20.109 Module 2

Lecture #5: **Introduction to screening: concepts & principles II:** *Phenotypic Screens*

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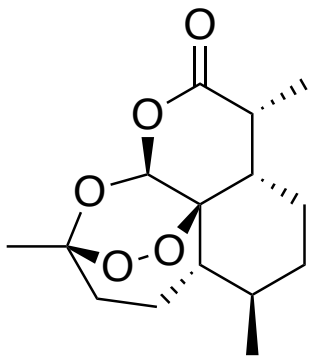
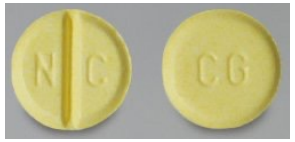
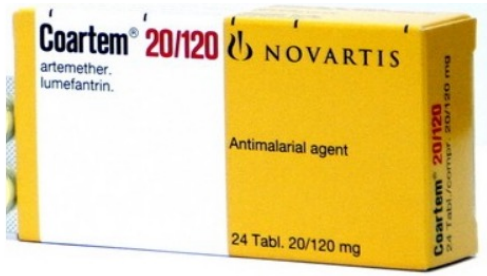






Modern framework for drug discovery and development

A. Basic science research and target identification

B. Target pharmacology and biomarker development

C. Lead identification

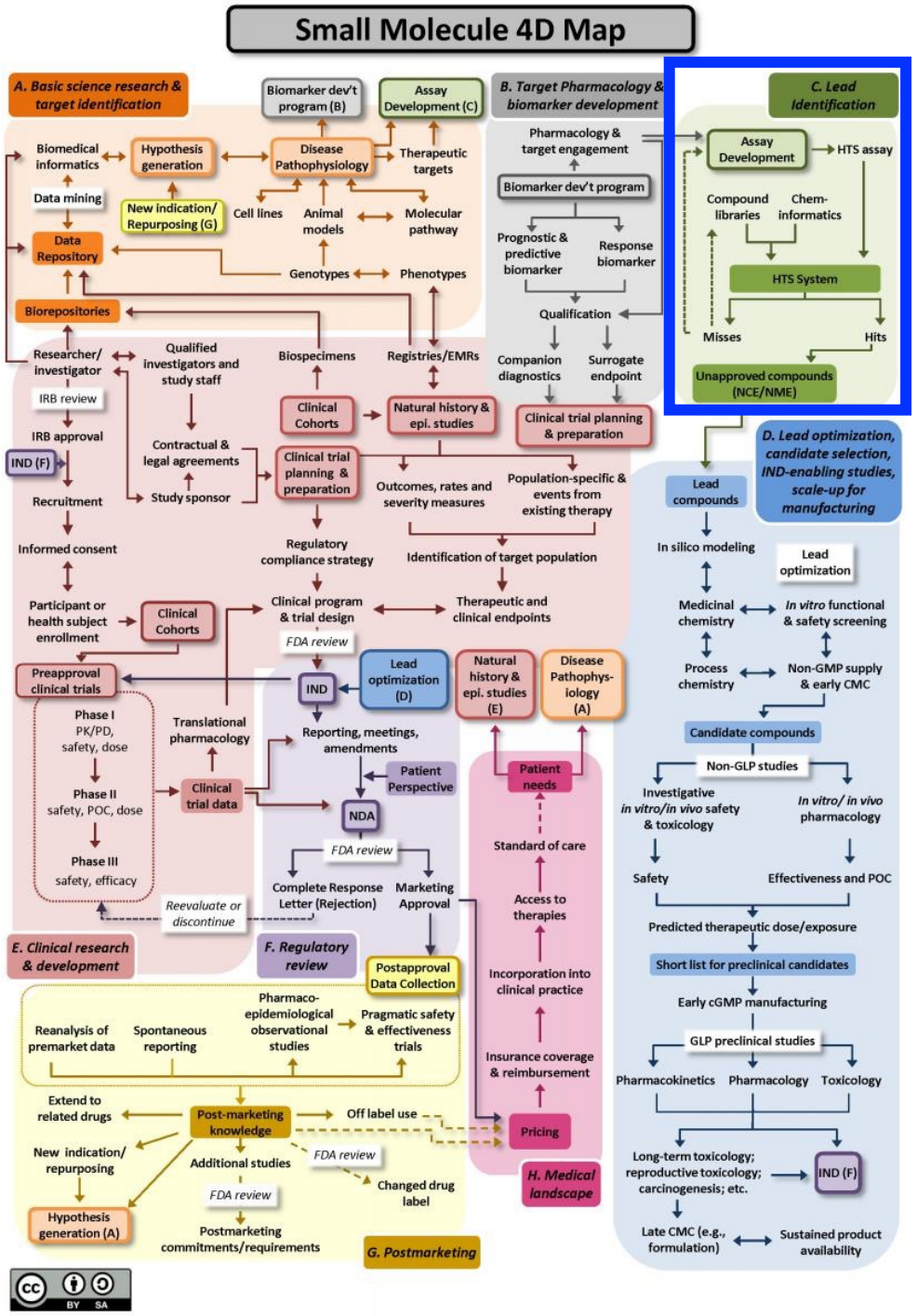
D. Lead optimization and candidate selection
- Improving pharmacologic, metabolic, safety profiles of lead toward use in humans

E. Clinical research & development
- Clinical trials to establish efficacy and safety

F. Regulatory review (FDA approval)

G. Post-marketing
- Surveillance (adverse effects)
- Repurposing
- Off-label use

H. Medical landscape

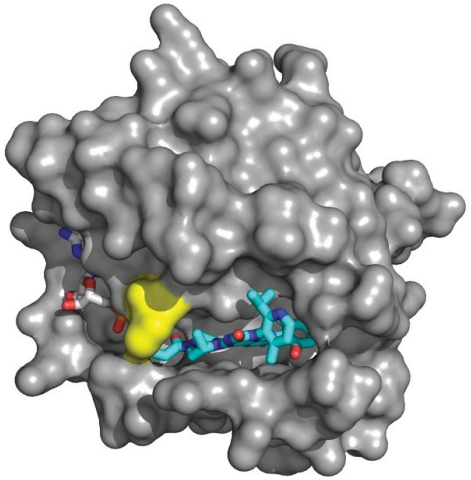


References:
1) Wagner et al; Nature Reviews Drug Discovery; 2018;
2) https://ncats.nih.gov/translation/maps
3) 4D Map (interactive): https://4dmap.ncats.nih.gov/#/

Target-based discovery recap…

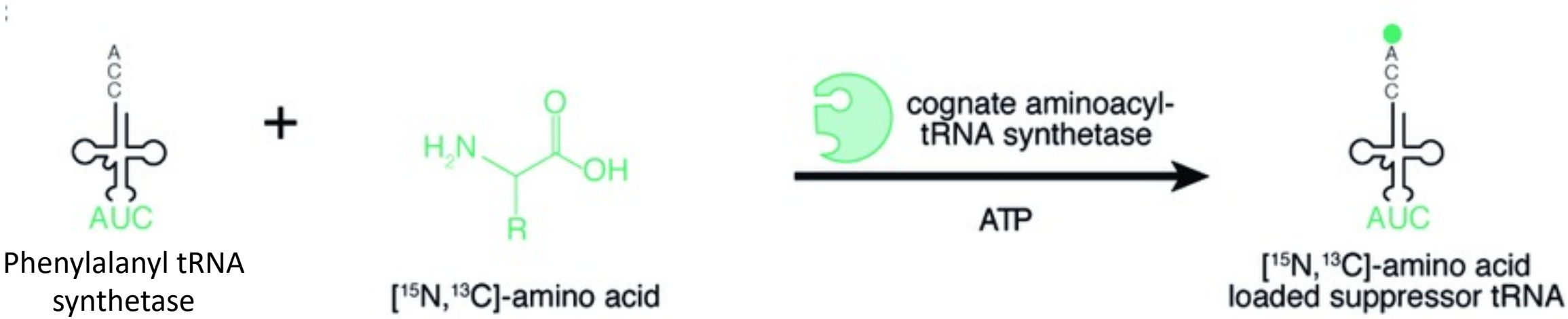
Target → Assay → Hit compounds

Compound library → Assay



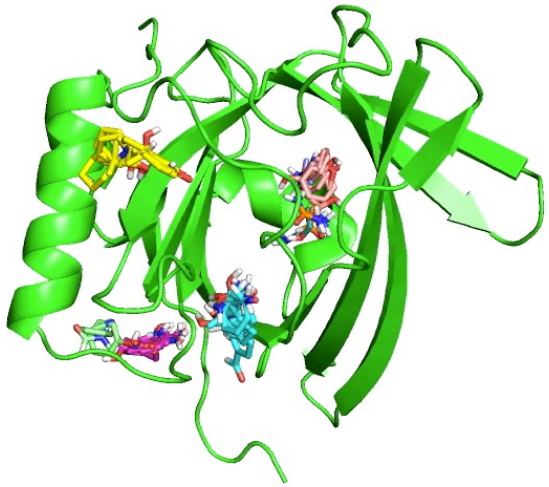
- **Target protein with surface features that can be selectively bound by small molecules that inhibit protein function**

Case Study 1: Discover inhibitors of the phenylalanyl tRNA synthetase enzyme



- Assay
  - Investigative procedure for qualitatively or quantitatively assessing the *presence*, *amount* or *functional activity* of a target entity
- Can be used in:
  - Discovery
  - Validation
- Components needed for an assay
  - Input
  - "operation" performed in a suitable "format"
  - Readout (to assess outcome)

Case Study 2: Discover inhibitors of an essential protein of unknown function



Cellular function – unknown, but **essential for survival**
Enzymatic activity -- unknown
Protein interactions -- unknown

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Learning Objectives

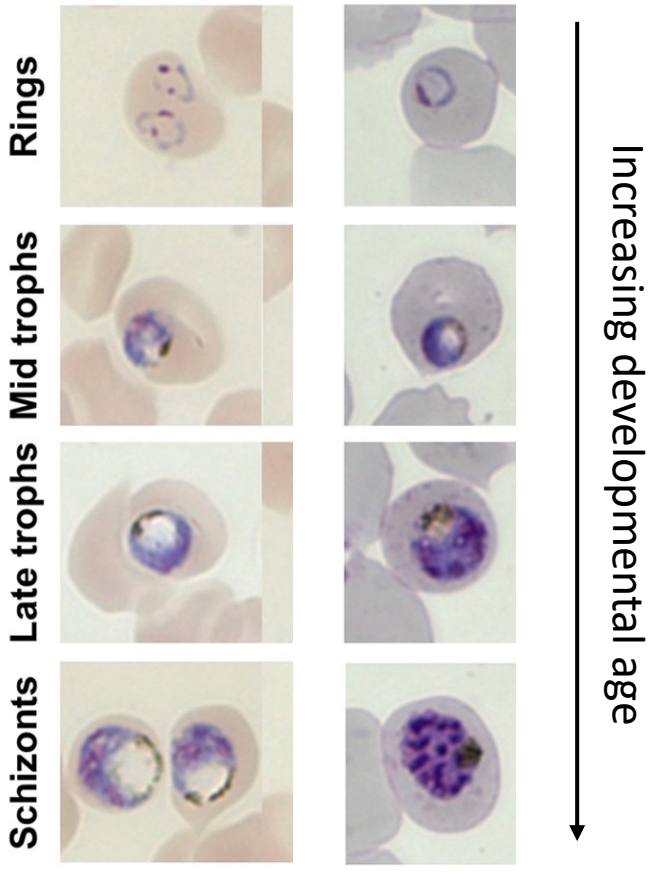
A. Discovering compounds (“hits”) using phenotypic screens
   A. What are phenotypic screens?
   B. What is required for a great phenotypic screen?
   C. What constraints are being placed on small molecules during phenotypic screens?

B. Comparing target-based and phenotypic screens
   A. Is one approach better than the other?

What do we mean by phenotype?

- Observable characteristics resulting from the interaction between genotype and environment

- Many different phenotypes to consider:
  - Cellular
    - Growth/ survival
  - Subcellular
    - Cell size, morphology
    - Organelle size, shape, distribution
  - Others?

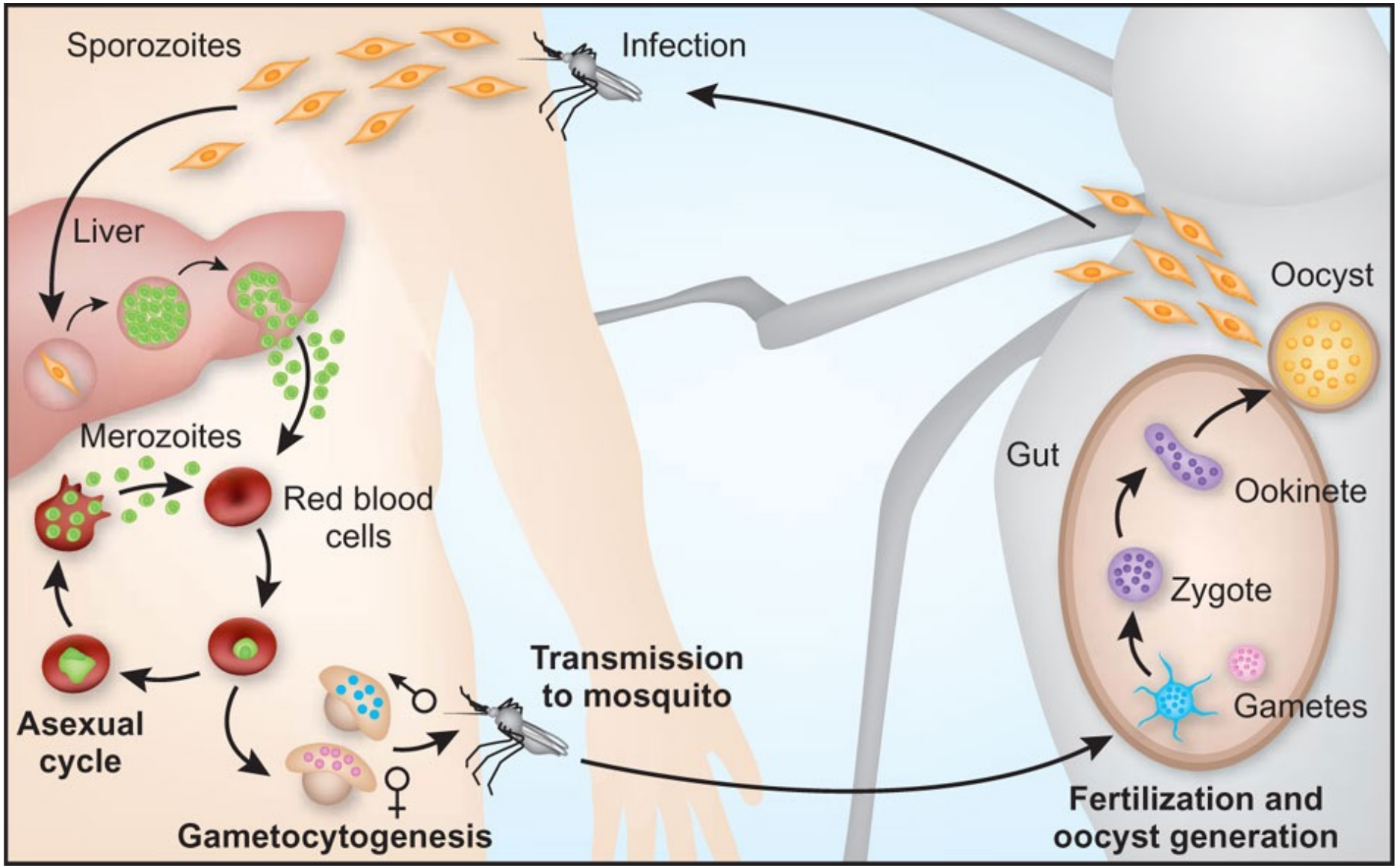


REF: Pulcini, S et al; Sci Rep 5, 14552 (2015). https://doi.org/10.1038/srep14552

How do you choose a phenotype for screening purposes?

P. vivax – a dormant form (hypnozoite) persists in the liver

All malaria symptoms associated with red blood cell infection



Devise some potential phenotypic assays based on the parasite biology described above

Pasvol, Nature Genetics (2010)

How do you choose a phenotype for screening purposes?

1. What biology do you want to manipulate:
    1. Infectious microbe?
    2. Vector?
    3. Host organism?

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1. What biology do you want to manipulate:
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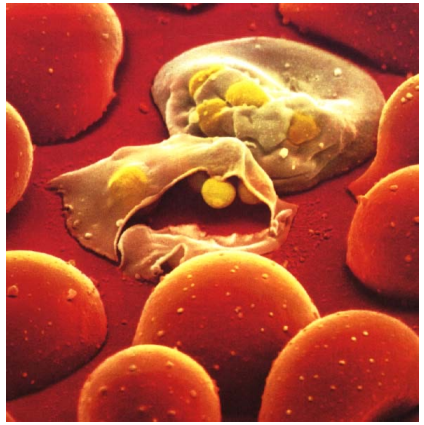
2. Can rigorously connect alterations in the selected phenotype with disruption of the disease process (e.g., infection/ disease pathogenesis)
    1. Direct adverse effects on the parasite;
    2. Indirect effects: altered phenotype disrupts critical interactions with host to prevent disease pathogenesis
        1. For example, cell death >> decreased risk of infection and disease;
        2. Non-lethal phenotype (e.g., altered cell surface protein display), BUT interferes with disease pathogenesis >> may still be reasonable.

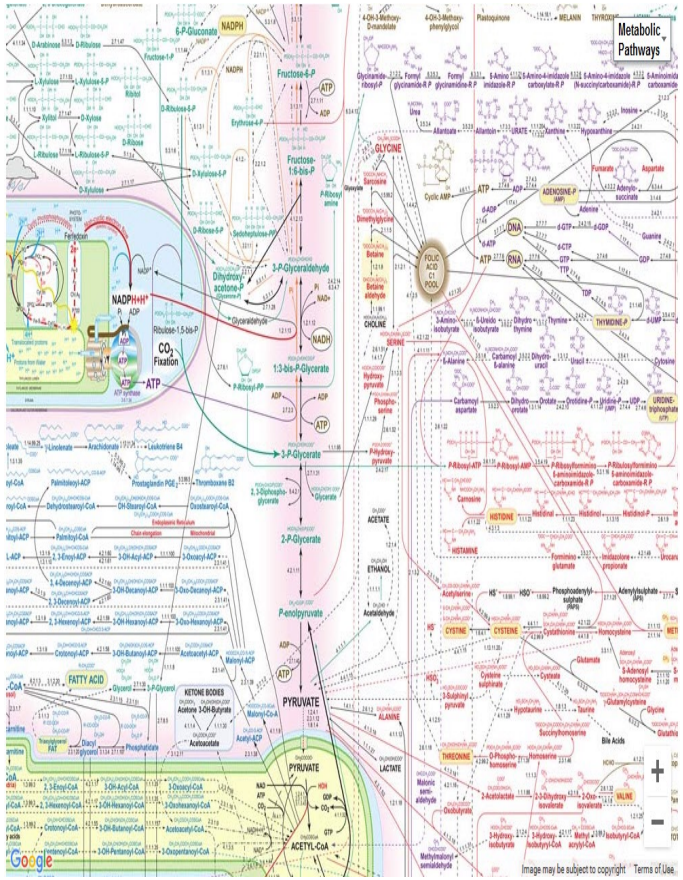
3. Should be assayable … preferably in high throughput [Input – operation --- output]
    1. *In vitro* cell culture (tractable)
    2. Animal model (… probably not!)
    3. Target organism (NOT!)

What question(s) are being asked in a phenotypic screen?

Compounds



Phenotypic Outcome (e.g., cell death)



Biochemical/ Metabolic pathways

- Enzymes
- Structural proteins

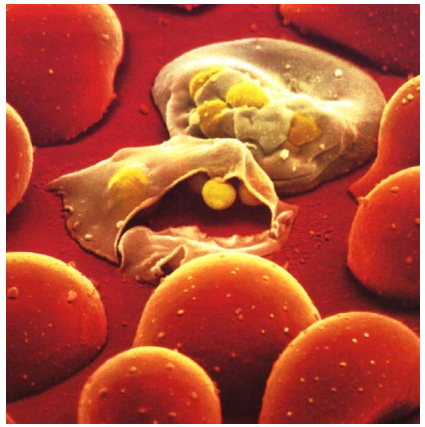
- Are there ***compounds that can enter the target cell*** to cause a measurable change in phenotype?
- Is there a process(es) that can be targeted by these compounds to disrupt cellular phenotype (survival)

What don't we immediately know from this screen?

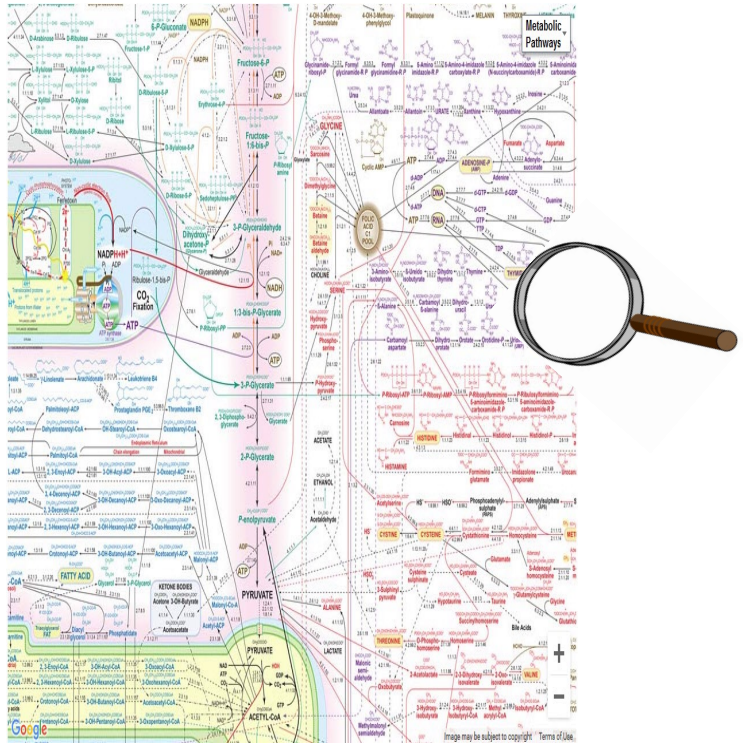
- The molecular pathways perturbed by phenotypically active compounds
- Single or multiple molecular target(s)?

Comparing target-based and phenotypic screens

Compounds



Phenotypic outcome
(e.g. survival)



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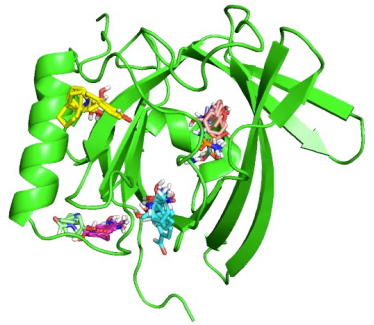

Target (e.g. protein)

Biochemical/ biophysical outcome
(e.g. binding; inhibition of enzymatic activity

1. What are some assumptions made in target-based versus phenotypic screens?

2. When would you select one option over the other?

Comparing target-based and phenotypic screens

	Target-based Screen	Phenotypic Screen
Biological		
Prior specification of precise molecular target required	Yes	No
Assumption/ knowledge of underlying biology required	Yes	No
Requires correlate between assay/ screening outcome and disease biology	Yes	Yes
Immediate requirement for biological components (e.g. cells) during initial screening process	No	Yes
Compounds		
Cellular permeability immediately required	No	Yes
Compound can be modified to enhance cell permeability, metabolic stability, etc.	Yes	Yes

Summary

- Small molecule therapeutic candidates can be identified using screens:
  - Target-based
  - Phenotypic

- Both types of screens require assays that:
  - Provide reliable and reproducible readouts of the effects of tested compounds
  - Are scalable to accommodate exploration of large chemical space to identify relatively rare ‘hits’

- These screening modes have non-overlapping pros and cons
  - Can be used in complementary ways