CRISPR/Cas9 – A Swiss Army Knife for the Identification and Validation of Novel Drug Targets
Is Target Validation the Key Bottleneck in Drug Discovery?

The costs of drug discovery have risen fast as the barriers for approval have risen. Less than 7% of clinical stage projects end in registration.

Failures peak at the Phase 2/3 transition: The 1st time the hypothesis is tested in the patient. Part compound quality; more often because reality wasn’t aligned to the hypothesis.
Is Target Validation the Key Bottleneck in Drug Discovery?

Reliance on literature for the key data to trigger projects limits diversity.
Most activity vs. kinases has been focused on <5% of the possible kinase targets.
Is Target Validation the Key Bottleneck in Drug Discovery?

Much of the academic literature is also hard to reproduce to a breadth and depth that justifies drug discovery

There are many structural reasons for this: e.g. John Ioannidis’ arguments “Why most published research findings are false”

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**No Cure**

When Bayer tried to replicate results of 67 studies published in academic journals, nearly two-thirds failed.

- **Fully replicated**: 20.9%
- **Partially replicated**: 11.9%
- **Not replicated**: 64.2%
- **Not applicable**: 3.0%

Source: Nature Reviews Drug Discovery

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**Repeat failures**

- 6 of 53 Cancer papers that Amgen could reproduce
- 14 of 67 Biomedical papers that Bayer completely reproduced
- 55% MD Anderson researchers who could not reproduce a published study
Target ID in oncology: new frontiers

**Good News:** Collapse in DNA sequencing costs has enabled large collaborative studies such as TCGA. We now have unprecedented knowledge regarding the identify and patterns of cancer driving mutations.

**Bad News:** These have revealed only a limited number of cancer driver genes are +ve acting oncogenes with an obvious small molecule inhibition approach.
Target ID in oncology: new frontiers

For a small subset of patients immunotherapy has given long lasting responses

Responses to immunotherapy are correlated with mutation: some cancers are unlikely to succumb to immuno-oncology approaches

Cancer genomics is dominated by undruggable oncogenes and tumour suppressor mutations.

Synthetic lethal approaches required to drug cancers with low mutation rates.
A cancer version of Fermi’s Paradox?

If there are lots of synthetic lethal targets, why haven’t we discovered them?
A cancer version of Fermi’s Paradox?

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1. They are few and far between
2. Our telescopes aren’t up to the job
A cancer version of Fermi’s Paradox?

If there are lots of synthetic lethal targets, why haven’t we discovered them?

1. They are few and far between
2. Our telescopes aren’t up to the job
RNAi: not a good telescope

Loss of function analysis using RNAi is inexpensive and widely applicable

However

- Incomplete knockdown
- Lack of reproducibility
- Off-target effects

Only partial KD

Little correlation between screens

Off-target effects

Total overlap only 3 genes

Brass et al. Science
273 genes

König et al. Cell
213 genes

Zhou et al. Cell Host Microbe
300 genes

HIV Host Factors

Problems with RNAi can result in false positives or negatives
CRISPR-Cas9: a better telescope?

*Pooled screening using CRISPR takes advantage of the nuclease activity of the Cas9 protein targeted to a precise genomic locus by a short guide sequence (sgRNA)*

- Cas9 + sgRNA introduces double strand breaks
- NHEJ repair and InDel editing of the locus
- The result: loss of the target gene ORF

Robust phenotypes due to complete loss of gene function

Anticipated to provide fewer off-target concerns than RNAi
CRISPR–Cas9 Screening

- Optimise cell lines for screening
- Determine functional MOI for each screen
- Maintain high library coverage during screen
- Collect samples & analyse by NGS
- Perform NGS and Screen QC
- Analyse results and nominate hits

Cell Line Optimisation

Growth rates, polybrene sensitivity, spinfection tolerance, antibiotic sensitivity, bulk TC tolerance & transduction relative MOI
CRISPR-Cas9 Screening

Resistance Screening
Identify resistance factors in sensitive cell lines
Uses a high dose of compound (IC₈₀)
Resistance genotypes accumulate in population

- sgRNA library → High Dose (IC₈₀) compound → Depleted targets confer resistance

✓ High quality data from unbiased analysis

Sensitivity Screening
Identify a priori resistance factors from GoF alterations
Uses a sub-toxic dose of compound (IC₂₀)
Analysis power is contingent on experimental design

- sgRNA library → - compound
+ compound

✓ Detect targets lost in treatment condition

Genetic Interaction Screening
Examine knock-out phenotypes in across multiple genotypically defined cell line panels
Whole genome vemurafenib resistance screen

Recapitulation of existing screens using new library identified all six previously identified hits and several additional targets.
What does the output of a screen look like?

Positive selection screens

Negative selection screens
Whole genome sensitivity screening in haploid cells

- **KBM-7**
  - Near-haploid (diploid chr8, chr15)
  - Isolated from CML patient
  - Myeloid lineage
  - Suspension cells
  - Andersson *et al.* Cancer Genet. 1986

- **HAP1**
  - Near-haploid (chr15)
  - Derived from KBM-7
  - Fibroblast like
  - Adherent cells
  - Carette *et al.* Nature. 2011

- **eHAP**
  - Fully haploid
  - Derived from HAP1
  - Patent EP 13194940.6
  - Essletzbichler *et al.* Genome Res. 2014

Mutation can be masked by second copy

Mutation leads to knockout
Whole genome sensitivity screening in haploid cells

Transduce Cells (122k whole genome sgRNA Library)

Baseline Sample

DMSO (11 days)

Control sample

24 hours starvation, then DMSO (13 days)

Drug treated sample

MA VoCK adjusted P-value

Mean Log2 Fold Change

1E-008
1E-007
1E-006
1E-005
1E-004
1E-003
1E-002
1E-001
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
-0.9
-1
-1.1

NDUFB10
NDUF5
TSC2
NDUFA8
NDUFB2
NDUFA2
NDUFB9
NDUFC2-KCTD14
ZFYVE1
MAPK14
AKT1
NDUFA5
FANCA
POLE
ACT3
MAPK4
ACT2
ACT1
Whole genome sensitivity screening in haploid cells

**eHap**

Transduce Cells (122k whole genome sgRNA Library)

- **DMSO (11 days)**
  - Control sample

- **2 nM paclitaxel (11 days)**
  - Drug treated sample

![Graph showing gene expression changes](image-url)
Hit validation in Hap1 cells

Availability of Hap1 KO cells enables rapid validation in orthogonal assays

Hap1 NDUFS2<sup>-</sup> clone was:

- Incapable of growth in low glucose media
- More sensitive to 2-DG when cultured in complete medium
- More sensitive to overnight glucose starvation in a colony formation assay
Identifying novel synthetic lethal interactions in colon cancer

Screening 2867 member subset library vs. 12 cell line CRC panel (+isogenics) with balance of APC, TP53, KRAS & PIK3CA mutations

<table>
<thead>
<tr>
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<th>ID</th>
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Subset library (2867 genes)
Identifying novel synthetic lethal interactions in colon cancer

Screening 2867 member subset library vs. 12 cell line CRC panel (+isogenics) with balance of APC, TP53, KRAS & PIK3CA mutations

<table>
<thead>
<tr>
<th>CRC Panel</th>
<th>Cell Line</th>
<th>KRAS status</th>
<th>PIK3CA status</th>
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<td>WT</td>
<td></td>
</tr>
<tr>
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<td>WT</td>
<td>P449T</td>
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Sub-library

Plasmid pool

Lentiviral library

Transduced cells

Transduced cell growth

Genes ranked by differential essentiality

DLD1 parental

(PIK3CA E545K/+)

selective essentiality

DLD1 isogenic

(PIK3CA +/-)

selective essentiality
Screening 2867 member subset library vs. 12 cell line CRC panel (+isogenics) with balance of APC, TP53, KRAS & PIK3CA mutations
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PIK3CA-activating mutant

WT PIK3CA
Identifying novel synthetic lethal interactions in colon cancer

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Heat-map view of MAGeCK MLE test generated β-scores as derived using the k-means clustering method (k=6). Only genes with β-scores among the 1% highest or lowest of all scores generated are shown.

Negatively selected genes in cell lines with PIK3CA-activating mutant allele
Target Validation with CRISPR/Cas9

In just the few years since the first demonstration of its utility for gene editing, the Cas9 endonuclease has been adapted for multiple purposes:

- **Cas9wt**
  - ds breaks

- **dCas9-KRAB**
  - CRISPRi

- **dCas9-p300**
  - Epigenome editing

- **dCas9-SunTag**

- **Cas9D10A**
  - +GFP Chromosome localization
  - +VP64 CRISPRa

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**Transcription Repression**

- Heterochromatinization
- KRAB
- dCas9

**Epigenome Editing**

- Cas9wt
- ds breaks

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**Genomic DNA**

- sgRNA
- Cas9 Nickase
- Site-specific ssDNA nick

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**Log fold change**

- dCas9-VP64
- dCas9-300 Core
- IL1RN isoforms
- KDR, FAM49A

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**Protein X**

- dCas9
- sgRNA
- Exon 1 of gene X

---

**Transcription Activation**

- RNA pol II promoter
- dCas9

---

25
Target Validation with CRISPR/Cas9

Tiled sgRNA screening allows the identification of domains important for protein function.

Cas9/sgRNA + an HDR donor allows knock-in of mutations. With tractable cell lines, all-allele KI mutants can readily be isolated.

LDLR E101K = GAG>AAG and KO clones

Oligo donor + 2 silent mutations

Transcript: ENSG00000130164

LDLR E101K/E101K

LDLR (E101K/+)

LDLR (E101K/-)

LDLR (-/-)

LDLR (+/-)
Target Validation: Making conditional KO lines in a single step

Sophisticated conditional alleles can be introduced by combining Cas9/sgRNA with large HDR donors.

NHEJ-mediated gene tagging can be deployed for rapid analysis of localization etc.
CRISPR-Cas9: Robust & Powerful

ROBUST
CRISPR-Cas9 can be deployed in most cell types

POWERFUL
Complete gene KO to enact maximum phenotypic effect

FLEXIBLE
Technology can be deployed in many modes

RAPID
Fast generation of complex models reduces the need for compromise in target validation & functional genomics
Your Horizon Contact:

Dr. Jonathan Moore
Chief Scientific Officer