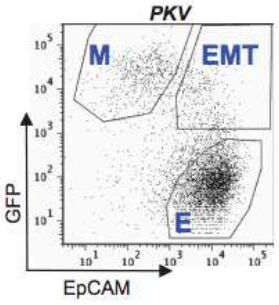
H1975, T=0

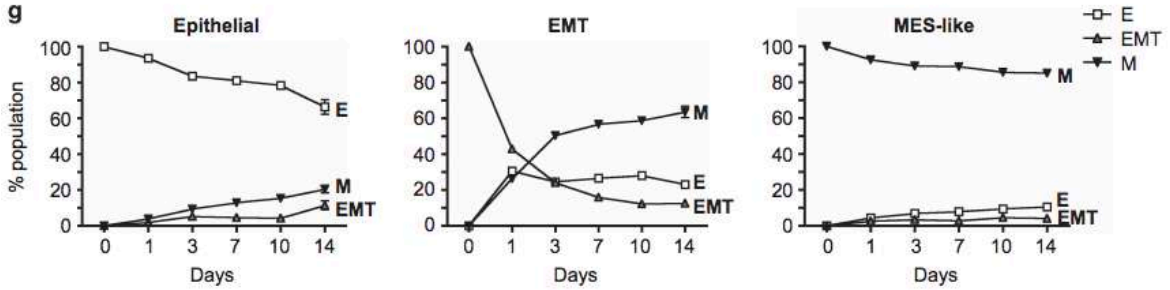


H1975, T=2 months



George*, Jolly* *et al.* Cancer Res 2017

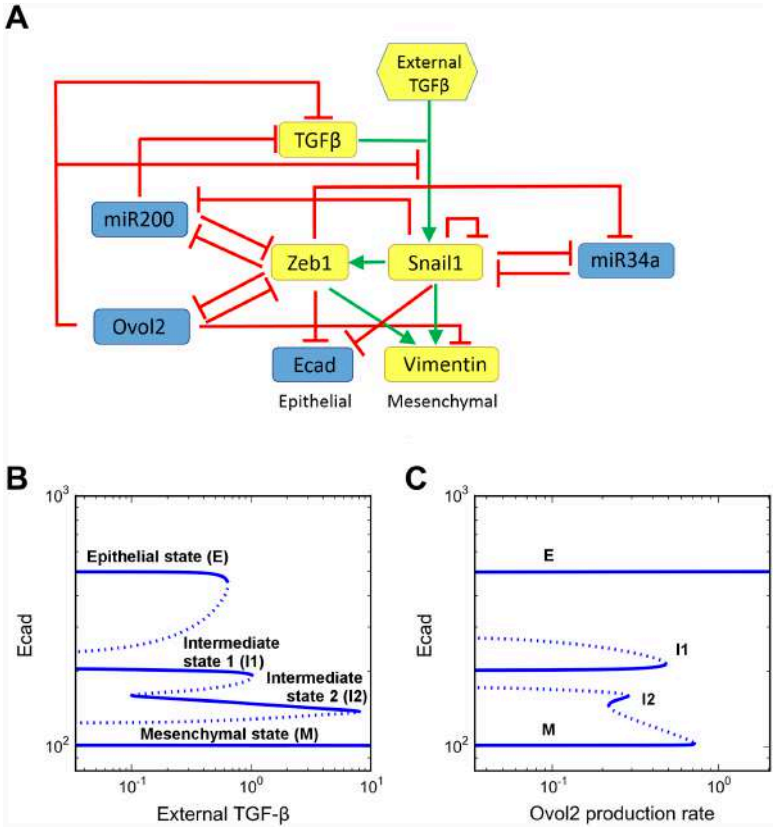


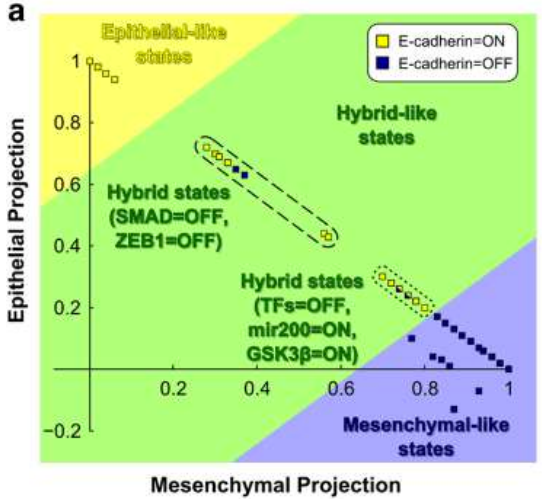


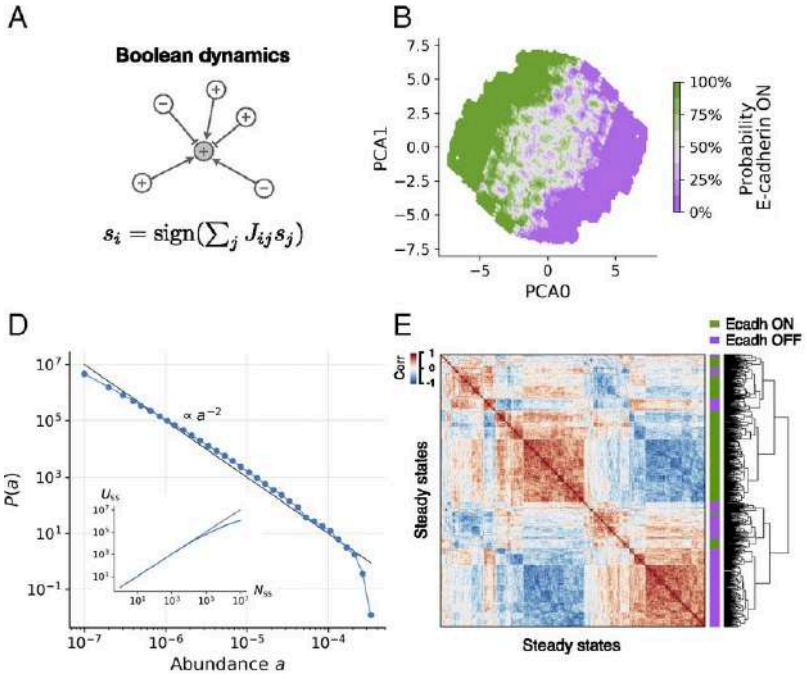
Jolly *et al.* Oncotarget 2016

Ruscetti *et al.* Oncogene 2016

Hybrid E/M phenotype(s) seen in other math models too

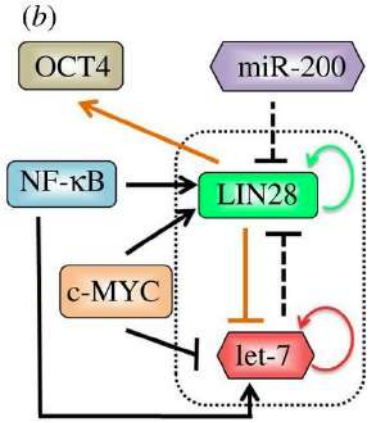


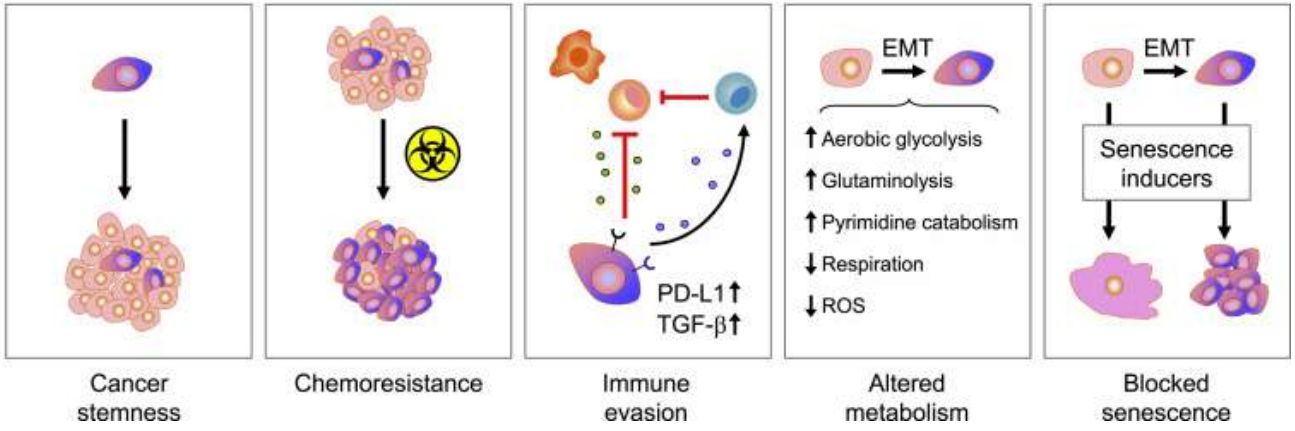


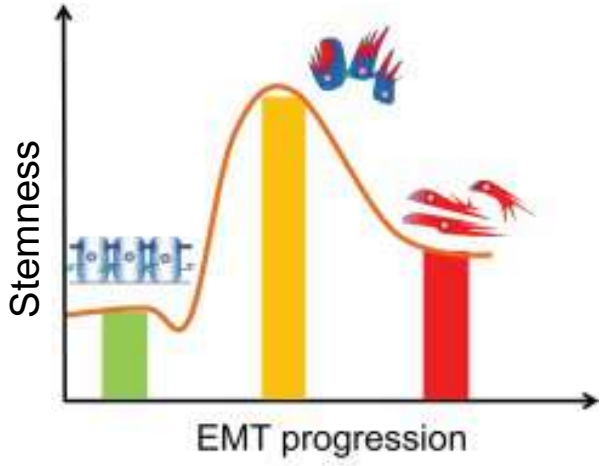


Xing *et al.* Biophys J 2013
Steinway *et al.* NPJ Sys Biol Appl 2015
Hong *et al.* PLoS Comp Biol 2015
Jolly *et al.* Oncotarget 2016
Huang *et al.* PLoS Comp Biol 2017
Font-Clos *et al.* PNAS 2018
Silveira *et al.* FEBS J 2019
Hari *et al.* NPJ Sys Biol Appl 2020

Hybrid E/M phenotype(s): 'fittest' for metastasis?



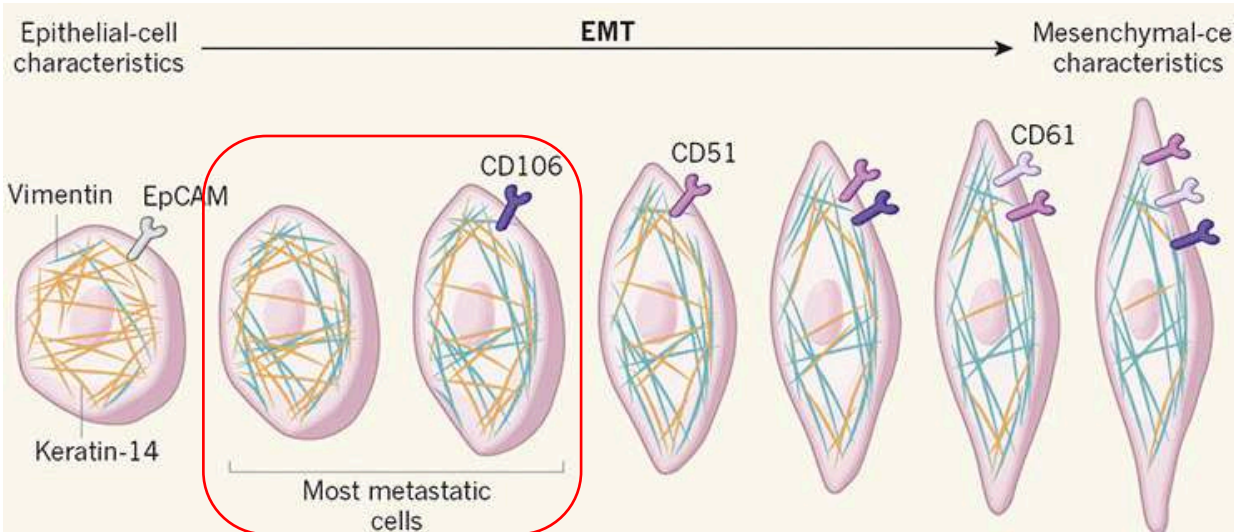




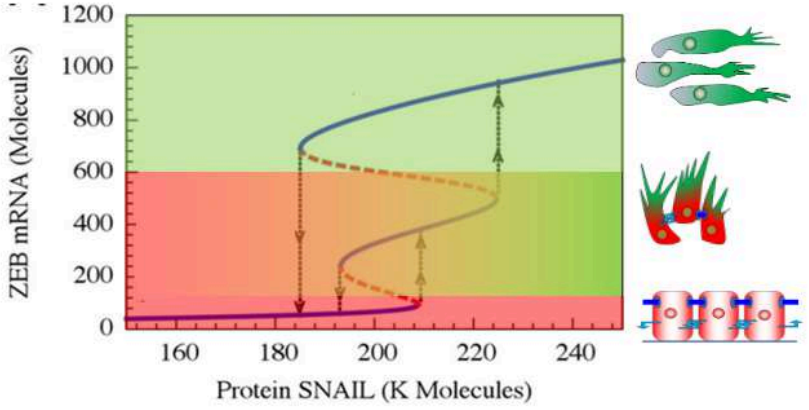
Jolly *et al.* J R Soc Interface 2014
Grosse-Wilde *et al.* PLoS One 2015
Bierie *et al.* PNAS 2017
Pastushenko *et al.* Nature 2018
Kroger *et al.* PNAS 2019
Lu & Kang, Dev Cell 2019
Pastushenko *et al.* Nature 2021

Acquisition of a hybrid E/M state is essential for tumorigenicity of basal breast cancer cells

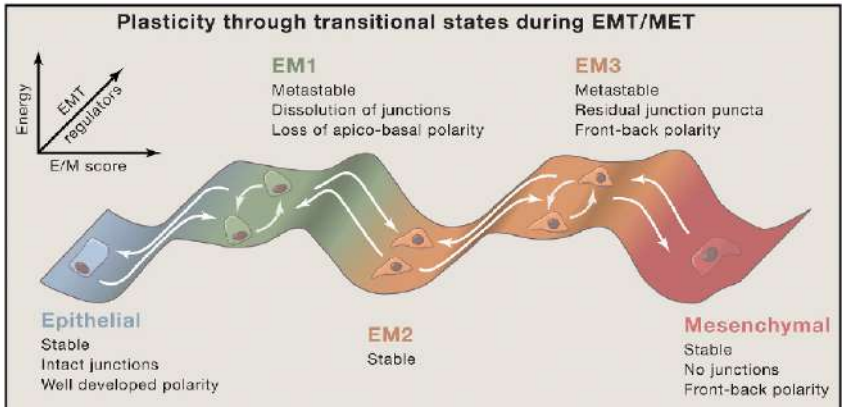
Cornelia Kröger[a], Alexander Afeyan[a,b], Jasmin Mraz[a,c], Elinor Ng Eaton[a], Ferenc Reinhardt[a], Yevgenia L. Khodor[d], Prathapan Thiru[a], Brian Bierie[a], Xin Ye[a,e], Christopher B. Burge[d], and Robert A. Weinberg[a,f,g,1]



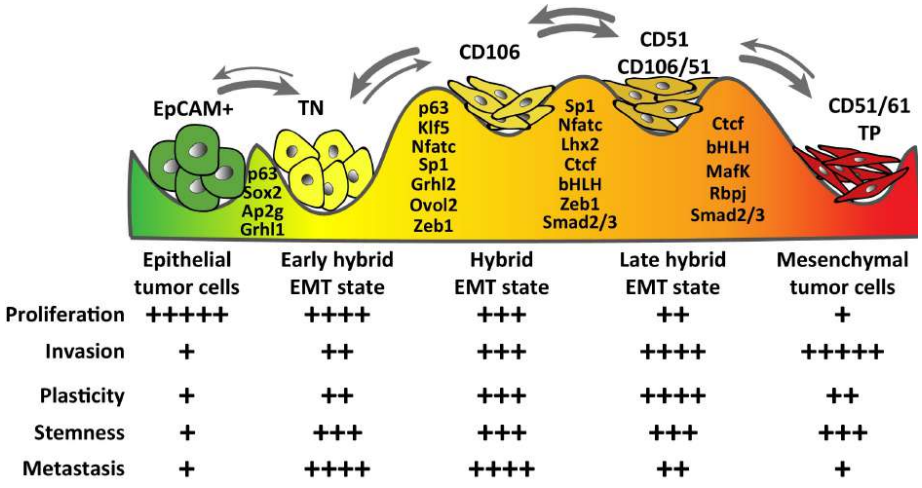
From EMT (2002-2012) to EMP (Epi-Mes Plasticity; 2013-now)



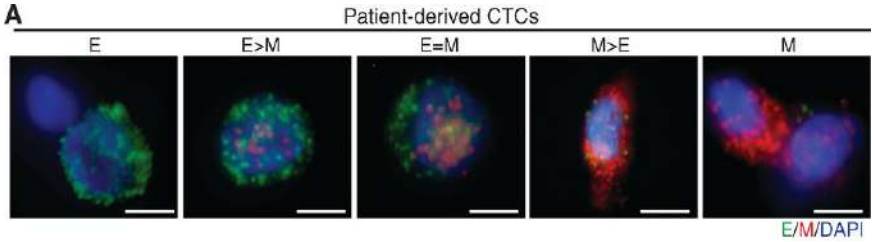
Lu#, Jolly# *et al.* PNAS 2013



Nieto *et al.* Cell 2016



Pastushenko & Blanpain, Trends Cell Biol 2019
Pastushenko *et al.* Nature 2018



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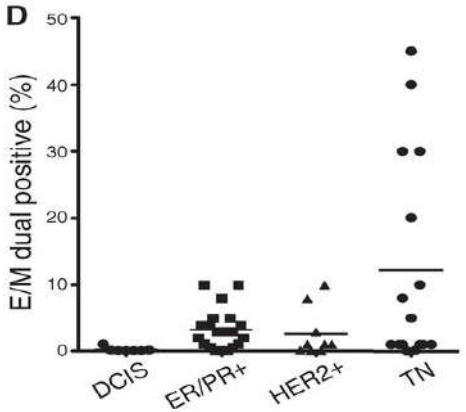
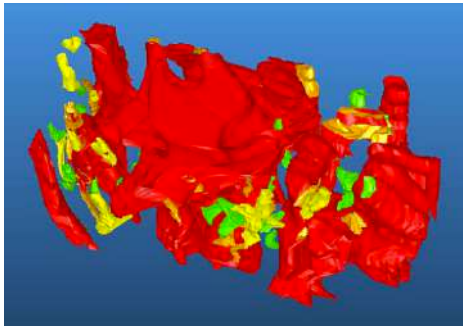
Yu *et al.* Science 2013
Kroger *et al.* PNAS 2019

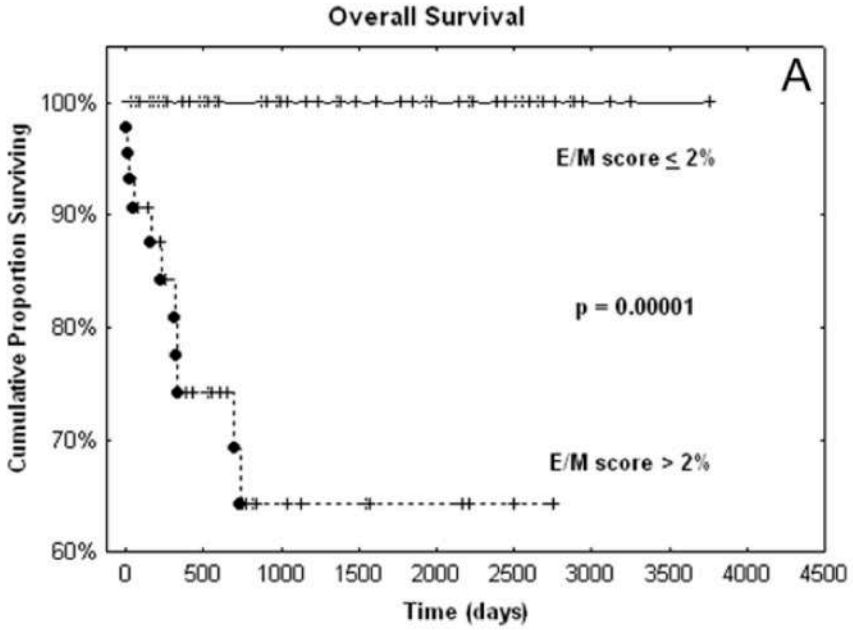
Clinical relevance of hybrid E/M phenotype(s)

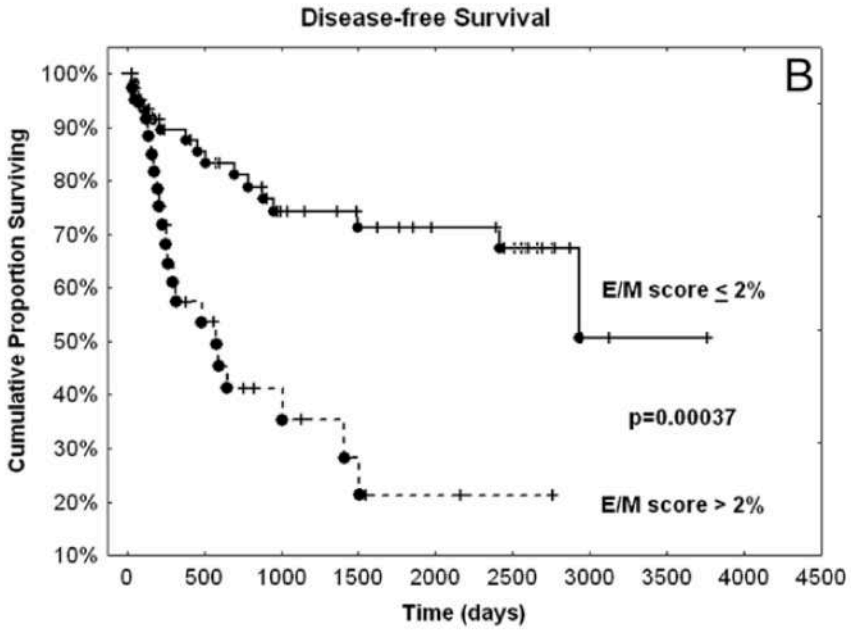




Single-cell migration is very rare, if any, in cancer

Co-expression of ZEB1 and membranous E-cad - a 'partial EMT' status of 'tumor buds'

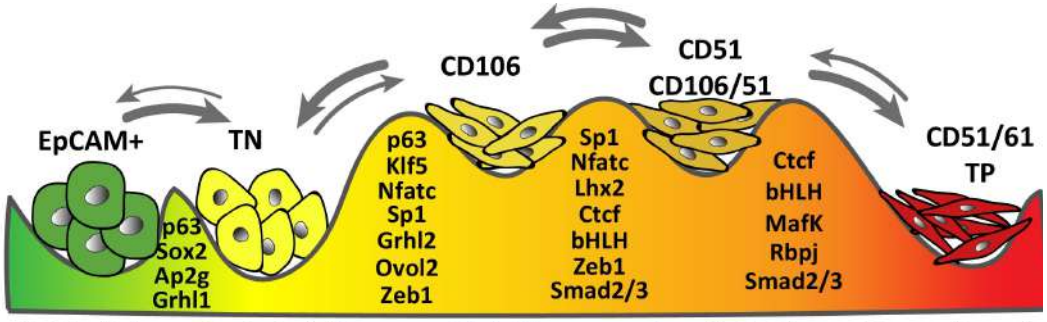




Godin *et al.* Cancers 2021; Bronsert *et al.* J Pathol 2014; Yu *et al.* Science 2013

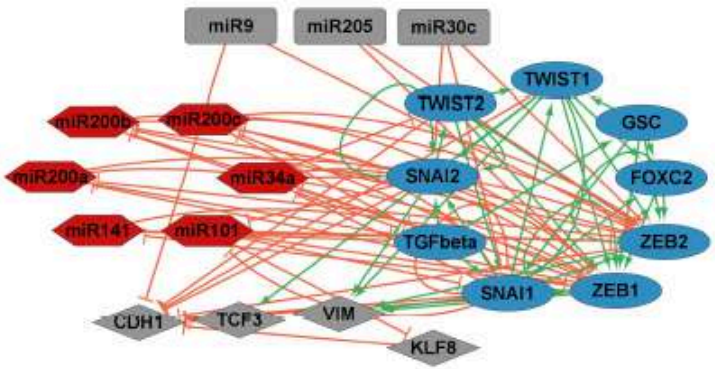
Ongoing questions…

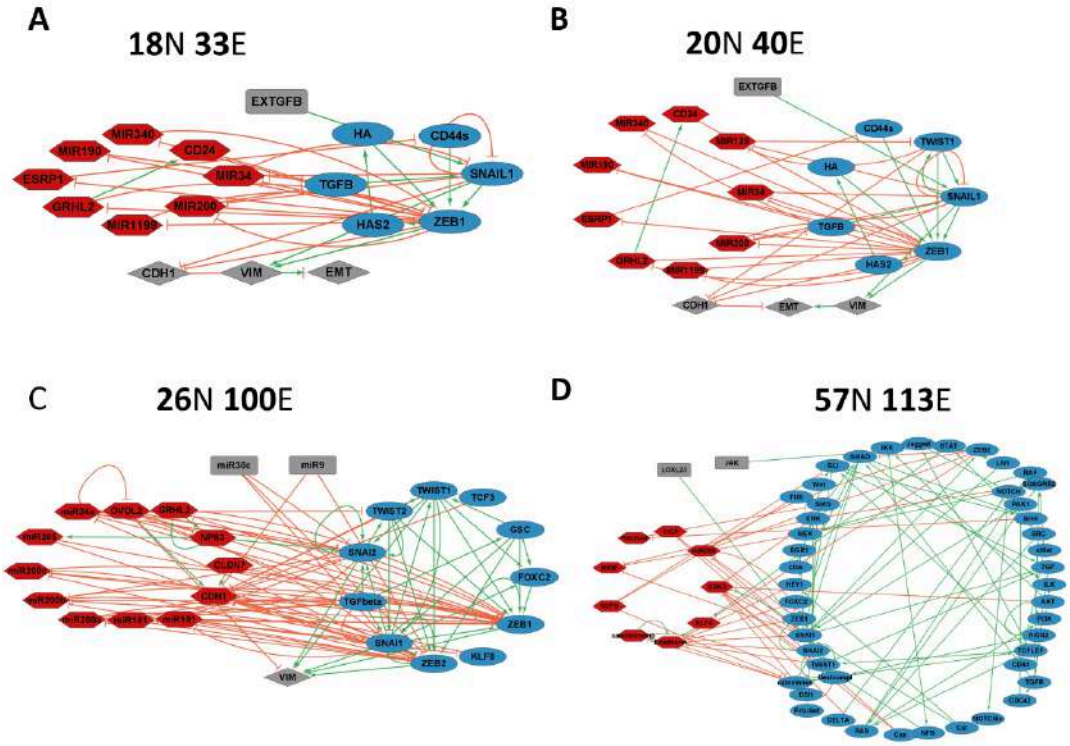
Why are hybrid E/M cells more plastic than E, M?



	Epithelial tumor cells	Early hybrid EMT state	Hybrid EMT state	Late hybrid EMT state	Mesenchymal tumor cells
Proliferation	+++++	++++	+++	++	+
Invasion	+	++	+++	++++	+++++
Plasticity	+	++	+++	++++	++
Stemness	+	+++	+++	+++	+++
Metastasis	+	++++	++++	++	+

EMP networks largely contain two “teams”





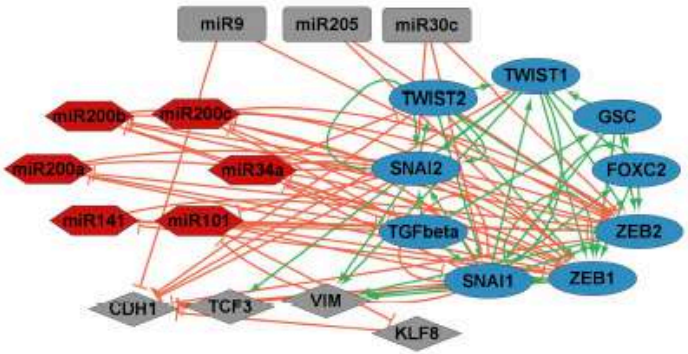
Huang *et al.* PLoS Comp Biol 2017
Jia *et al.* Phys Biol 2019
Font-Clos *et al.* PNAS 2018
Silveira *et al.* FEBS J 2019; JRSI 2020

- Nodes: Epithelial, Mesenchymal, Input/output (Signal/Effector)
- Edges: Activation, Inhibition

- Mostly activation within a “team”, but inhibition across the two “teams”

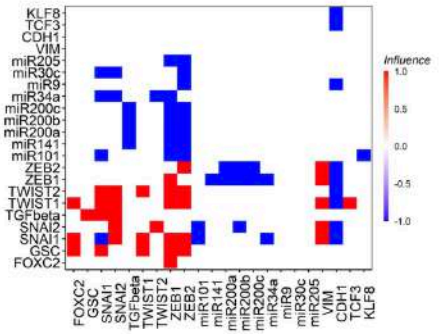
Hari *et al.* bioRxiv 2021: 472090

Presence of teams is specific to EMP networks

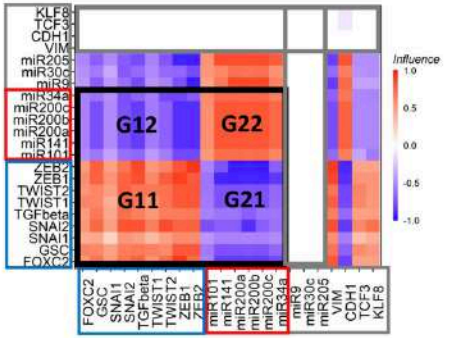


22N 82E

22N 82E
Adjancency Matrix



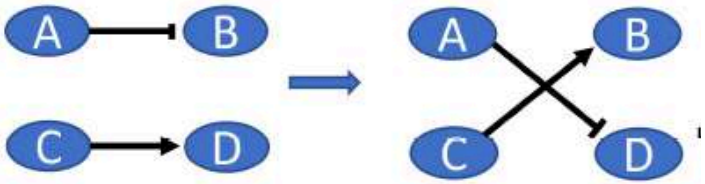
22N 82E
Influence Matrix

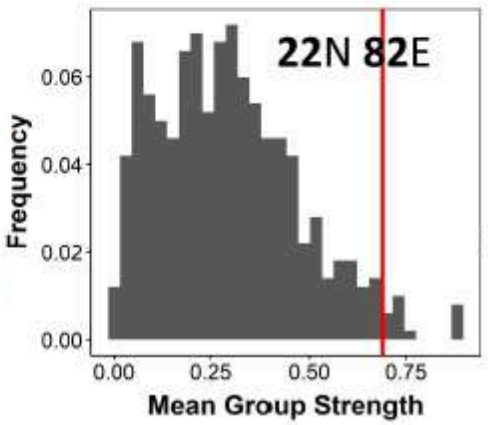


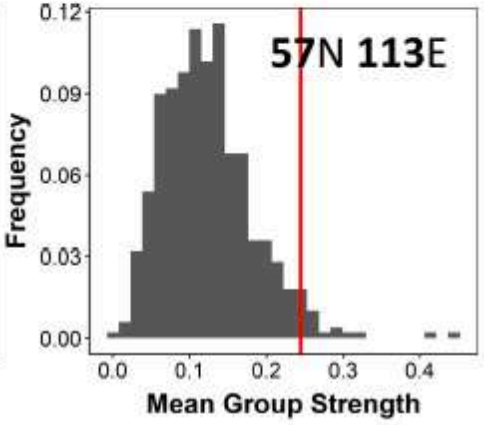
$$Infl = \frac{\sum_{l=1}^{lmax} \frac{Adj^l}{Adj^l_{max}}}{lmax}$$

$$G_{IJ} = \frac{\sum_{i \in I, j \in J} Infl_{ij}}{n_{II}}$$

$$G_S = \sum_{i,j \in \{1,2\}} |G_{ij}|$$







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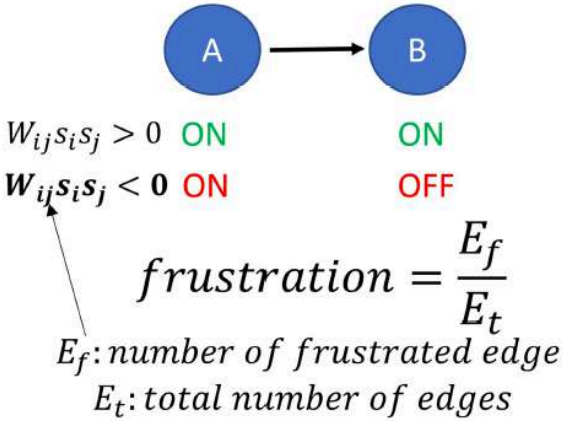
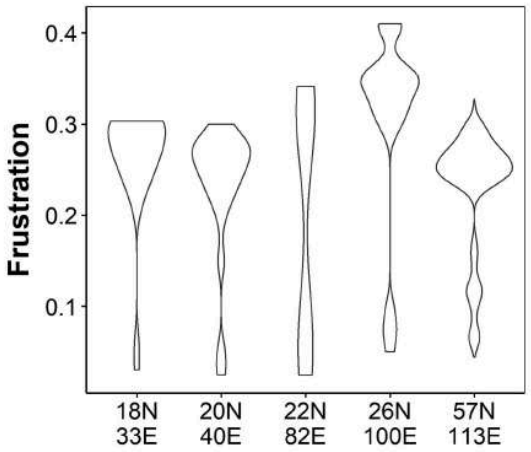
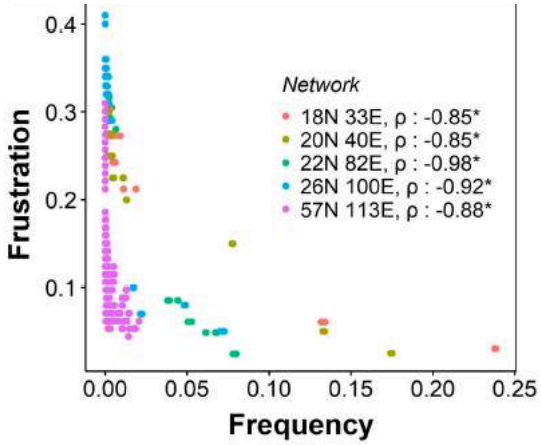
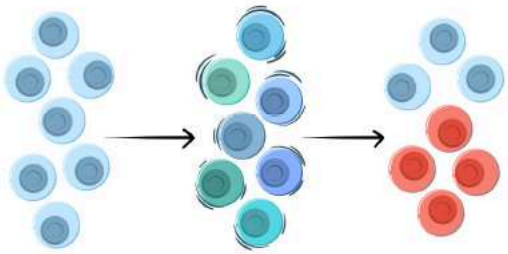
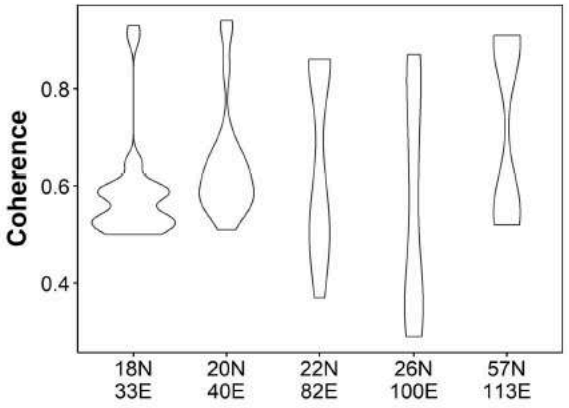
EMP networks give rise to two types of states

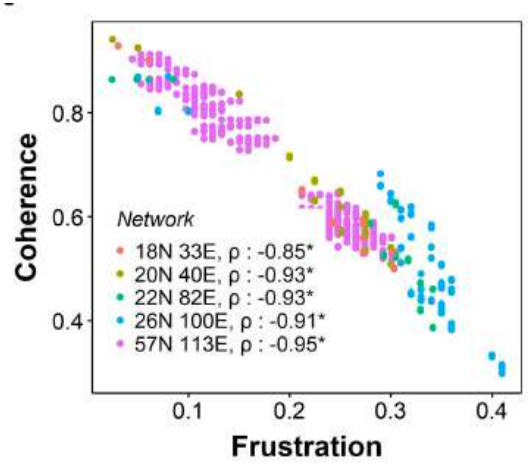












Which phenotypes (E, M, hybrid) are more frustrated or coherent?

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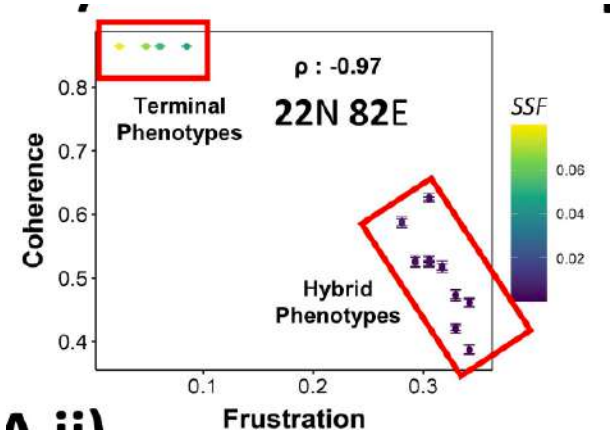
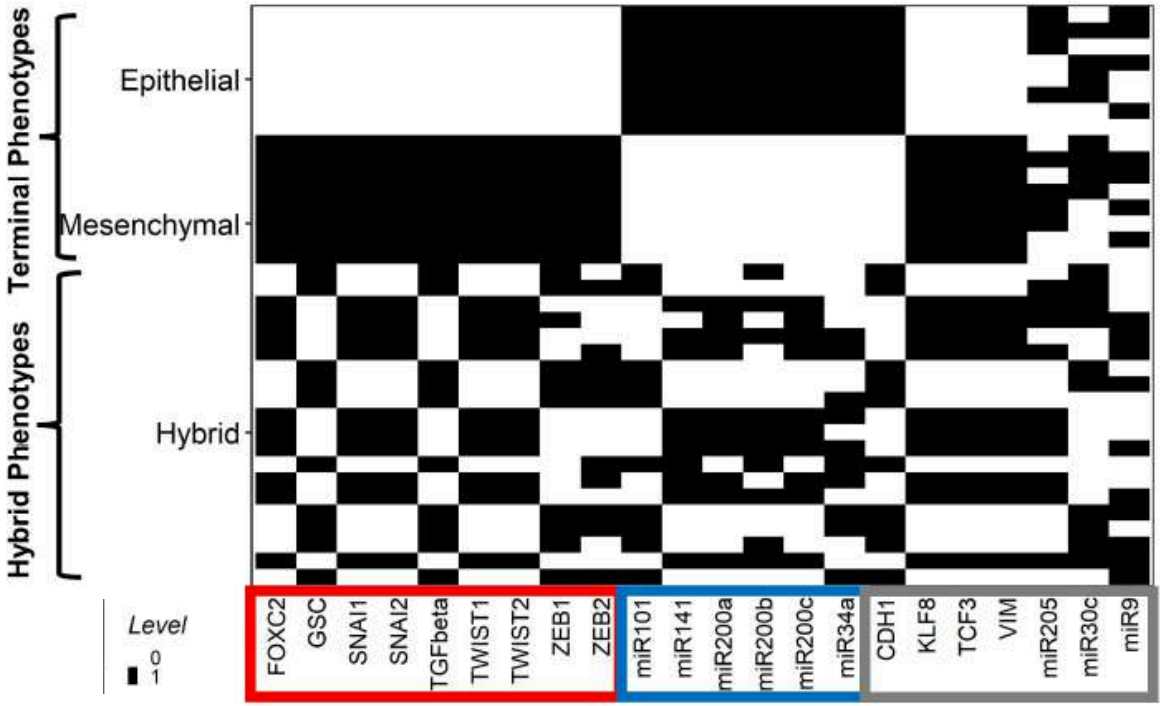
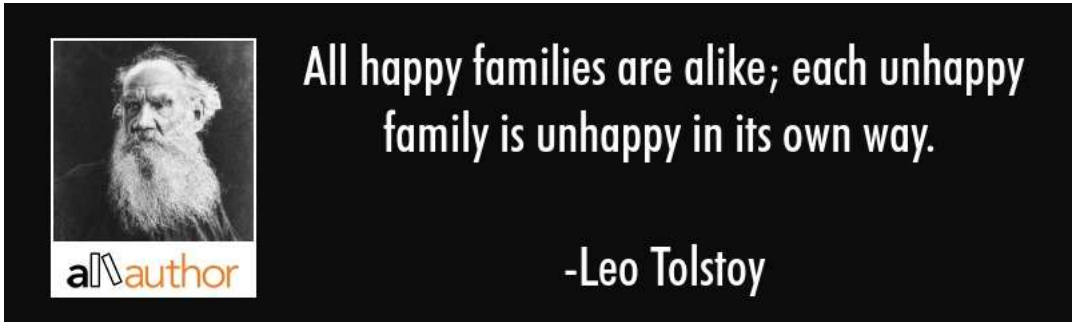
Terminal states (E, M) more stable than hybrid E/M



Terminal state more coherent, frequent; less frustrated than hybrid





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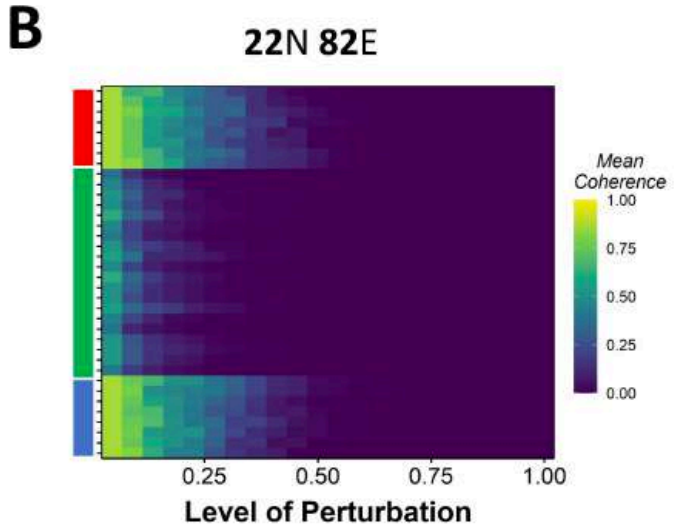
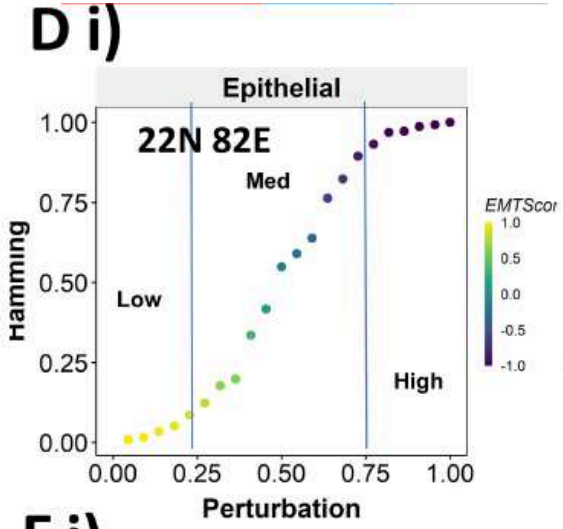
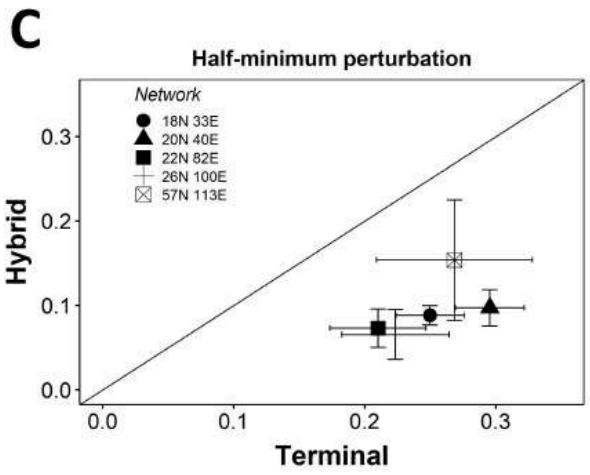
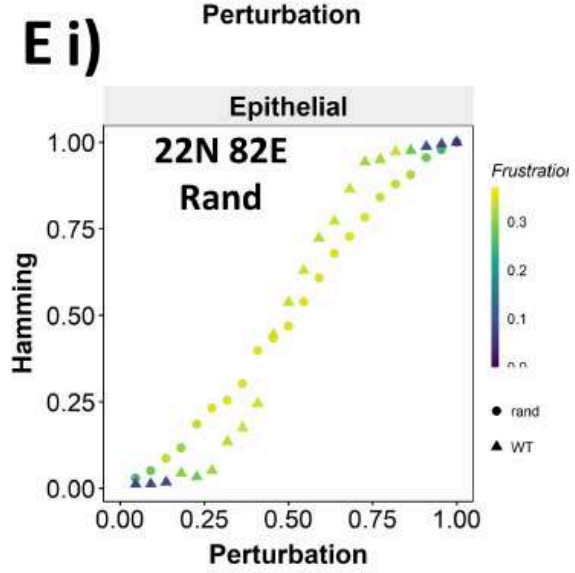
“Teams” stabilize terminal states (E, M) specifically









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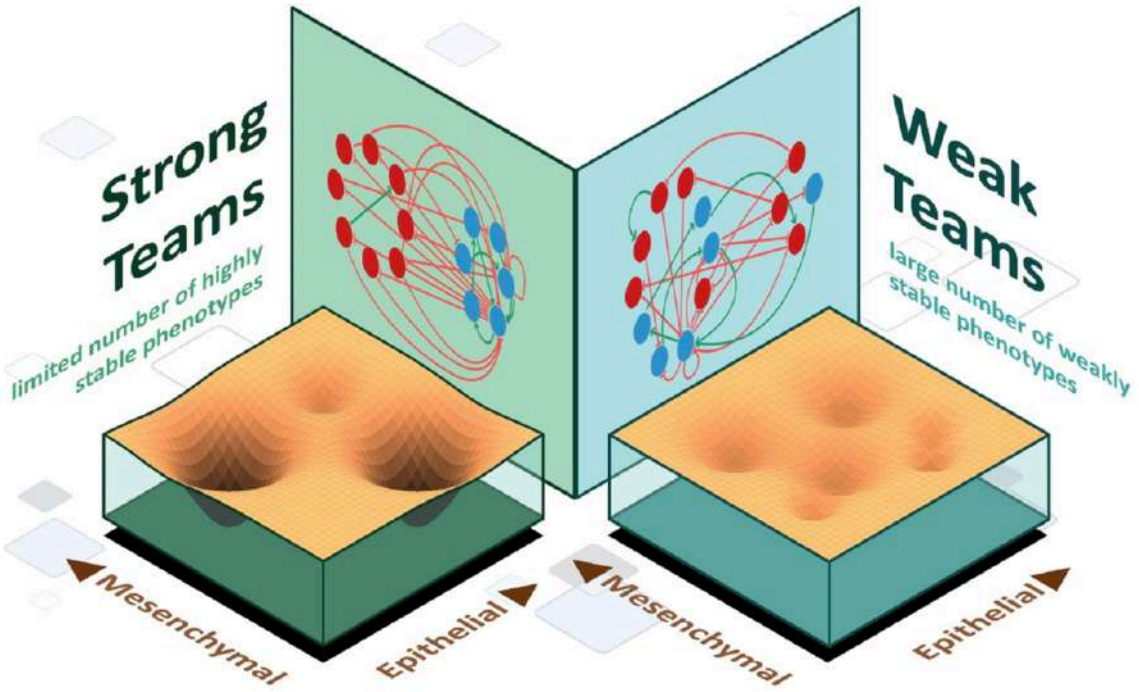
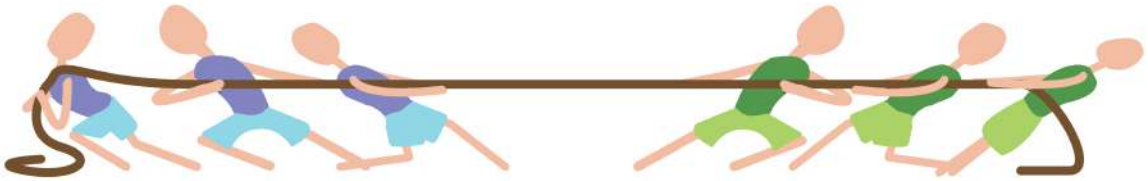
Summary (Teams in EMP networks)



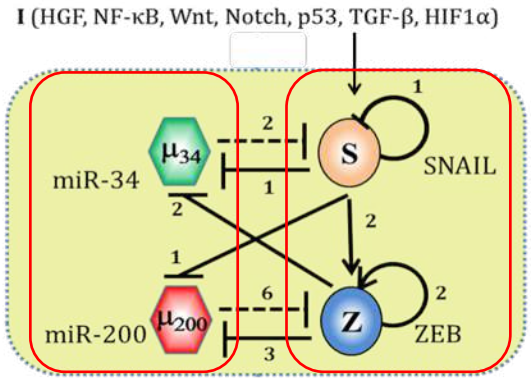
"Teams" shape the landscape enabling higher plasticity and heterogeneity of hybrid E/M phenotypes

Hari *et al.* bioRxiv 2021: 472090

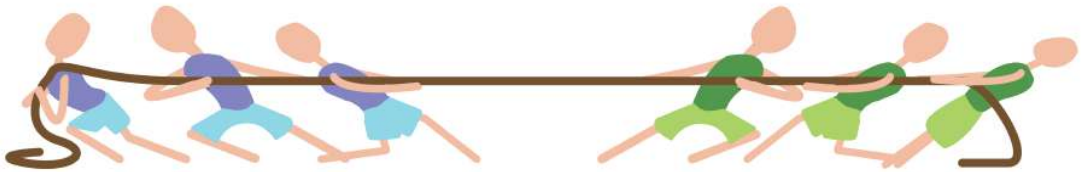


Kishore Hari

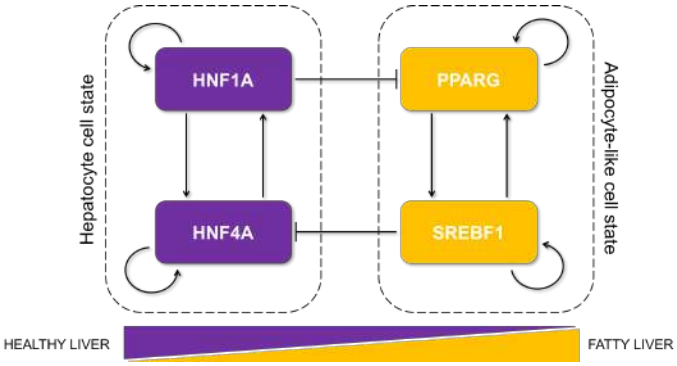
‘Teams’ seen in other cell-state switching networks?



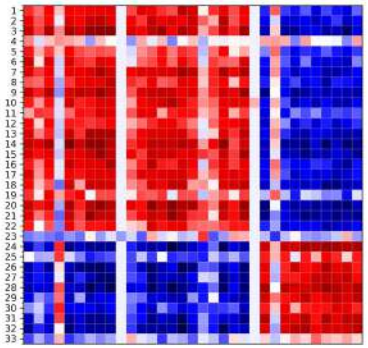
Lu*, Jolly* *et al.* PNAS 2013



Artwork Credit: Atchuta S Duddu

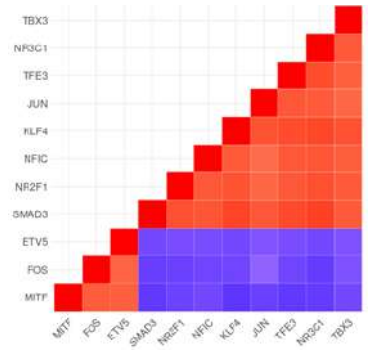


Sahoo*, Singh* *et al.* J Clin Med 2020



Small Cell Lung Cancer (SCLC)

Chauhan*, Ram* *et al.* eLife 2021



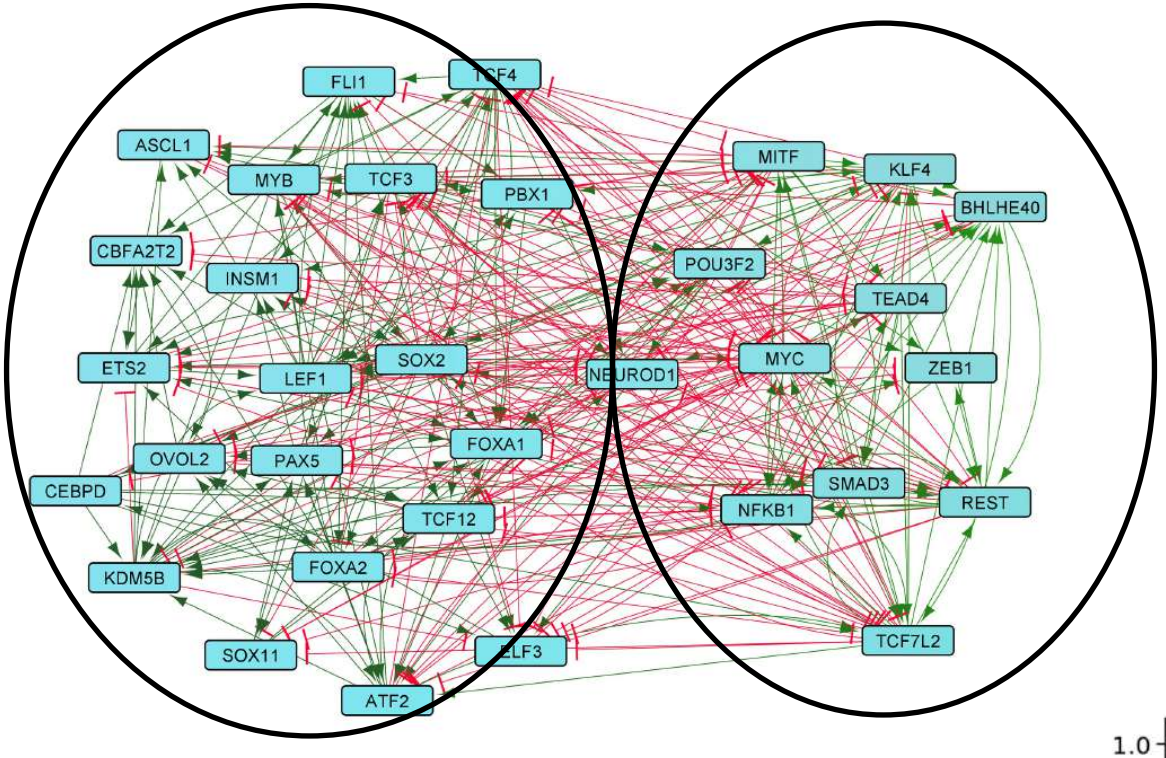
Proliferative-invasive switch in melanoma

Pillai & Jolly, iScience 2021

Divyoj Singh (BS/MS, IISc, Phy)

Sarthak Sahoo (BS/MS, IISc, Bio)

Why do ‘teams’ exist?

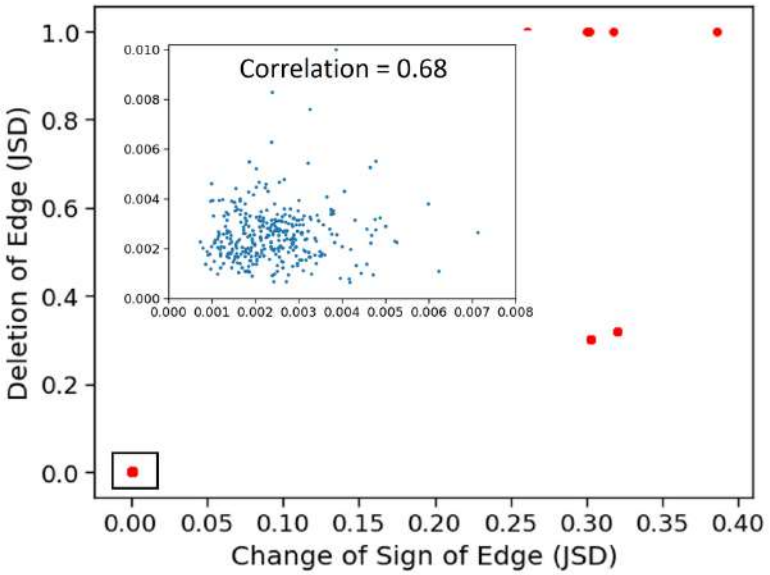


33 nodes, 357 edges - **SCLC**

This network gave rise to 4 states, each with ~25% frequency.

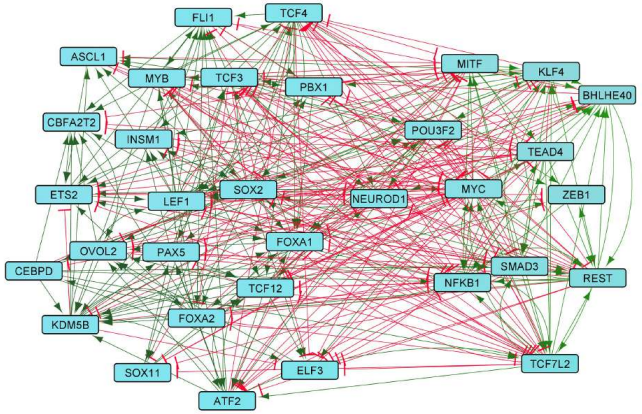
What if we delete an edge (in silico CRISPR)?

Offer robustness (Deletion of 345 out of 357 edges – one at a time – have minimal effect on SCLC cell states)



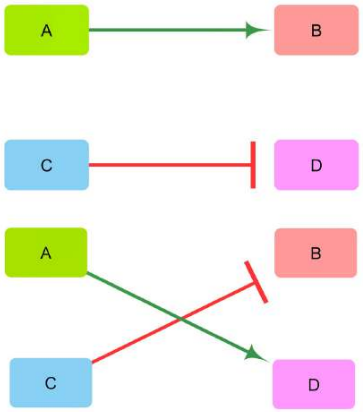
Chauhan*, Ram*, Hari & Jolly MK, eLife 2021

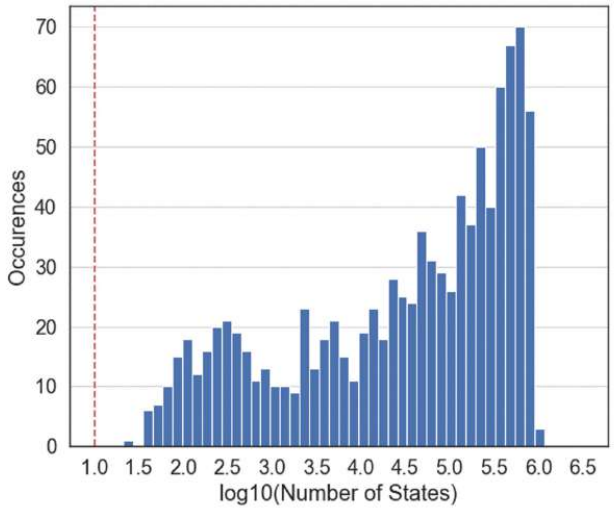
Why do ‘teams’ exist?

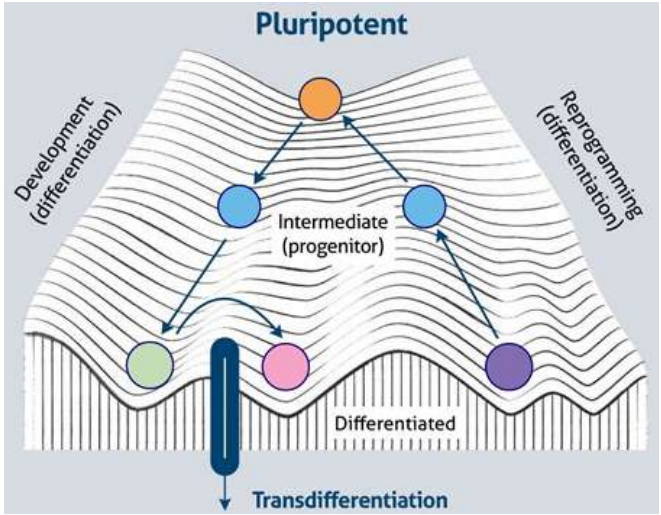


33 nodes, 357 edges - **SCLC**

What if we shuffle edges in the entire network (thus breaking teams)?







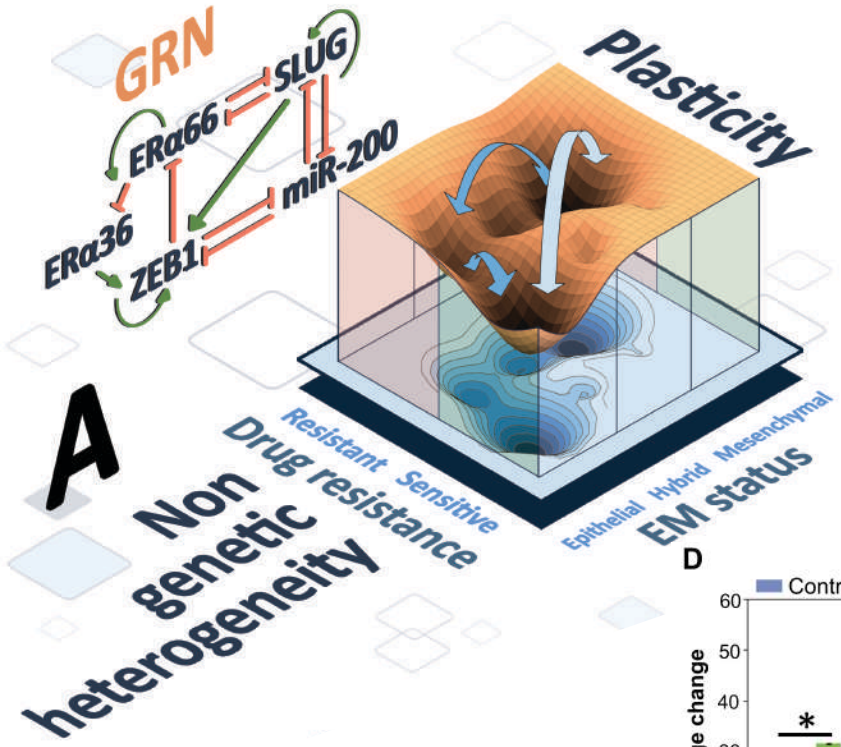
Allow limited number of cell-states

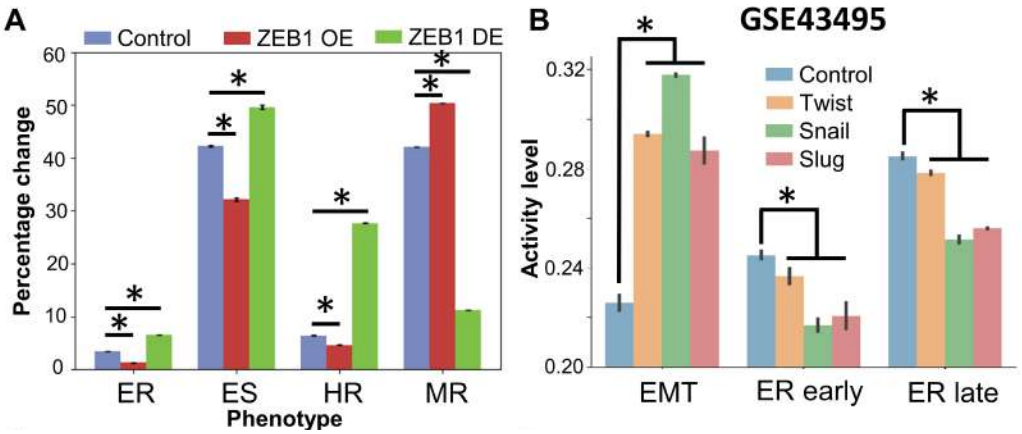
(“Controlled enthusiasm”)

Chauhan*, Ram*, Hari & Jolly MK, eLife 2021

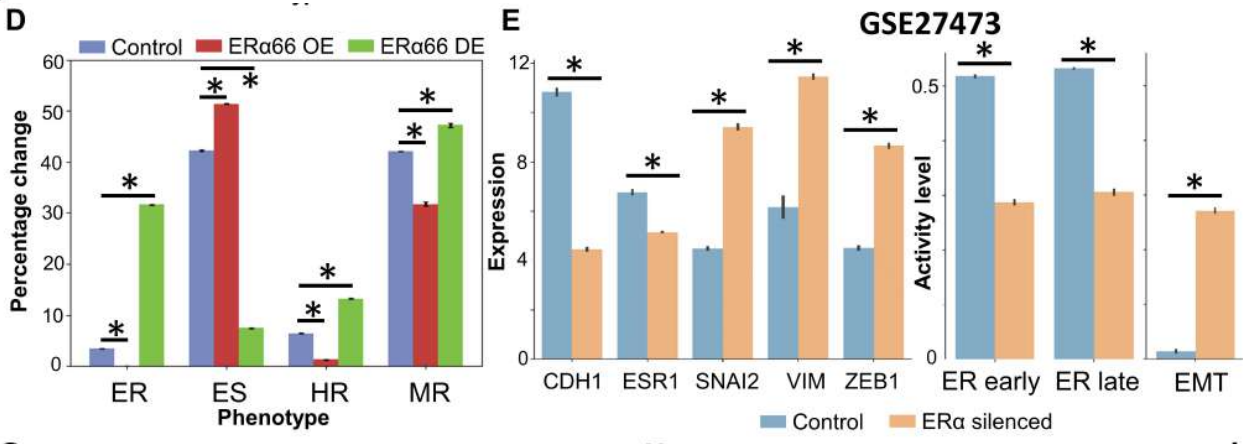
Why do ‘teams’ exist?

Couple the axes of plasticity: EMP and drug resistance in ER+ breast cancer





EMT can drive resistance to tamoxifen

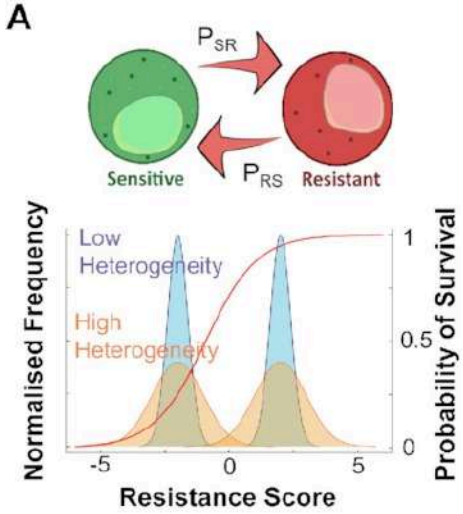


Tamoxifen resistance can drive EMT

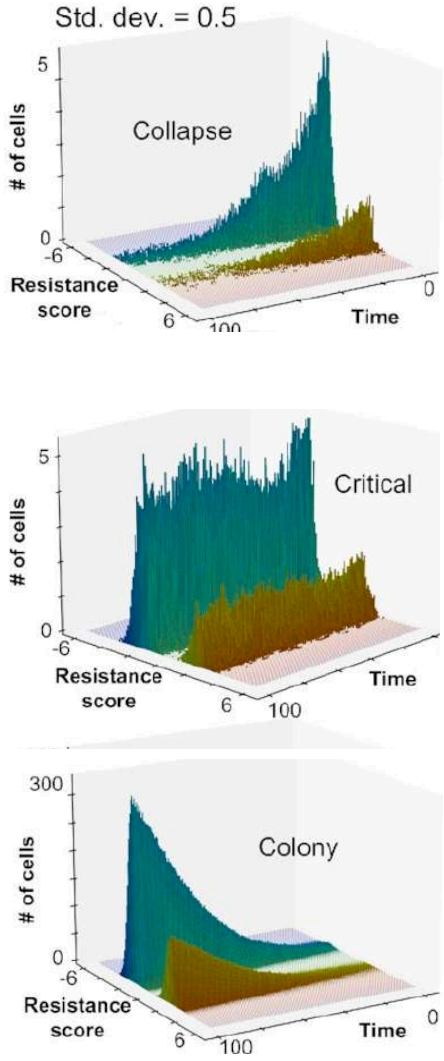
Sarthak Sahoo

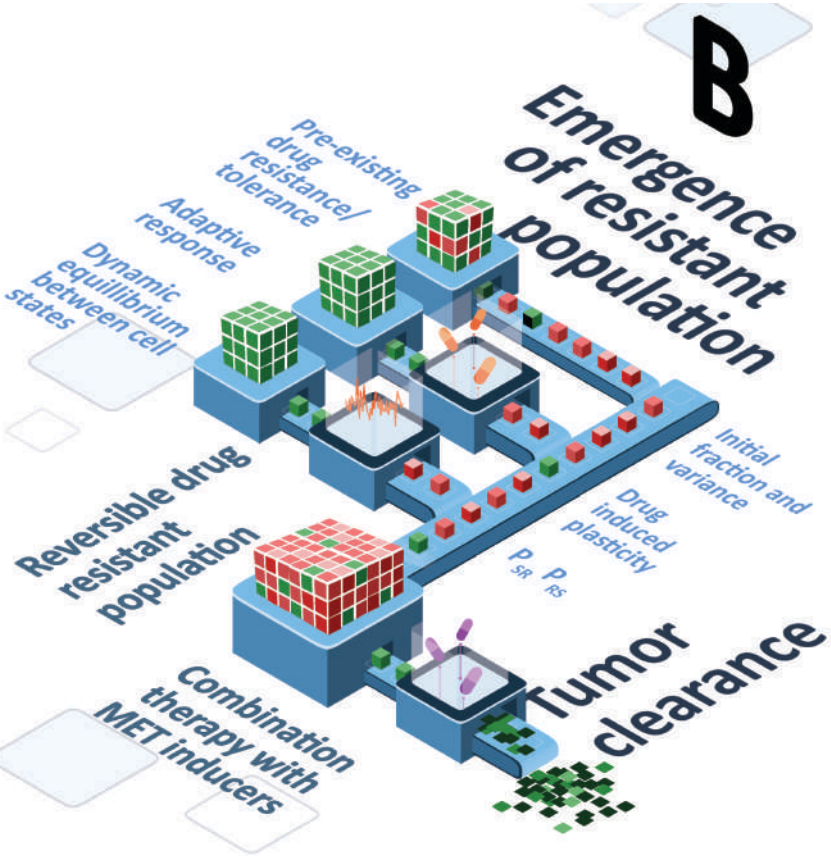
Sahoo *et al.* NAR Cancer 2021

Suggesting combinatorial therapies for ER+ breast cancer



Sahoo *et al.*
NAR Cancer 2021





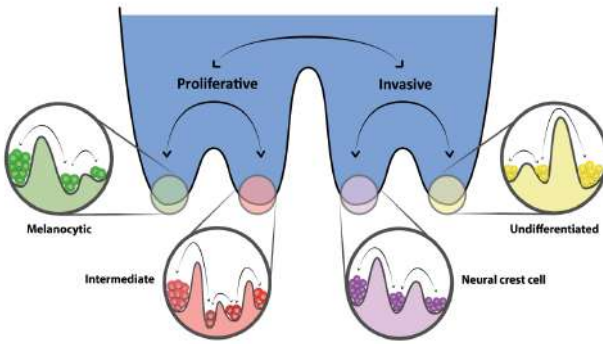
Model predictions currently undergoing experimental validation

Summary

- Multistable dynamics of underlying networks driving cell-state switching

⇒ Phenotypic plasticity

⇒ Non-genetic heterogeneity



Pillai & Jolly, **iScience** 2021

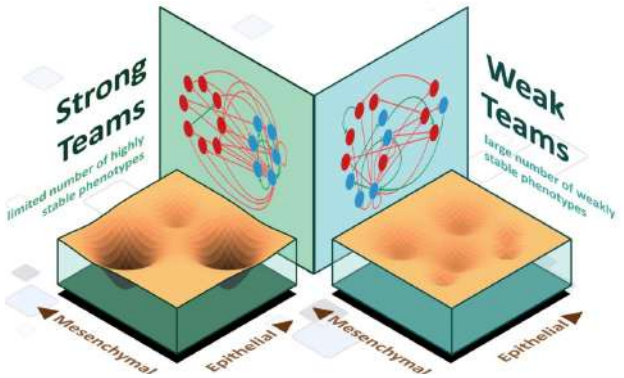
- 'Design principles' of such networks:

1. "Teams" exist in multiple such networks
2. "Teams" offer canalization of phenotypes

Chauhan*, Ram* *et al.* **eLife** 2021

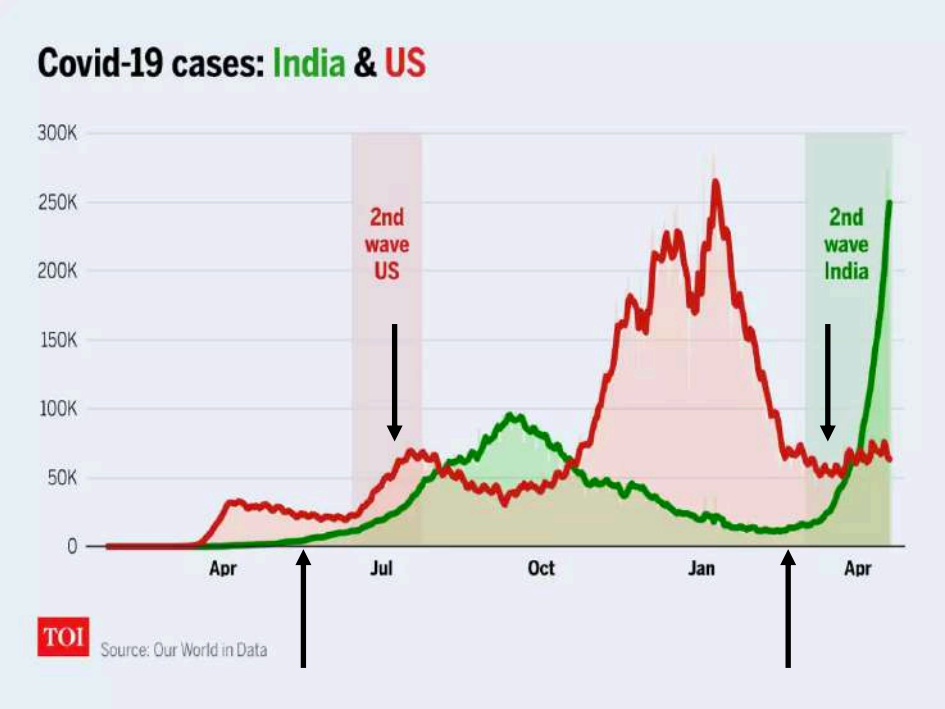
- These networks can explain adaptive, heterogeneous response to drug treatment

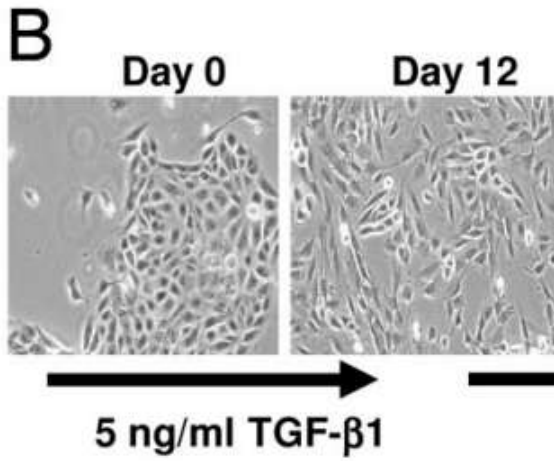
- These networks as platforms to predict combination and sequence of therapies?



Hari *et al.* **bioRxiv** 2021: 472090

Dynamical vs. Static hallmarks of Cancer





Mani *et al.*
PNAS 2007

Cancer is a complex, dynamic, adaptive system
(and therefore needs to looked at such).

Math models, coupled with experimental data, have steered our understanding of such systems (weather predictions, finance etc.)

Acknowledgements: Cancer Systems Biology Lab

Any questions/comments/ suggestions welcome!

mkjolly@iisc.ac.in

Background of CSB members

Biotechnology, Engineering (Electrical, Mechanical), Bioinformatics, Physics, Mathematics, Biology, Chemistry