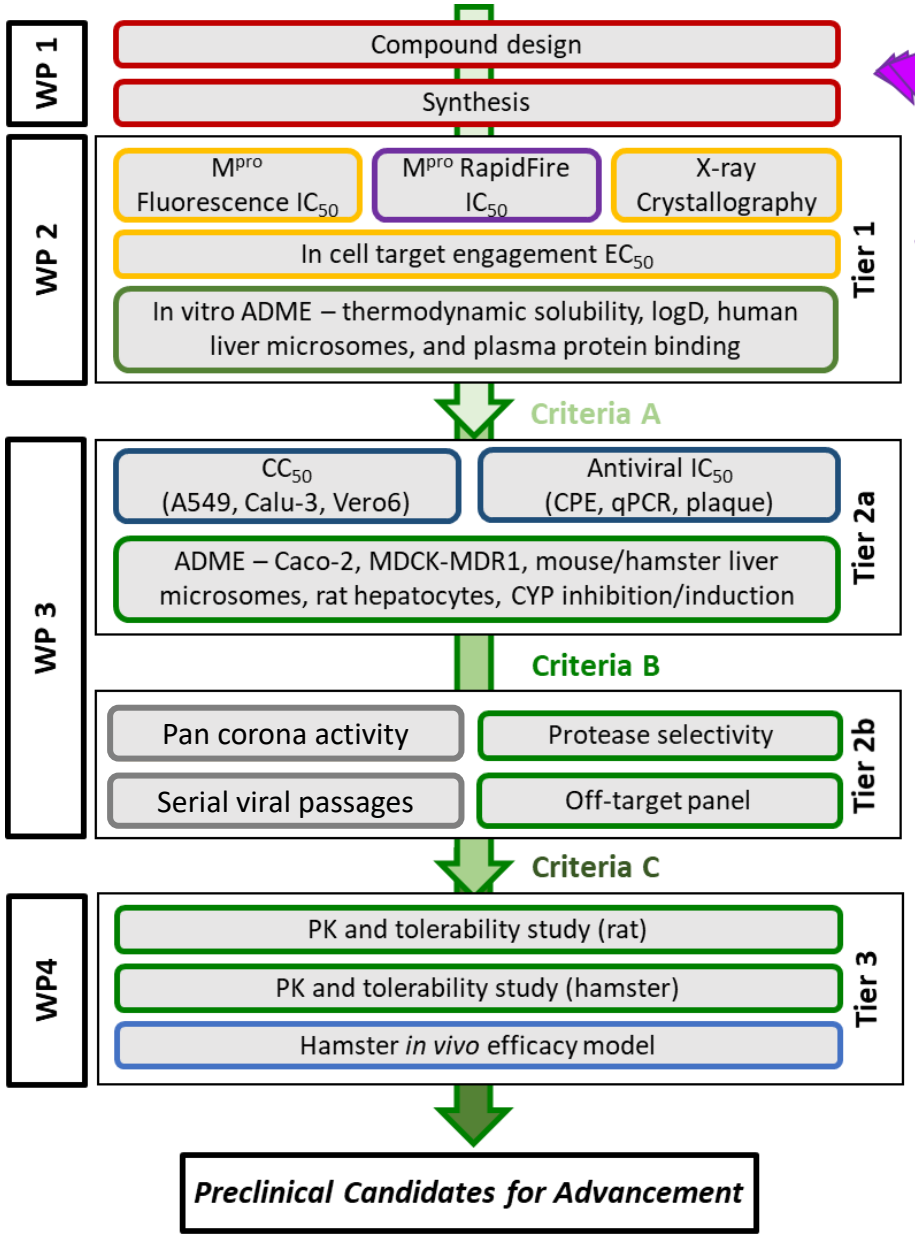
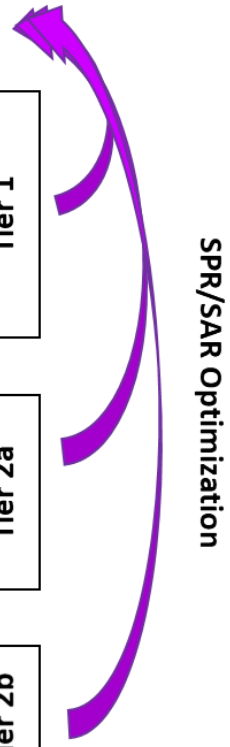
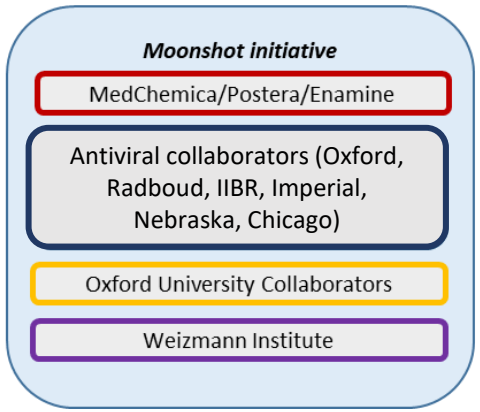
Established pre-clinical assay cascade and criteria for compound development







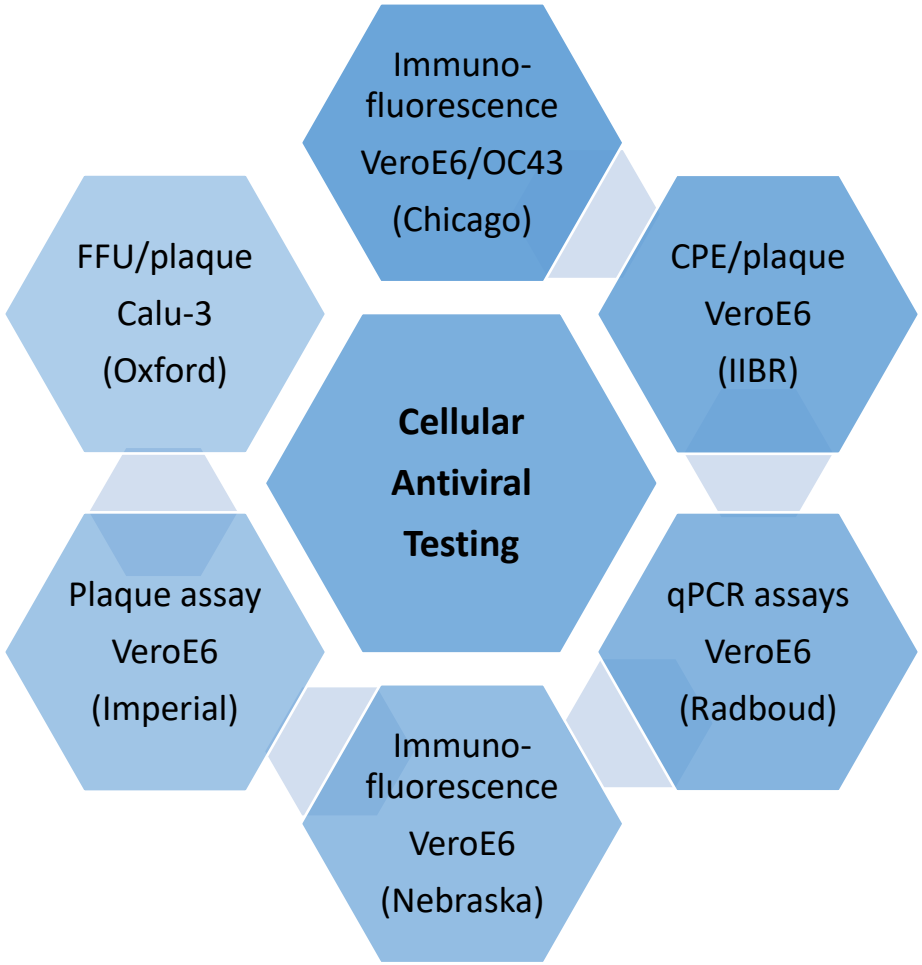
Outsourced

Public Health England & others

	Assay	Measurement	Expected range
Criteria A			
	Biochemistry assays		
	Fluorescence assay	IC50 (uM)	<1uM
	RapidFire assay	IC50 (uM)	<1uM
	Tier 1 ADME		
	thermodynamic solubility,	Solubility mg/ml	*>10 µM*
	logD		
	Plasma protein binding	Free fraction (rat)	>1%
	Human liver microsomal stability	CLint (µg/min/mg protein)	<10
		t 1/2 (min)	>120
Criteria B			
	Cytotoxicity		
	Calu-3 cells	CC50 (uM)	>100uM
	(VeroE6)	CC50 (uM)	>100uM
	(A549 cells)	CC50 (uM)	>100uM
	Antiviral assay data 2a		
	VeroE6 (CPE)	IC50 (uM)	<5uM
	VeroE6 (CPE)	CC50 (uM)	<5uM
	Calu-3 (plaque assay)	CC50 (uM)	<5uM
	Tier 2a ADME		
	MDCK-MDR1	P app (10^-6 cms-1)	>10
	Rat liver microsomal stability	CLint (µg/min/mg protein)	<10
		t 1/2 (min)	>60
	Hamster liver microsomal stability	CLint (µg/min/mg protein)	<50
		t 1/2 (min)	>30
	CYP inhibition and induction	µM	>30
Criteria C			
	Antiviral assay data 2b		
	Protease selectivity	Human cystein proteases	>100x
	Pan-corona efficacy	IC50 (uM)	<5uM
	Serial viral passages		TBD
	Tier 2b ADME		
	hERG	IC50	>100uM
	Ames		Inactive

Antiviral assays

Cellular assays (established collaborations)



Thank you!

Logistical difficulties

- Work intense assay
- BSL3 work – aerosol virus
- Working during lock-down

Why do you run so many assays?

We take up any offer - we have many compounds to profile.

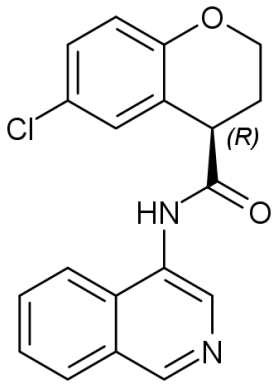
Different cell types?

- VeroE6 cells are easy to grow and enable a quick readout
- Calu-3 cells provide a more physiological readout, but difficult to handle

Redundancy for assay readouts?

- Some assays are more high-throughput/easier to run
- High variability between cellular assay results

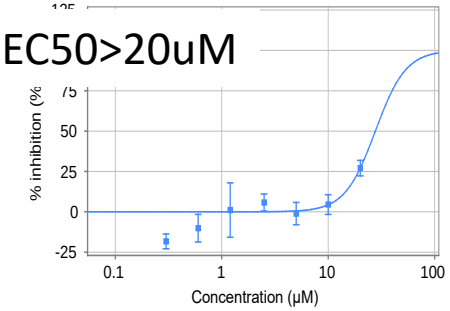
Antiviral efficacy (example current lead isoquinoline):



	Cell type	Readout	IC50 (uM)	CC50 (uM)
Chicago	VeroE6	Immunofluorescence	>20	>20
IIBR	VeroE6	CPE	1.57	>100
Radboud	VeroE6	qPCR	0.232	>25
Oxford	Calu-3	FFU	TBD	>100

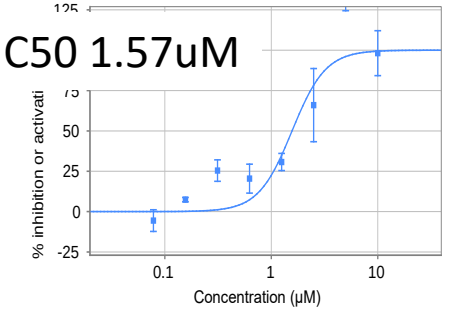
Chicago (OC43)

EC50>20uM



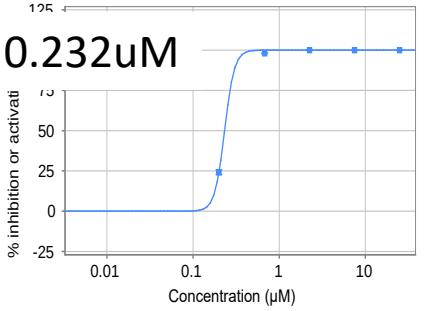
IIBR (SARS-CoV-2)

IC50 1.57uM



Radboud (SARS-CoV-2)

IC50 0.232uM

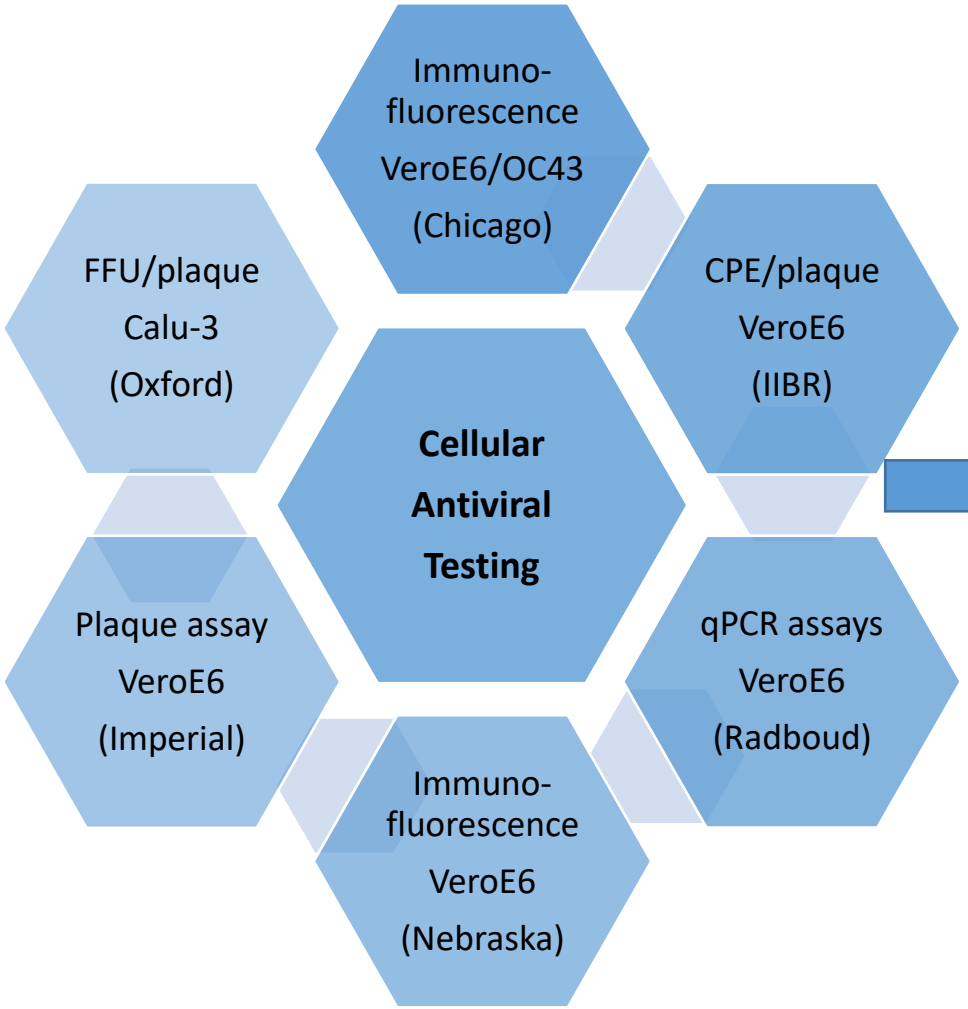


Different viral strains?
Assay differences?
Timing differences?
Solubility issues?

Antiviral assays

Cellular assays (established collaborations)

Animal efficacy (ongoing discussions)



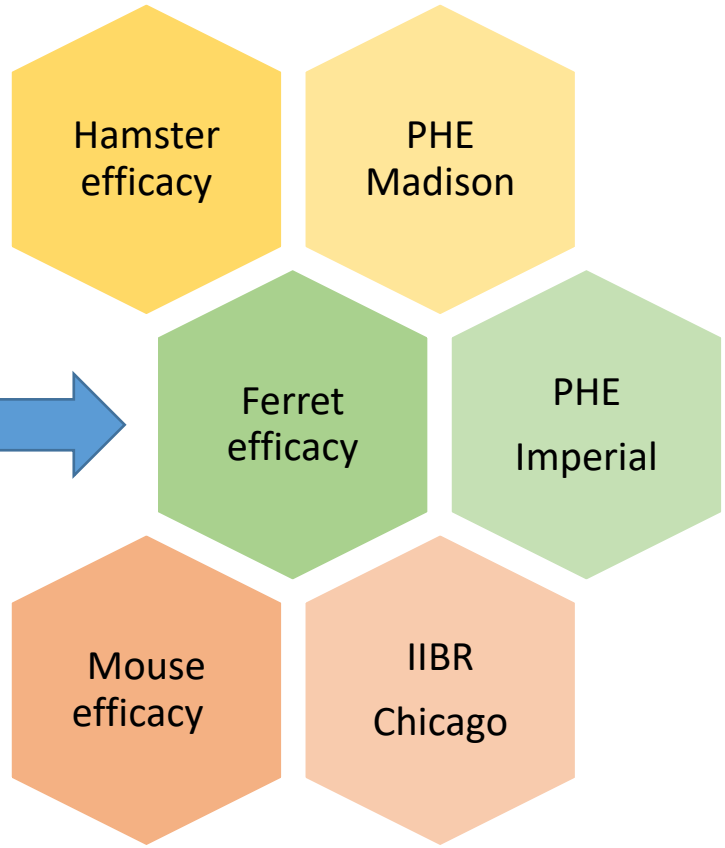
Antiviral efficacy

<10nM biochemical assays

<1uM antiviral assays

(3 assays, 2 cell types)

- Cytotoxicity >100uM
- Good solubility
- Good metabolic profile
- Oral exposure



How do we get to Phase 1?

Task Name	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8
Nominate compound and back up (Preparation for regulatory phase)								
Efficacy Hamster								
Safety index (receptor profiling, safety panels)								
In Silico DEREK / UCB D2P2								
Safety pharmacology (Ames, Genotox)								
Hepatotoxicity cellular assay								
Patch clamp cardiomyocytes								
Safety 44 panel receptor binding panel								
Check CNS exposure - initial assessment (Irwin test)								
Acceptable drug interaction profile								
Compound synthesis for 7 day tox								
7 d ascending dose (non GLP) tox rat								
Process chemistry assessment of scale up feasibility								
Large scale synthesis for GLP tox								
Forced degredation study								
Stability in capsule								
Stability (3 - 6 months)								
Bioanalysis validated (ICH), rat, dog, human								
Regulatory phase								
Dose Range Finding (DRF) pilot toxicology - rat								
Dose Range Finding (DRF) pilot toxicology - dog								
GLP tox - 1 month dog (assume 5 days dosing)								
GLP tox - 1 month rat (assume 5 days dosing)								
Acceptable PK (with a validated bioanalytical method)								
PK scaling and Dose to Human prediction								
Acceptable safety margin (toxicity in rodents or dogs when appropriate)								
GMP manufacture feasibility								
GMP manufacture								
Develop clinical endpoints								
Regulatory assessments								
Clear IND regulatory path								
HPOC/CPOC plan is acceptible to regulatory agency								

Efficacy
CMC
Pharmacokinetics
Regulatory
Clinical
Safety