Tumor-genome informed approaches to identify drugs that enhance R-CHOP response in DLBCL

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Diffuse large B-cell lymphoma (DLBCL)

- B cell cancer
- 30-40% of Non-Hodgkin lymphomas
- ~22,000 new diagnoses/year
- Incidence is increasing
- Uniformly treated with R-CHOP
- ~45% cure rate (pre-Rituximab era), ~65% cure rate currently
- ~13,000 deaths/yr in North America

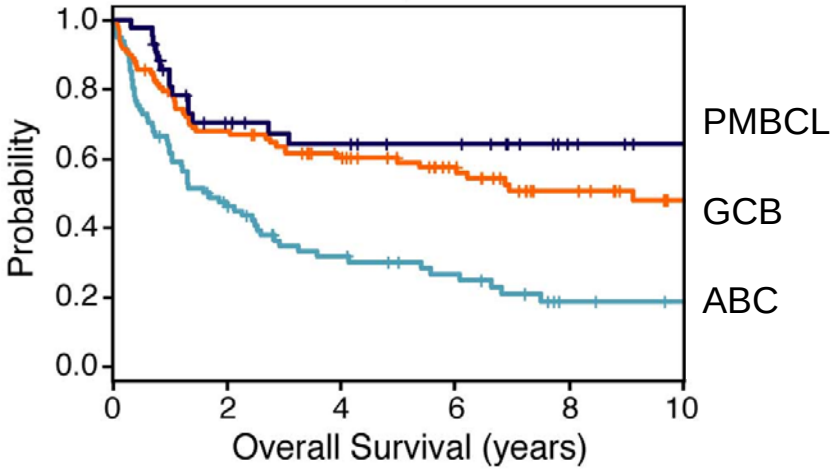


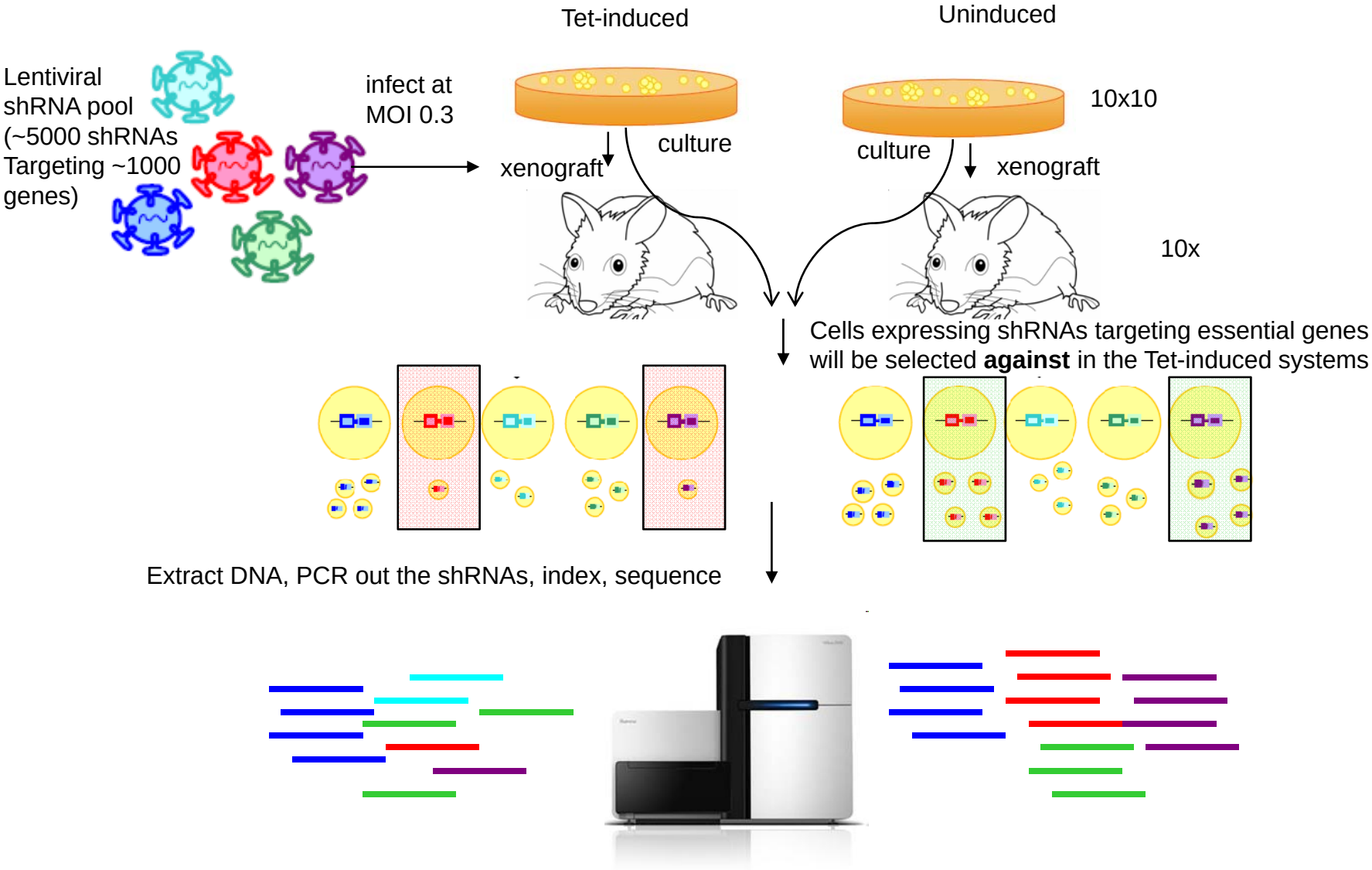
Image from Staudt & Dave, Adv Immunol. 2005

- Can be classified into at least 2 molecular subtypes: Germinal Center B-cell (GCB) and Activated B-cell (ABC)
- Molecular drivers of malignancy beginning to be revealed
  - Which are suitable drug targets (oncogenes)?
  - What oncogenic proteins, when targeted, can improve current treatments?

Objective

To collectively employ genomic, in vitro, and in vivo experiments to identify drug targets and ultimately novel therapeutics that act synergistically with R-CHOP therapy

shRNA screens to identify cancer drivers

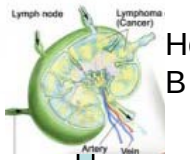


Quantify reads corresponding to each shRNA; detect shRNAs differentially represented between the Tet-induced and uninduced cells using Fisher exact test

Aims

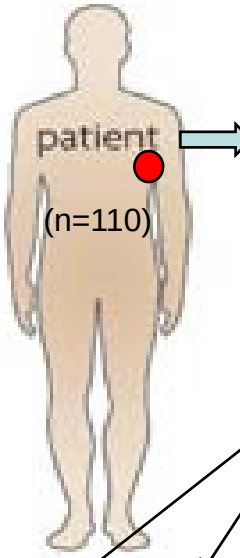
1. Genomic characterization to identify mutated/dysregulated genes.
2. shRNA to identify oncogenes from (1) in tumor cells.
   Three model systems:
   1. cancer cell lines
   2. cancer cells xenografted into immune-deficient mice
   3. Transplanted cancer cells in a mouse model of DLBCL
3. Identification of novel therapeutics to inhibit oncogenic proteins identified in (2) using:
   1. In silico screening for potential inhibitory small molecules
   2. Validation in vitro and in vivo

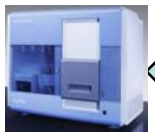
Gene selection strategy



Healthy B cells

DLBCL cell lines (n=10)





RNA/DNA sequencing

Protein-altering point mutations

Recurrent structural aberrations

Fusion transcripts

Tumour-restricted alternative splicing

High expression correlated with (lack of) treatment response

Examples:

EZH2, MEF2B

BCL2

BCL6

???

HGAL

DrugBank

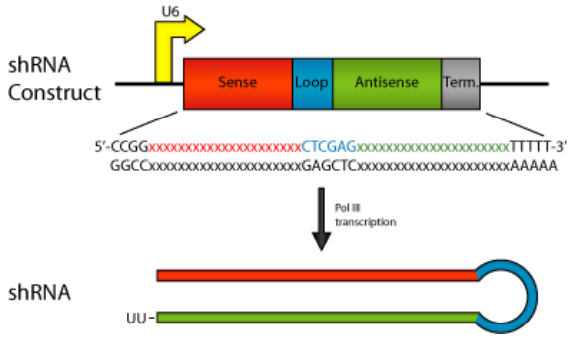
PubMed

Drug targets (expressed in DLBCL)

Genes associated with lymphoma

RCSB PDB PROTEIN DATA BANK

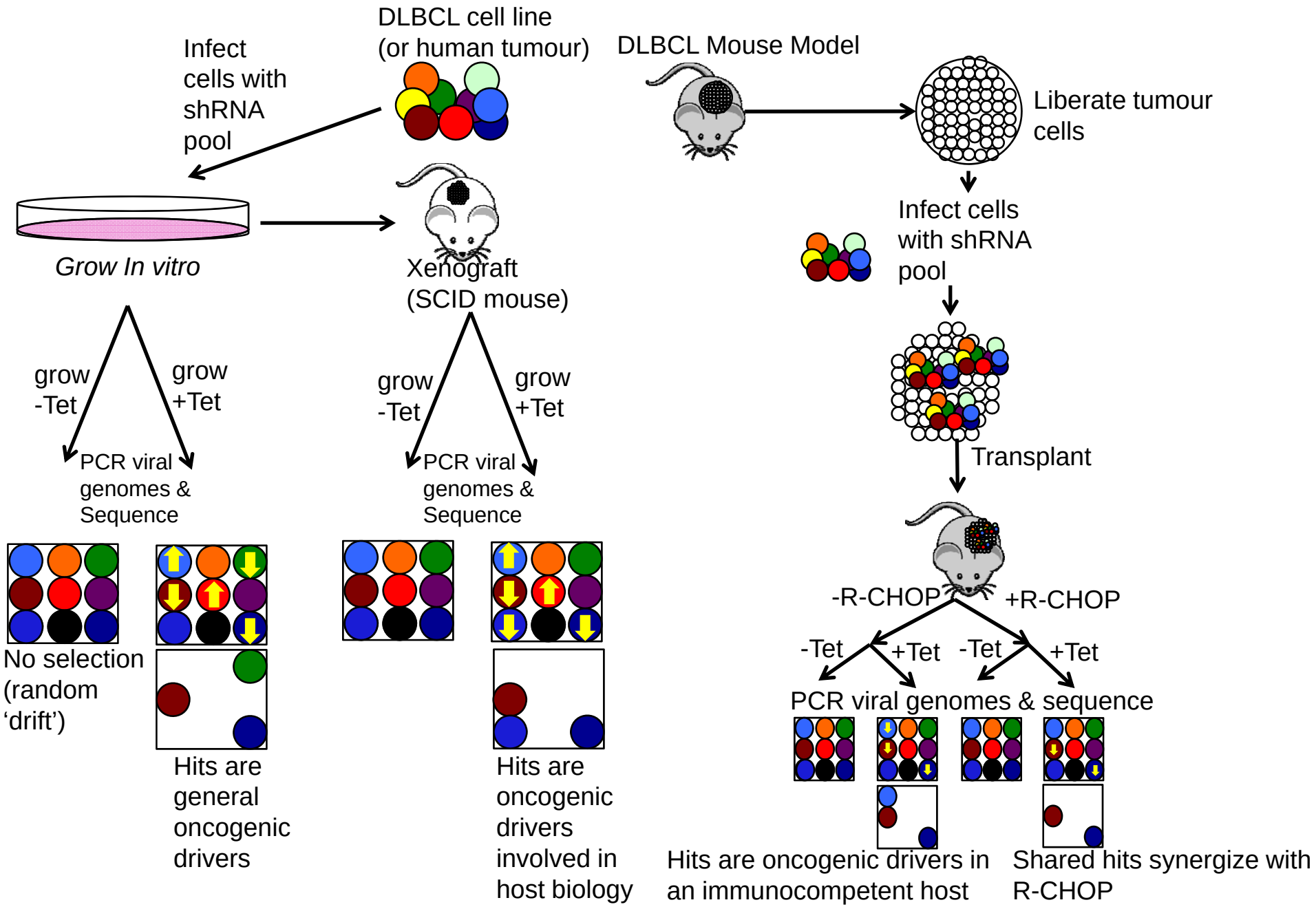
Kinases with solved structure



shRNA pool

~5000 shRNAs targeting ~1000 genes

Experimental work flow



Analysis

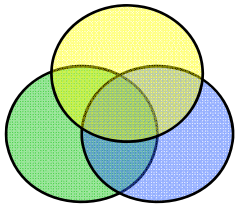
Common hits
across all lines?

Subtype-restricted
hits?

Cell line

Do hits correlate with known mutations?

Shared hits?
Hits only in
xenografts?



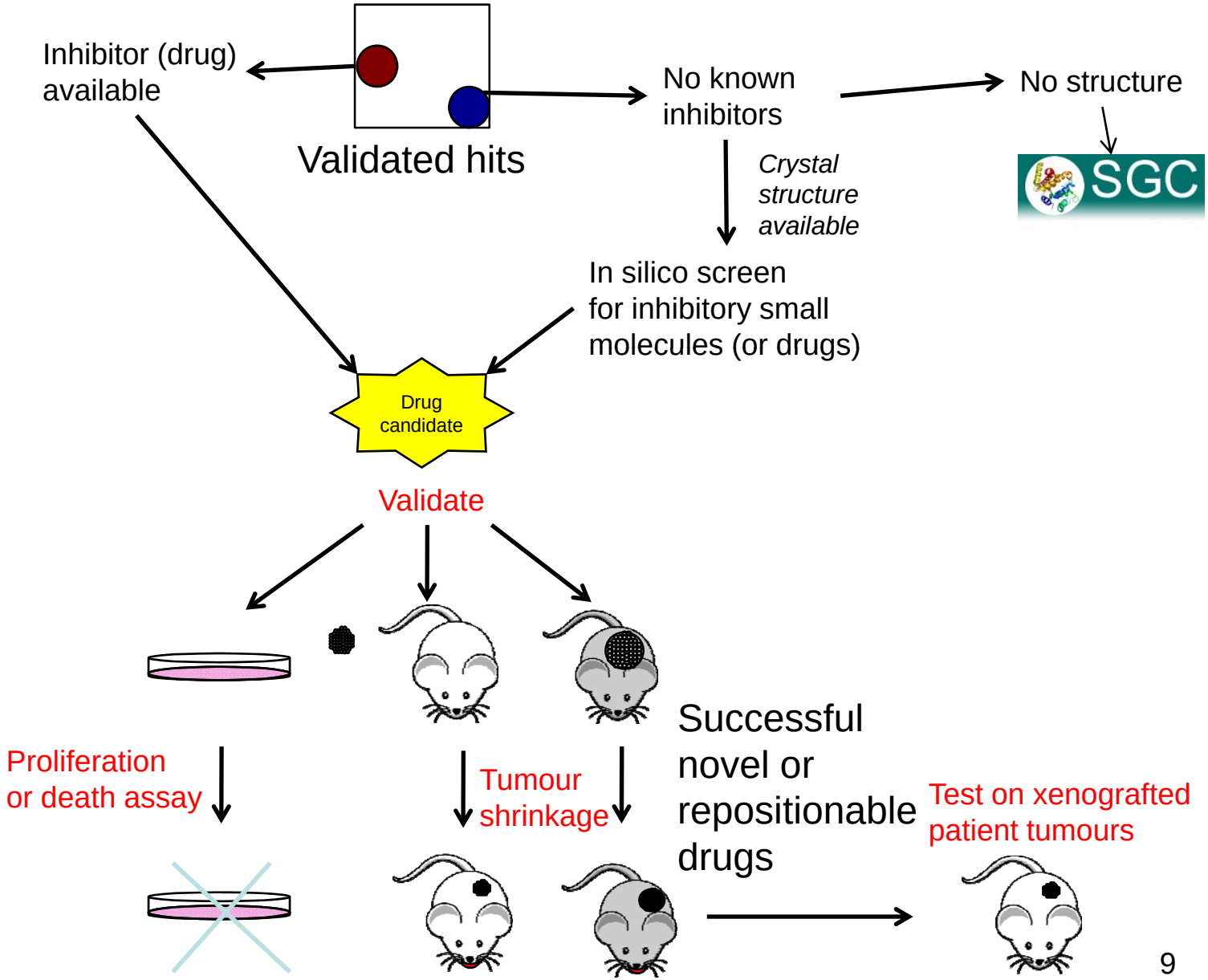
Shared hits?
Hits only in
mouse model?

Xenograft

Mouse
model

Hits that are relevant only in
combination with R-CHOP treatment?

Translational Strategy



Expected Outcomes

Proteins whose inhibition results in decreased viability in DLBCL

- *In vitro*
  - general pro-survival genes
  - known and novel oncogenes
- *In vivo*
  - genes involved in angiogenesis, microenvironment interaction or cell motility
- In vivo, immunocompetent drug-treated host
  - Proteins involved in immunoevasion
  - Drug metabolism/resistance proteins

Novel or readily available therapeutics that selectively inhibit these proteins

Timeline

Gene/shRNA selection (6 months)
- Sequence analysis
- Database/literature mining

shRNA pool & xenograft testing (6 months)
- Packaging virus
- Optimizing infection and growth conditions

shRNA scale-up, data collection (1 year)
- Many concurrent experiments
- Sequence shRNA libraries in multiplex

Data analysis & validation (1 year)
- Identification of significant ‘hits’
- Hit validation in vitro and in vivo

Drug identification and screening (1 year)
- Identify candidate inhibitors for individual hits
- Screen in vitro or in vivo
- Apply any valid hits to xenografted patient samples

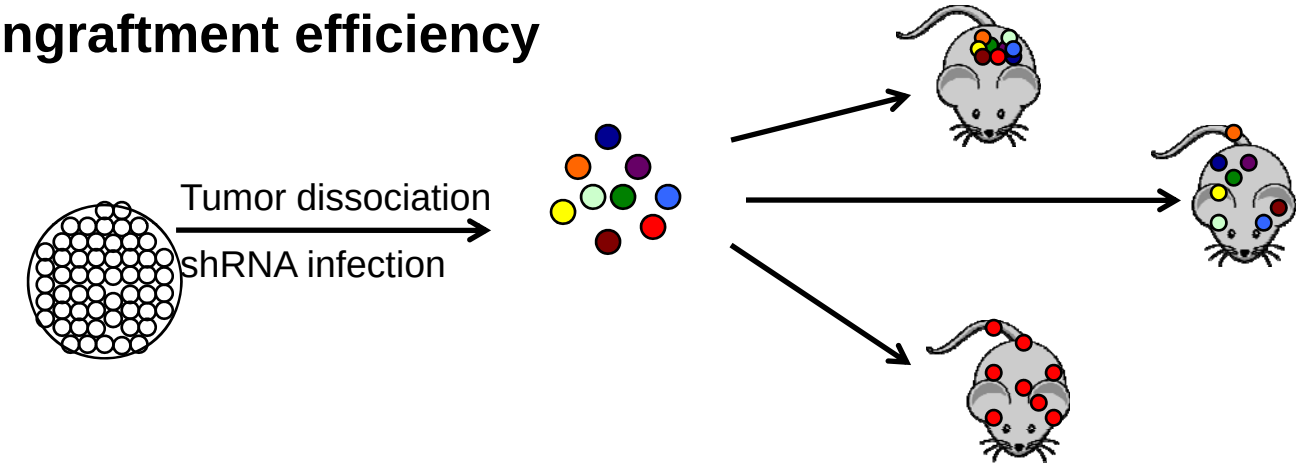
$750,000 to be spent over 4 years

Significance

- Many somatic alterations occur in DLBCL
- Standard therapy has not been modified to leverage information from genomic study of this disease
- Genome characterization only provides indirect evidence for drivers
- Direct identification of proteins that promote tumor cell survival *in vitro* and *in vivo* should greatly accelerate the translation of this knowledge
- Novel small molecules and known drugs that target these drivers synergistically with R-CHOP could quickly be translated to improved treatment outcomes

Problems and Solutions

Poor engraftment efficiency

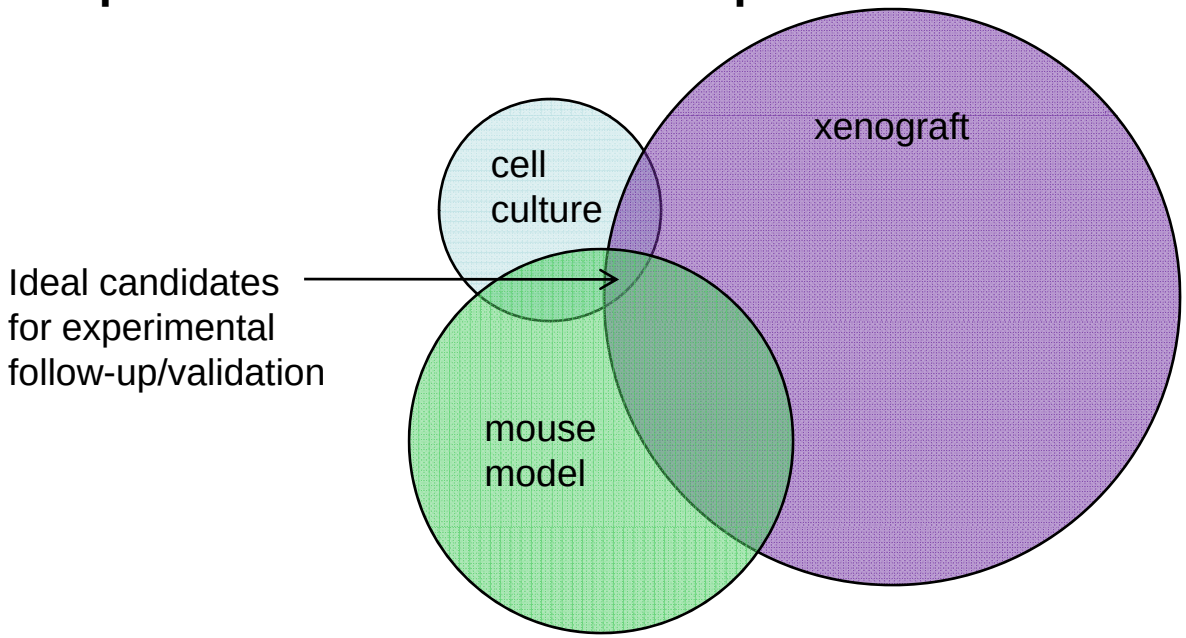


- **Detectable by :**
  - **GFP labeling of shRNA infected cells**
  - **Analysis of diversity of shRNA sequences in uninduced tumor controls**
- **If unsatisfactory:**
  - **Use Eμ-myc mouse which has been shown to have sufficient tumor cell engraftment for this system instead of ImHABCL6, which is a better model of DLBCL**

Problems and Solutions

Shared Gene Targets

- **We expect some shRNAs to be depleted in all three experimental systems**



- **If no overlapping gene targets are identified, we will prioritize targets from the xenograft and mouse models for validation as these are most biologically relevant**

Questions?