USE OF RESEARCH INFRASTRUCTURES TO IDENTIFY THERAPIES FOR COVID-19

Philip Gribbon – Discovery Research, Fraunhofer ITMP
Partner site of EU-OPENSCREEN
European RI for Chemical Biology and early Drug Discovery

- Long-term funding from 8 member countries: CZ, DE, DK, ES, FI, LV, NO, PL
- Distributed RI with 24 partner sites across Europe
- Services offered:
  - Screening platforms (17)
  - Medicinal Chemistry groups (6)
  - FAIR Database hosting (1)

Open-access database:
- FAIRification of data
- Collaborative data sharing environment
  - maximise re-use of FAIR data
  - data available without restrictions on use
  - optional embargo period up to 36 months
  - data links to other databases (e.g. ChEMBL)

https://ecbd.eu/
Why drug repurposing for COVID-19, when vaccines are available?

- Multiple vaccines authorized worldwide
- May face challenges in
  - Safety in certain groups
  - Efficacy in certain groups
  - Duration of effectiveness
  - Production scale up
  - Clinical roll out
  - Patient Access
  - Virus mutation effects leading to vaccine escape / resistance
- No high efficacy and safe curative therapy in place yet

![Image of table comparing Covid-19 vaccines](https://wellcome.org/news/what-different-types-covid-19-vaccine-are-there)
Clinical trials in Europe, China, US, Japan, Australia..

Some successes

- Approved Therapeutics
  - Dexamethasone in the United Kingdom and Japan;
  - Avigan (favilavir) in China, Italy and Russia;
  - Veklury (remdesivir) in the United States, Japan and Australia.

- Emergency use authorizations (EUAs) in the US.
  - Convalescent plasma, initially authorized by the FDA
  - Eli Lilly and Company’s monoclonal bamlanivimab,
  - Regeneron monoclonals casirivimab and imdevimab
  - Combination of Veklury and the JAK inhibitor Olumiant (Eli Lilly and Company)

- Emerging pre-clinical and clinical stage therapeutics
  - PF-07321332 Phase 1 clinical start for 3-CL/M Pro inhibitor (NSP-5)
  - EMA has provided advice given on 57 possible therapies (@Feb ‘21)
Repurposing compounds (> 6000) and screening infrastructure

Indications

Target s

Screening Infrastructure
Anti-Cytopathic Effect phenotypic assay with Caco-2 cells

1. Read the images
2. Identify cells
3. Calculate morphology, intensity and distance parameters

(with Ciesek group GuF)
Anti Cytopathic effect phenotypic assay with Caco-2 cells

Dissemination (EU H2020 European Open Science Cloud – LIFE)
Publication @ Ellinger et al Nature Scientific Data
https://doi.org/10.1038/s41597-021-00848-4
Bioactivity data @ ChEMBL data sets
https://www.ebi.ac.uk/chembl/document_report_card/CHEMBL4303101/
Bioactivity data @ ECBD (imminently)
https://ecbd.eu/
Primary images for re-analysis @ IDR
https://idr.openmicroscopy.org/webclient/?show=screen-2603
Remdesivir data @ IDR
http://idr.openmicroscopy.org/mapr/compound/?value=Remdesivir
Jupyter Notebook linking data to analysis workflows
https://github.com/IDR/idr0094-ellinger-sarscov2
E4C Study 1  Anti Cytopathic effect phenotypic assay with Vero_E6 cells (KUL)
Anti Cytopathic effect phenotypic assay with VeroE6_GFP Cells

- **Primary screening**
  - 1-point at 10 µM
  - 8702 compounds

- **Active molecules**
  - >18% inhibition
  - 184 compounds

- **Confirmed actives**
  - Activity in DRC triplicates
  - 132 compounds

- **Relevant drugs**
  - IC50 inhibition <10 µM
  - 75 compounds

- **Selection**
  - Safety index > 5
  - 23 compounds

Data can be found here: [https://www.ebi.ac.uk/chembl/document_report_card/CHEMBL4495565/](https://www.ebi.ac.uk/chembl/document_report_card/CHEMBL4495565/)
Raloxifene active ingredient of Optruma®,

- Raloxifene 60 mg oral tablets.
- Available on the market as branded (Optruma® Eli Lilly; Evista® Daiichi Sankyo) or generic (Raloxifene Teva) medication.
- Registered in EU with CP since 1998 for the treatment and prevention of osteoporosis in postmenopausal women.

* www.ema.europa.eu
Achievements in EXSCALATE4COV (E4C)

**Project Results in Numbers**

1. **ongoing clinical trials**: RALOXIFENE
2. **Clinical Trial in preparation**
3. **>400** Molecules found to be active in experimental assays
4. **30,000** Biological experiments performed
5. **5** Biological assays developed

- **>1 Trillion** Molecules simulated
- **45 + 20** Proteins simulated in molecular dynamics experiments
- **>60 million calculation hours** Used for molecular dynamics simulations
- **38** Crystallographic structures generated
- **28+1*(154)** Number of publications
  - *Published on E4C Special Issue (Impact Factor)
- **4** Number of patents
- **18** Number of partners
- **35** League members
- **3+1** milioni Project budget

- **>30,300** Results for “exscalate4cov” in Google Search

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Europoscreen

ITMP
Massive X-ray screening against SARS-CoV-2 main protease

High-throughput X-ray synchrotron crystallography

Cooperation led by Alke Meents and Sebastian Günther, DESY /Hamburg SARS-CoV-2 X-ray Screening Initiative
X-Ray studies - more than 100 Contributors ...
from 25 different institutions

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Najeeb Ullah
Sven Falke
Bruno Alves Franca
Martin Schwinzer
Hévila Brognaro
Vasundara Srivinasa
Christian Betzel

**物理化学**

**Biography**

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Cromarte Rogers
Arwen R. Pearson

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**Dompé Farmaceutici SpA**
Andrea R. Beccari

**DESY. | Massive X-ray Screening | Sebastian Günther**
Potential SARS-CoV-2 protein targets
Non-structural proteins essential for virus replication

From: Gordon et al., Nature 2020

Structure solved by X-ray crystallography at BESSY

From: Zhang et al., Science 2020
Hamburg SARS-CoV-2 screening project

Idea: High-throughput screening of a 5700 compound re-purposing library against three SARS-CoV-2 relevant target proteins with X-ray crystallography

> 100 contributing scientists

Further partners:
- HZB
- University of Hannover
- SLAC / Caltech
- EMBL

Experimental workflow

Feedback from experiment
Workflow for High-throughput X-ray screening

Establishing a screening platform during a pandemic

Protein production and purification

Protein crystallization

Crystal harvesting and flash-freezing

Structure refinement and ligand identification

Data processing

Synchrotron X-ray data collection
Hits in active site and beyond

29 M\textsuperscript{pro}/ligand structures
From screening to hits

- 7857 crystals, incl. apo/other ligand

<table>
<thead>
<tr>
<th></th>
<th>unique</th>
<th>Fraunhofer</th>
<th>Dompe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total compounds</td>
<td>5953</td>
<td>5632</td>
<td>607</td>
</tr>
<tr>
<td>Total crystals mounted</td>
<td>6976</td>
<td>4777</td>
<td>2199</td>
</tr>
<tr>
<td>Unique compounds w/xtals</td>
<td>3757 (63.1%)</td>
<td>3367 (59.8%)</td>
<td>58 (96.5%)</td>
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<tr>
<td>Dataset in hit finding</td>
<td>3757</td>
<td>2545</td>
<td>1212</td>
</tr>
<tr>
<td>Unique compounds in hit</td>
<td>2381 (40%)</td>
<td>1984 (35.2%)</td>
<td>491 (80.9%)</td>
</tr>
<tr>
<td>Identified hits</td>
<td>36 (0.6%)</td>
<td>25 (0.44%)</td>
<td>11 (1.8%)</td>
</tr>
</tbody>
</table>
From compounds to hits
With antiviral activity – our study

Some promising hits

EC\textsubscript{50} = 1.25 \mu M

EC\textsubscript{50} = 0.072 \mu M

pelitinib [\mu M]

calpeptin [\mu M]

\% of control

SARS-CoV-2 vRNA  SARS-CoV-2 titer  cell viability

In parallel studies @ Pfizer

PF-07321332

Pfizer begins early stage clinical trial testing oral antiviral drug for Covid

KEY POINTS

- Pfizer said it has begun an early stage clinical trial testing its experimental oral antiviral drug for Covid-19.
- The company said the phase one trial of the drug – called PF-07321332 – is being conducted in the United States.
Conclusions and next steps

- Identification of several hits which can serve as **new lead structure** for future drug developments
- **Fast response**: Project start to completion of data collection took months rather than years
- High Scientific impact studies and FAIR data dissemination

- Next steps: progress clinical studies to identify optimal treatments
- EU-wide: Five drugs selected for investment, will be chosen from a long list of (57) candidates drawn up by the European Medicines Agency (EMA),
Lessons learnt

- Serendipity has a great part to play, but not coincidence.…
- Repurposing collection - rare disease
- Informed by SARS CoV expertises (Hilgenfeld)
- In-silico workflows in E4C from Ebola project
- Utilizing Cross Infrastructure workflows was essential to success
  - Chemical screening + structural biology + data-analytics
  - Image analysis + bioinformatics databases + large scale ‘omics + systems biology tools
  - High Biosafety labs + animal models + medicinal chemistry
- FAIR data working is essential to reach full understanding of the virus function and roles:
Acknowledgements

Phil Gribbon
Maria Kuzikov
Markus Wolf
Jeanette Reinshagen
Bernhard Ellinger
Carsten Claussen
Gerd Geisslinger

Kristoff Rieken

Pieter Leyssen
Laura Vangeel

Katja Herzog

Enzo Tramontano
Angela Corona
Francesca Esposito

Andrea Beccari
Carmine Talarico
Daniela Jaconis
Candida Manelfi
Extra slides
EU-OPENSSCREEN Libraries

European Chemical Biology Library (ECBL)

**Diversity library**
- 99.096 structurally highly diverse compounds
- Average MW=350 g/mol
- 0.0005 % of PAINS

Horvath D. et al., ChemMedChem 2014, 9, 2309

**European Chemical Biology Library (ECBL)**

**Pilot library**
- 2.464 bioactives: active against 1039 different targets, contain 654 approved drugs and 368 highly selective probes
- 2.464 representative compounds of the diversity library
- 88 assay interference compounds in 4 dilutions

The European Academic Compound Library (EACL)

**Novel compounds sourced from chemists worldwide**
- Target is 40,000 compounds
- Regulated and confidential access (e.g. MTA)
- IP stays with the chemist
- Embargo period up to 3 years

Fragment Library NEW!

**Set of low MW and ultra-low MW fragments**
- 968 fragments with HAC > 8 in DMSO-d₆
- 88 so called "minifrags" with HAC < 8 in DMSO-d₆
  (O'Reilly M. et al., Drug Discov. Today 2019, 24, 1081)
- Derived from the fragment space of the ECBL
- Collaboration with INSTRUCT/iNEXT-Discovery sites
EU-OPENSSCREEN Fragment Library

- Set of low MW and ultra-low MW fragments (1,056 compounds)
- Derived from the fragment space of the ECBL
- Designed by Andrea Zaliani, Fraunhofer ITMP and Jordi Mestres, IMIM (manuscript in preparation)
- Library composition:
  - 968 fragments with HAC > 8 in DMSO-d6 (c = 100 mM)
  - 88 so called “minifrags” with HAC < 8 in DMSO-d6 (c = 1 M) (O’Reilly M. et al., Drug Discov. Today 2019, 24, 1081)
  - 131 fluorinated fragments
  - Consistent with a ‘rule of three’
  - QC performed at the EU-OPENSSCREEN Central Compound Management Facility (LC-MS)

- Available at 6 EU-OPENSSCREEN-DRIVE partners
  - Latvian Institute of Organic Synthesis (NMR, SPR, ITC, X-Ray)
  - Príncipe Felipe Research Center/ Health Research Institute Hospital La Fe (NMR, SPR, ITC, biochemical experiments)
  - Technical University of Denmark (19F NMR, Thermal Shift)
  - University of Bergen (SPR, BLI)
  - Natural Science Research Center of the Hungarian Academy of Sciences
  - Fraunhofer ITMP in collaboration with DESY Hamburg (X-Ray)

- FBDD projects on COVID-19 targets (e.g. Nsp14-nsp10 complex using NMR), viral enzyme endonucleases of the negative strand RNA viruses (X-Ray) and antibiotic targets (BLI).
EU-OPENSSCREEN Fragment Library

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- Available at 6 iNEXT-Discovery partners
  - EMBL Grenoble - High-Throughput Crystallisation Laboratory
  - Diamond Light Source - XChem facility
  - Helmholtz Zentrum Berlin - macromolecular crystallography at the BESSY-II electron storage ring
  - Goethe University Frankfurt- Center for Biomolecular Magnetic Resonance (NMR)
  - Netherlands Cancer Institute NKI (biophysical methods)
  - FragMAX facility at MAX IV Laboratory (X-ray)

- X-ray FBDD project on Nsp3 Macrodomain 1 of SARS-CoV-2 at Diamond: Science Advances, 2021, 7 (16), eabf8711
EU-OPENSCREEN Screening Campaigns using the ECBL

- A total of 25 screening campaigns incl. 13 screening projects funded by EU-OPENSCREEN-DRIVE and 3 SARS-CoV-2 related projects

- Researchers from:
  - France, Germany, Italy, Spain, Switzerland, The Netherlands, United Kingdom

- Research fields (among others):
  - Cancer and neurodegenerative disorders
  - Infectious diseases
  - Rare diseases
  - Spinal muscular atrophy
  - Prion disease

- Publication in focus: