HORIZON DISCOVERY

THE CASE FOR A SYNTHETIC-LETHALITY FOCUSSED NEWCO

Jon Moore, CSO
Dutch Life Sciences Conference, November 24th 2016

horizon

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The Cell Builders: Powering genomic research and personalised medicine

What we do

How we do it

We deploy a powerful and flexible gene editing platform to…

…to develop cell models that drive understanding of disease

…to create novel molecular, cellular and gene therapies

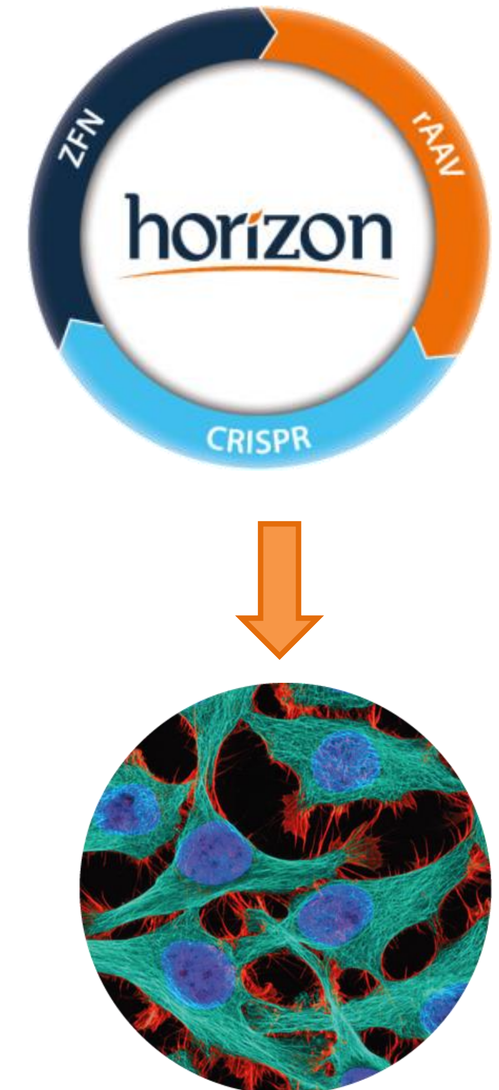
Strong business fundamentals

- c275 FTEs located in Cambridge, UK; Boston, Philadelphia and St Louis, USA; Vienna, Austria
- $175M raised since listing on London Stock Exchange (AIM; HZD.L) in March 2014
- Provide products, services and research programs to >1400 partners in over 50 countries
- Translational research base has driven several industry-leading paradigms

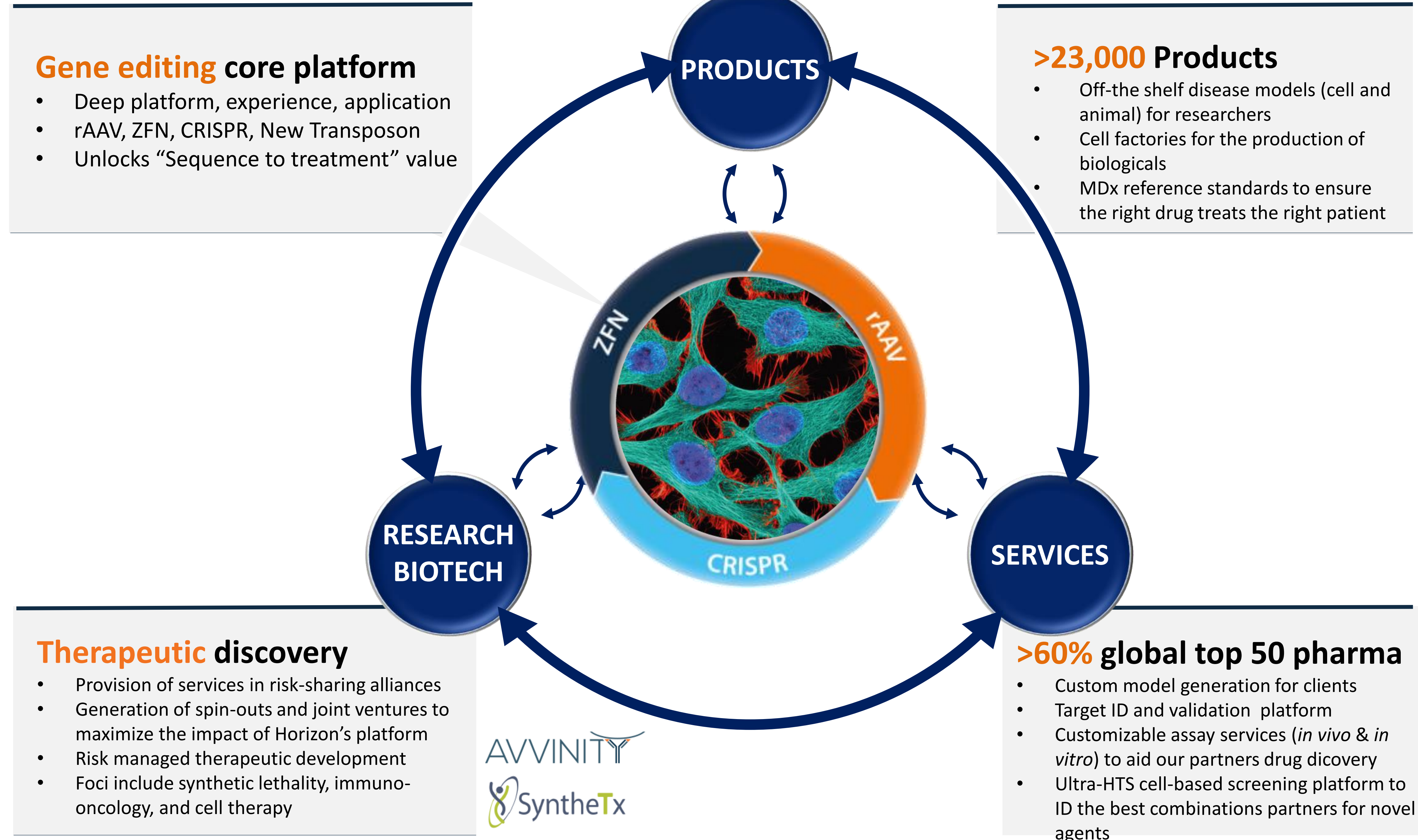
Horizon is an acknowledged leader in gene editing

Horizon are 'cell builders', using rAAV, ZFN and CRISPR technologies, as appropriate to deliver the job at hand

Wide range of IP	**Ground-breaking scientific publications**	**World-class gene-editing advisers in SAB**	**Extensive practical experience**
– Exclusive license to rAAV for research applications – Multiple commercial licenses to important CRISPR patent portfolios for a variety of applications – Exclusive license for the use of ZFNs for *in vivo* applications and non-exclusive for *in vitro* – Exclusive license for haploid gene editing and CRISPR-based screening - Patent filed on new cut and paste transposase gene editing method	– First knockout, knockin and conditional gene edited rats – Characterisation of the essential haploid genome – Cited in over 200 peer reviewed journals	– Dr. Feng Zhang (MIT/Broad, founder EDITAS) – Dr. Emmanuelle Charpentier (Max Planck Institute, CRISPRTx) – Professor David Russell (University of Washington) – Professor Eric Hendrickson (University of Minnesota) – Dr. Keith Joung (Harvard/ Mass. General, EDITAS) – Dr. Sebastian Nijman (University of Oxford)	– A decade of industrial application and experience – Over 4,000 genes editedin a wide range of cells with virtually every possible type of modification – High throughput, low cost haploid cell line generation engine



Flywheel commercial model serves multiple partners

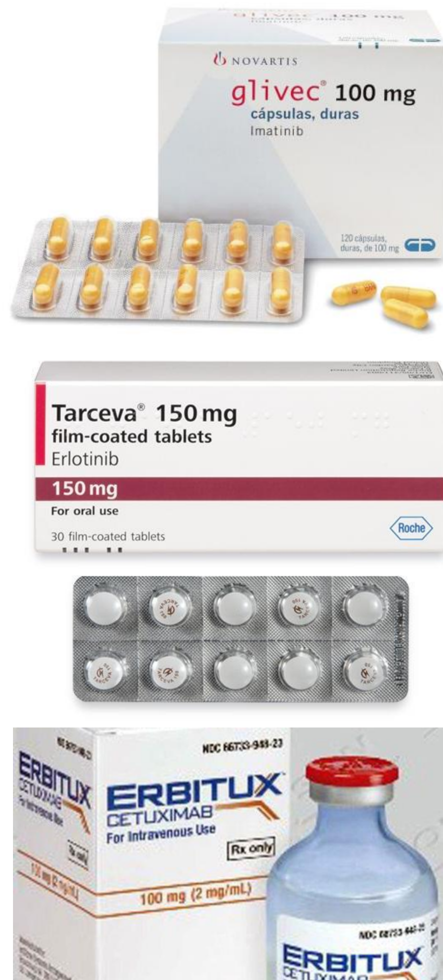


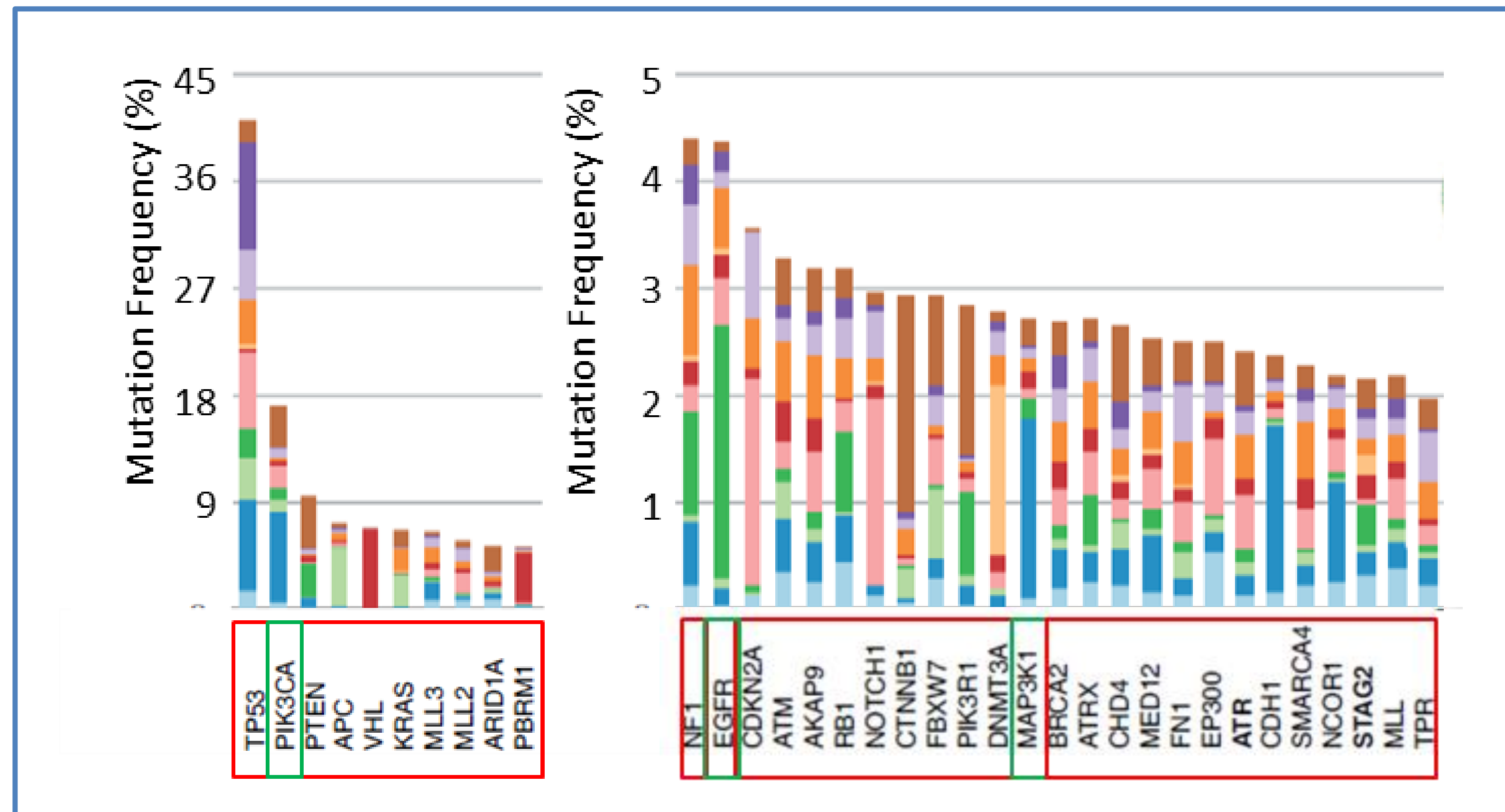
Oncology: State of play 1

Conventional cytotoxics remain the mainstay of therapy for many cancers

Molecular targeted agents have concentrated on RTKs (EGFR etc); responses are typically restricted to cancers with either amplification or activating mutations in the target gene

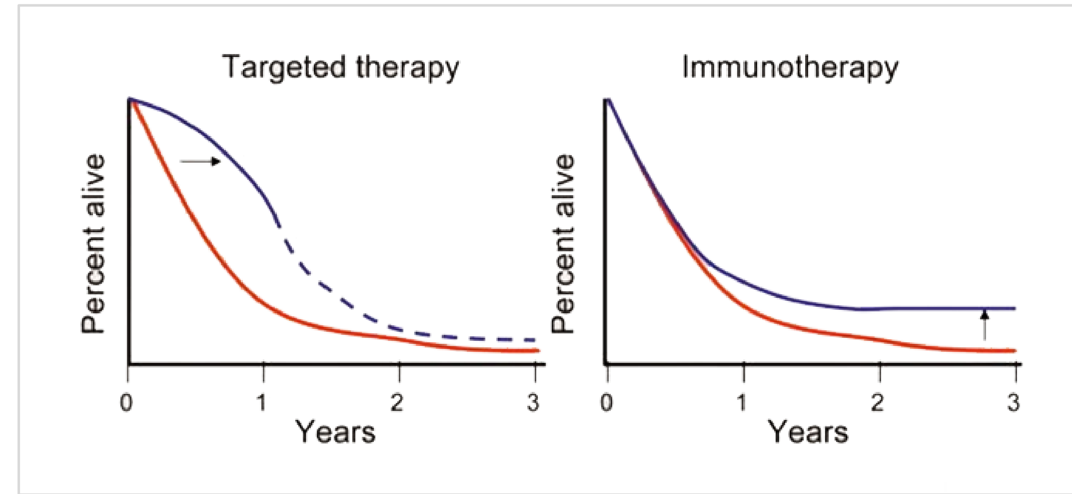
Cancer sequencing projects indicate that frequently-mutated targets adhering to this paradigm are now mined out





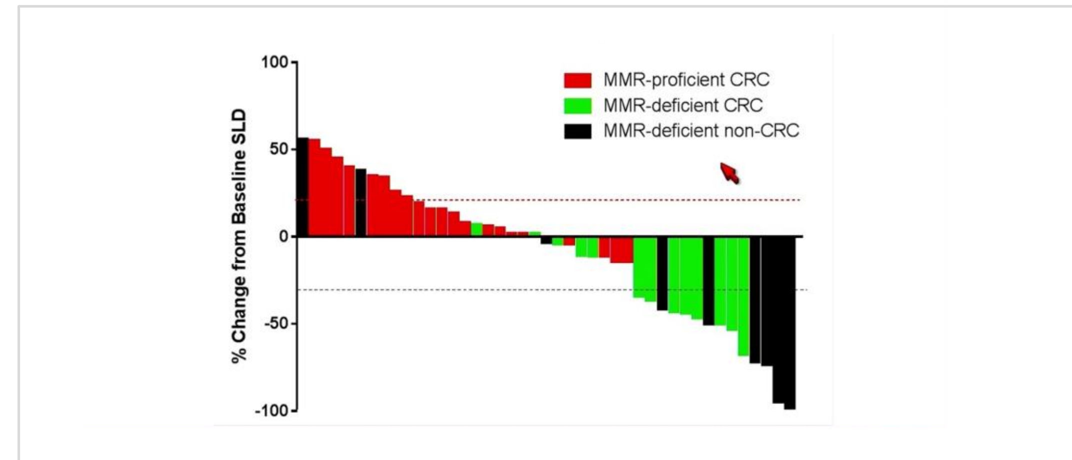
Oncology: State of play 2

For some cancer patients immunotherapy has given long lasting responses

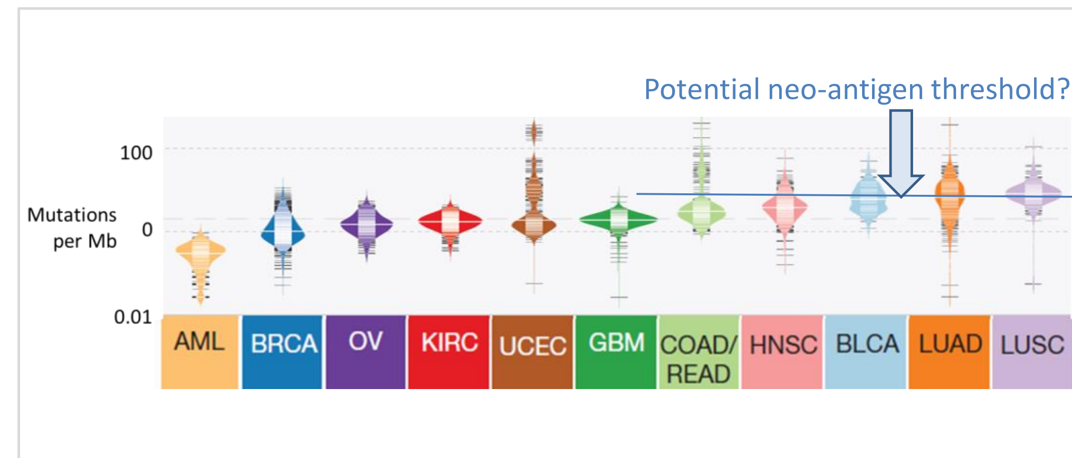


Responses to immunotherapy appear correlated with neo-antigen load

Responses in colon cancers treated with pembrolizumab are generally restricted to the MMR-deficient minority that have high mutation rates (and therefore neo-antigen load)



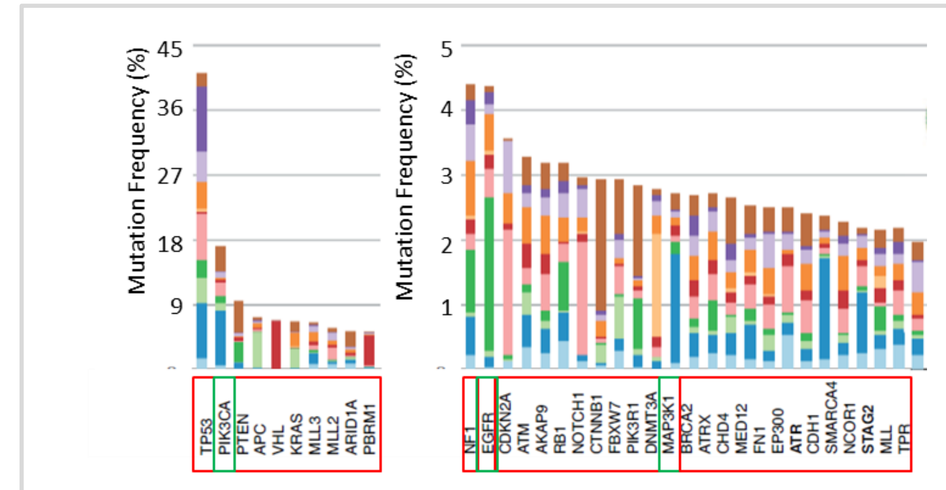
Cancer sequencing data indicates mutation rates vary dramatically: a large fraction of tumours may not have sufficient mutations to respond to immunotherapy

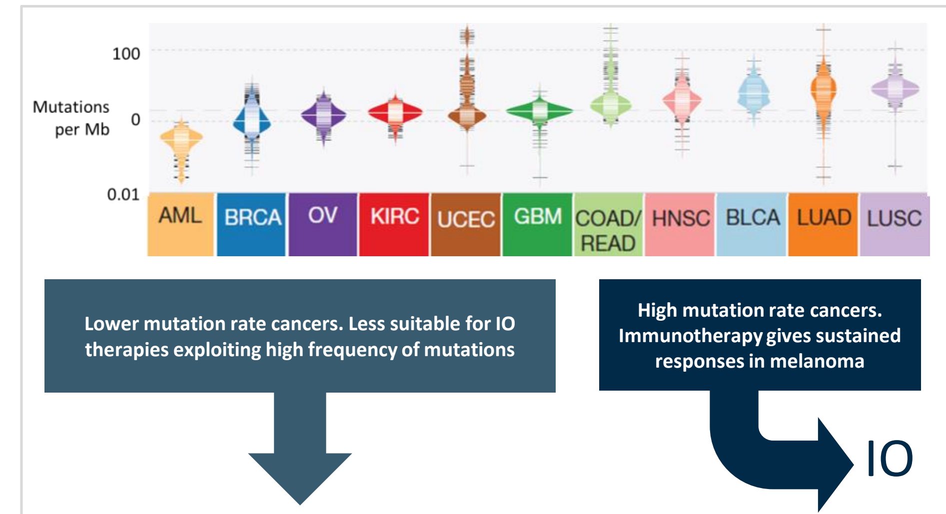


Target ID in oncology: new frontiers

The cancer mutation landscape is dominated by mutations in hard to drug oncogenes (e.g. KRAS) and loss of function mutations in tumour suppressors.

How can we serve the unmet medical need for new therapies vs cancers from low-mutation rate histologies that are driven by “undruggable” oncogenes and tumour suppressor mutations?





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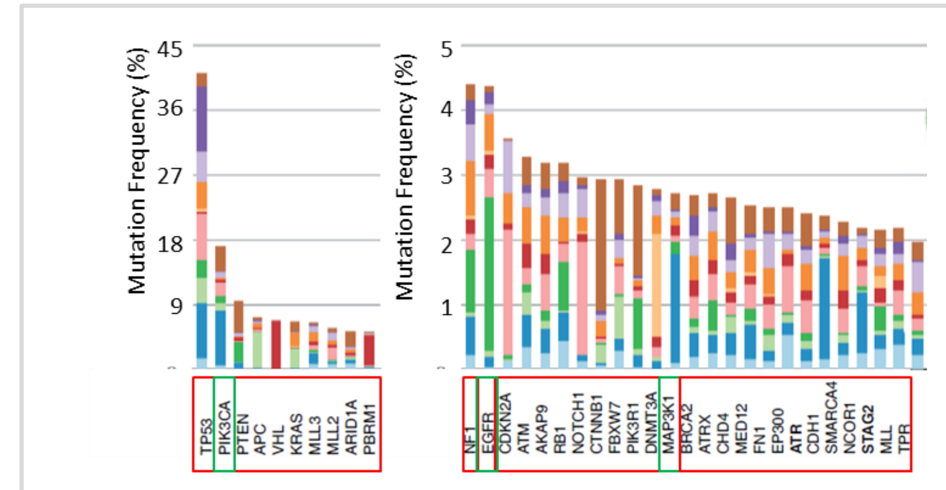
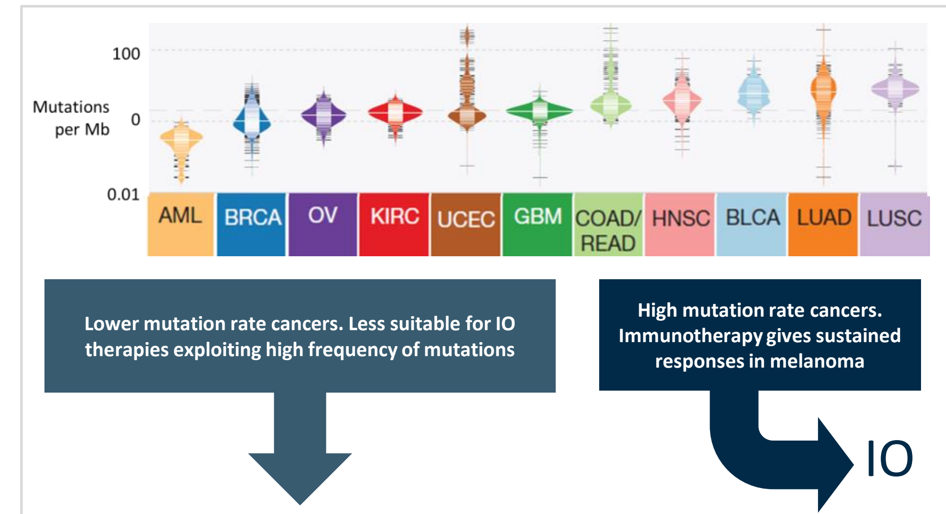
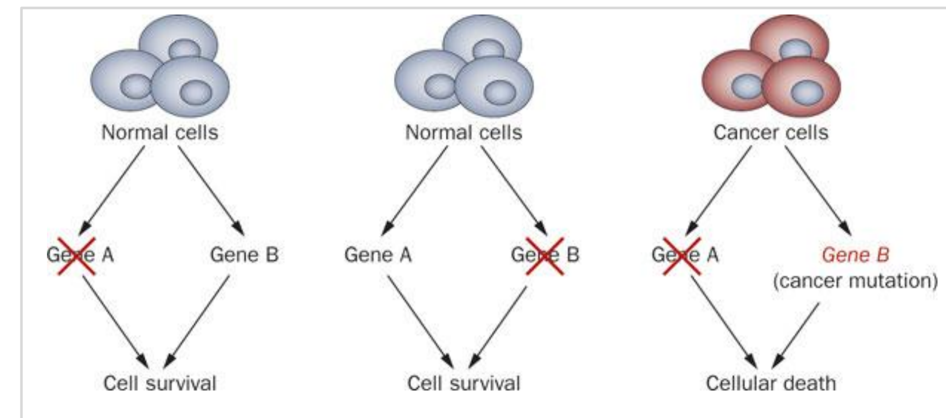
Answer:

Synthetic lethality. Exploiting targets that become essential in the presence of a non-druggable cancer-driver gene

Exemplified by the PARP inhibitor, olaparib, for which AZ predict peak sales in excess of >$2 billion p.a.







On Synthetic Lethality

Dhobzansky coined term in 1940s to describe non viability of Drosophila bearing certain combinations of otherwise tolerated mutations

Used extensively from 1990s by yeast geneticists to understand genetic networks

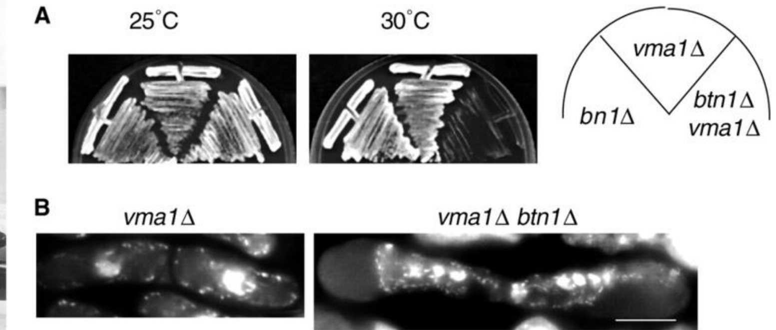
Proposed as a new route to oncology targets by Hartwell *et al*. in 1997

Explosion in literature in last 10 years as functional genomics became accessible in cancer cells

Several putative synthetic lethal targets for cancers with various mutations published in prominent journals (e.g. STK33)

Considerable resources were applied to exploit these opportunities, but target validation was not achieved and few drugs other than olaparib have emerged

Why?



Integrating Genetic Approaches into the Discovery of Anticancer Drugs

Leland H. Hartwell, Philippe Szankasi, Christopher J. Roberts, Andrew W. Murray, Stephen H. Friend*

The discovery of anticancer drugs is now driven by the numerous molecular alterations identified in tumor cells over the past decade. To exploit these alterations, it is necessary to understand how they define a molecular context that allows increased sensitivity to particular compounds. Traditional genetic approaches together with the new wealth of genomic information for both human and model organisms open up strategies by which drugs can be profiled for their ability to selectively kill cells in a molecular context that matches those found in tumors. Similarly, it may be possible to identify and validate new targets for drugs that would selectively kill tumor cells with a particular molecular context. This article outlines some of the ways that yeast genetics can be used to streamline anticancer drug discovery.

The recent remarkable progress in identifying molecular alterations in human tumor cells has unfortunately not been paralleled in the field of anticancer drug discovery. The shortage of effective anticancer drugs is due in part to the fundamental difficulties associated with the development of any safe effective drug. For example, it remains a formidable task to design small molecules that alter the function of macromolecules with both sensitivity and specificity (for example, an enzyme with a small active site). It is even more difficult to inhibit protein-protein interactions mediated over a large surface, or to restore function to a defective protein (such as an inactive tumor suppressor protein). Even when successful, massive efforts are required—often measured in years to decades—from dozens of chemists, biochemists, and toxicologists.

There are also many difficulties specific to anticancer drug discovery programs. An effective chemotherapeutic must selectively

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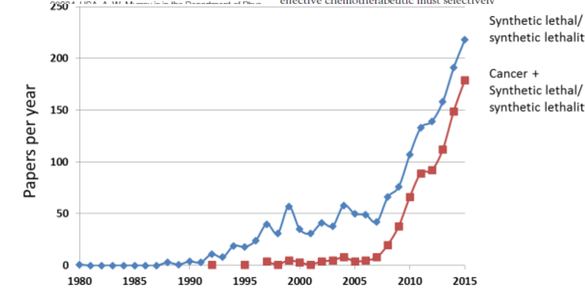
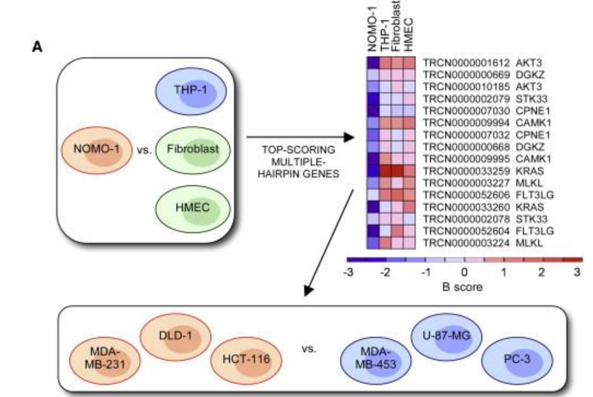
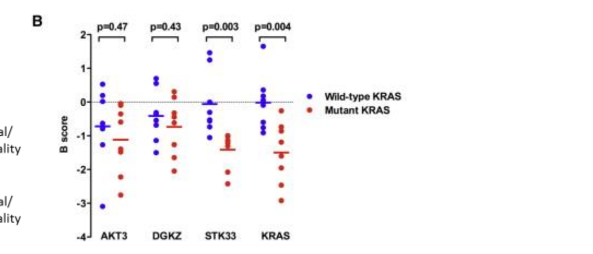
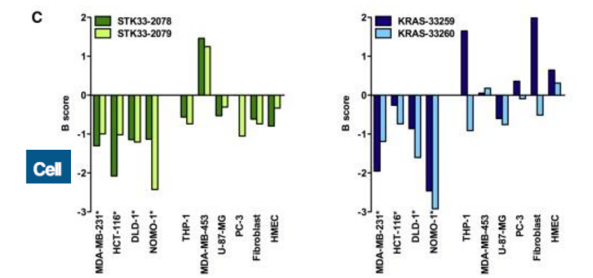








Synthetic Lethal Interaction between Oncogenic *KRAS* Dependency and *STK33* Suppression in Human Cancer Cells

Claudia Scholl,[1,15] Stefan Fröhling,[1,15] Ian F. Dunn,[2,3,4,5,6] Anna C. Schinzel,[3,4,5,6] David A. Barbie,[3,4,5,6,7] So Young Kim,[3,4,5,6] Serena J. Silver,[6] Pablo Tamayo,[6] Raymond C. Wadlow,[7,8] Sridhar Ramaswamy,[6,7,8,9] Konstanze Döhner,[10] Lars Bullinger,[10] Peter Sandy,[11] Jesse S. Boehm,[6] David E. Root,[6] Tyler Jacks,[6,11,12] William C. Hahn,[1,3,4,5,6,*] and D. Gary Gilliland[1,3,6,9,13,14,*]

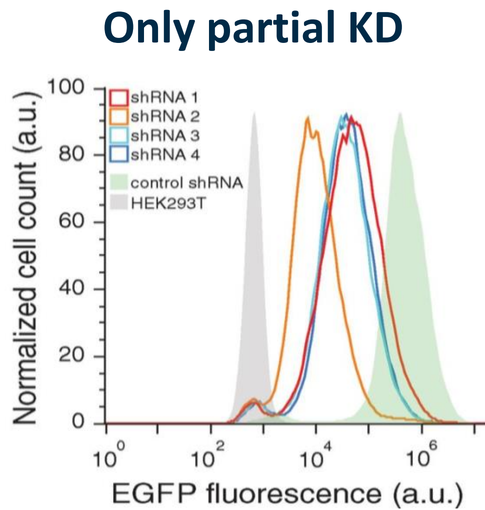
Target ID: The problem with RNAi

Loss of function analysis using RNAi is inexpensive and widely applicable

However

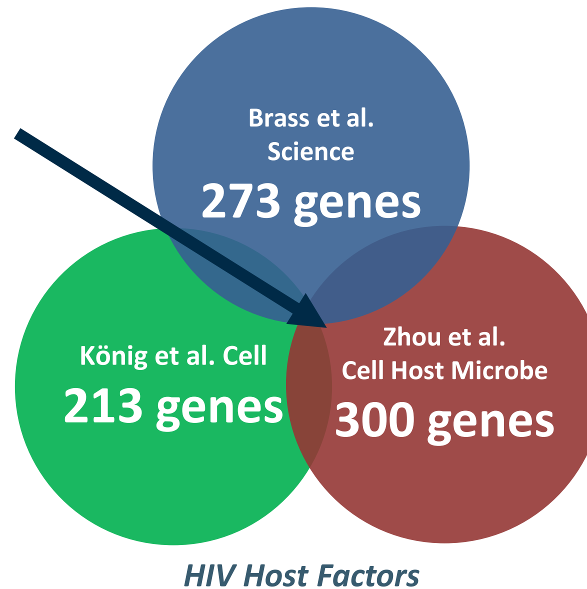
- **Incomplete knockdown**
- **Lack of reproducibility**
- **Off-target effects**

Only partial KD



Shalem et al Science 2014

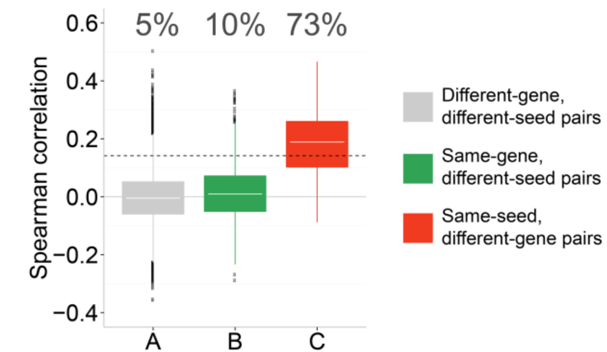
Little correlation between screens



HIV Host Factors

Off-target effects of RNAi driven by seed sequence

shRNAs vs same target exhibit very poor correlation of effects on morphology; effects of shRNAs sharing a seed sequence are well correlated



Problems with RNAi can result in false positives or negatives

The CRISPR-Cas9 Gene Editing Platform

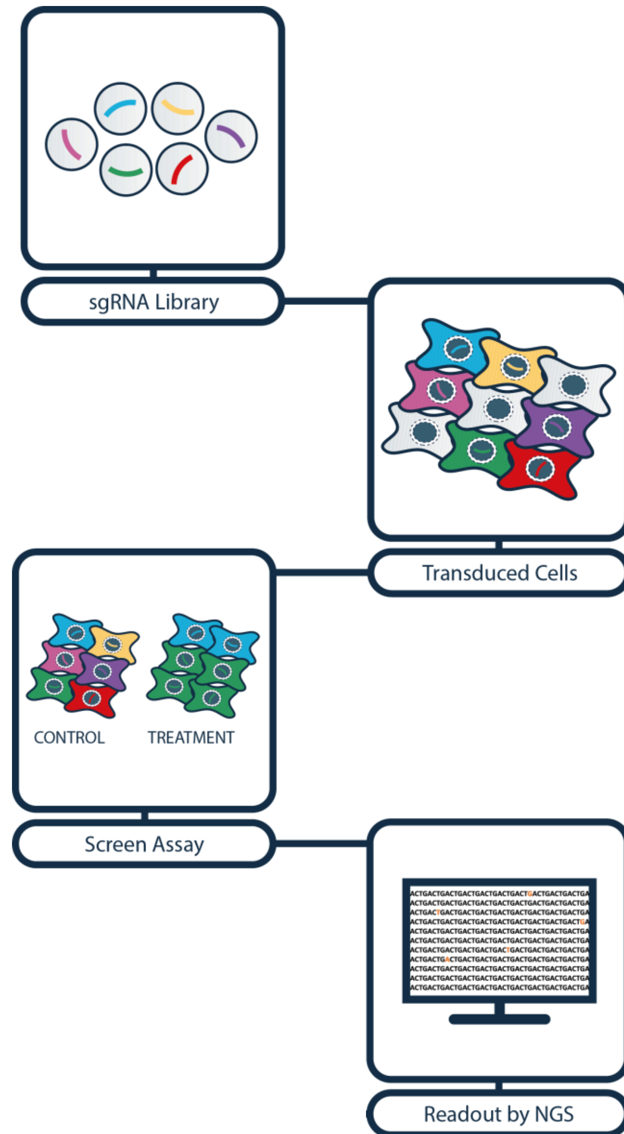
*CRISPR (**C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats) takes advantage of the nuclease activity of the **Cas9** protein targeted to a precise genomic locus by a short **guide** seq*

Cas9 enacts knock-out of target gene

Robust phenotypes due to complete loss of gene function

Anticipated to provide fewer off-target concerns than RNAi

CRISPR-Cas9 Screens use sgRNA sequences as barcodes to ID selected genotypes



1. Select genes to target and design suitable sgRNA library

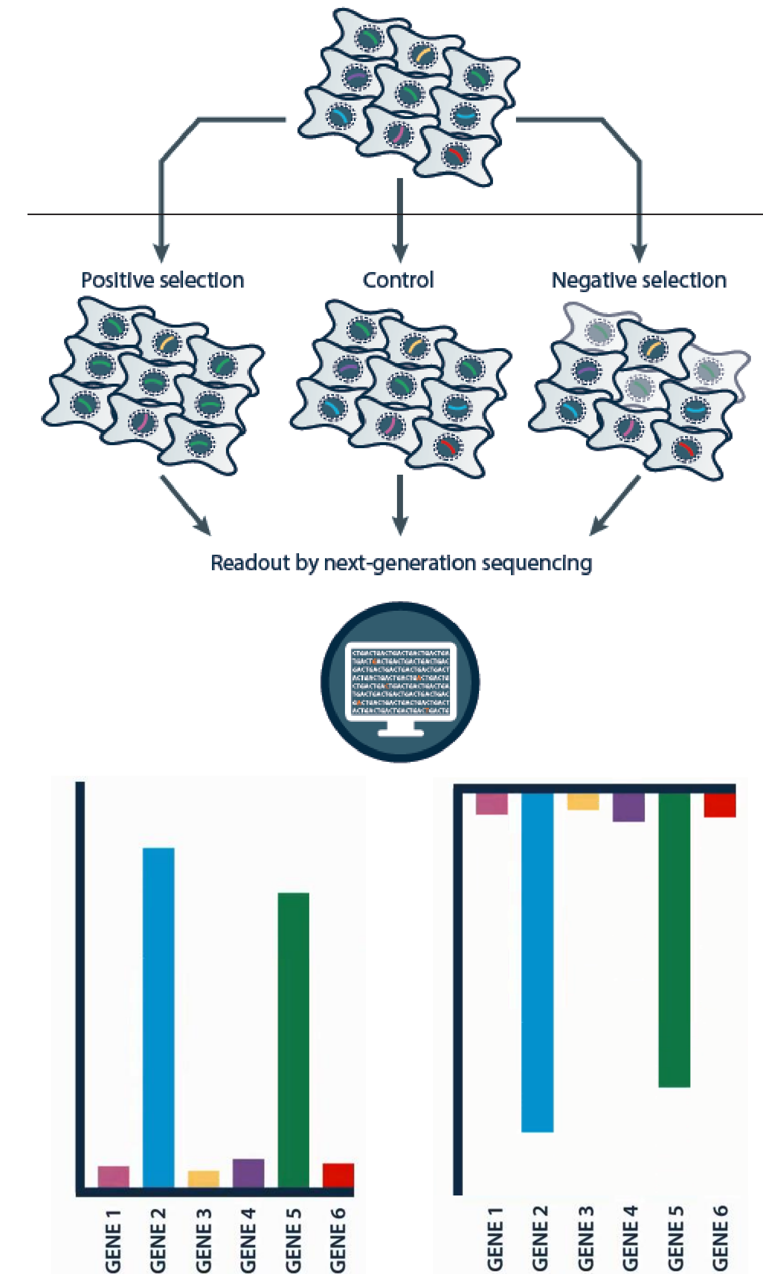
2. Optimise cell culture conditions and then transduce with pooled lentivirus library

3. Select transduced cells then apply assay conditions (e.g. +/- drug)

4. Isolate genomic DNA, amplify sgRNA from lentiviral insert then perform NGS

   lentiviral expression cassette

5. Track changes in sgRNA abundance between samples; aggregate data to identify selected genes

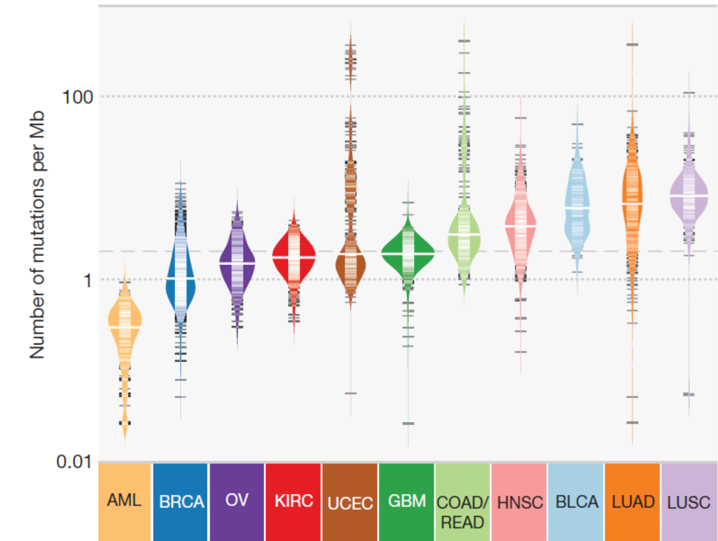


Genetic Interaction Screening in colon cancer

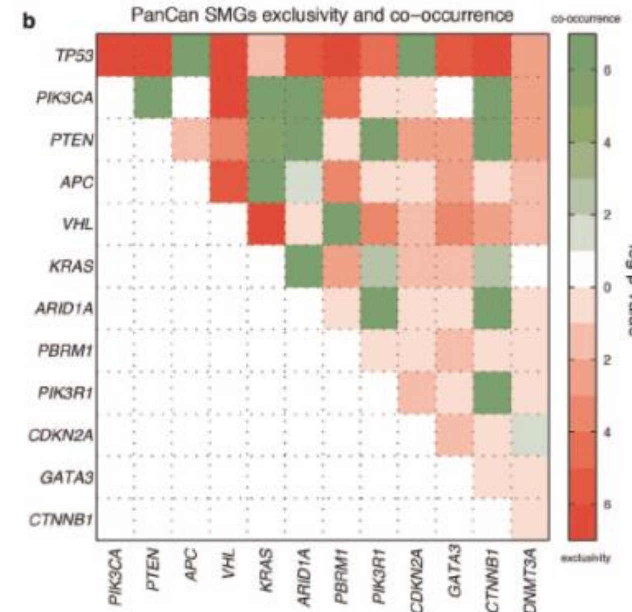
Our efforts have focussed on five frequent genotypes in colon cancer: APC; TP53; KRAS; PIK3CA, FBXW7

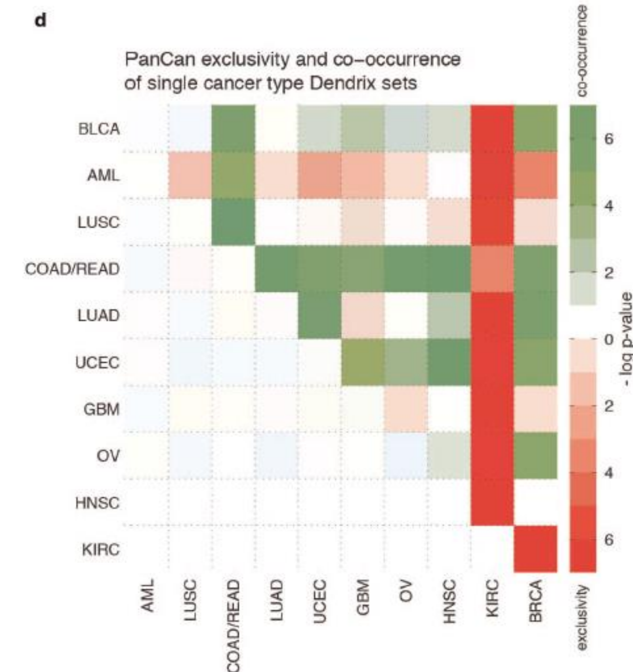
Colon cancer was selected due to:

- Horizon's scientific heritage (founded by Vogelstein lab alumni)
- The most prevalent forms of colon cancer have modest neo-antigen load, so immunotherapies are unlikely to provide a solution for patients
- Mutation co-occurrence in colon has similarities with other histologies such as lung and breast



Gene	Mutation frequency
APC	82%
TP53	59%
KRAS	45%
PIK3CA	17.6%
FBXW7	11.4%
SMAD4	9.8%
NRAS	8.8%
SMAD2	5.7%
ATM	5.7%
ARID1A	5.7%





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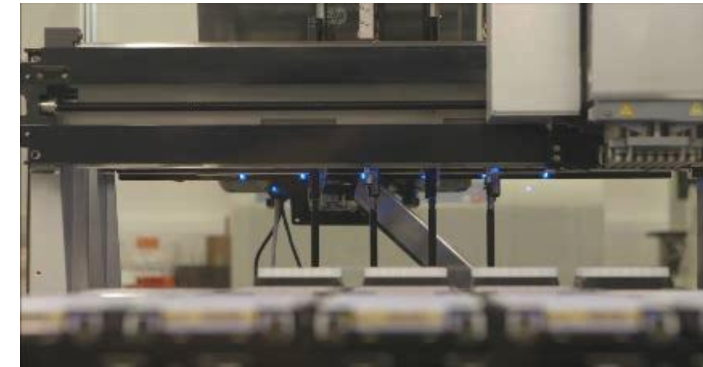
Our program has 3 components

- Arrayed siRNA screening of isogenic/non-isogenic cell line panels



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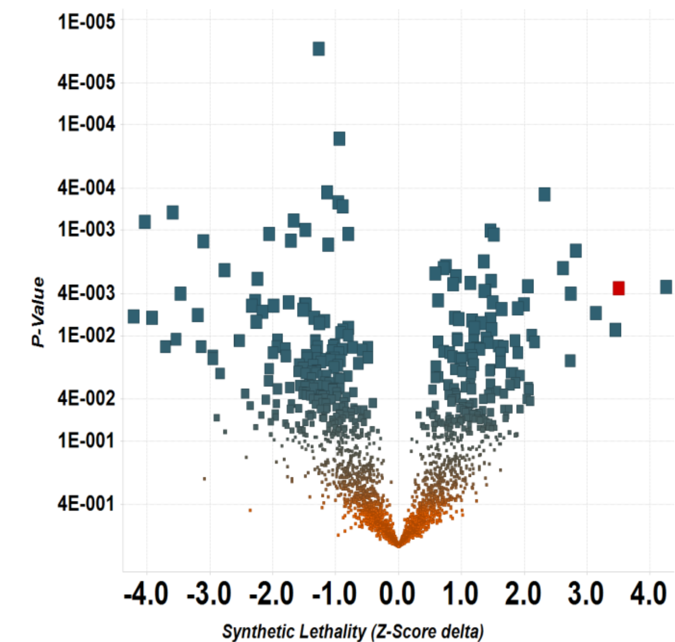

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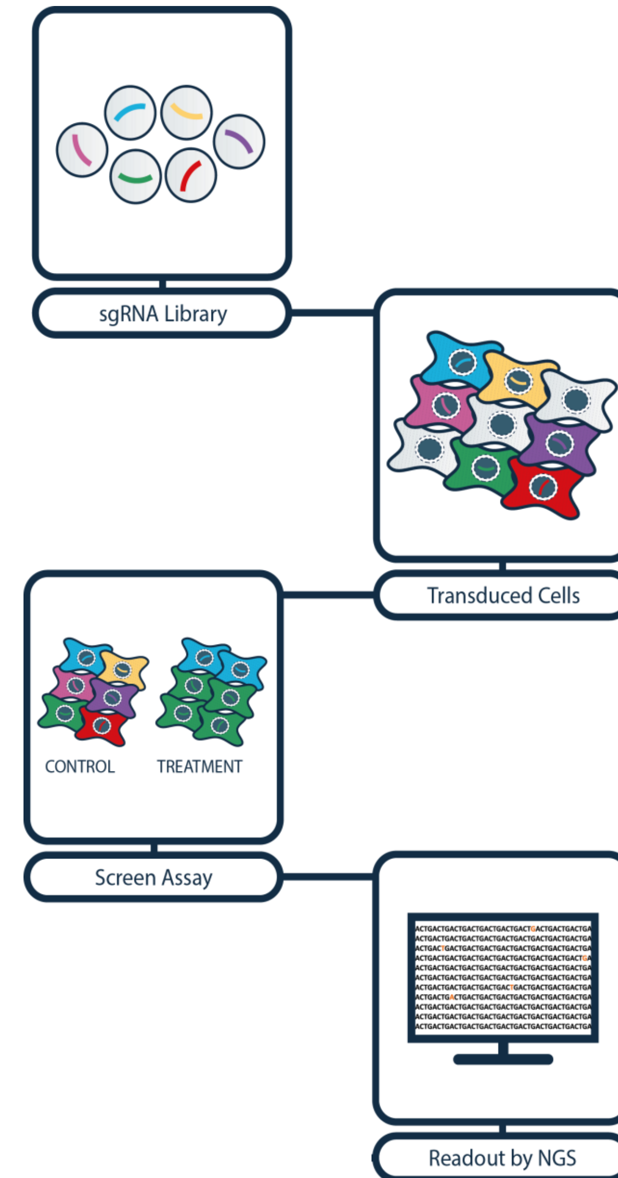

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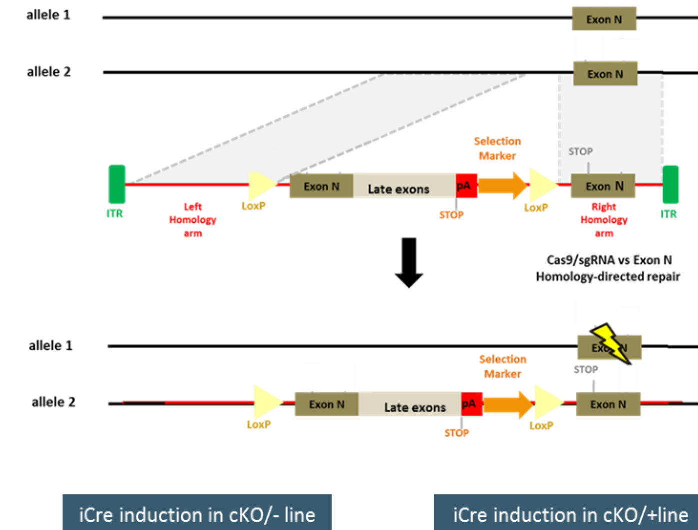

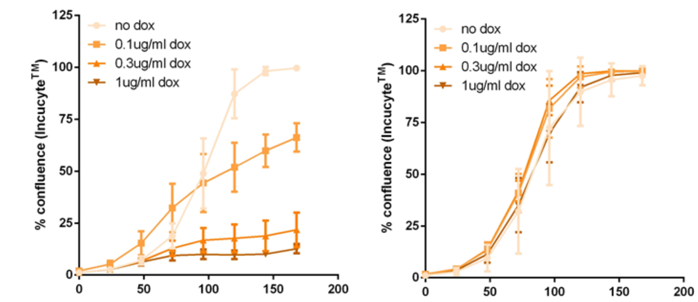

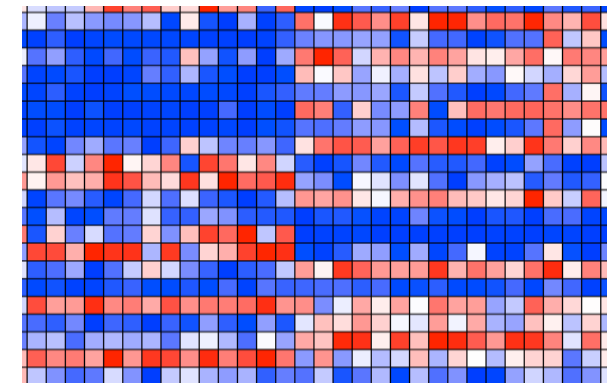

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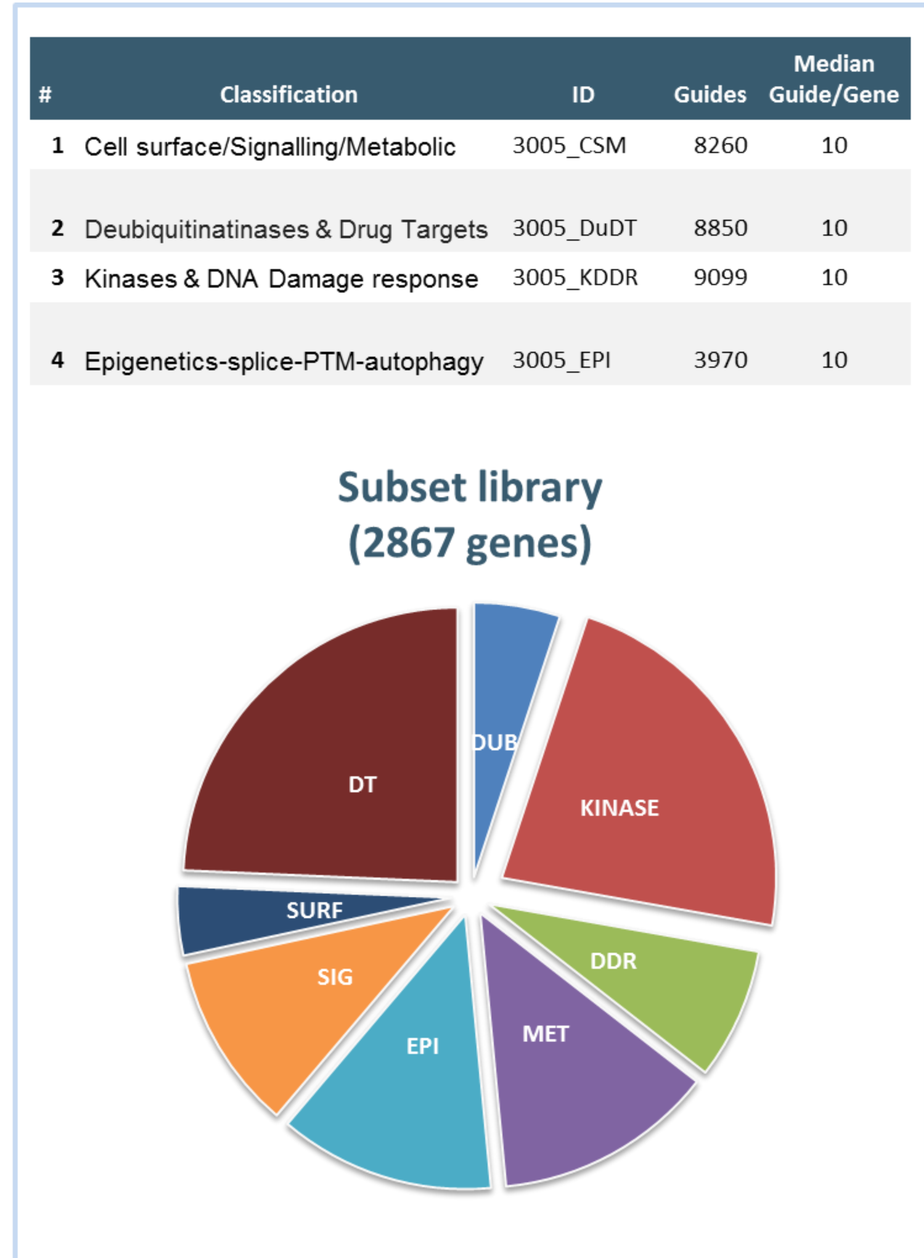
- sgRNA library generation followed by pooled drop-out screening in colon cancer cell line panels to ID essential genes
- Target validation: confirming hits and understanding MOA
- Both the siRNA & sgRNA workflows used a similar subset library targeting 2200-3000 genes

- Horizon has also performed sgRNA library screens with a panel of predominantly lung cell cancer cell lines

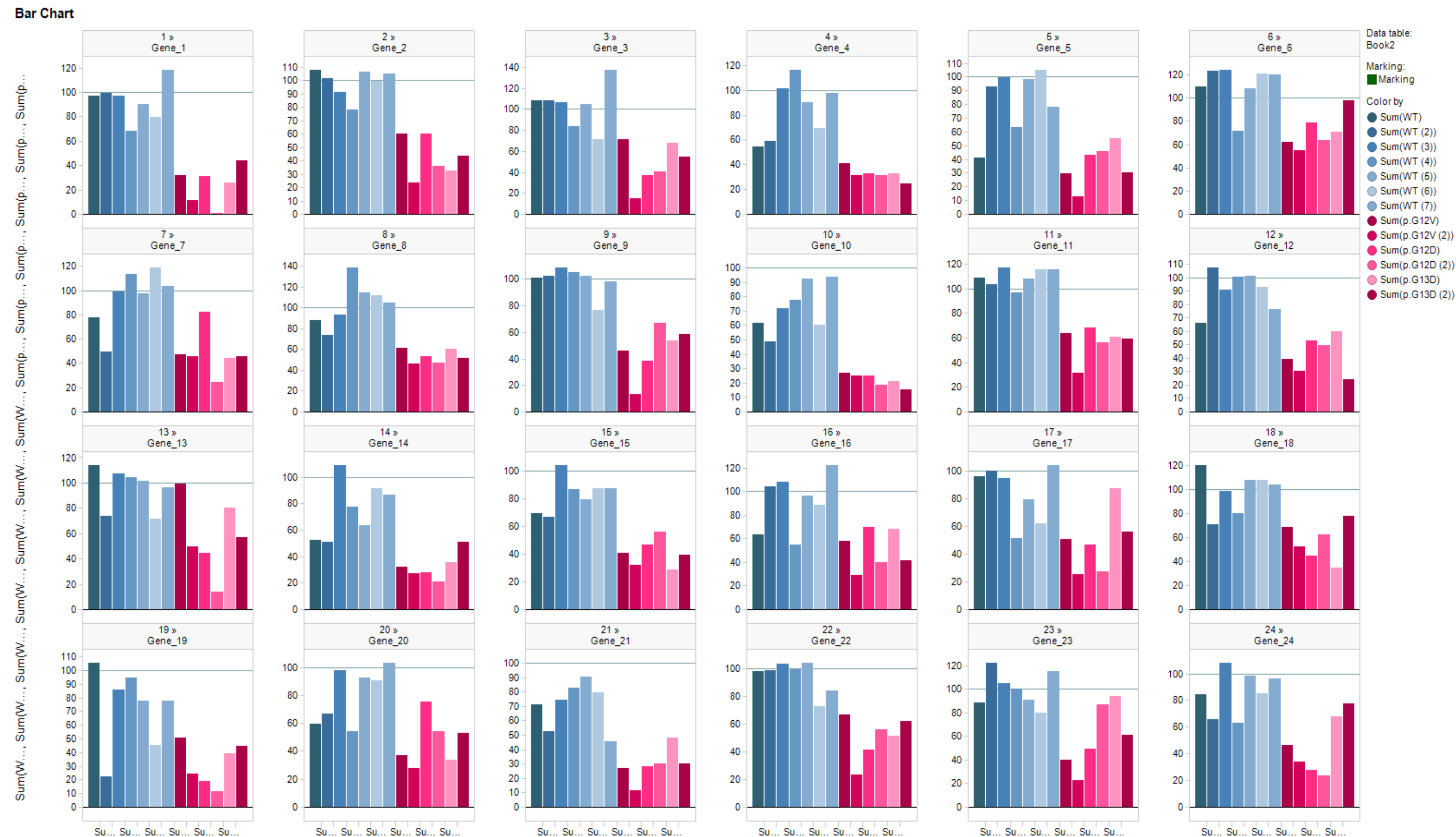


#	Classification	ID	Guides	Median Guide/Gene
1	Cell surface/Signalling/Metabolic	3005_CSM	8260	10
2	Deubiquitinatinases & Drug Targets	3005_DuDT	8850	10
3	Kinases & DNA Damage response	3005_KDDR	9099	10
4	Epigenetics-splice-PTM-autophagy	3005_EPI	3970	10

Are synthetic lethal hits from siRNA screens verified by CRISPR?

siRNA results from non-isogenic panel was encouraging. This figure shows anonymised data for the targets that most closely fit the ideal of blockading growth of KRAS mutant lines (red) but not KRAS wild-type lines (blue);

Gene_1 is KRAS itself

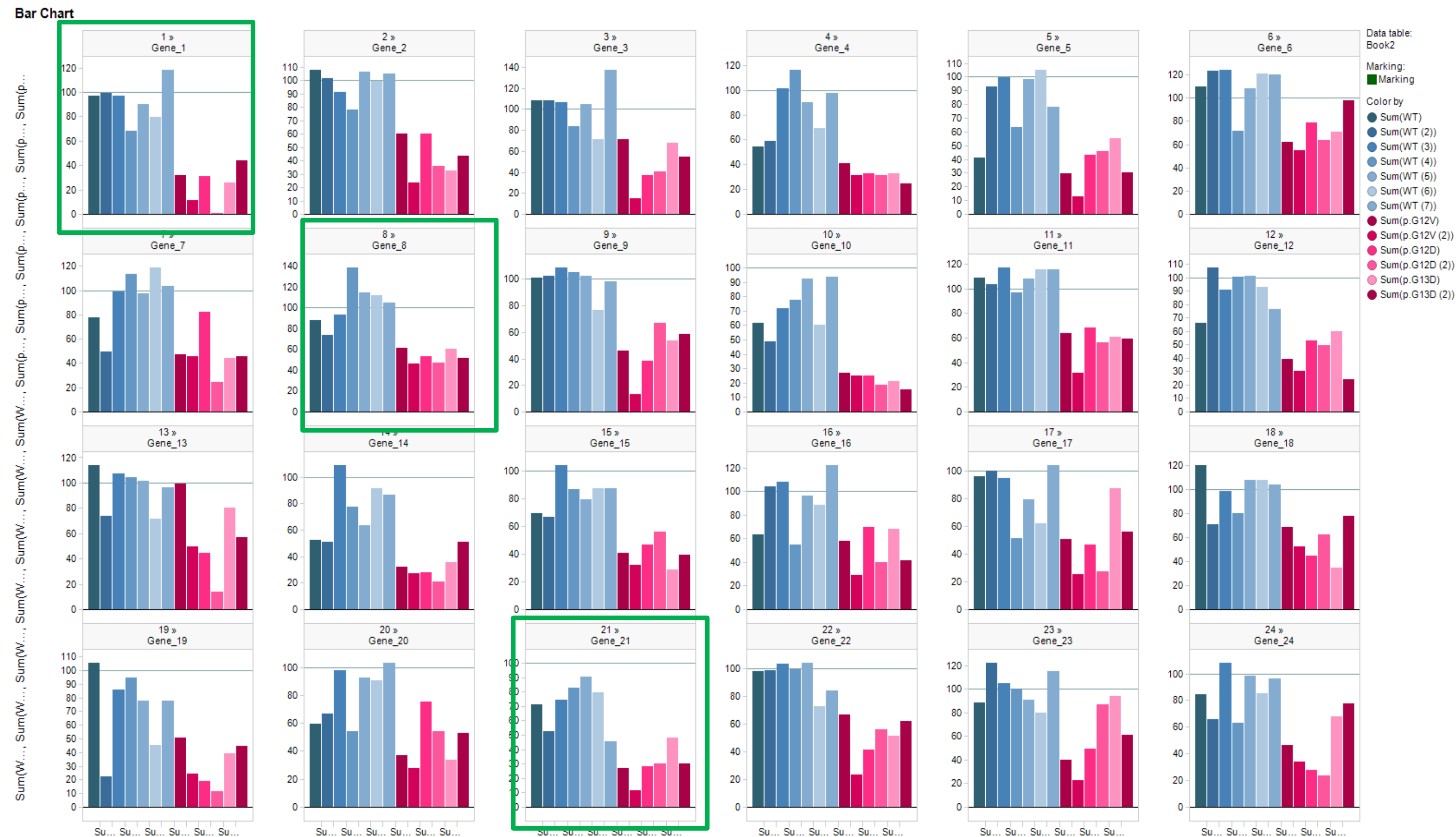


Are synthetic lethal hits from siRNA screens verified by CRISPR?

Generally no! Few siRNA hits were confirmed by CRISPR/Cas9: we only saw support for genes highlighted in green boxes.

Interestingly, for most siRNA SL hits we see no fitness defect in either genotype via CRISPR.

But for a minority if the siRNA-derived putative SL targets, wide-spread essentiality was observed.



Bayes Factor Analysis

Hart's analysis returns a BF for each sgRNA, which takes the log_2FC change through the screen and returns the relative chances of the sgRNA being a member of the set of sgRNAs targeting pre-defined essential genes *vs*. being a member of the set of sgRNAs targeting the pre-defined set of non-essential genes

Gene level log_2BFs are recovered by adding the log_2 of all the BFs defined above for the sgRNAs targeting the gene in question

For a high quality screen with the improved sgRNA library, results look as shown on the right

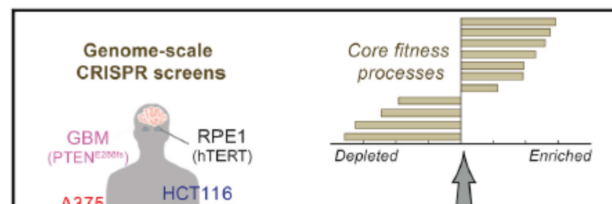
We typically use a log_2BF of 3 to call a fitness phenotype

Resource

Cell

High-Resolution CRISPR Screens Reveal Fitness Genes and Genotype-Specific Cancer Liabilities

Graphical Abstract



Authors

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Correspondence

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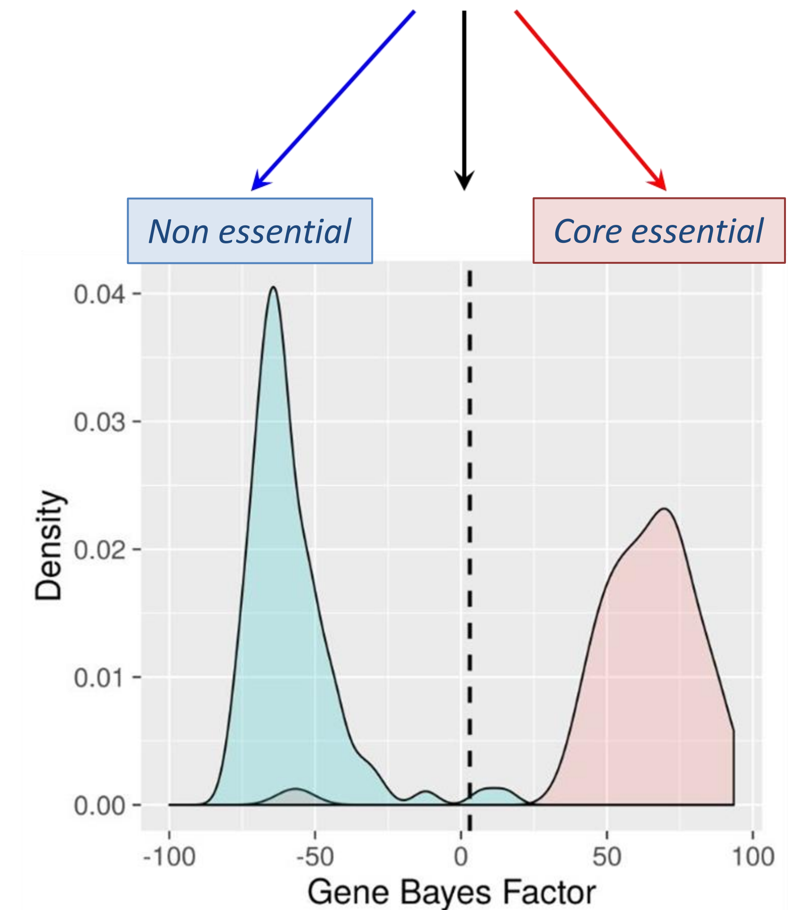
Hit selection based on logFC → Bayes Factor analysis



BFs are quoted as log2 K. A BF of 3 indicates that the gene in question is 8x more likely to be a fitness gene than it being not subject to selection

CRISPR screens find novel synthetic lethal targets

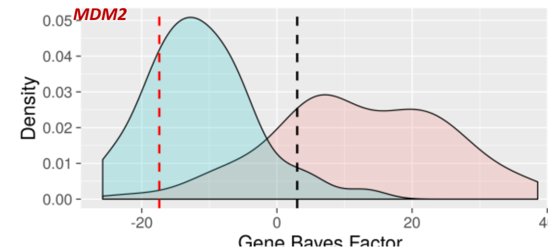
Our CRISPR screens have been analysed by various methods including Hart's Bayesian approaches

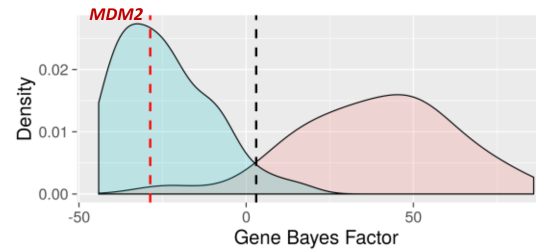
Putative synthetic lethal hits have been identified in the FBXW7, PIK3CA, TP53 and KRAS genotypes

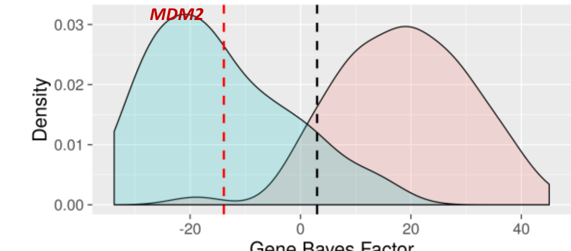
We have confirmed some known interactions: e.g. MDM2 is essential in TP53 wild-type cancers

	TP53 mutant lines									*TP53 wild-type lines*			
GENE	C2BBE1	DLD1	HT115	HT29	HT55	KM12	SNU1197	SNUC5	SW480	GP2D	HCT116	RKO	SNU407
MDM2	-14.9	-34.7	-8.5	-27.6	-4.4	-11.8	-14.1	0.7	-11.4	21.4	41.8	29.0	30.6
Phosphatase													
CHEK1													
STK33													
Kinase R													
GTPAse													
Metabolic enzyme													
Lysine demethylase													
Rb binding partner													
Kinase S													
CUL3													
Helicase H													
Proteosome subunit													
Kinase T													
Lipid oxidase													
Tripartite motif protein													

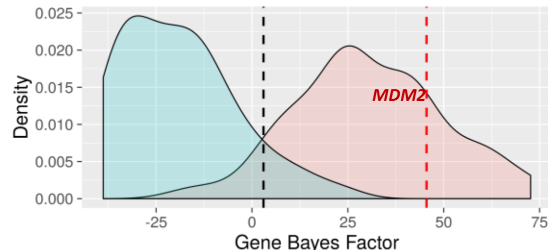
TP53 mutants

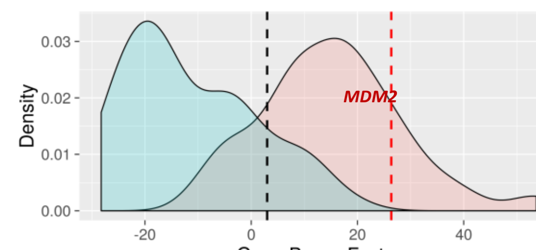


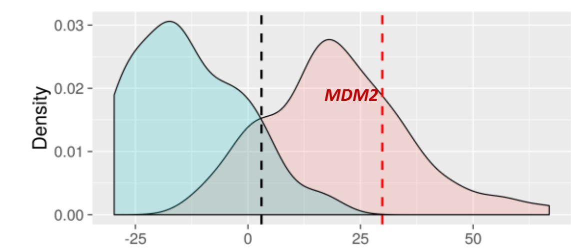




TP53 wild-types







BFs are quoted as log2 K. A BF of 3 indicates that the gene in question is 8x more likely to be a fitness gene than it being not subject to selection

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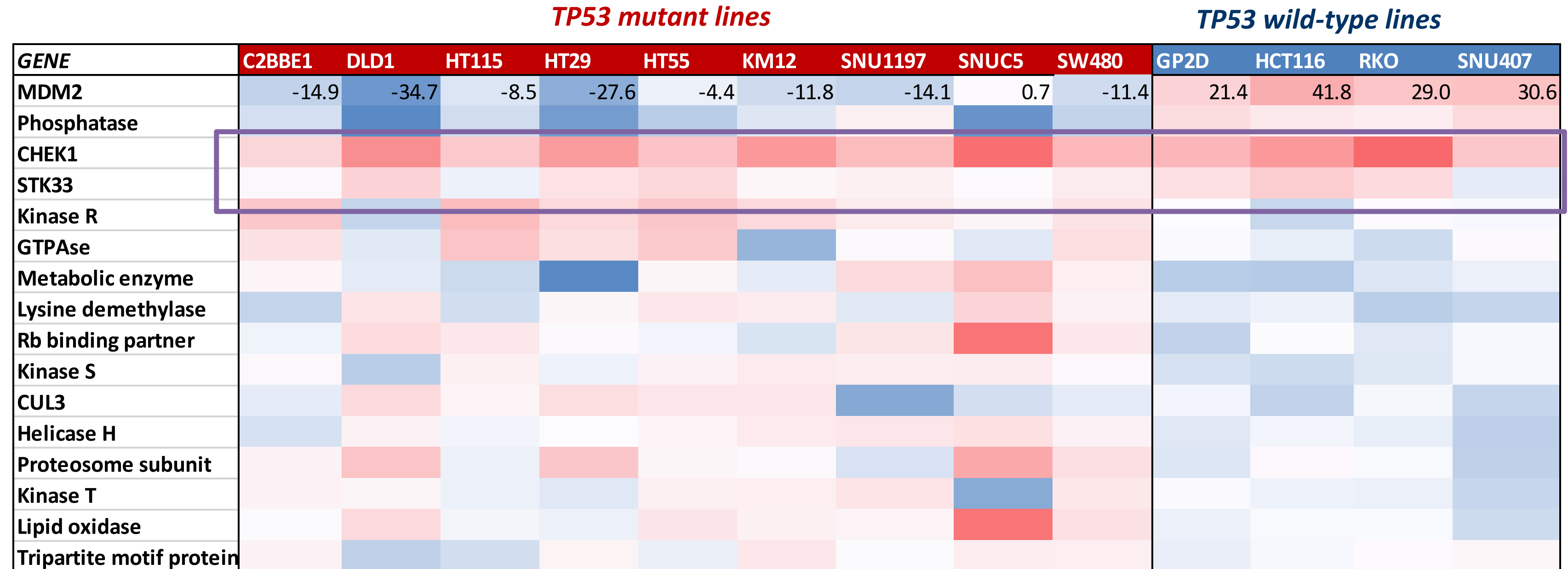
Some of our data may be relevant to the failure of first generation synthetic lethal targets.

CHEK1 (shown below) and STK33 are essential in the great majority of cell lines tested.



	TP53 mutant lines								*TP53 wild-type lines*			
GENE	**C2BBE1**	**DLD1**	**HT115**	**HT29**	**HT55**	**KM12**	**SNU1197**	**SNUC5**	**SW480**	**GP2D**	**HCT116**	**RKO**	**SNU407**
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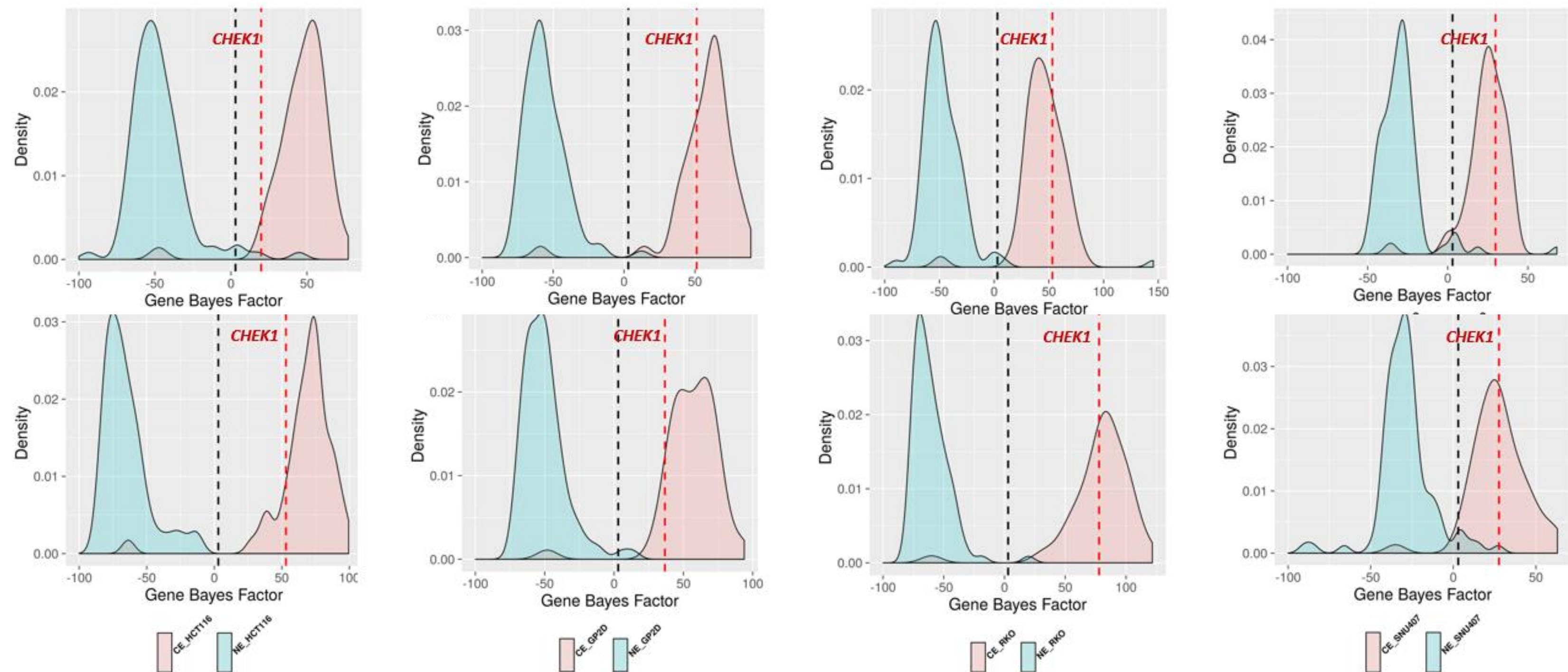

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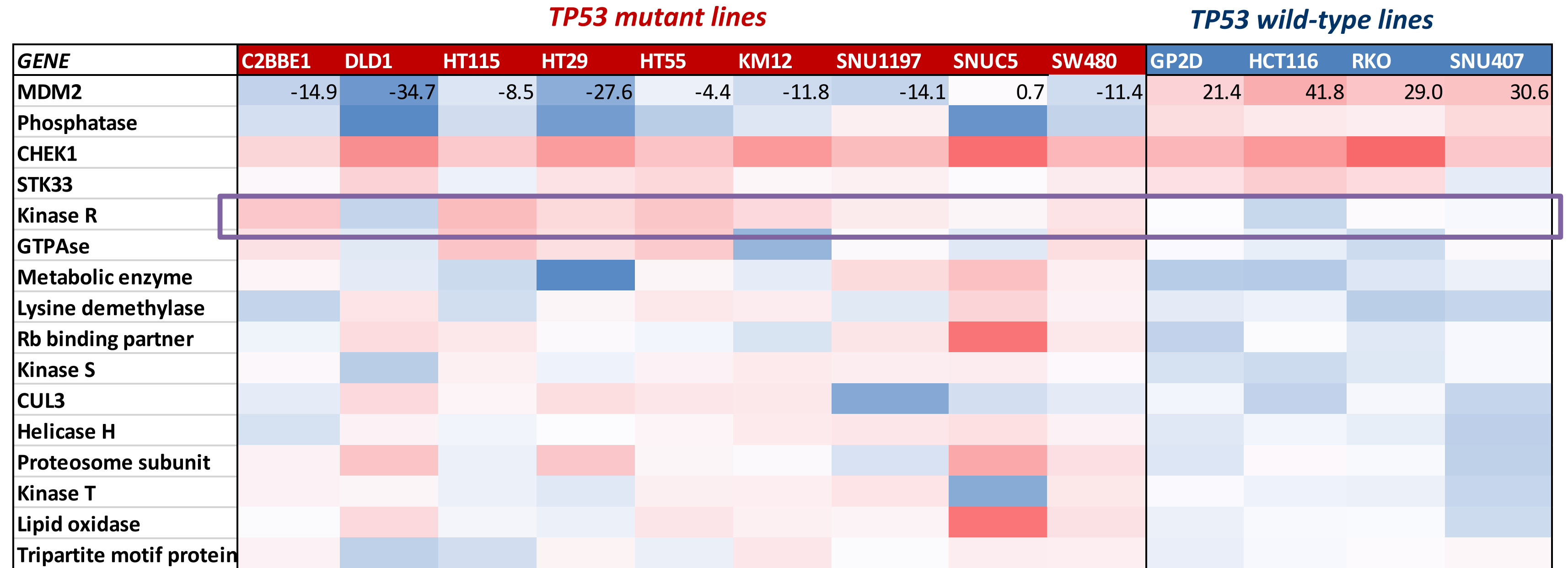
NB CHEK1 BFs are from expt with DDR library in improved sgRNA backbone

CRISPR screens find novel synthetic lethal targets

We also find many novel potential SL relationships.

“Kinase R” is one of several novel & potentially tractable targets that may be selectively essential in TP53 mutant colon cancers



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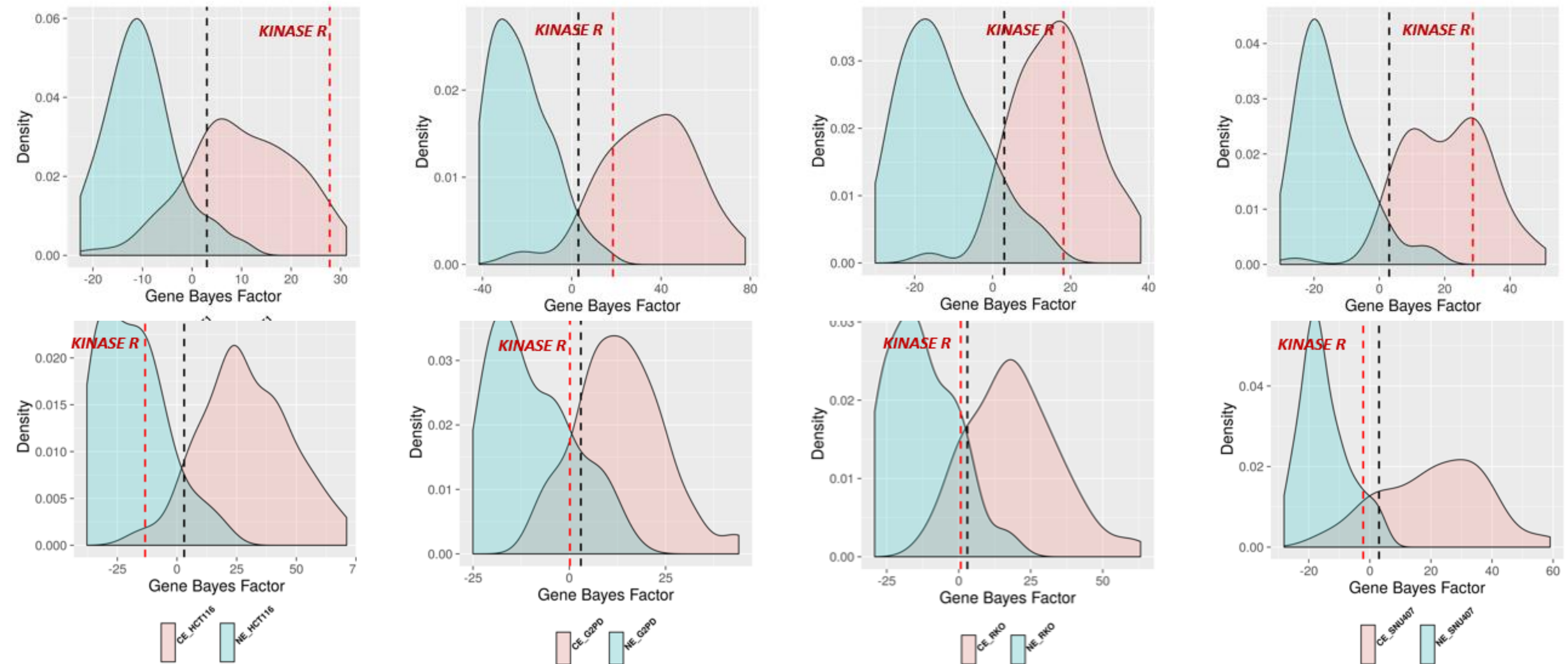
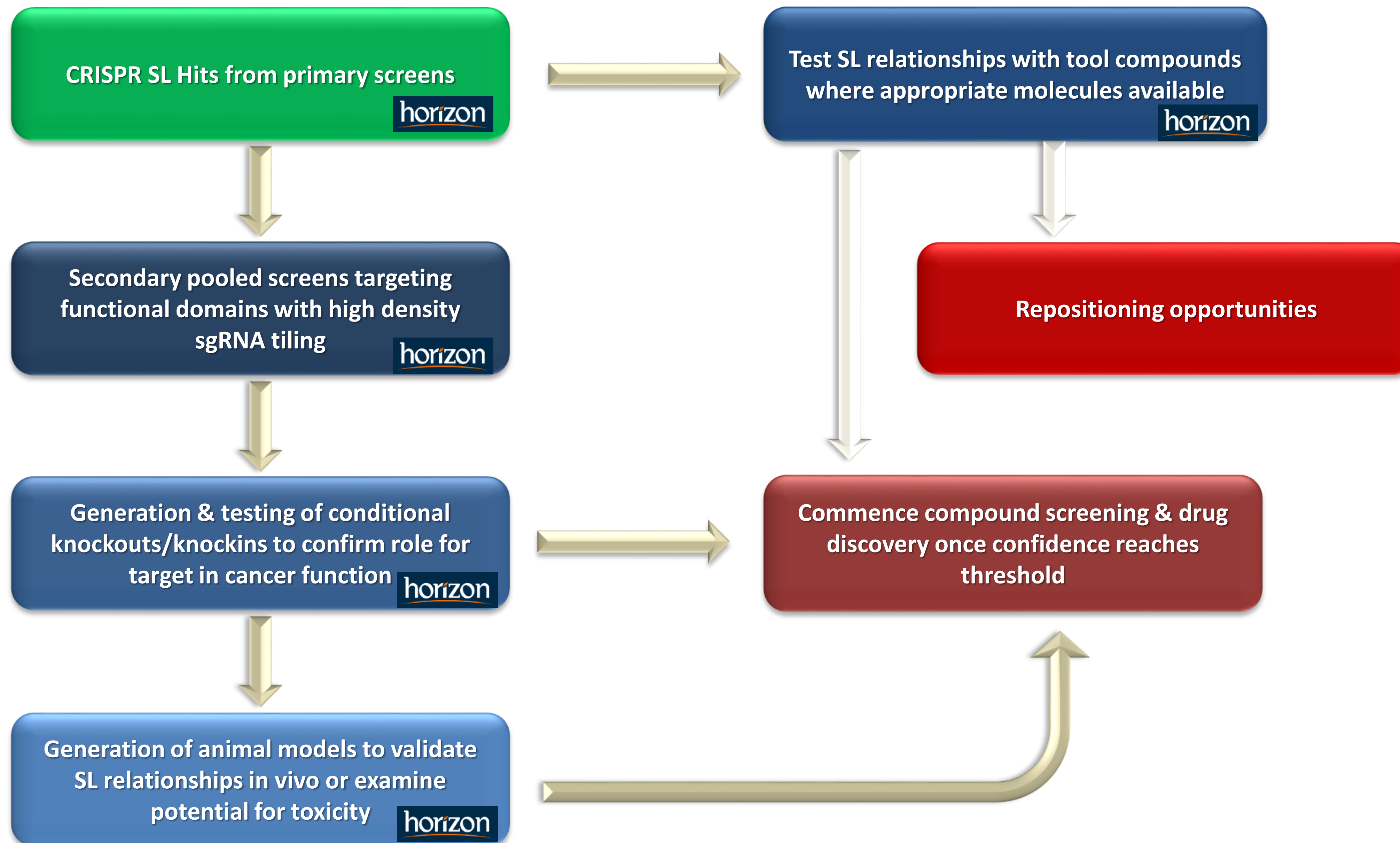


Horizon's Target Validation Capabilities



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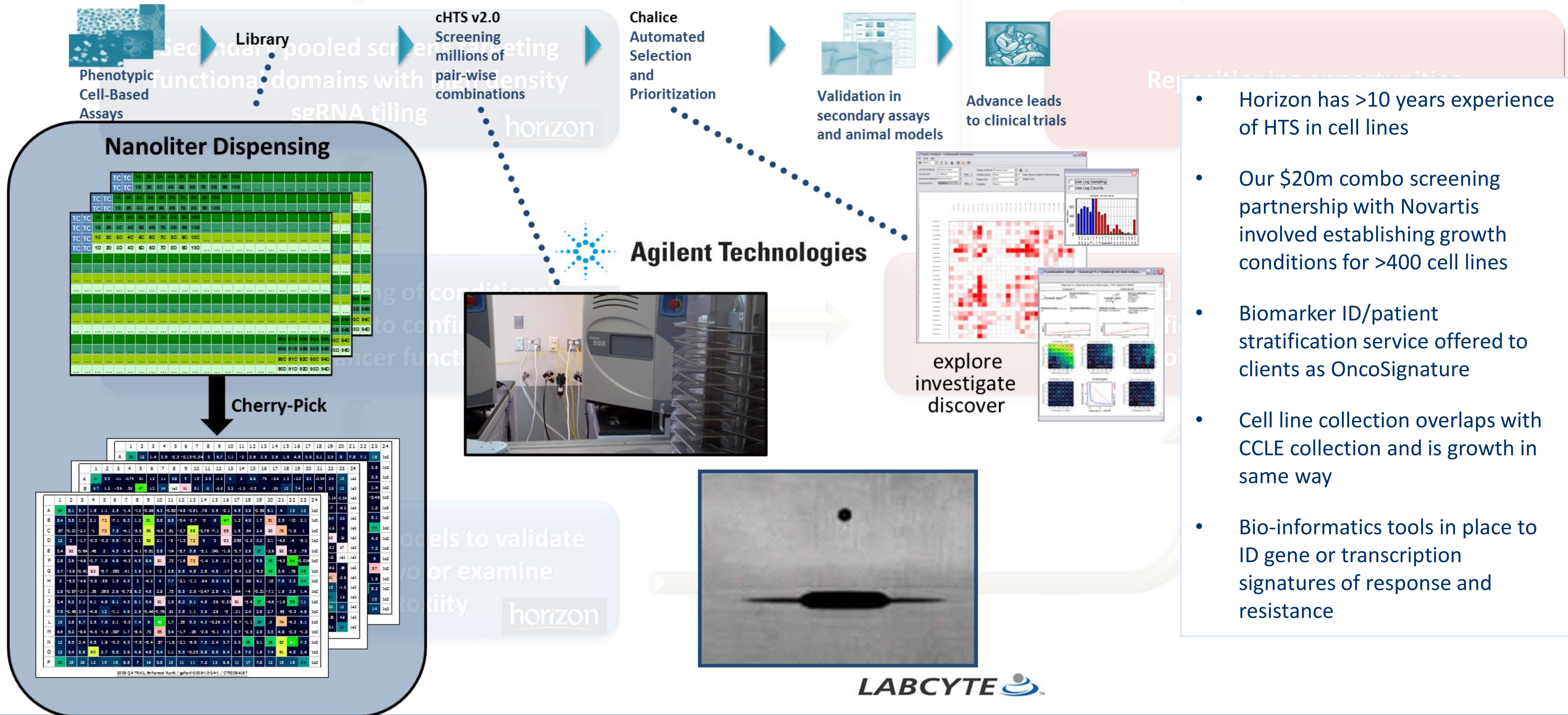
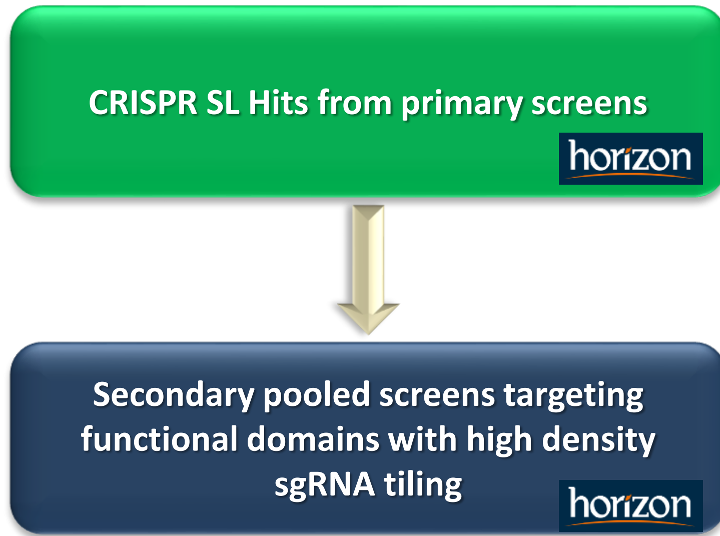


- Horizon has >10 years experience of HTS in cell lines
- Our $20m combo screening partnership with Novartis involved establishing growth conditions for >400 cell lines
- Biomarker ID/patient stratification service offered to clients as OncoSignature
- Cell line collection overlaps with CCLE collection and is growth in same way
- Bio-informatics tools in place to ID gene or transcription signatures of response and resistance

Horizon's Target Validation Capabilities



- One issue with CRISPR technology is that a fraction of cuts are resolved as in frame edits/substitutions that may leave gene function intact
- However, Christopher Vakoc's lab found that sgRNAs targeted vs important functional domains where there was little tolerance for mutation were depleted far more effectively
- Horizon has used ultra-deep pooled CRISPR screens to validate its siRNA hits
- The putative SL hit on the right from an siRNA screen, proved essential in all cell lines tested. The blue guides target a coiled-coil region involved in complex formation
- This provides a high throughput way of validating hits

Discovery of cancer drug targets by CRISPR-Cas9 screening of protein domains

Junwei Shi[1,2], Eric Wang[1], Joseph P Milazzo[1], Zihua Wang[1], Justin B Kinney[1] & Christopher R Vakoc[1]

nature biotechnology

Brd4

Fold depletion (GFP% d2/GFP% d6)

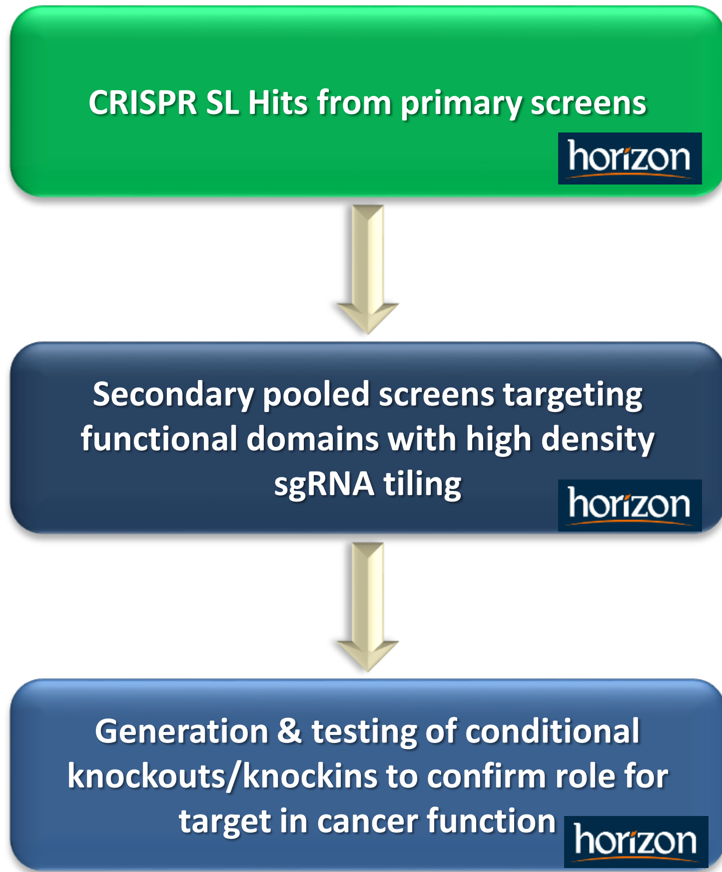
BD1 BD2 ET CTM

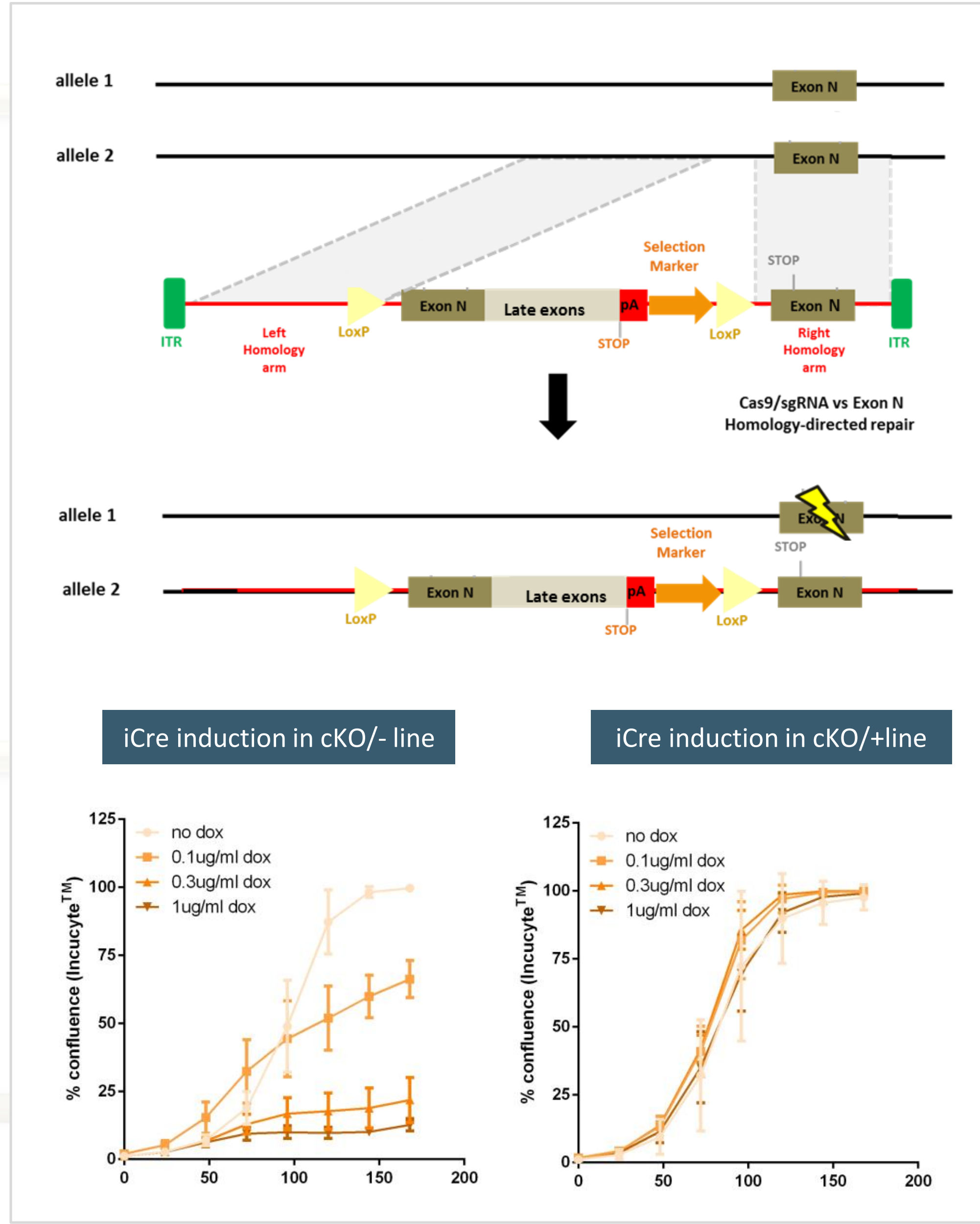
C2BBE1 GP2D HCT116 KM12 RKO SW480

sgRNA log fold change (end-point versus plasmid)

Amino acid cut position

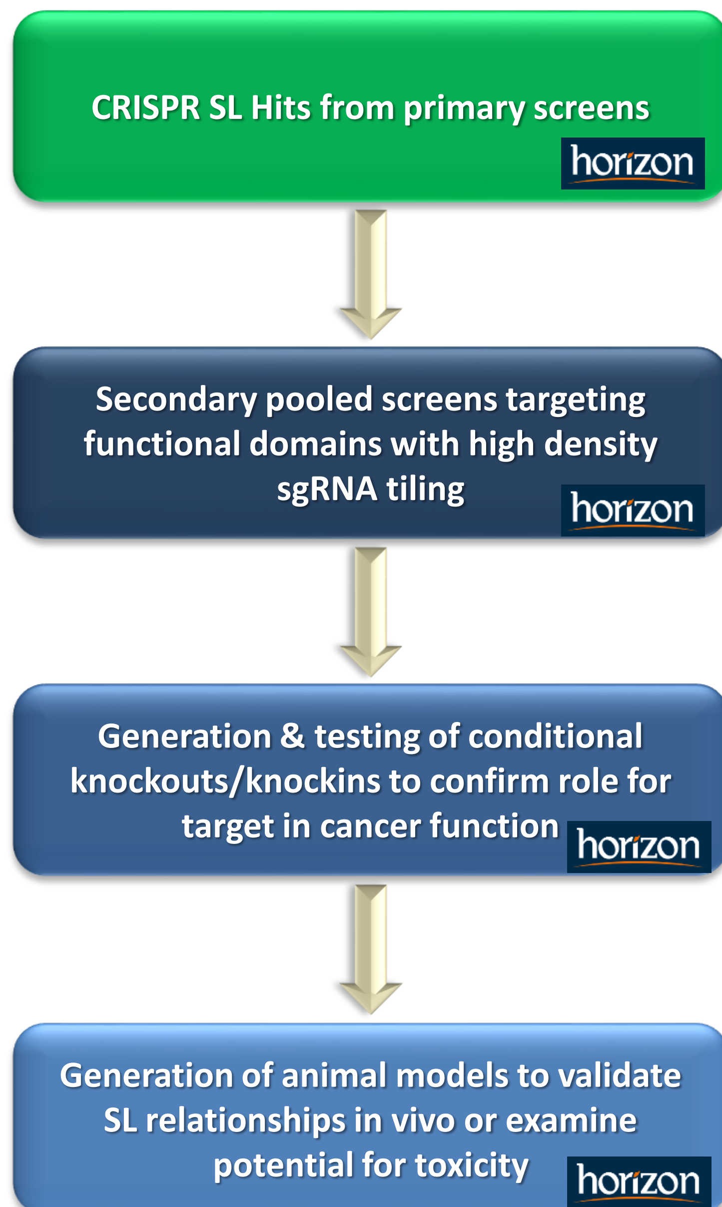
Horizon's Target Validation Capabilities



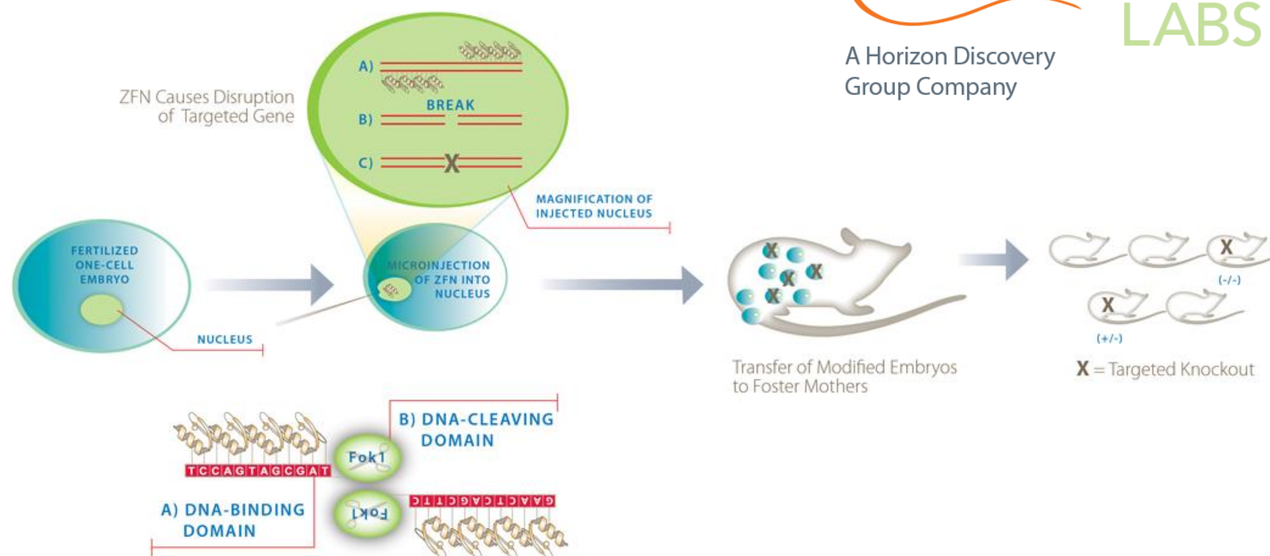


- Horizon has the expertise to perform ambitious gene editing experiments, such as the one-step generation of conditional KO cell lines
- We can tag genes, make activity-dead mutations, assess the ability of a pool of mutant proteins to complement the phenotype of a knockout
- This can be coupled with extensive assay development experience relevant to understanding signal transduction and now also immuno-oncology

Horizon’s Target Validation Capabilities



SAGE LABS
A Horizon Discovery Group Company



- Through its St Louis/Boyertown In vivo Centre of Excellence (formerly SAGE labs) Horizon has the expertise to generate sophisticated animals models using gene editing and ES cell approaches
- SAGE achieved numerous firsts in establishing the technology:
  - First KO Rat — *Science* 2009 **325**(5939): 433
  - 6-month KO Mouse — *Genetics* 2010 **186**(2): 451-2
  - First KI Rat — *Nat Biotechnol* 2011 **29**(1): 64-7
  - First Conditional KO Rat — *Nat Methods* 2013 **10**(7): 638-40
- Gene editing affords significant increases in speed for creation of mouse models over ES cell based approaches
- Horizon also offers in vivo oncology services with PDX models and has the capacity to also run xenografts of standard cancer cell lines
- Our St Louis facility therefore provides an in-house platform for in vivo target validation to de-risk drug discovery vs novel targets

The screens so far have just scratched the surface

Horizon has screened just 35 cell lines with its 2999 member library for **survival** phenotypes

There are untapped opportunities for discovery of targets that:

- **Shut down the output of signal transduction pathways** dysregulated in cancer & haematological malignancies **using flow cytometry** based readouts
- **Overcome the innate resistance many cancers have to drugs** such as PIK3CA inhibitors that have reached the clinic but not achieved registration
- **Shut down expression of immuno-suppressive cytokines**

Furthermore, improved CRISPR technology will **increase screen productivity**

- Horizon has reported a lentiviral CRISPR-Cas9 system with a modified tracRNA region that increases the proportion of sgRNAs driving efficient target knock-out

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Increasing the performance of pooled CRISPR–Cas9 drop-out screening

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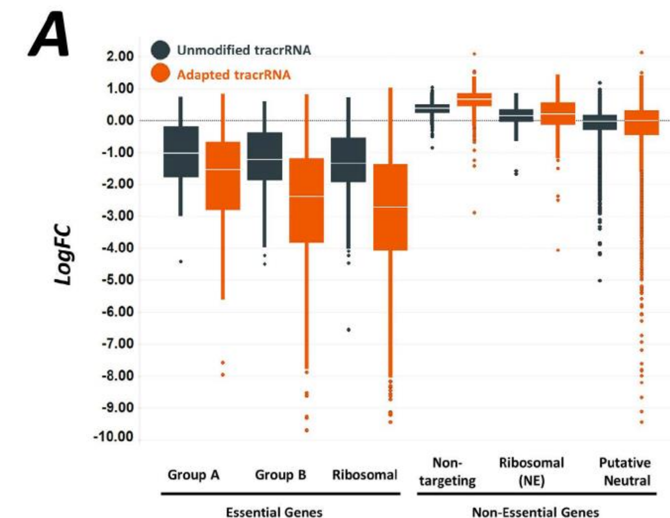

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