



**Northumbria  
University**  
NEWCASTLE

# **Resolving Cytosolic Molecular Therapy for Airways Disease: Targeting, Delivery, Bioanalysis, Diagnostics**

**Dr Sterghios A. Moschos FRSC FIBMS FHEA MRSB**

Associate Professor, Cellular and Molecular Sciences

Chief Scientific Officer, PulmoBioMed Ltd.

@docmoschos



# Overview

- Introduction
  - Nucleic Acid Based Therapeutics
  - Key success paradigms
  - Treating *the* lung vs. *via* the lung
- Group efforts in:
  - Diagnostics: exhaled breath
  - Targeting: oligosaccharides
  - Delivery: biovesicles
  - Targets: Idiopathic Pulmonary Fibrosis
- Conclusions for the Post-C19 world

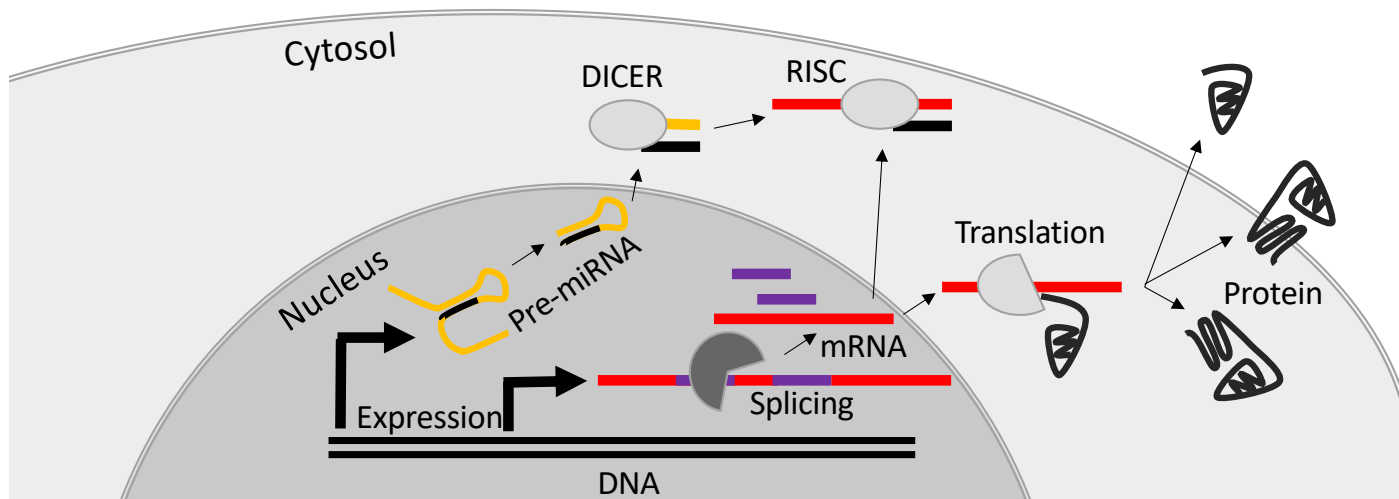
# Introduction: Oligonucleotide Rx

**SINGLE STRANDED: 8 - 40 nt**  
Antisense, Aptamers, ssRNA and ssDNA  
Decoys

**DOUBLE STRANDED: 19 – 30 nt**  
siRNA, microRNA mimics, dsDNA decoys

mRNA  
microRNA  
Splice Junctions  
ncRNA  
Proteins

mRNA  
ncRNA  
Splice Junctions



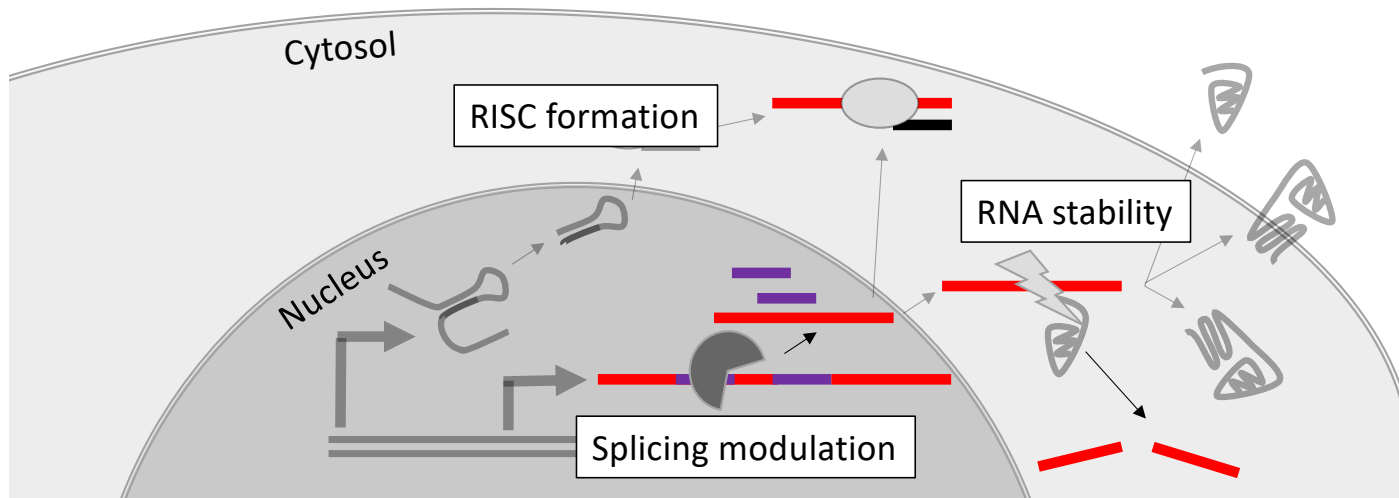
# Introduction: Lung Studies in Animals

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Decoys

**DOUBLE STRANDED: 19 - 25 nt**  
siRNA, microRNA mimics, dsDNA decoys

mRNA  
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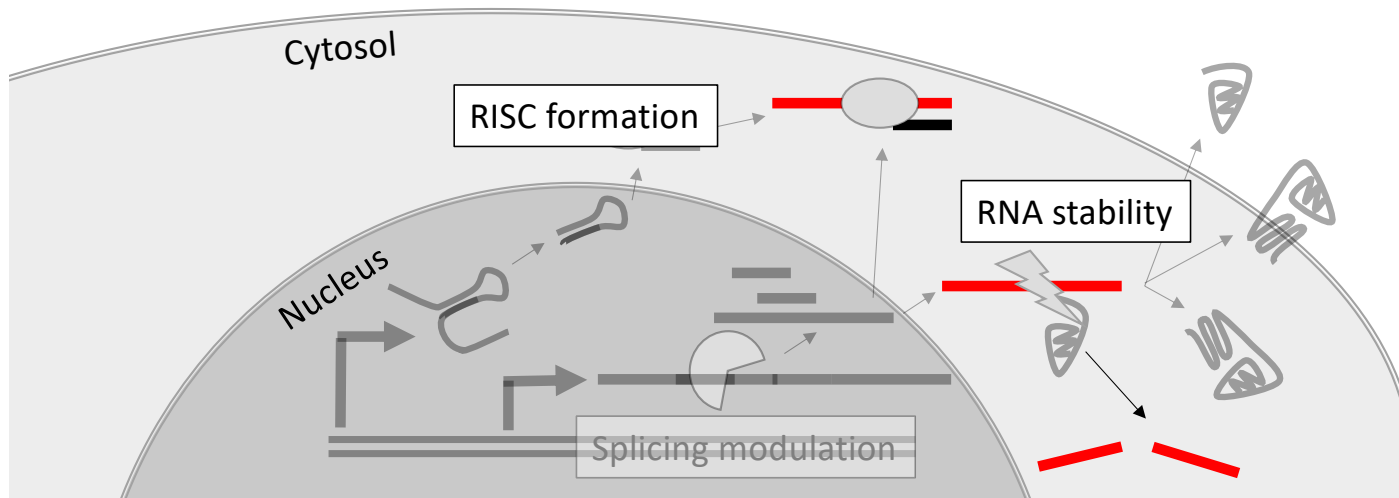
# Introduction: Lung Studies in the Clinic

**SINGLE STRANDED: 20 - 30 nt**  
Antisense, Aptamers, ssRNA and ssDNA  
Decoys

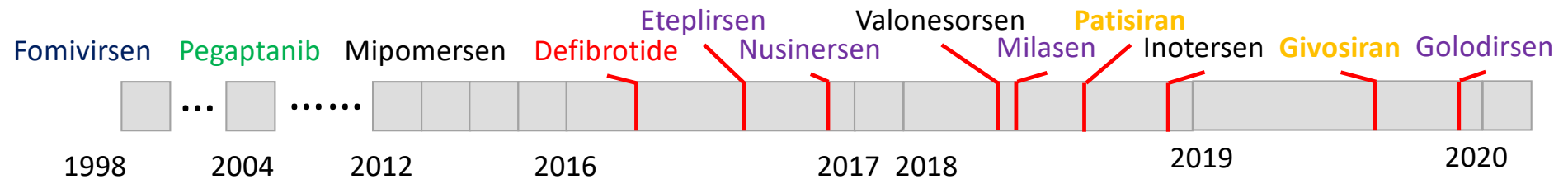
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Proteins

mRNA  
ncRNA  
Splice Junctions



# Clinical Progress: State of the Art.



## Mipomersen: hepatocyte targeting antisense

- Phosphorothioate ASO (PK).
- FDA ✓ but not EMA.
- Platelet loss.

## Eteplirsen: myocyte targeting antisense.

- Splice modulation: restores dystrophin.
- <1% of normal.
- Low expert confidence.

## Nusinersen: Motor neuron targeting antisense.

- Splicing modulation.
- Intrathecal treatment.
- 3x increase in correct RNA.

## Givosiran: small interfering RNA+GalNAc.

- Aminolevulinic acid synthase 1 knockdown.
- Enhanced hepatic uptake.

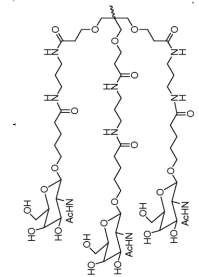
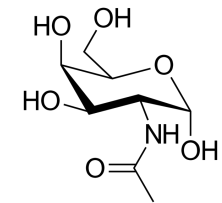
# Targeted delivery: GalNAc and ASGPR

## Asialoglycoprotein receptor

- Hepatocyte-specific.
- High density (1-5 e4/cell).
- High affinity  $10^{-6}$ –  $10^{-9}$  based on valency.
- 10-50x higher for GalNAc

## GalNAc conjugates

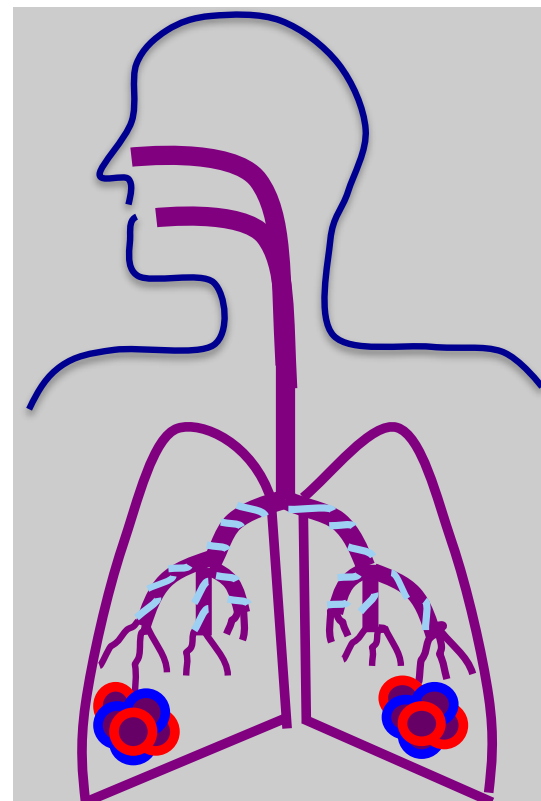
- Antisense, siRNA.
- 1-2 log lower  $IC_{50}$ .
- 1-2 log lower dose.
- Phase II efficacy.
- Multiple oligo classes.



# Does the lung need delivery?

The lung as a target for biotherapeutics:

- Complex anatomy.
- Multiple cell types.
- Variable disease mechanisms.



Moschos *et al.* *Pharmacology & Therapeutics* 2017

# Does the lung need delivery?

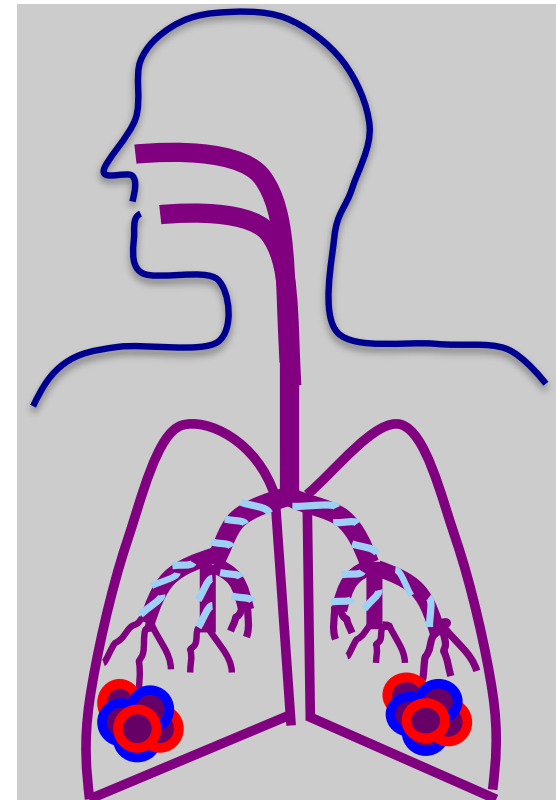
## The lung as a target:

- Complex anatomy.
- Multiple cell types.
- Variable disease mechanisms.

## Gene therapy and oligonucleotides.

### Key assumptions:

1. Local dosing = adequate exposure, lung specific delivery.
2. Tissue loading = target engagement.
3. Target modulation = on target action (~50%).
4. Disease biomarker modulation = on target action.

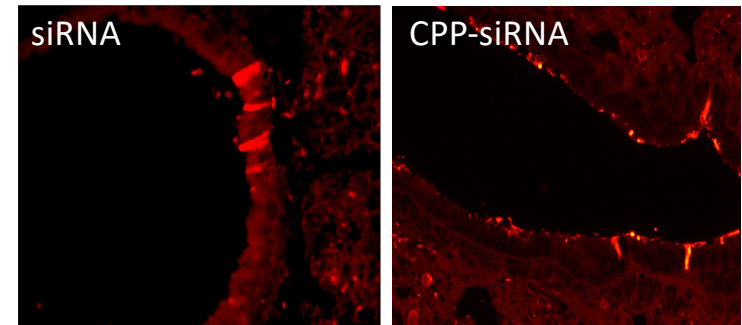


Moschos et al. *Pharmacology & Therapeutics* 2017

# Does the lung need delivery?

## The lung as a target:

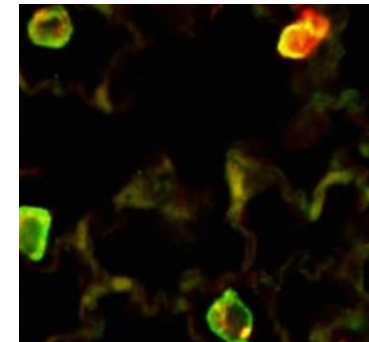
- Complex anatomy.
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- Variable disease mechanisms.



## Gene therapy and oligo efforts to 2010.

### Key assumptions:

1. Local dosing = adequate exposure, lung specific delivery.
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*Moschos et al. 2007*

# Better *in vivo* pharmacology...

## Tissue analysis:

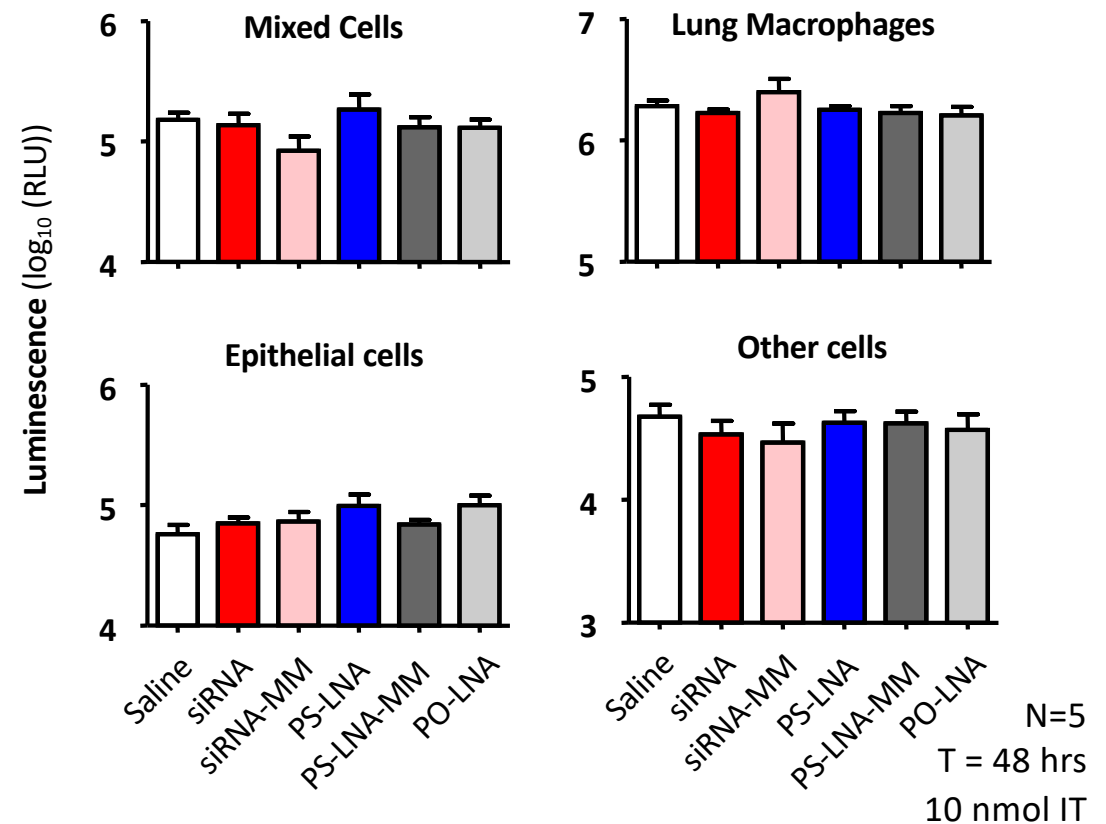
- Tissue digestion.
- Mixed cell population.
- Sorted cells.

## Improvements:

- Lower variability.
- Higher statistical power.
- 66% less animals.

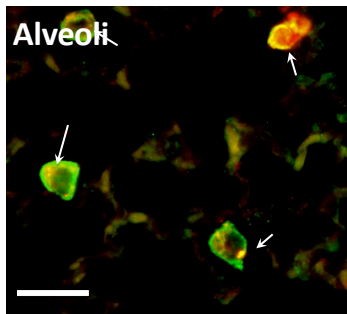
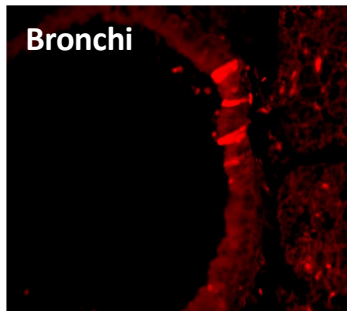
**No statistically significant effect at the protein level.**

Moschos *et al.* 2011

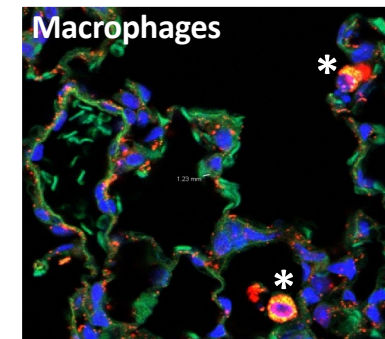
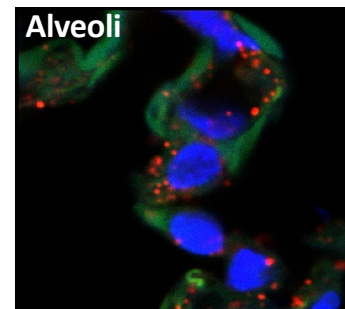
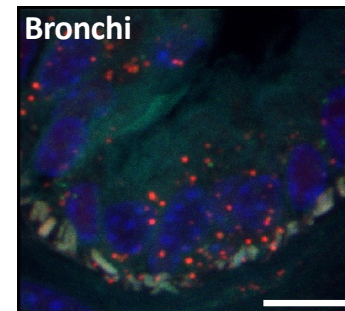
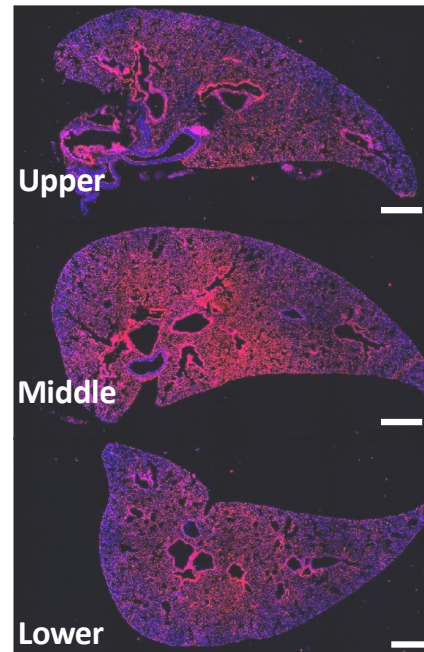


# Better *in vivo* pharmacology...

Lipophilic dye-oligos



Low  $\Delta C_{\log D}$  dye-oligos (PS-LNA)



Moschos *et al.* 2007

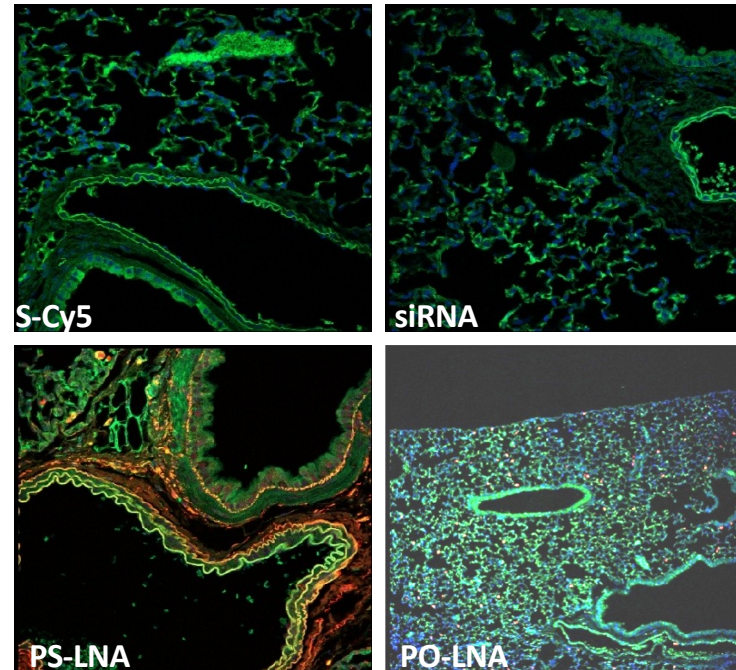


# ...leads to better drug design...

## Confirmed achievable targets:

- Minimal chemical modification needed.
- Topical dosing.
- Uniform distribution.

### Oligonucleotide chemistry & retention



Moschos *et al.* 2011

# ...leads to better drug design...

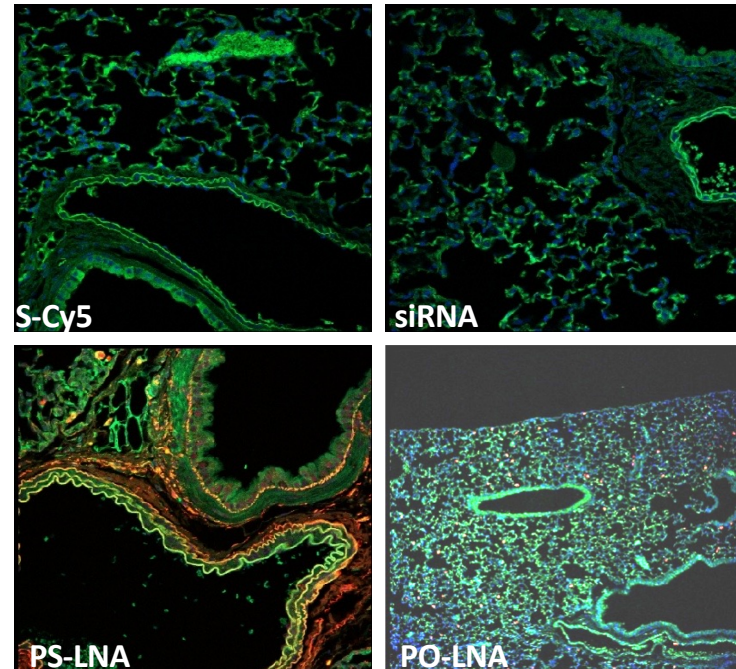
## Confirmed achievable targets:

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- Uniform distribution.

## Improvements needed:

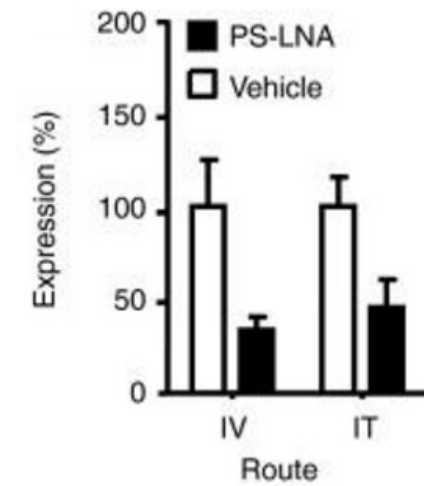
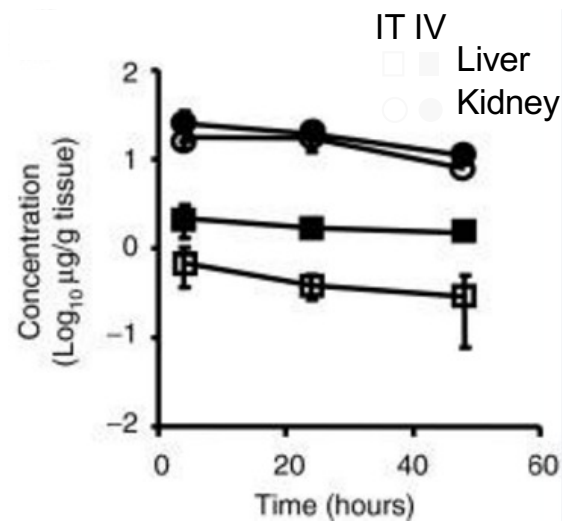
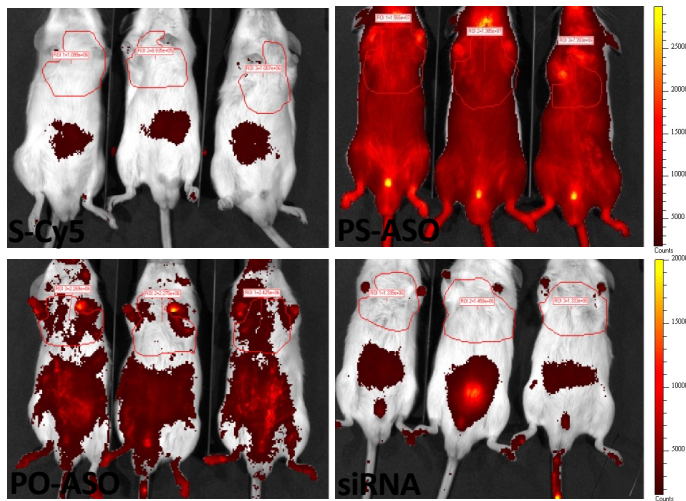
- Delivery through targeting.
- Macrophage avoidance.
- Directed cytosolic access.

### Oligonucleotide chemistry & retention



Moschos *et al.* 2011

# ...or to new opportunities!

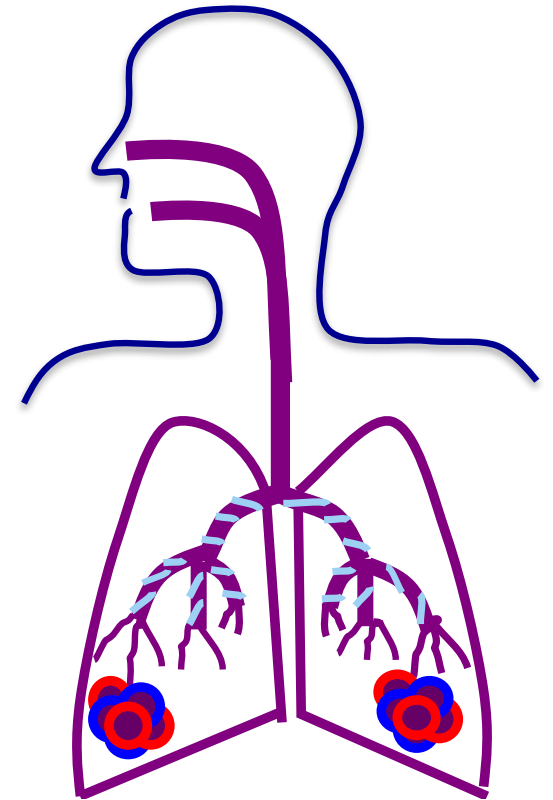


Moschos *et al.* 2011

Methodology and data independently validated by Merck: Ng B *et al. Mol. Ther. Nucleic Acids* 2019 and others

# Does the lung need delivery? Yes.

1. Ionis antisense ~50% effective (CF, IPF).
2. Merck modified siRNA effective at RNA level (only), also liver.
3. ProQR ASO also systemically bioavailable.
4. IVT-RNA therapeutics rely on lipid nanoparticles.
5. NP & EXOs load alveolar macrophage (foamy phenotype).
6. Contradictions on oligonucleotide immunogenicity.
7. Phosphorothioate platelet effects.



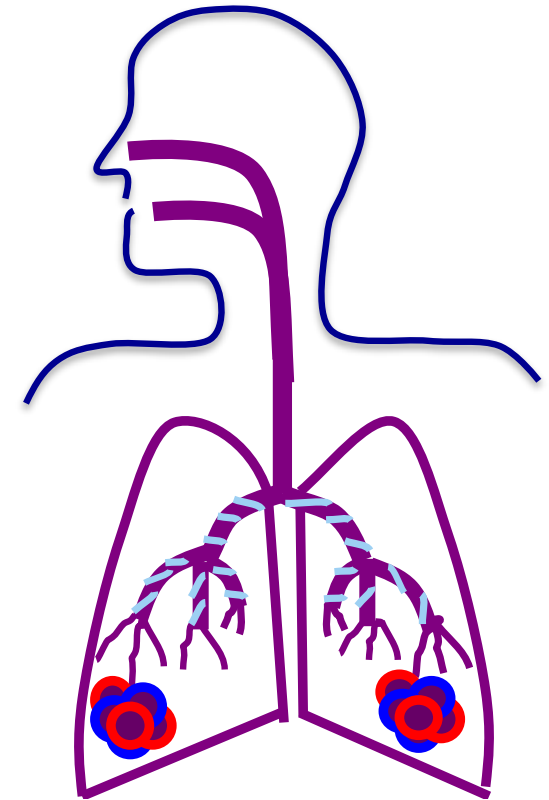
# Does the lung need delivery? Yes.

## Clinical failures:

1. 3x antisense: asthma.
2. 1x siRNA: respiratory syncytial virus.

## Reasons:

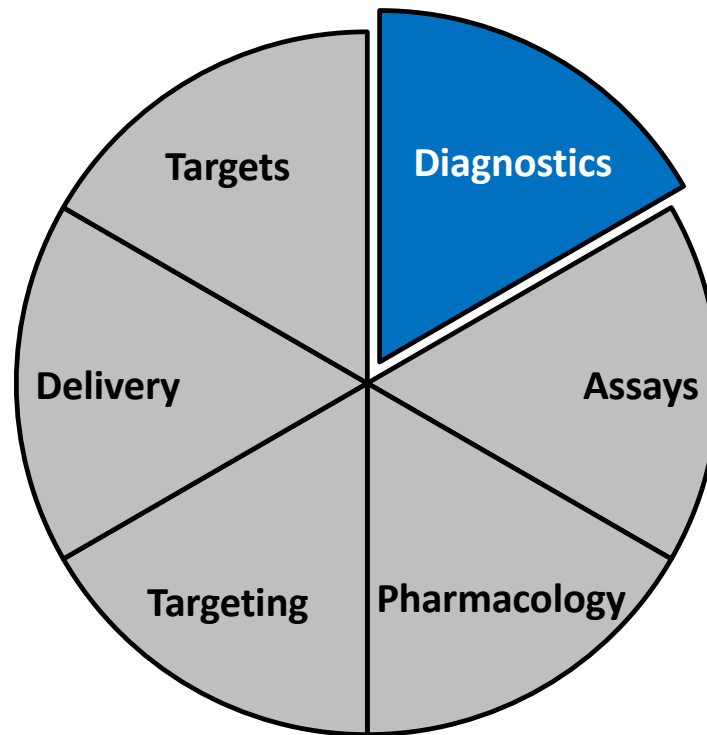
- Poor mechanism of action data, clinical endpoints, statistics.
- Poor targeting & delivery: foamy macrophages.
- Immunomodulation.



# The solution ecosystem



# The solution ecosystem



# Better sampling for the lung.

## Lung biology methods:

- Invasive.
- Unpleasant.
- Risk to patients with lung disease.

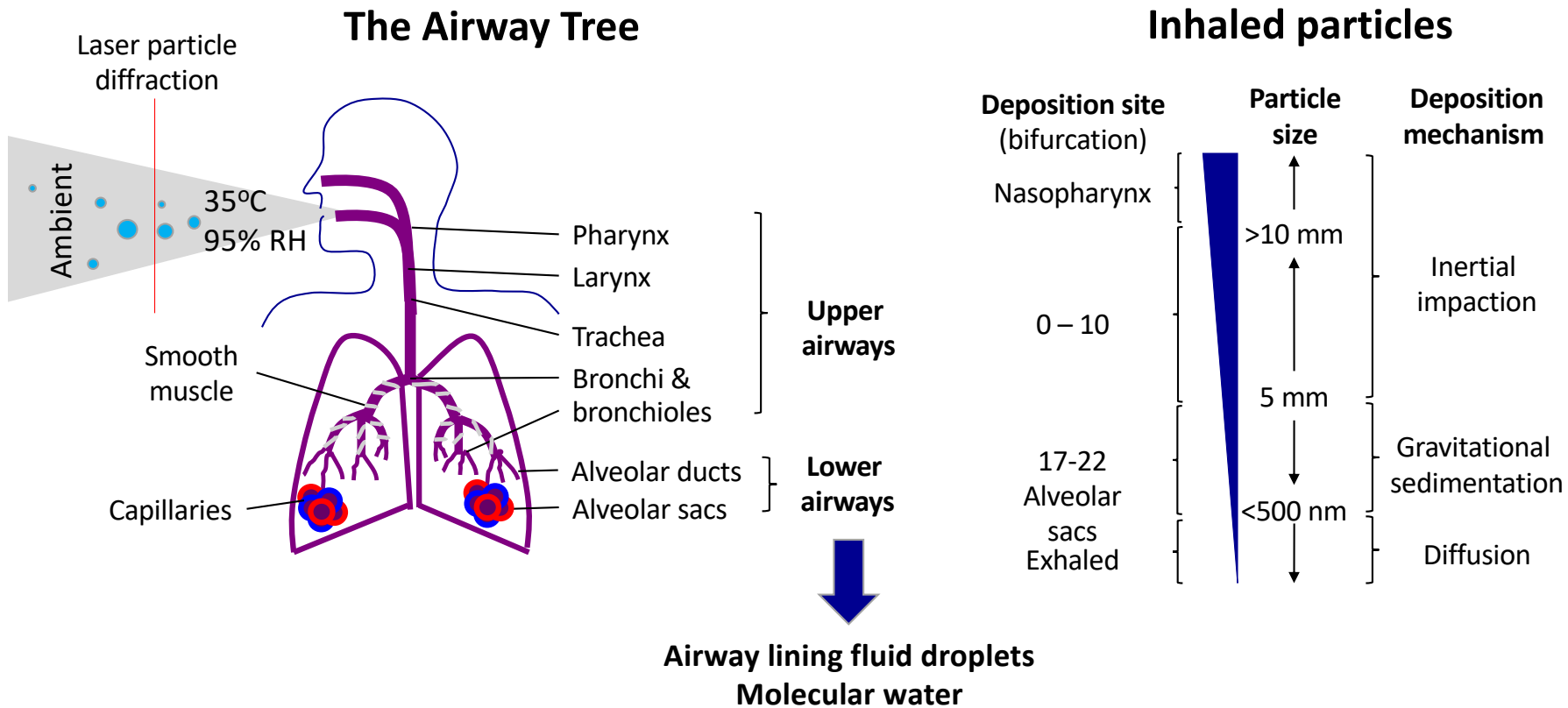
## Non-invasive bioanalytics?

- Surrogate biomarkers.
- Transoral / nasal sampling.
- Exhaled volatile compounds.
- Exhaled breath condensate (EBC).





# EBC: origin and physics



# Sampling Exhaled Breath

## Background

A number of firms already offer breath analysis products:

➤ Respiratory Research, Inc. (USA)

- Rtube

➤ FILT GmbH (Germany)

- EcoScreen

➤ Owlstone Medical Ltd (UK)

- ReCIVA

➤ BreathOmix (NL)

- eNose / SpiroNose



RTube



EcoScreen

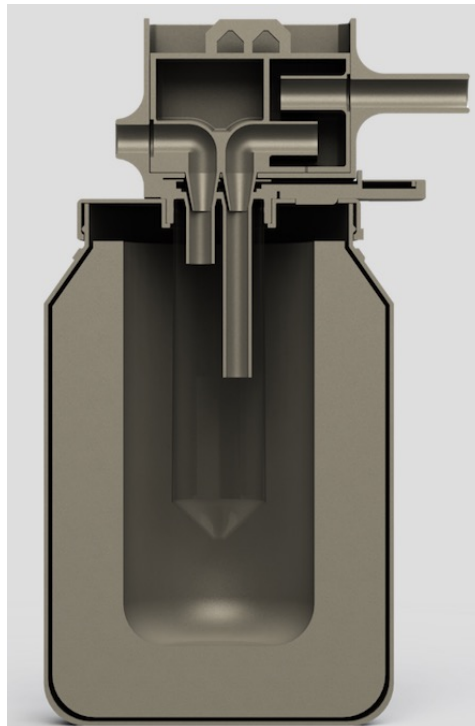


ReCIVA

**ALL existing products suffer from one or more of the following limitations:**

- Poor sample reproducibility
- Contamination (saliva / ambient)
- Sample loss
- Safety
- Upper and deep lung separation
- and, importantly, inability to analyse all classes of disease indicators.

# PBM-HALE™: the platform



## EBC collector:

- Volatiles and
- Proteins.
- DNA.
- RNA.
- Lipids.
- Medications

## Solves key problems:

- Reproducibility.
- Contamination.
- Sample loss.
- Safety.

## Cold Chain Dependent:

- Uses dry ice powder (CO<sub>2</sub>) to collect sample reliably.
- Sample needs PON test, or frozen transfer to lab.

**WO2017153755A1:** exhaled breath collector –granted; **WO2019053423A1:** cascade impactor array – published.

# PBM-HALE™: the platform



## EBC collector:

- Volatiles and
- Proteins.
- DNA.
- RNA.
- Lipids.
- Medications.

## Solves key problems:

- Reproducibility.
- Contamination.
- Sample loss.
- Safety.

## Path to removing the cold chain:

- Proprietary coating to remove need for dry ice.
- Stabilisation material to remove freezer storage.

**WO2017153755A1:** exhaled breath collector – granted; **WO2019053423A1:** cascade impactor array – published.

# PBM-HALE™: the platform

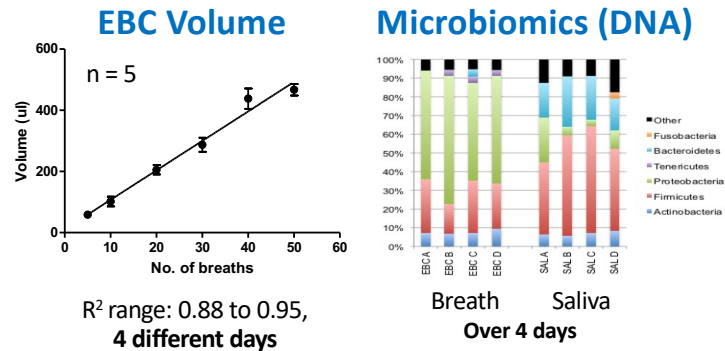
## Biomarker capability & performance.

### ➤ All key classes of disease indicators can be measured:

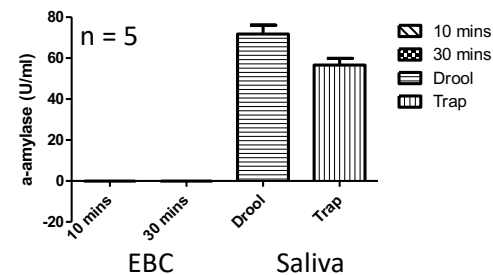
- Volatile organic compounds (VOC's).
- Protein (0.75 mg/ml), RNA (7.0 mg/ml), DNA (0.5 mg/ml).
- Short-to-long chain fatty acids ( $C_6$ - $C_{24}$ ).
- Glycans, medications, drugs of abuse.

### ➤ Highly consistent sampling, without salivary contamination.

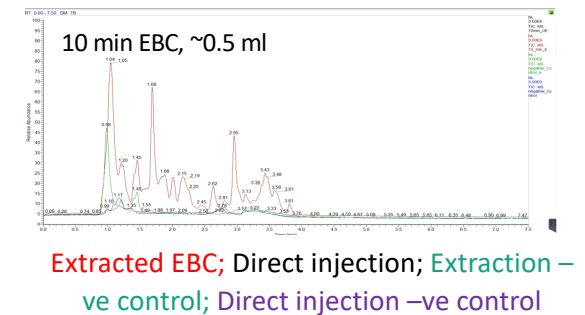
#### Sample consistency



#### No saliva contamination

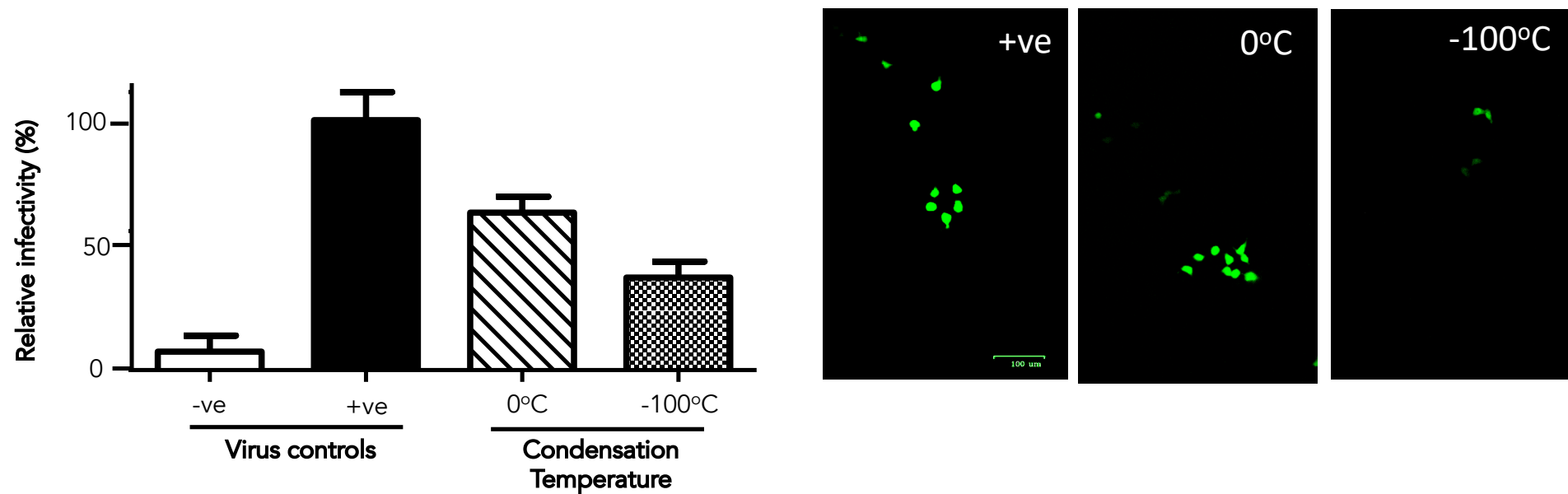


#### Metabolomics



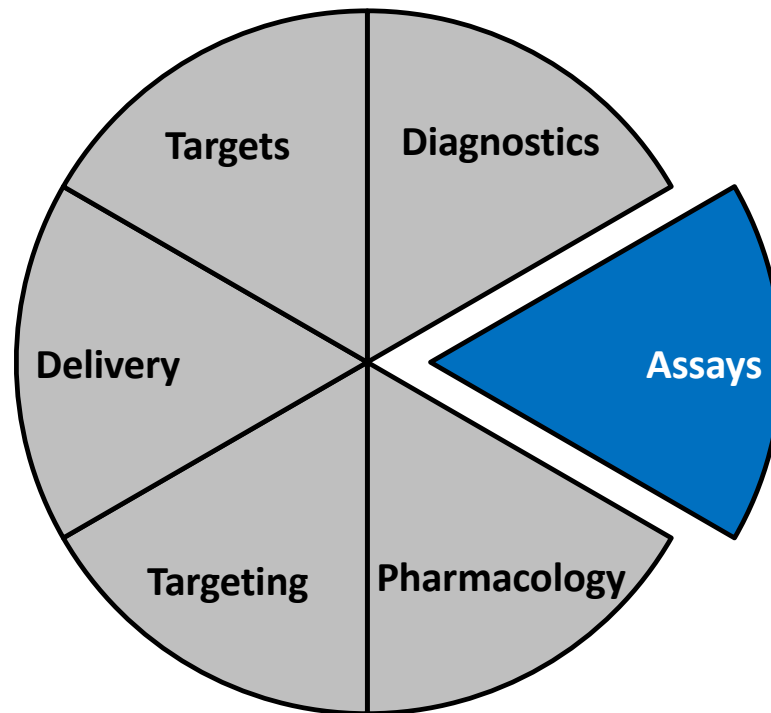
# Prototype: Detection of aerosolized virus

## Efficient capture of aerosolized virus.



GFP-expressing VSV-pseudotyped lentivirus nebulized using PARI TurboBoy SX and captured using PBM-HALE™ (15 min). Condensates seeded on 10,000 HEK-293T's and GFP expression measured at 72hrs by FACS, visualized by fluorescent microscopy. Bar = 100 µm

# The solution ecosystem



# Reliable RNA testing

- Significant manual error in clinical trial data.
- No methods for high sensitivity and frequency RNA [target] or [oligo] analysis.
- Ebola is an RNA virus, diagnosed by RT-PCR on blood.

**Can we make RT-PCR a single manual step process?**



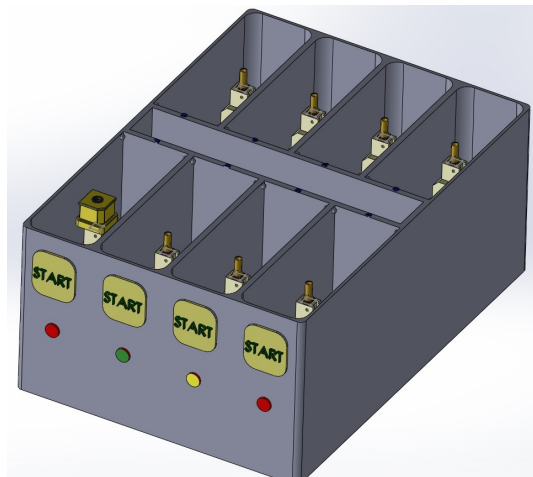
# Reliable RNA testing in rural Africa



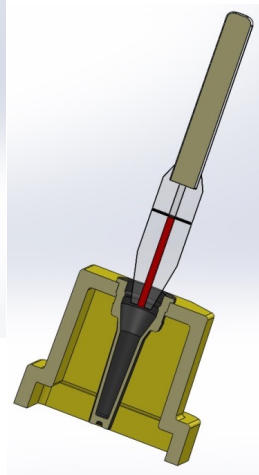
No electricity, no running water, no windows

# EbolaCHECK: £620K, 1 year, 4 questions.

## QuRapid System



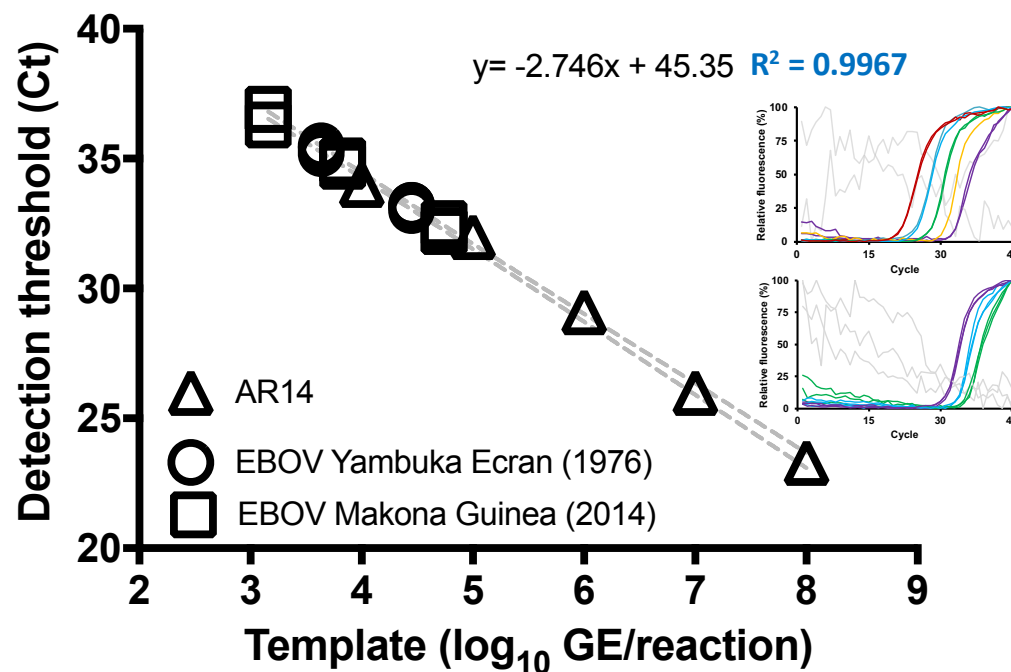
Reaction chamber



1. Can we perform RT-PCR in the presence of blood?
2. Can we access viral genomes in blood by controlled freeze-thaw?
3. Can we build an instrument and process suited to field use?
4. Will this be as reliable as a laboratory-based test?



# Measures genomes...



2 separate operators.

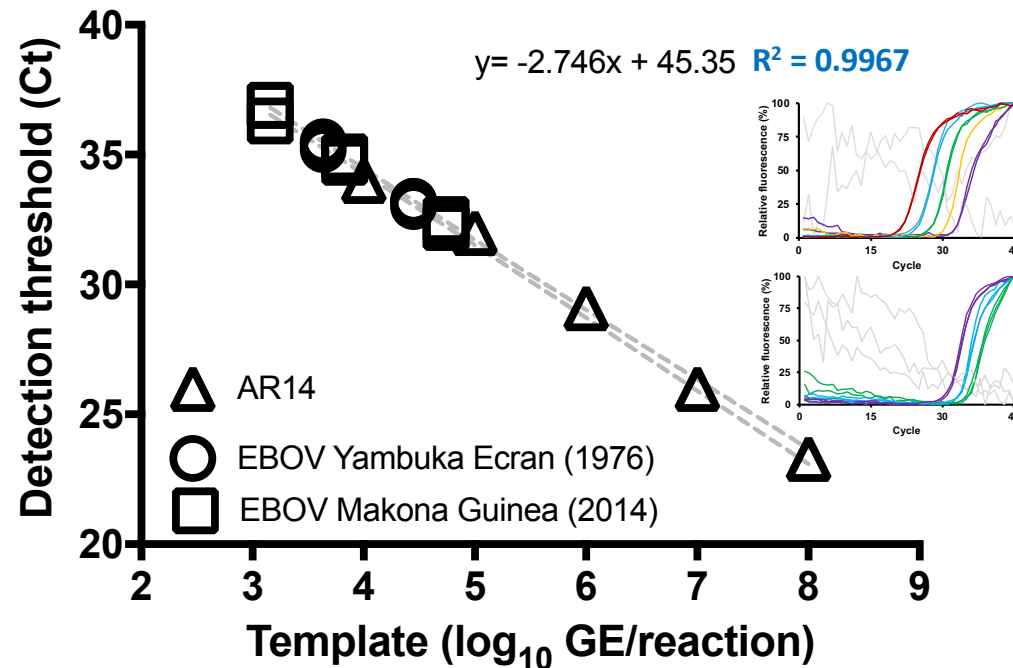
2 different batches of live viruses.

Experiments across 5 days.

99.96% Probability of Single Curve Fit for All Data.

Akaike's Informative Criteria test

# and discriminates between filoviruses...



2 separate operators.

2 different batches of live viruses.

Experiments across 5 days.

**No false positives with 4 phylogenetically close filoviruses.**

Sudan Ebolavirus, Bundibugyo Ebolavirus, Tai Forest Ebolavirus, Marburgvirus (Ravn virus).

as well as the gold standard diagnostic process.

Target	Ebolavirus amount		Genome/virion ratio (log GE/log PFU)		
	Live virions (log PFU/ml)	Genomes (log GE/ml)	ABI*	QR**	
Ebola 1976	6.85	9.68	9.39	1.41	1.37
Ebola 2014	8.32	9.26	8.27	1.113	0.994

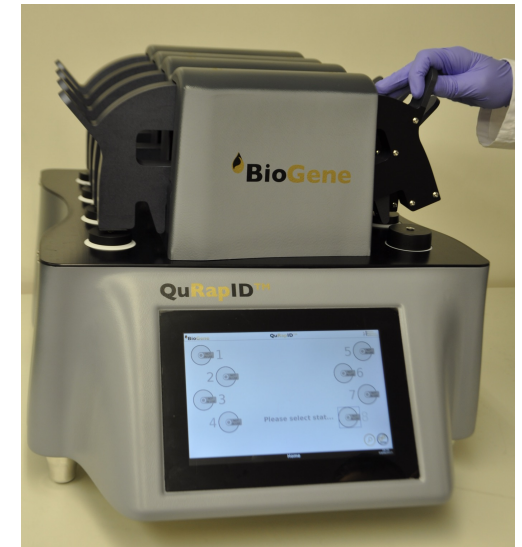
\* Calibrated against quantified AR14 on the ABI7500 (ABI)

\*\* Calibrated against quantified AR14 on the QuRapID (QR)

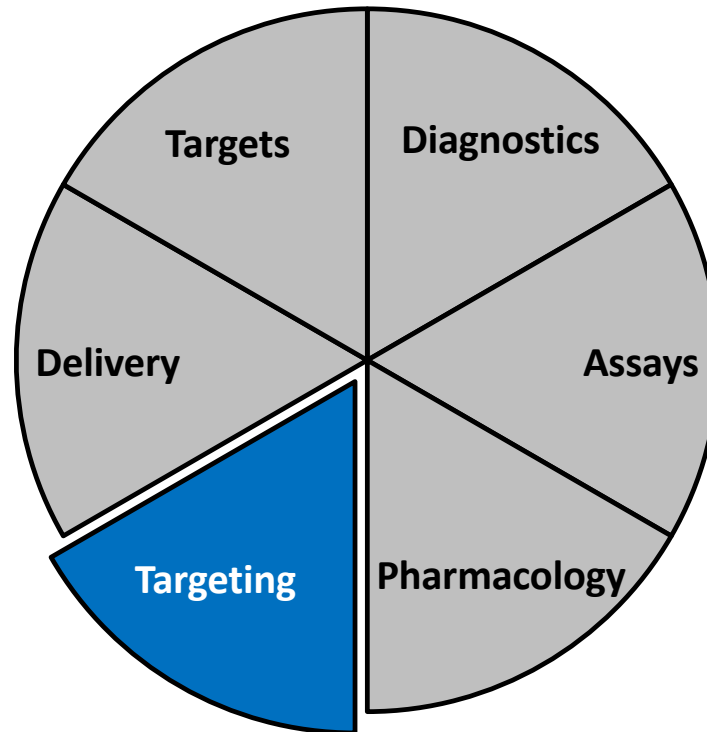
**Comparable unknown genome quantification between lab and Point of Need platforms**

# Impact

- International NAAT standards for Ebolavirus diagnostics (NIBSC HIV).
- Far-red RT-qPCR in blood in a single reaction.
- Validated on:
  - 11 other haemorrhagic fever viruses.
  - Bacteria, exosomes, malaria, cfDNA, veterinary diseases.
- >£5m investment in biotech partners.
- Field tested in 3 continents.
- Commercial release in 4Q2020.
- Mass screening solution for SARS-CoV-2 (Moonshot programme).



# The solution ecosystem



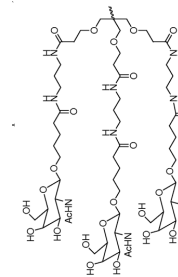
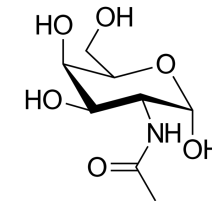
# Back to lung delivery: GalNAc and ASGPR

## Asialoglycoprotein receptor

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## GalNAc conjugates

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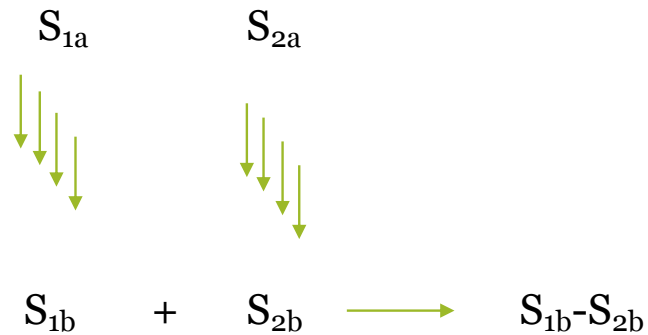
***Can we Deliver the GalNAc of the Lung?***



# Building novel oligosaccharide targeting ligands

## Chemically

Complex sugar protection strategy



## Biocatalytically

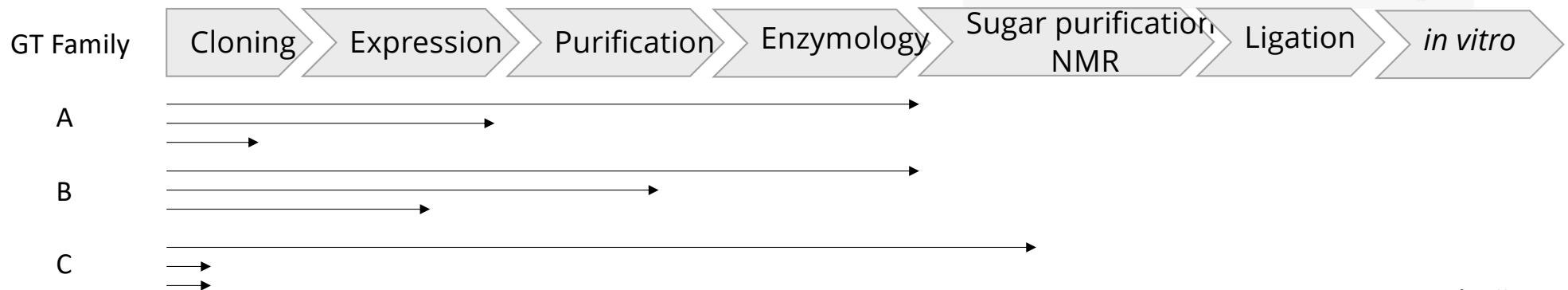
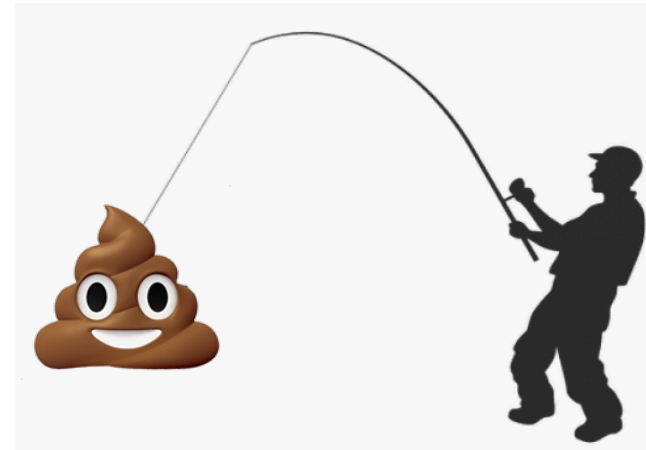
Glycosyltransferases (GT)



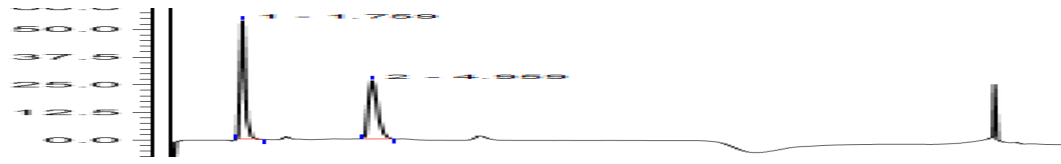
One protein will selectively link the sugars in the chosen conformation

# Resourcing GT

- Purification from faecal matter
- Cloning from enteric bacterial strains.

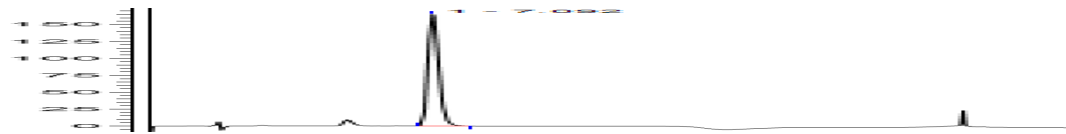


# GT Biocatalysis - purification

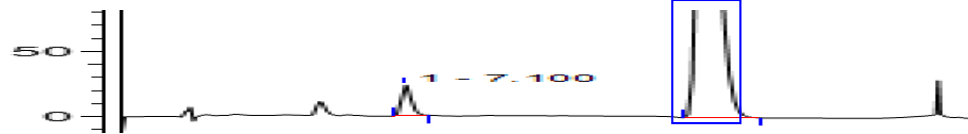


## Sugar substrates

UDP-Hexose 1  
[Donor]

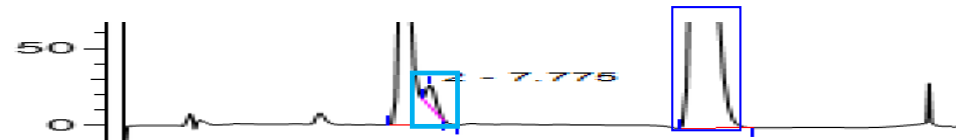


Hexose 2  
[Acceptor]

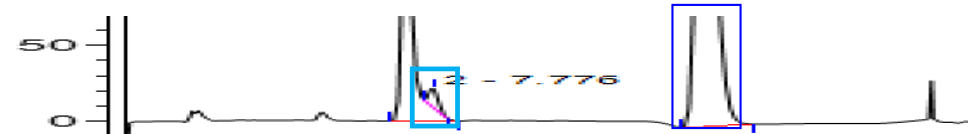
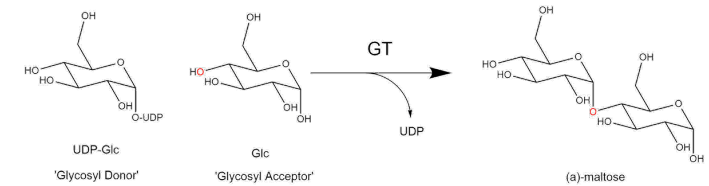


## Reaction products

4°C Enzyme formulation  
storage temperature



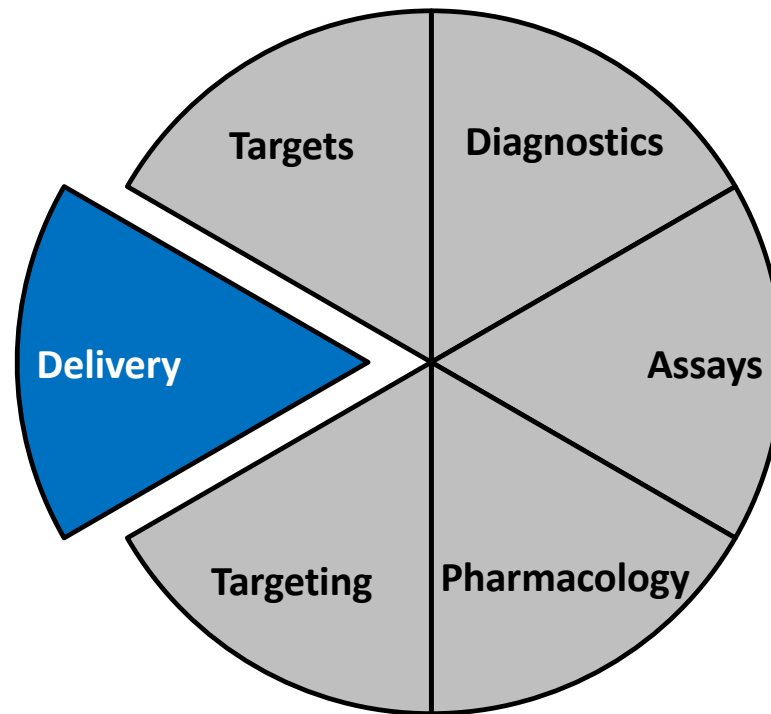
-20°C



-80°C

Mr Joe P. O'Sullivan

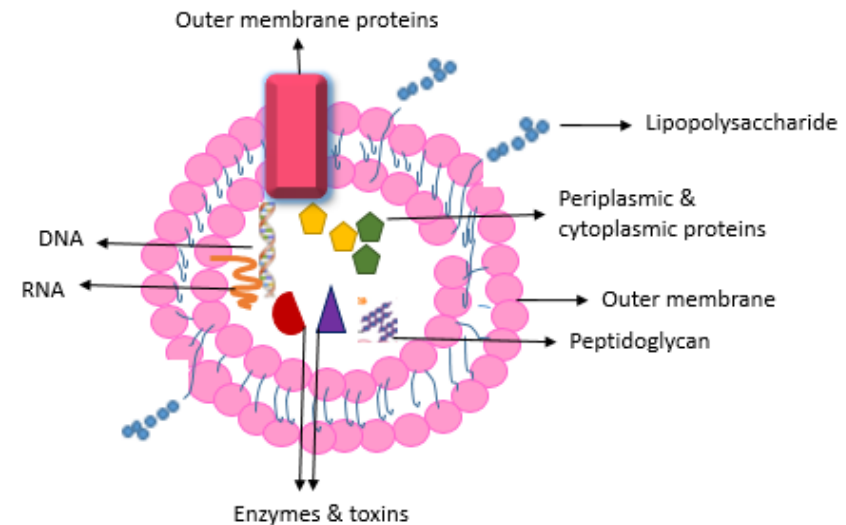
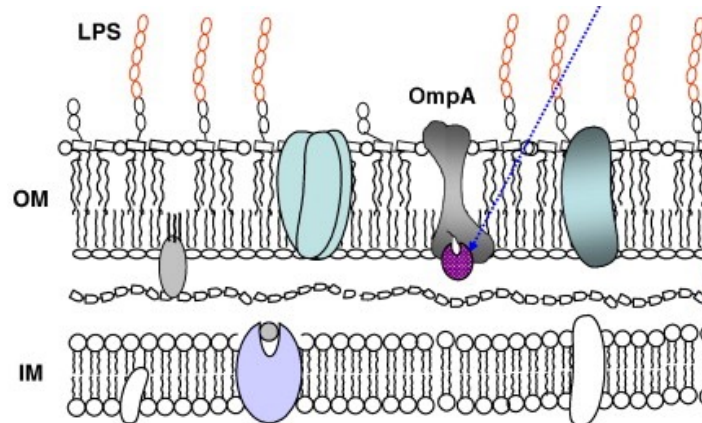
# The solution ecosystem



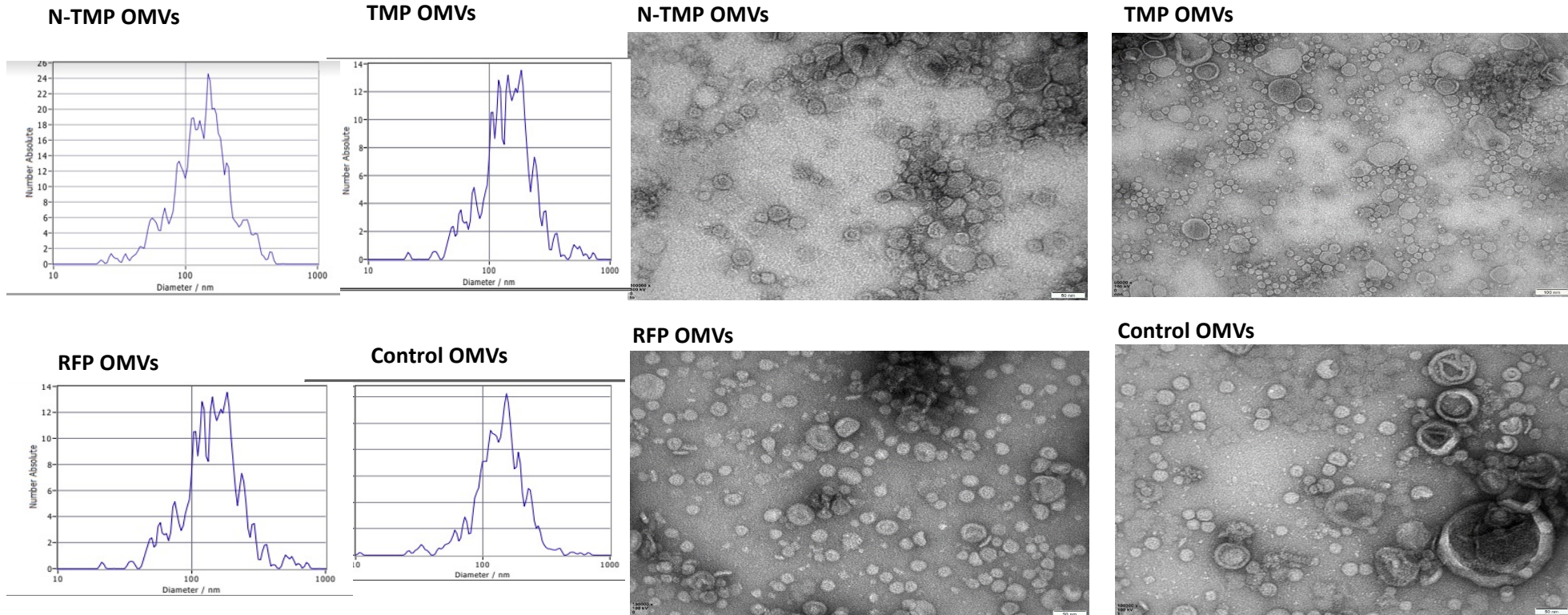
# Targeting using transmembrane proteins (TMP): outer membrane vesicles (OMV)

## Complex composition, biology.

- DNA, RNA, Protein, metabolites.
- Toxin removal, immune evasion, bioadhesion, nutrient shuttling, etc.
- [Inter-kingdom cross-talk.](#)



# TMP does not change OMV structure

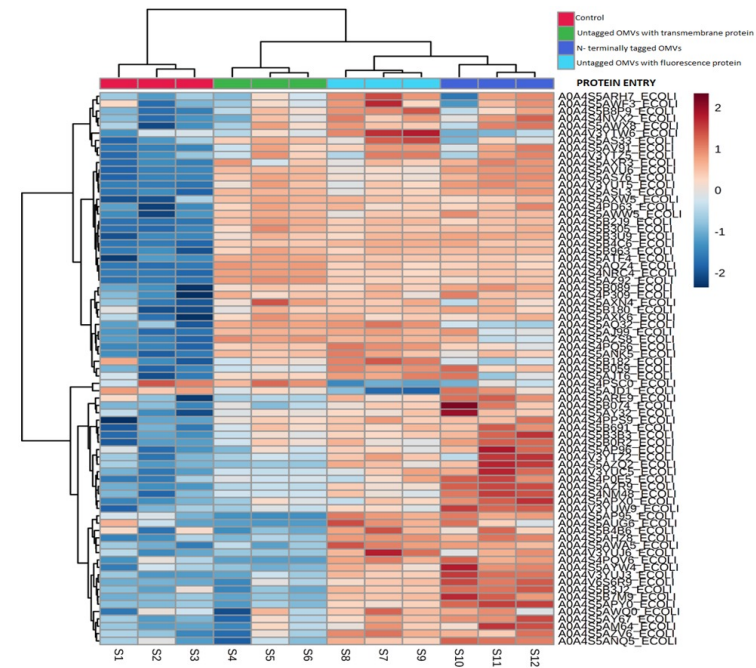
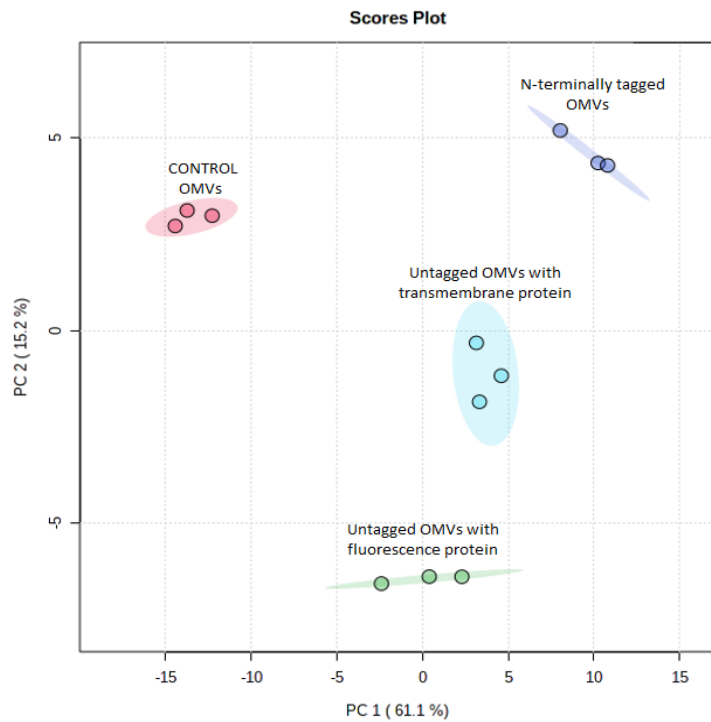


NTA analysis

100000 x, scale bar 50nm, 100 kV.

Miss Elena Eftychiou

# Consistent proteomic profiles



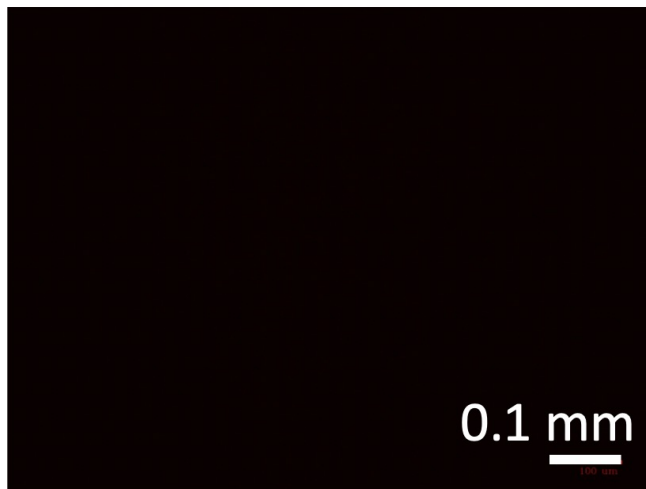
1100 proteins detected across all samples; 400 proteins differ by OMV class (top 50 shown).

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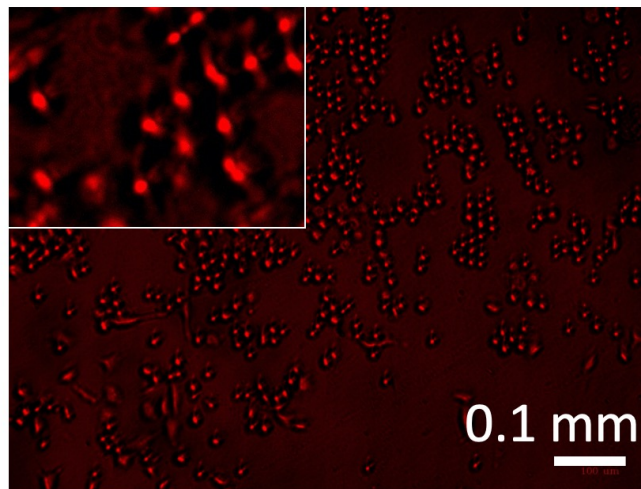


# TMP drives A549 loading

OMV (-C)



N-TMP OMV



RFP OMV



40x magnification.  
10,000 cells/well  
10,000 OMV/ cell  
30 min internalisation at 37oC.

Representative of 3 independent biological experiments  
carried out independently in 2 laboratories.

Miss Elena Eftychiou



# The solution ecosystem

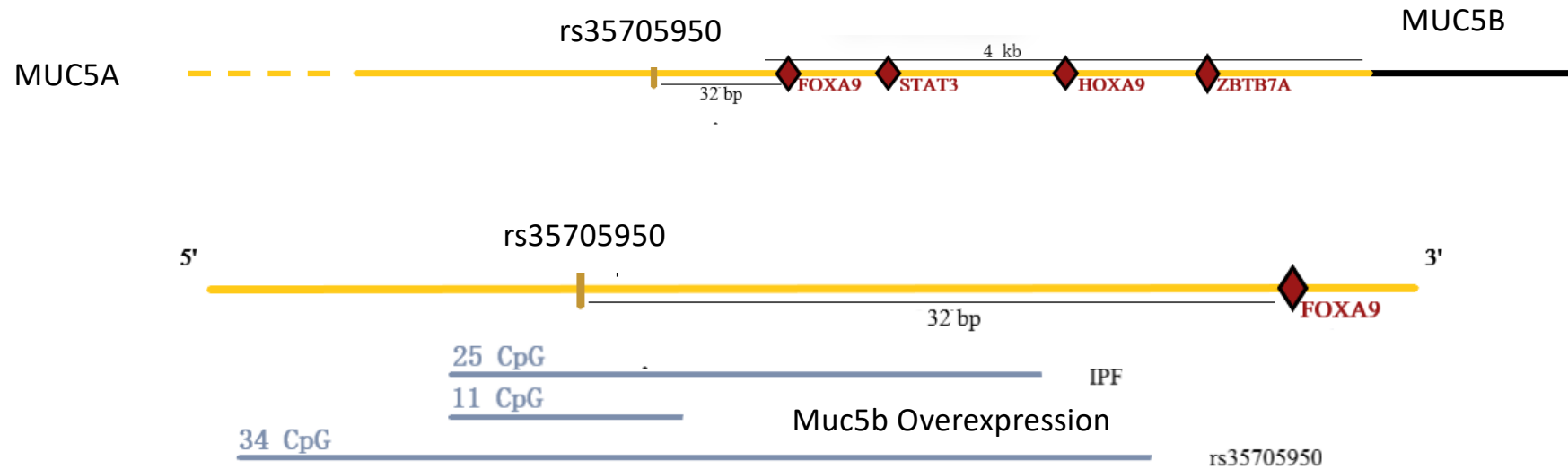


# Idiopathic pulmonary fibrosis

- Middle-to-late onset interstitial pneumonia.
  - Severe scarring of the lung.
  - Dyspnoea and dry cough.
  - 2-3 year survival, poor QoL.
  - Minimal effect from antifibrotics/steroids
- Males
- 16-18 / 100,000 prevalence; <30% of ILD.
- Significant genetic association.



# Chronic epithelial damage and stress



**Endoplasmic Reticulum stress in A11; low damage threshold; fibrosis.**

**Is there a promoter non-coding RNA transcript across rs3570950?**

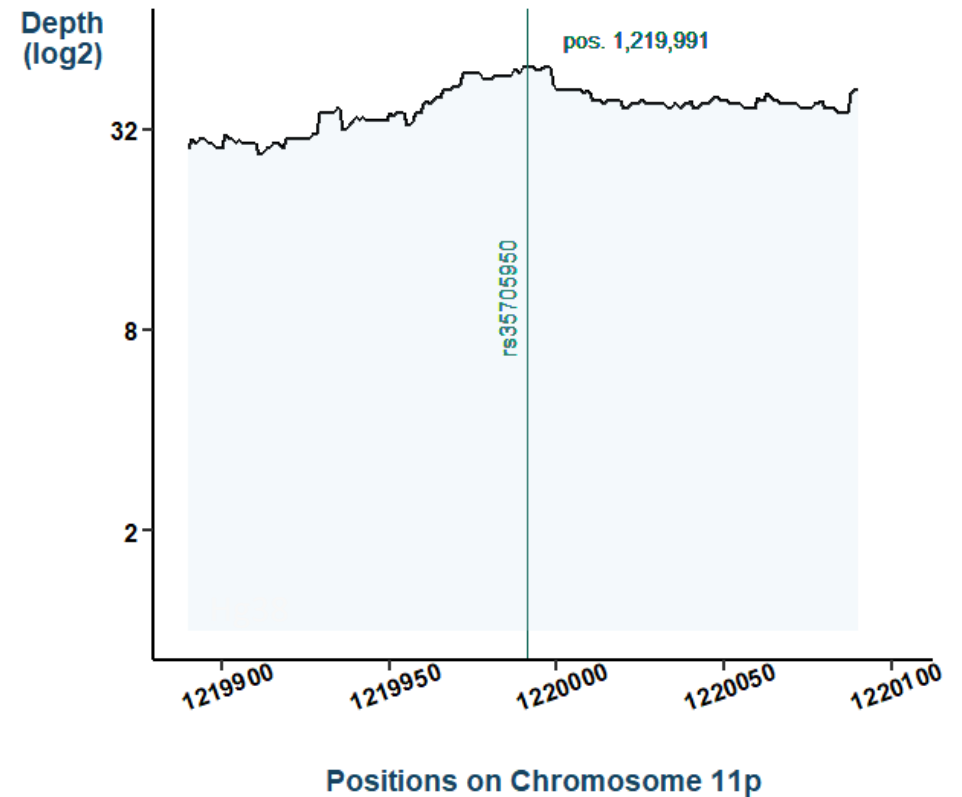
# RNA-SEQ data meta-analysis

- Human lung (IPF, ILD, other).
- Lung epithelial cell lines.
- 20 studies, 2.75 TB.
- Custom pipeline.



# RNA-SEQ data meta-analysis

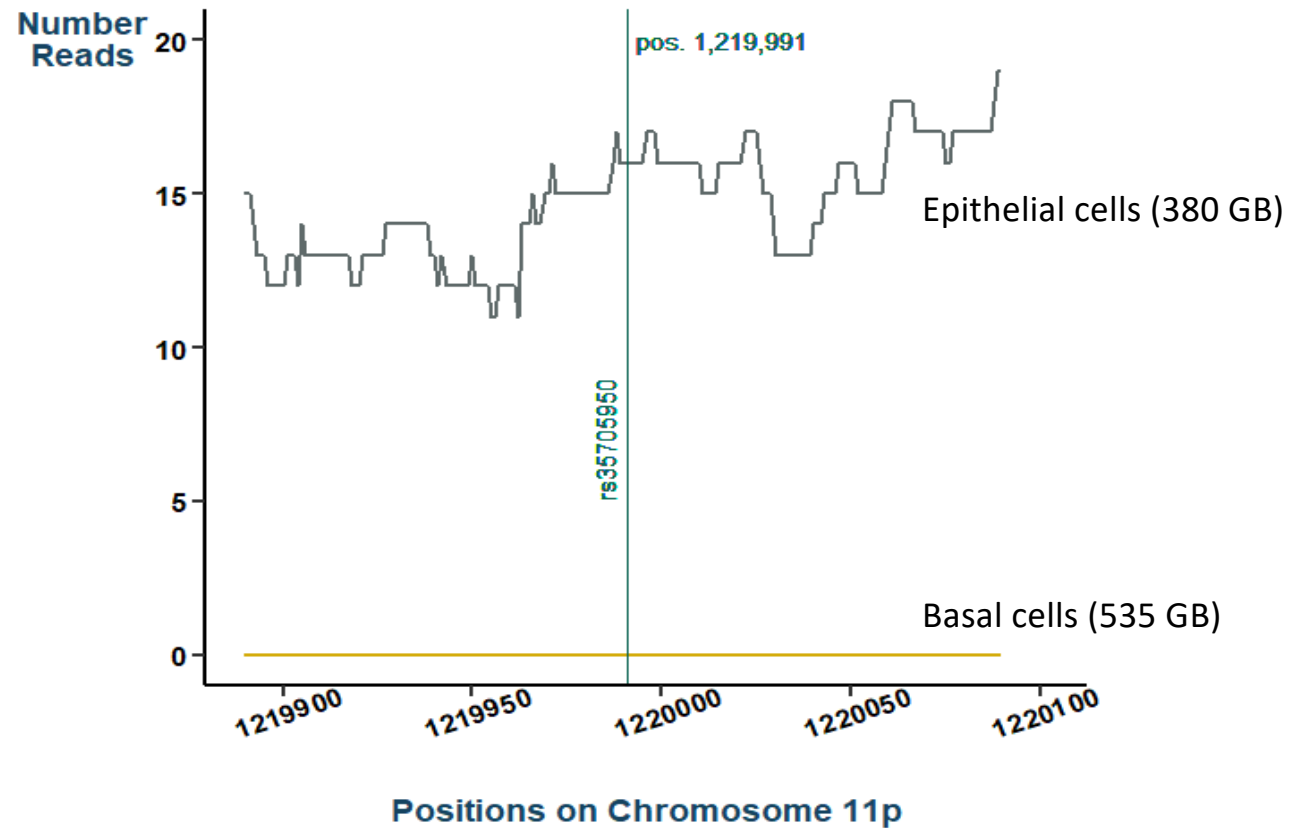
- Human lung (IPF, ILD, other).
- Lung epithelial cell lines.
- 20 studies, 2.75 TB.
- Custom pipeline.
  
- Low copy number transcript



# RNA-SEQ data meta-analysis

Epithelial-specific expression

Validation experiments under Way.



Data from Zhang et al 2018 Oncotarget

Miss Ruxandra Neatu

# Intervention options

- MUC5b knockdown (siRNA, antisense).
  - Essential mucin.
  - Non-ATII effect risks?
- paRNA knockdown (siRNA, antisense).
  - Unknown potency/efficacy.
  - Off target effects
- Genome editing?

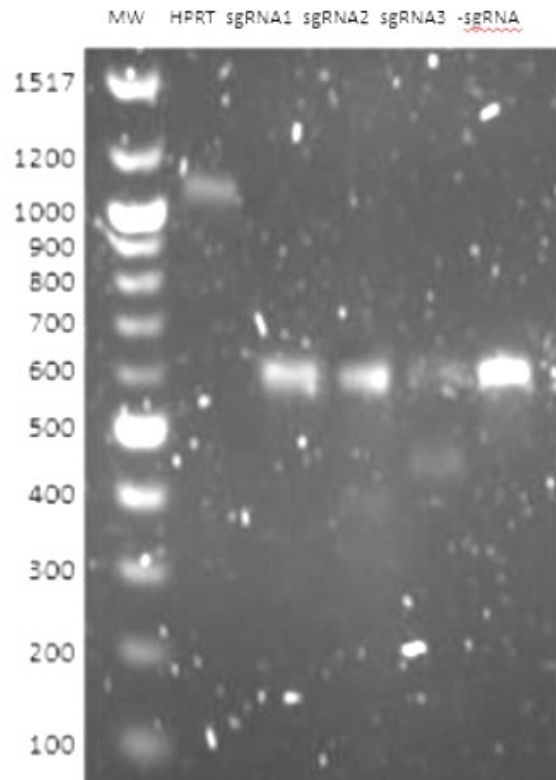


**Lifetime prophylactic treatment**



**Single shot preventative gene therapy**

# rs'950 ncRNA genome editing



- Active sgRNA identified.
- HDR template testing.
- ATII and Bronchial rs'950 cell lines under development.
  - siRNA and antisense testing.
- Novel delivery system testing *in vitro* and in 1ry cells.



# The post-COVID19 world



# Acknowledgements



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