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Evotec

Integrated Drug Discovery in Antimicrobial Resistance (AMR)

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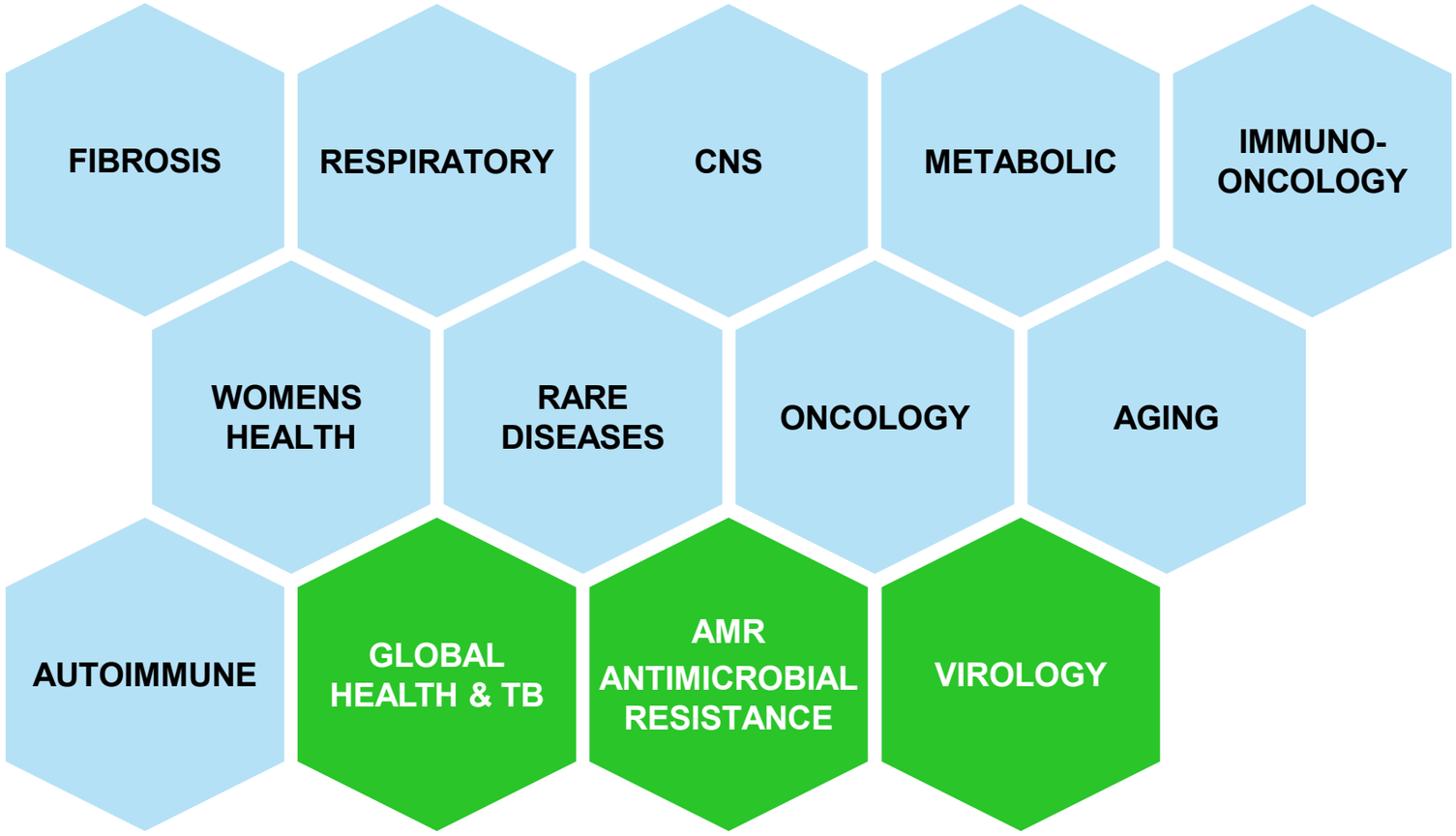
Evotec focuses on diseases with high unmet medical need

Broad and deep range of therapeutic area expertise at Evotec



Relevant, seamless and state of the art

Integrated value chain

PDC

Best-in-class Drug Discovery & Early Development

- Translation from idea to development candidate
- Drug discovery solutions based on multidisciplinary capabilities across biology and chemistry

Preclinical studies & INDiGO

- Translation from *in vitro* to patient
- Seamless transition from development candidate through to pre-clinic and IND

Small molecules high-end CMC

- GMP manufacturing for clinical development programmes and niche scale commercial API and Drug Product

Biologics Molecule Optimization – J.MD™

- ML based sequence evaluation for lead selection of best molecules for rapid and efficient manu-facturability and formulation stability

Biologics process development – JP3®

- Formulation design from clinical to commercial with powerful analytical, ML and high-throughput tools

Biologics high-end CMC – J.POD®

Modular and flexible clinical and commercial manufacturing at highest quality and low costs

- Seamless integration

Leader in infectious diseases discovery platforms

A global partner delivering new anti-infectives

Viral infections

- Evotec has supported NIH-led initiative Accelerating COVID-19 Therapeutic Interventions and Vaccines (“ACTIV”)
- Building world-leading footprint in antivirals focussing on respiratory viruses and HBV
- Building a unique pandemic **P**reparedness and **R**apid **R**esp**O**nse **TEC**hnology **P**la**T**form (PRROTECT)

Bacterial infections

- Partnerships to discover novel anti-biotics (Forge, GNA-NOW, COMBINE, IMI ENABLE, WTF AMR, AMR Industry Alliance, Novo REPAIR, …)
- CARB-X funding for development of a novel broad spectrum antibiotic project
- Alliance with Liverpool School of Tropical Medicine (LSTM): IICON, organoids and PK/PD

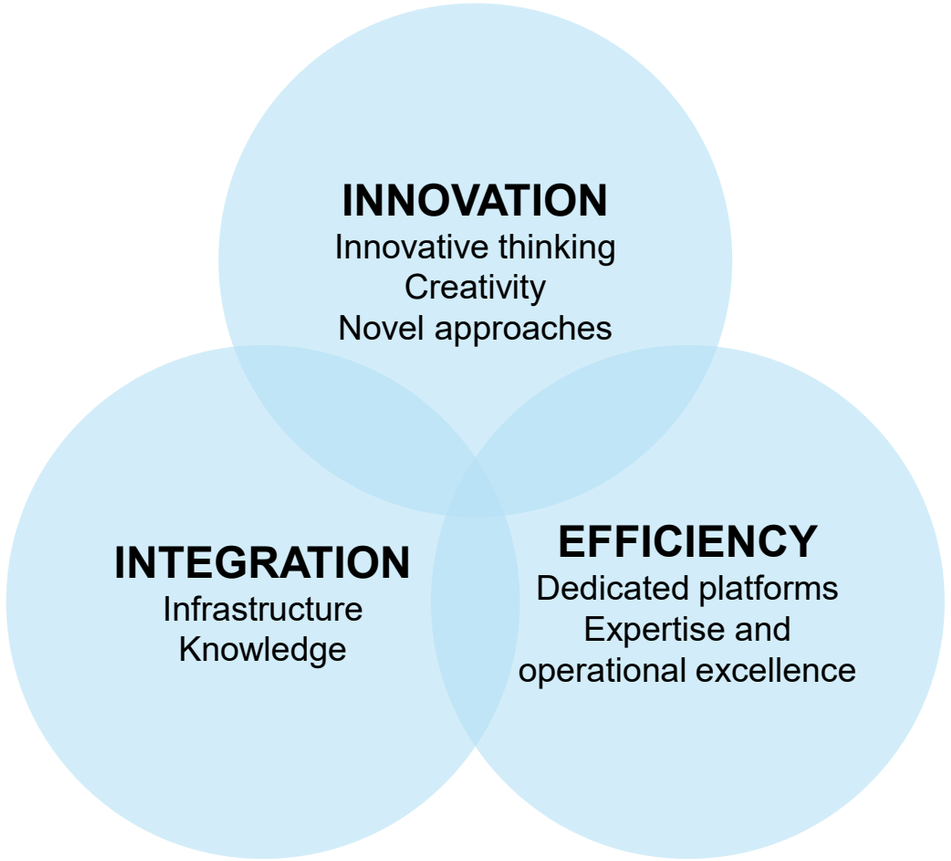
Global health

- Multiple programs
  - BMGF: TB Hollow Fibre Systems to model (quadruple!) drug combinations
- New opportunities in Non-TB mycobacteria (CF, etc.)
- New TB initiatives (e.g. PAN-TB, ERA4TB)

State of the art, multimodality anti-infective discovery platform and world-leading expertise

Evotec is confronting the renewed challenge in Infectious Diseases

Innovation and operational excellence



~200 Scientists dedicated to ID across multiple functions

Fully integrated drug discovery and disease biology

Combining world-class expertise and platforms

- **Deep rooted heritage in ID:** High calibre scientists
- **Strong expertise:** Dedicated technologies and scientific platforms
- **Global partner network:** Foundations, BRIDGES, KOLs
- **EvostrAIn™:** Highly valuable collection of ~10,000 strains from clinic and reference collections
- ***In vivo* pharmacology and biomarker solutions:** Efficacy, PK/PD, *ex vivo* platform

- **Designing a TPP-driven strategy & screening cascade**
  - From HTS to Lead identification and optimization
  - Clear criteria for progression
  - Link between *in vitro* activity and *in vivo* efficacy
- **Supporting the development of multiple therapeutic modalities**
  - From small molecules to biologics and large molecules
  - Traditional and non-traditional target
  - Full integration with DMPK, Immunology and Toxicology platforms
- **Development of disease models representing pathological conditions**
  - Use of clinical isolates from seriously ill patients
  - Qualify the model on the basis of host infection biomarkers
  - Apply imaging technologies for real time evaluation of progression and infection distribution

Overall summary: A comprehensive portfolio of microbiology and translational disease biology capabilities

Cross-functional chemistry & biology platform from concept to clinical PoC in AMR

Microbial Biochemistry	*In vitro* Profiling	Target Validation	*In vivo* and translational biology	
Generation of engineered bacteria	**MIC, MIC_{50}, MIC_{90}** (CLSI Guidelines)	**Functional Genomics (TnSeq)**	**Septicaemia**	**Chronic Lung Infection**
Target based assay development	**Combination Studies**	**Genomics (WGS)**	**Thigh Infection** (immunocompetent)	**Human Foreign Body**
Whole-cell based assay development	**Time Kill Kinetic**	**Transcriptomics**	**Thigh Infection** (neutropenic)	**Endocarditis**
Protein overexpression & purification	**Post-Antibiotic Effect**	**Proteomics**	**Nasal Colonization**	**Tissue Cage**
Enzymology of ß-lactamases	**Fitness Studies**	**Thermal shift assay in live cells**	**Lung Infection – Acute** (immunocompetent)	**Urinary Tract Infection**
Enzymology of PBPs	**Resistance generation/FoR**	**Fluorescence Microscopy, BCP**	**Lung Infection – Acute** (neutropenic)	**Osteomyelitis**
MoA Studies by MMS	**Mutant Prevention Concentration** (MPC)	**Genome engineering**	**IV sepsis survival**	***Clostridium difficile* Infection** (CDI)
Label-free bacterial intracellular compound accumulation assay	**MBEC**	**Cellular Target Profiling / Chemoproteomics**	**Anti-fungal models**	**GI tract infection**
Phenotypic Microarray (Biolog)	**Biofilm Production Assessment**	**Photo-affinity labelling/MS**		

Hollow fibre models – Pharmacokinetics – Mathematical modelling
Preclinical PK/PD – Dose prediction – Clinical stage PK/PD

The anti-infective Autobahn: from discovery biology to the clinic

Seamless program progression from discovery to development

Discovery biology

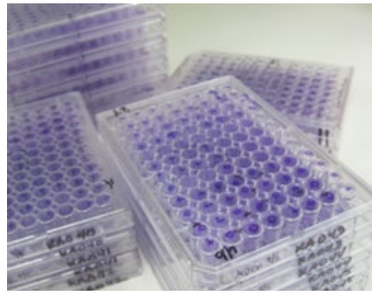
- Standard microbiology on a large strain collection (MIC, MIC_{90}, TKC, FoR, PAE, resistant mutants characterization, etc.)
- Target identification
- MoA/MoR determination and molecular profiling
- Omics and sequencing technologies
- Generation of engineered bacteria
- Target or Whole-cell based assay development

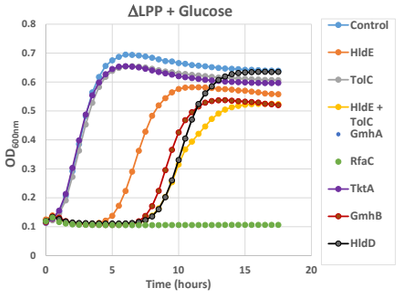
Integrated Drug Discovery

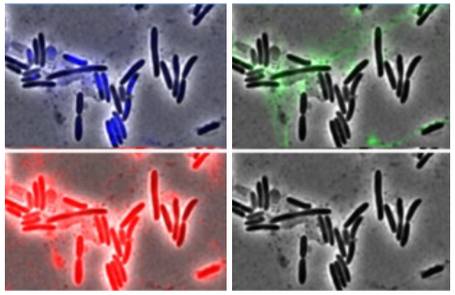
- Label-free bacterial intracellular compound accumulation assay
- Phenotypic screening (Biolog)
- Vivo-mimetic screening
- Target-based screening including fragment approaches
- Medicinal chemistry
- Computational chemistry and structure based drug design
- Highly efficient DMTA cycles

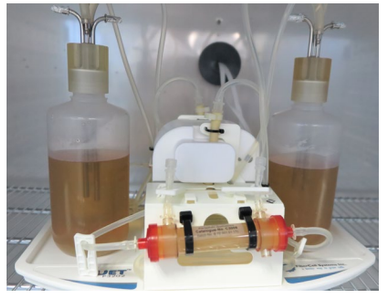
Translational microbiology and PK/PD

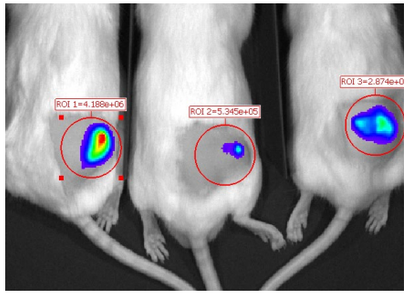
- State of the art *in vivo* DMPK
- *In vivo* microbiology for efficacy profiling
- *In vivo* and *in vitro* PK/PD platforms including Hollow fibre systems
- Mathematical modelling and simulations
- Translation of discovery data to the clinical setting

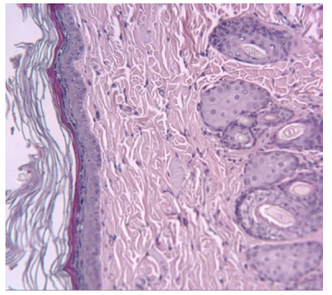












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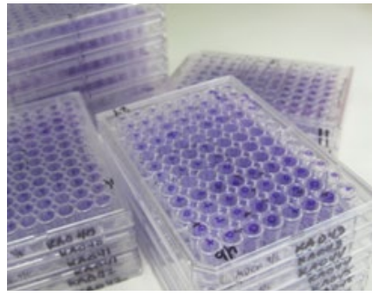
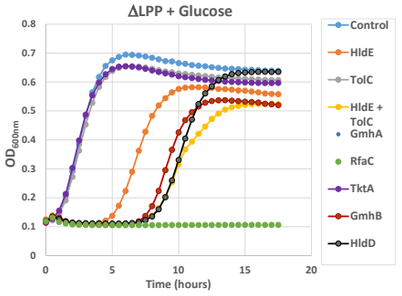
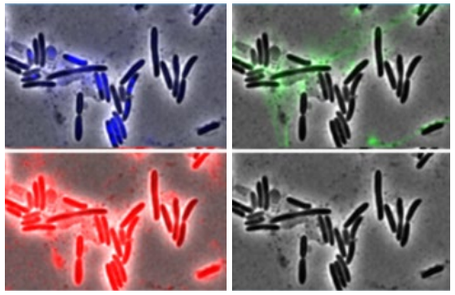
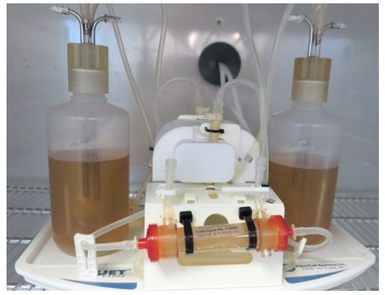
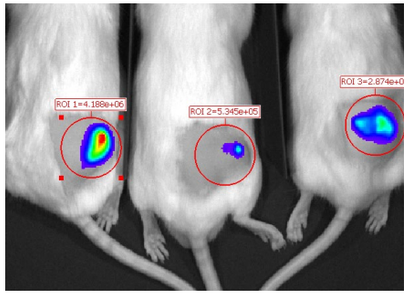
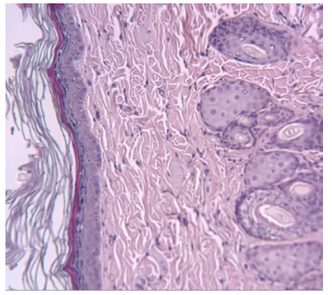








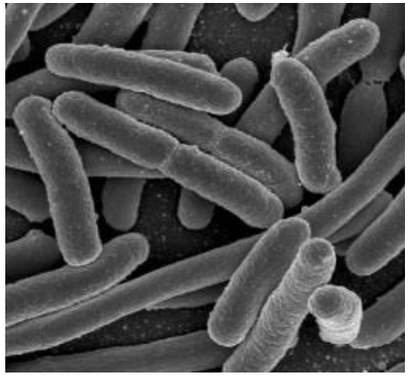


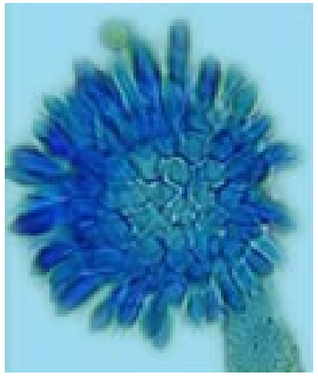


EvostrAIn™: a dedicated resource for AMR programs

Evotec's collection of characterised strains and clinical isolates

- Constantly evolving resource of thousands of primary clinical isolates and reference strains
- Broad collection of bacteria, fungi, viruses & parasites
  - ~10,000 strains from the clinic and culture collections
  - Global, recent sources with continual refreshment
  - High degree of characterization (susceptibility profiles, mechanisms of resistance and *in vivo* drug response, genome sequences available)
  - Includes isogenic mutant strains and mutant libraries
- Used to establish spectrum of activity & potential clinical use of new antimicrobials
- Rapidly build bespoke panels
  - Guide SAR
  - MoA / MoR (target validation & identification)
  - TPP validation
  - Translational activities





EvostrAIn™

Bacteria, fungi, viruses

Bacteria: Gram-positive pathogens

- *Staphylococcus aureus* including MRSA, VISA & VRSA strains
- β-Haemolytic *streptococci* groups A, B, C & G
- *Streptococcus pneumoniae* (including penicillin, macrolide, fluoroquinolone, cephalosporin and MDRSP resistant strains)
- Vancomycin Resistant *Enterococci* (VRE)
- *Bacillus* species
- *Listeria* species
- *Corynebacterium* and *Propionibacterium* species
- *Clostridium difficile* (multiple ribotypes incl. 012, 027 & 078)
- Other *Clostridia* (including C. *perfringens*)
- Constituents of gut microbiota

Other

- Mycobacteria (*Mtb* & non-*MTb* BSL2)

Bacteria: Gram-negative pathogens

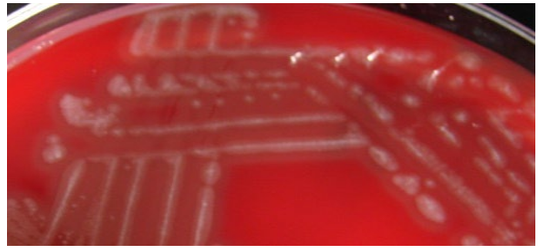
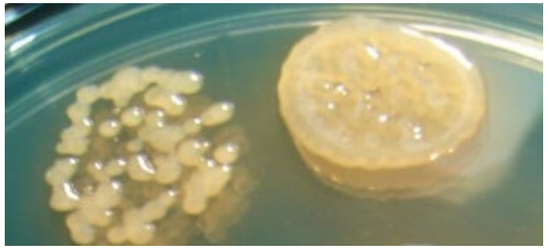
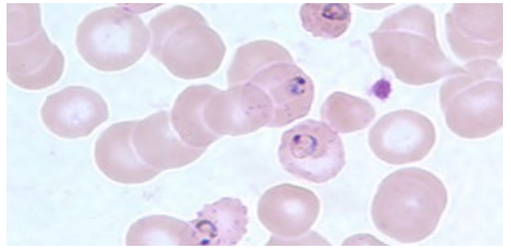
- *E. coli* including Extended Beta lactamase producing strains
- *Klebsiella pneumoniae* Carbapenemase producing strains (KPCs & MDR XDR)
- *Acinetobacter baumannii* incl. MDR XDR
- *Pseudomonas* spp. including MDR XDR
- *Haemophilus influenzae*
- *Bacteroides* spp.
- *Neisseria gonorrhoeae*
- Intestinal pathogens: *Vibrio* spp, *Campylobacter* spp incl. pylori, *Salmonella* spp, *Shigella* spp, *Yersinia* spp.
- *Legionella* spp. and Chlamydia
- Other Enterobacteriaceae: *Enterobacter, Proteus, Citrobacter, Serratia, Providencia & Morganella*
- *Burkholderia* and *Stenotrophomonas*

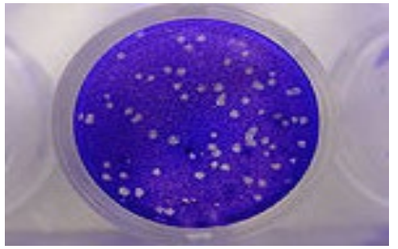
Fungi

- *Aspergillus* spp.
  (resistant to azoles, polyenes and echinocandins)
- *Candida* spp.
  (resistant to azoles, polyenes and echinocandins)
- *Mucorales*
- *Cryptococcus*
- Dermatophytes (*Fusarium*)

Viruses

- SARS-CoCV2 (all VOC)
- Influenza virus
- Respiratory syncytial virus
- Human rhinovirus
- Human parainfluenza virus
- Human metapneumovirus
- Hepatitis B virus
- SV-40



Case study

Selection of a *P. aeruginosa* strain panel

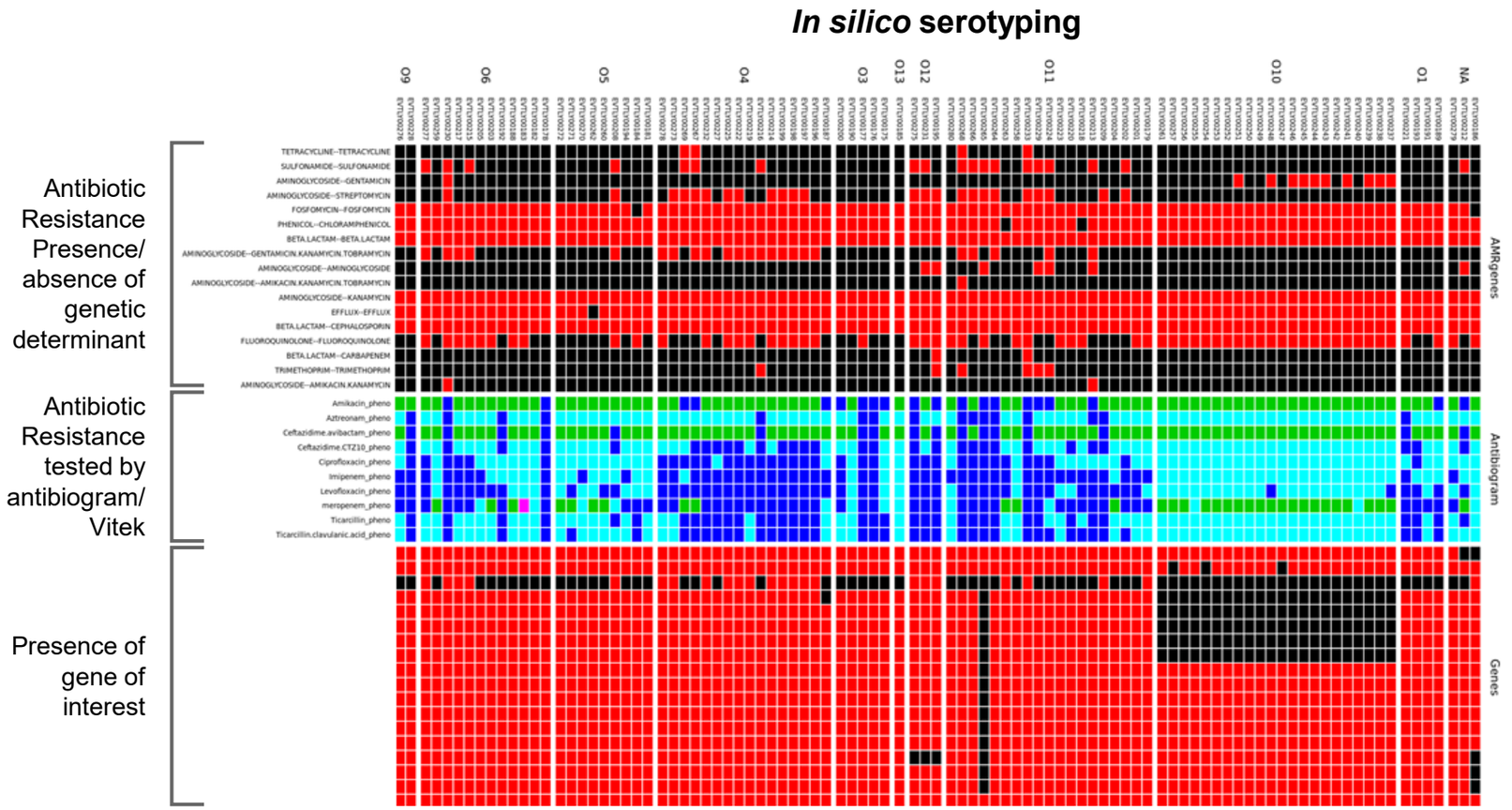
The strain panel can be compiled based on

- Presence/absence of gene of interest
- *In silico* serotyping
- Predicted antibiotic resistance
- Tested antibiotic resistance

Strain library

StrainSeq

Antibiograms / Vitek



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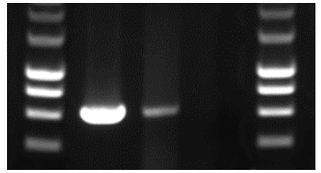
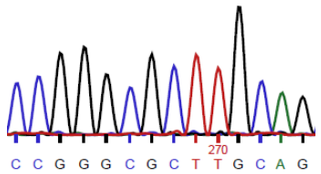
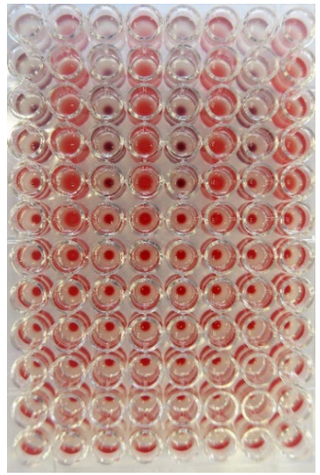
Highly validated and bespoke studies for target identification and molecular profiling

Mechanism of action determination (MoA)







Mutant generation:	Resistant mutants, spontaneous generation and serial passage
Genomics:	Whole-cell random mutagenesis of bacteria Determination of mutation frequencies/stability or mutation WGS of mutants Verification mutation correlates with resistance incl. *in vivo* Mutations recreated in WT where tools are available
Genome engineering:	Generation of targeted gene knockout and essential gene modulation (CRISPRi) Reporter strain construction Gene cloning and vector construction, expression Site-directed mutagenesis or random mutagenesis of target genes RT-PCR analysis of gene expression, use of reporter constructs
Phenotypic screens:	Screening of mutant libraries for hyper-susceptibility or resistance Phenotypic profiling using fluorescent markers (microscopy-cytometry-plate reader) Phenotypic counterscreens
Transcriptomics:	State-of-the-art pan-omics (RNAseq) *in vitro/in vivo*
Proteomics:	Whole cell proteome and biomarker platforms
Metabolomics:	Characterization of compound metabolic signatures

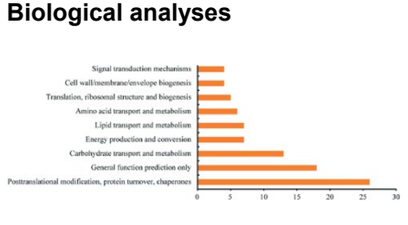
Utilizing the power of genomics & transcriptomics

Next Generation Sequencing (NGS) for MoA, MoR and Translation

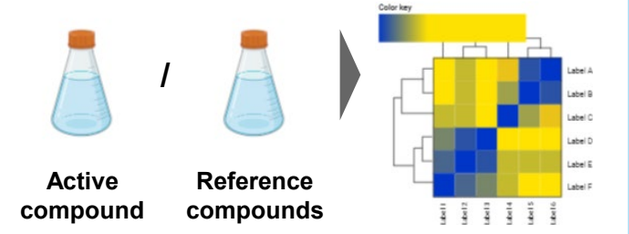
Antibiotic MoA / MoR / Cpd profiling (SAR)

WGS (high throughput)
Characterise resistance-associated mutations

RNAseq (high throughput)
Identify bacterial pathways regulated by active compounds



TnSeq (low throughput) – *E.coli*, *K.pneumoniae*
Comparing gene fitness in presence of sub-lethal (for MoA) or lethal (for MoR) concentrations of antibiotics and clustering by fitness profile vs. reference compounds

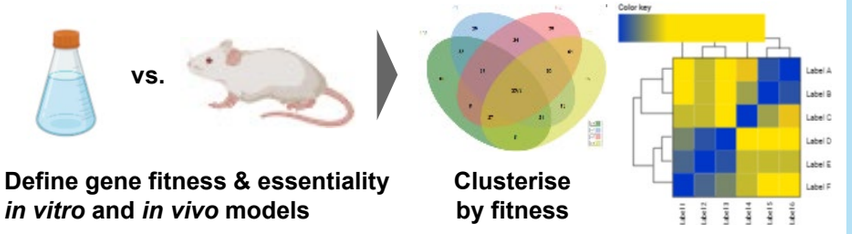


Active compound **Reference compounds**

Translatability of *in vitro* models

TnSeq: use genome-wide transposon mutagenesis to link genotype to phenotype & compare *in vitro* vs *in vivo* models

RNASeq: compare gene expression levels in *in vitro* vs *in vivo* models



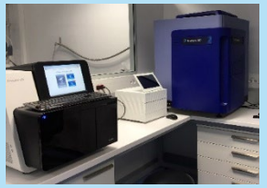
Define gene fitness & essentiality *in vitro* and *in vivo* models

Clusterise by fitness

Genomic sequence collection

Search engine / GWAS approach in development

Applications	WGS	TnSeq	RNASeq
Target ID	X	X	–
MoA	–	X	X
MoR	X	X	–
Translation	–	X	X
SAR	–	X	–



Collection of TnSeq reference compound profiles

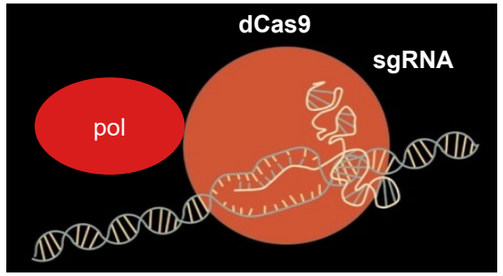
Full support from dedicated Bioinformatics team

State-of-the-art genetics

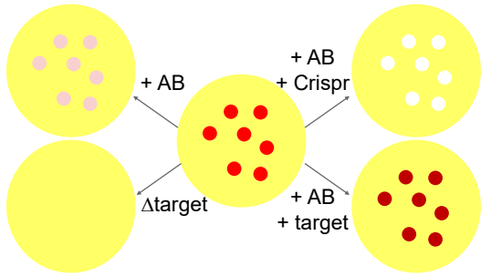
Strategic implementation of genetic tools

- Gene inactivation
- Fine-tuned gene expression
- Construction of genetic tools (plasmids, reporters, fluorescent markers)
- Multiple techniques including CRISPR-cas9
- Available in principal key pathogens (Gr+/-, *Mycobacterium*)

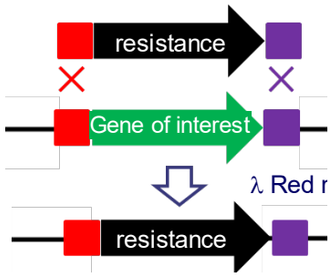
CRISPRi



Genetics / Phenotypes

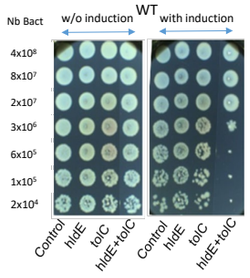


Target validation

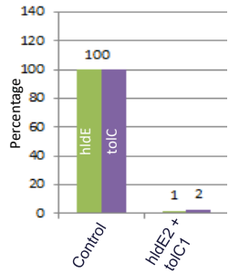


- Essential genes by CRISPi
- Library of the sgRNAs directed (~300 genes)
- Multiplex approach (several genes modulated in the same strain)
- Investigation of synthetic lethality

CRISPRi: 1 week

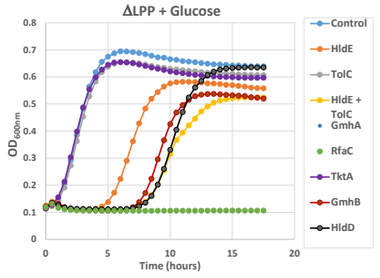


Multiplex CRISPRi: No viability



ddPCR: >98% inhibition of expression

CRISPRi: Several genes at once



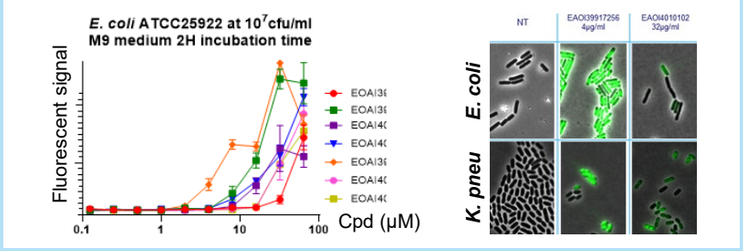
Several genes tested at the same time in one mutant: various levels of viability

Deep knowledge from MoA signature to MoA phenotyping

Multiscale phenotypic approach by microscopy-cytometry-plate reader

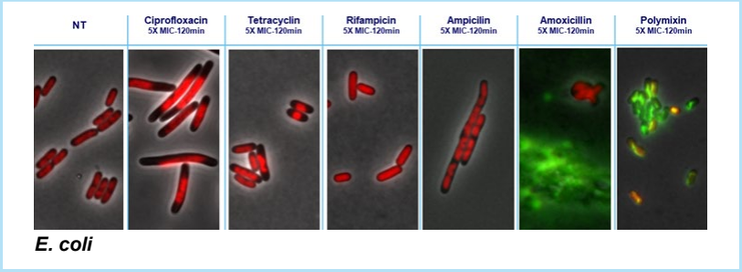
MoA guided screen / counterscreen / validation assay

- Fluorescent-based assay for any relevant pathway (specific reporter): e.g. permea-bilisation of membranes, SOS response, RNA transcription
- Assay development on demand: e.g. Alon library (GFP transcriptional fusions)



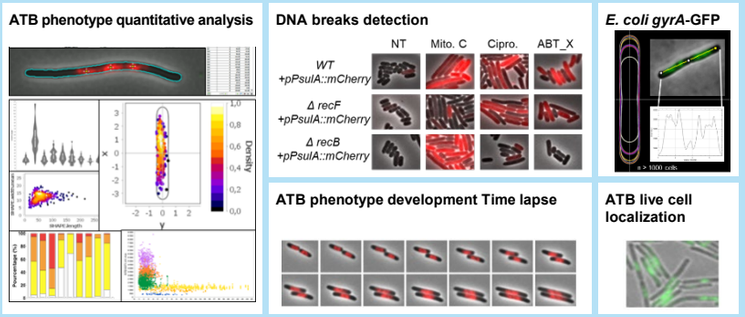
Hit selection and MoA profiling

- Bacterial Cytological Profiling (BCP)
- Reporter specific BCP (rsBCP)
- Qualitative or quantitative MoA profile attribution
- Family / subfamily MoA studies
- Reference panel comparison on demand

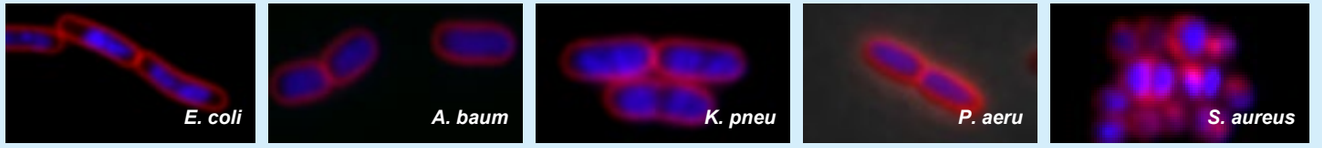


Specific MoA studies and target validation

- MoA specific reporters and assays
- Qualitative/quantitative analysis
- Compound live cell localisation
- Target live cell localisation
- Space and time resolution



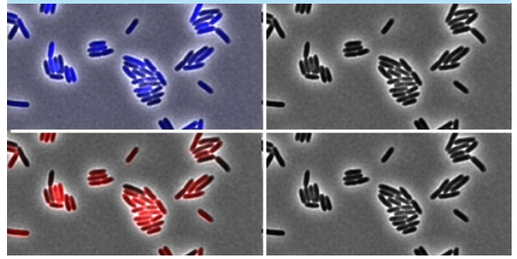
- In rich/poor media or *in vivo mimetic media* – In WT, tolC or any background (combination with e.g. KEIO – CRISPr)
- Developed for *E. coli*, on-going development with ESKAPE spectrum



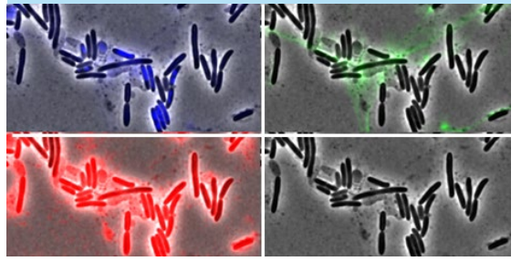
Pseudomonas aeruginosa MoA profiling

Bacterial Cytological Profiling approach (BCP)

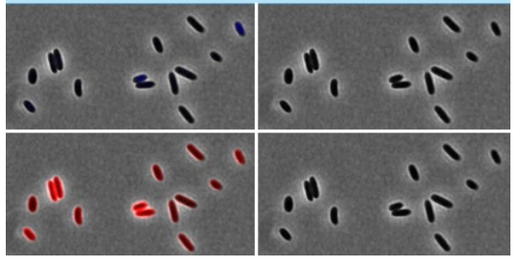
NT



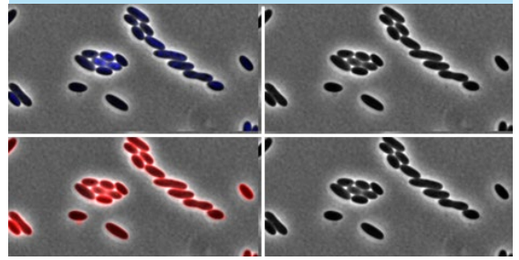
Ciprofloxacin



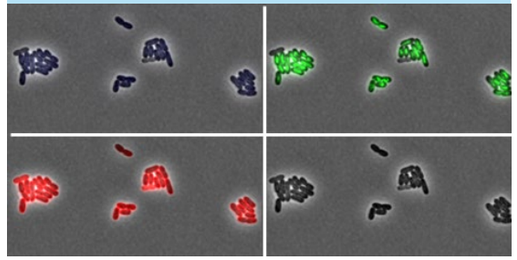
Rifampicin



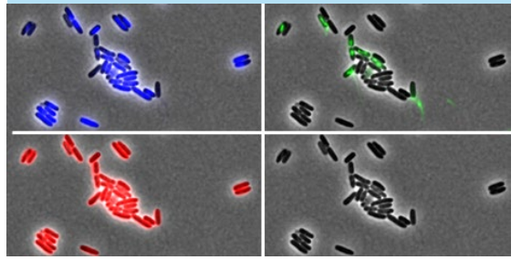
Tetracyclin



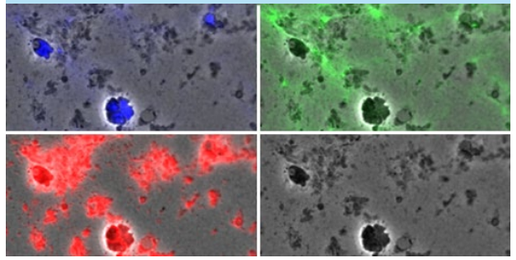
Colistin



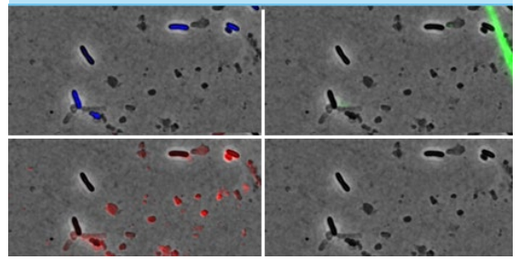
Gentamicin



Imipenem 90 min



Imipenem 60 min



BCP allows

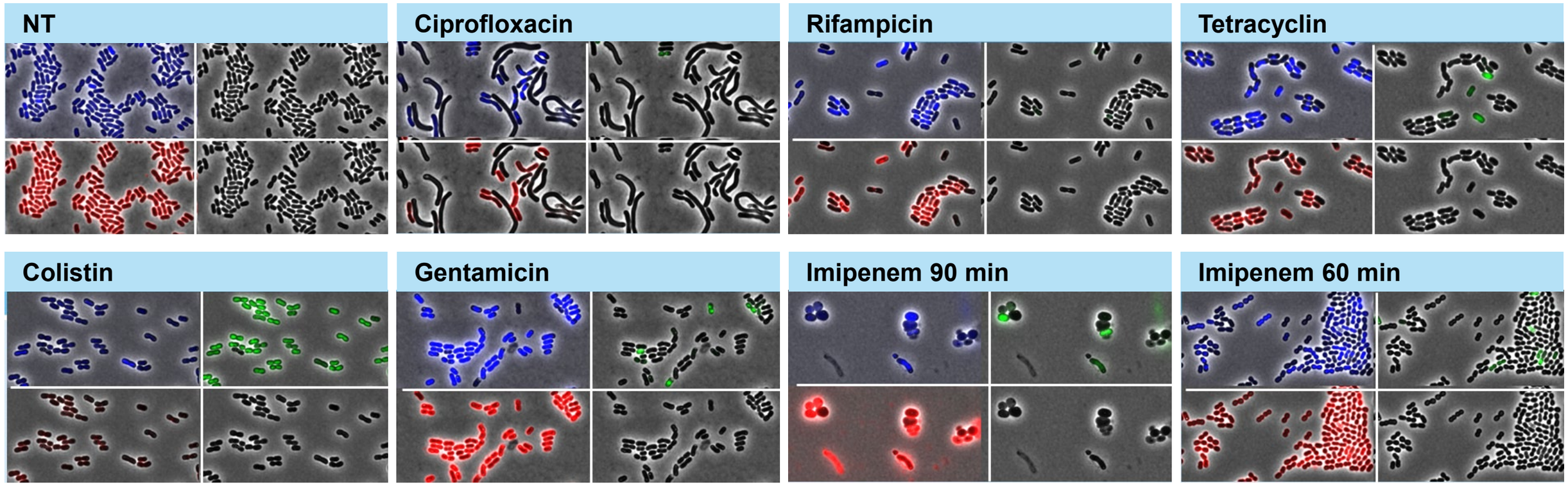
- To attribute a MoA to a molecule based on the profiling of the morphological changes induces
- To derisk off target effects

One profile can be attributed to one MoA

Cell shape + membrane FM4-64 + DNA DAPI + permeabilization Sytox Green = morphological profile

Acinetobacter baumannii MoA profiling

Bacterial Cytological Profiling approach (BCP)



One profile can be attributed to one MoA

Cell shape + membrane FM4-64 + DNA DAPI + permeabilization Sytox Green = morphological profile

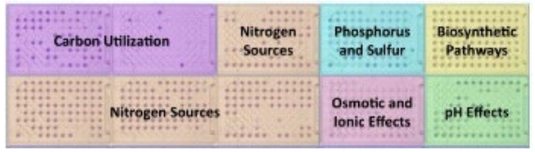
Modulation of activity by growth media to inform MoA, MoR and *in vitro* / *in vivo* translation

Unlocking the power of phenotypic microarrays for global compound evaluation

- **Robustness of a compound / translation *to vivo***
  - Medium dependent modulation of compound activity and resistance
- **MoA pathway insight**
  - From metabolism / growth medium dependency

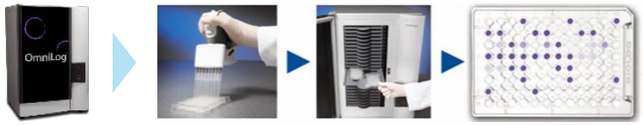
Assess global metabolism, osmotic conditions & pH sensitivity

10 x 96 wells microplates = 960 media
Compound dose effect
Growth on 20h



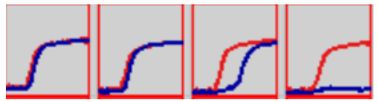
- **Bespoke plates**
  - To resume long and fastidious classic assays (salts, cations, BSA, pH, etc.)
  - To test complex media (serum, urine, etc.)

Collect the data **ΔT = 15 min on 24h**



Metabolic profiles comparison

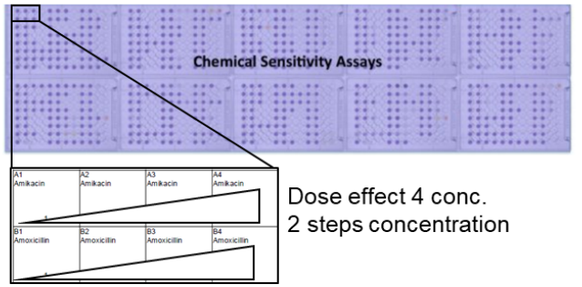
All	Plate Type	Field 1
1	PM11C	NT
2	PM11C	VUF15259



- **Plates with 240 marketed antibiotics and chemicals to assess compound's**
  - Synergy, potentiation & antagonism
  - Cross-resistance

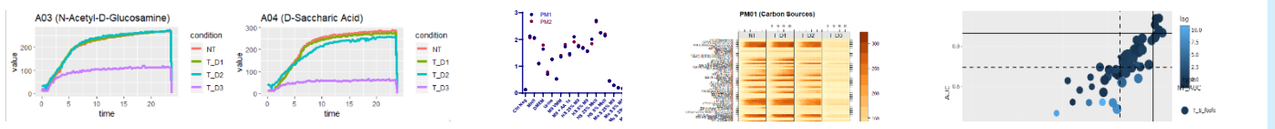
10 MicroPlates to assess chemical interactions

PM11 to 20: Rich medium + 240 ATB and chemicals - Dose effect 4 conc.



Dose effect 4 conc.
2 steps concentration

QC and data analysis pipeline development by bioinformatics team



The anti-infective Autobahn: from discovery biology to the clinic

Seamless program progression from discovery to development

Discovery biology

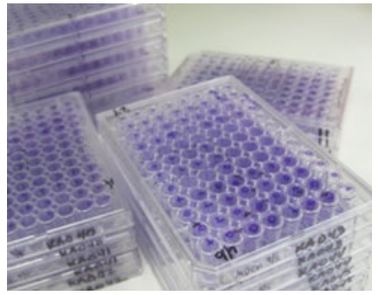
- Standard microbiology on a large strain collection (MIC, MIC_{90}, TKC, FoR, PAE, resistant mutants characterization, etc.)
- Target identification
- MoA/MoR determination and molecular profiling
- Omics and sequencing technologies
- Generation of engineered bacteria
- Target or Whole-cell based assay development

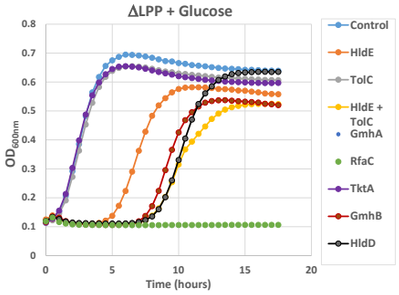
Integrated Drug Discovery

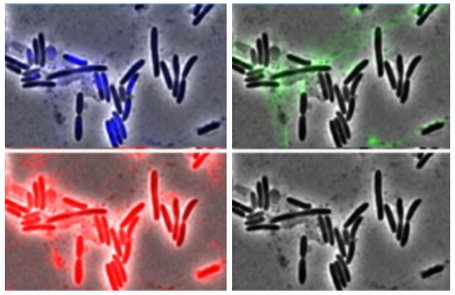
- Label-free bacterial intracellular compound accumulation assay
- Phenotypic screening (Biolog)
- Vivo-mimetic screening
- Target-based screening including fragment approaches
- Medicinal chemistry
- Computational chemistry and structure based drug design
- Highly efficient DMTA cycles

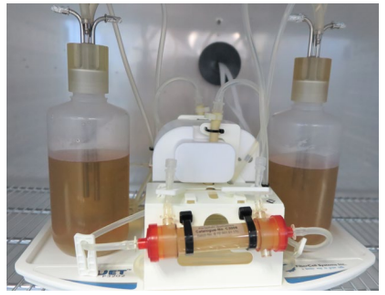
Translational microbiology and PK/PD

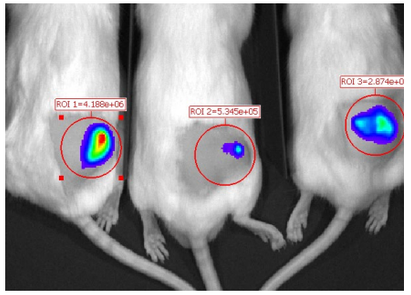
- State of the art *in vivo* DMPK
- *In vivo* microbiology for efficacy profiling
- *In vivo* and *in vitro* PK/PD platforms including Hollow fibre systems
- Mathematical modelling and simulations
- Translation of discovery data to the clinical setting

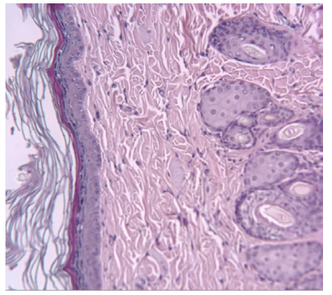










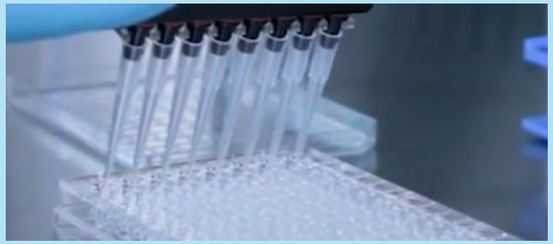


Compound quantification in bacteria

Breaching the bottleneck in the development of new Gram- antibiotics

Whole-Cell Assay

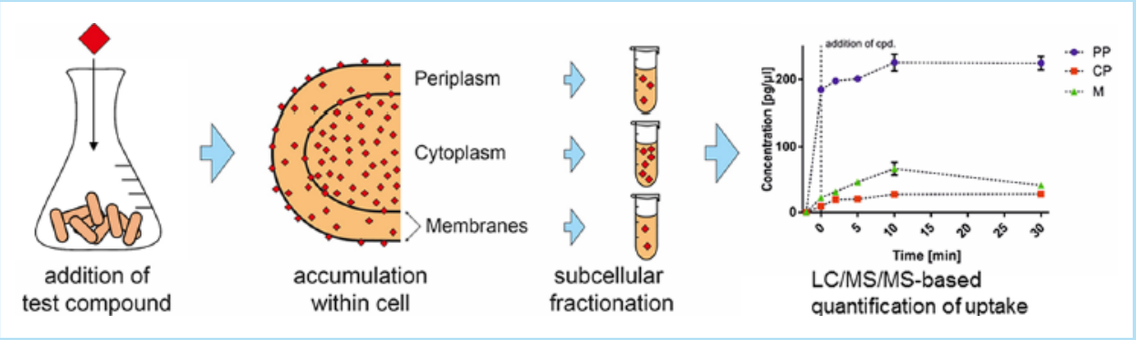
- Determine the compound concentration in the whole cell
- Medium throughput (96-well plate assay)
- Up to 32 compounds
- Sufficient throughput for SAR studies
- Development entirely in-house driven
- Applicable to different pathogens (*Ec, Kp, BCG, Mtb, …*)
- Applicable to different growth media
- Label-free mass spectrometry – based drug quantification



→ Correlate SAR and bacterial accumulation within a chemical series
→ Compare different chemical series, hits ranking

Subcellular Fractionation Assay

- Determine the compound concentration in the different cell compartments
- Low throughput (2-3 compounds)
- Proof of concept done on *E. coli*
- **Assay development started on *A. baumannii***
- Label-free mass spectrometry – based drug quantification



→ Variable level of activity between different strains/mutants of the same species (MoA and MoR)

Understand drug permeability into bacteria to support rational design of novel drug candidates

H. Prochnowb *et al.*, Analytical Chem., 2019

Compound quantification in bacteria

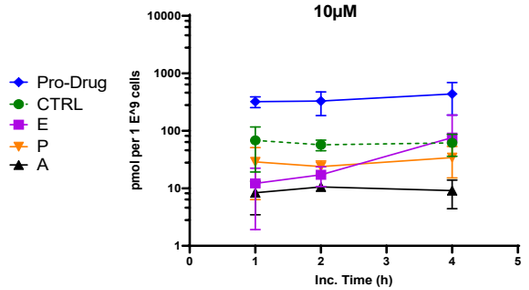
Assay development and case studies

Whole-Cell Assay – Quantification of compound uptake & metabolization in *Mtb*

Compound uptake in *Mtb* H37Rv

Mtb ATCC 27294 IC80 µm

A	Pro-Drug	E	P	RIF
1	1	0.13	0.054	0.11



Cpds uptake:
A < E ~ P < CTRL << ProDrug

***In cellulo* compound metabolization**

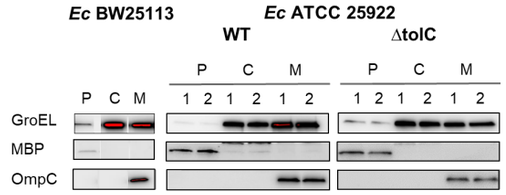
Dose 10µM

	inc. time (h)	cpds distribution (%) Parental	A	Potential Metabolite (inactive)
Pro-drug	1	61%	20%	18%
	2	53%	30%	17%
	4	39%	47%	14%
E	1	88%		12%
	2	88%		12%
	4	88%		12%
A	1	68%		32%
	2	68%		32%
	4	65%		35%
P	1	90%		10%
	2	90%		10%
	4	90%		10%

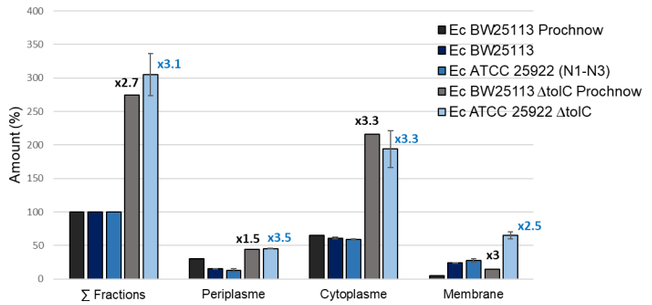
- Pro-drug is cleaved once in *Mtb* H37Rv
- Degradation of cpds into an inactive metabolite
- Compounds P and E are less impacted by this degradation

Subcellular Fractionation Assay – PoC on *E. coli*

Evaluation of the fractionation efficiency (Western blots)



Repartition of CIP in subcellular compartments of *E.coli* WT and ΔtolC



Ranking of compounds within a chemical series based on:

- Compound accumulation within *Mtb*
- Compound metabolization intra-*Mtb*

Fractionation assay validated for *E. coli*:

- CIP accumulation 3-fold higher in the Δ*tolC* strain *vs* the parental
- Results in line with published data

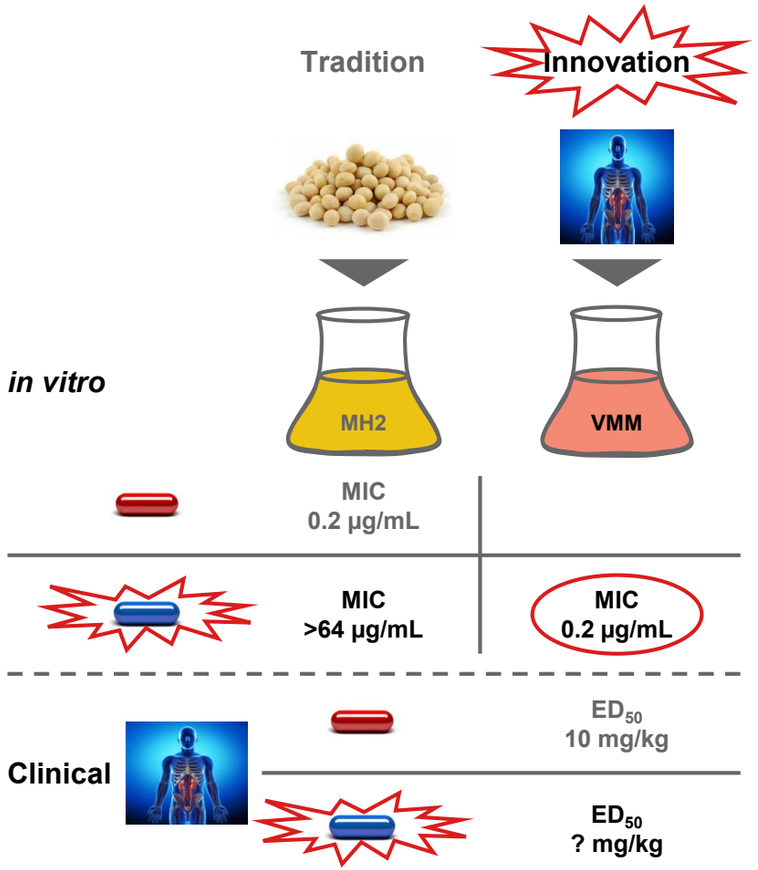
State of the art platforms for multiple hit-finding approaches

MTS and HTS for drug discovery programs

- >15 years of expertise in screening, from assay development to hit finding and hit profiling with >30 HTS campaigns in the anti-infective space
- Primary screening for identification of actives
  - Screening against BSL 2 biological agents: human cells & micro-organisms
  - Assay development and miniaturisation
  - HTS in 384 and 1536 well format
  - Screening collection adapted to the targets or approaches (25K to 900K)
- Characterisation of actives/hits: diverse range of secondary assays
- State-of-the-art robotic platforms
  - HTS: ET-1 and ET-2 robotic platforms (up to 100 plates/run, 30-100K/run)
  - MTS: 1 Beckman robotic system & 1 Agilent workstation (42 plates/run, 15K/run)
- BSL 2/3 cabinet and containment
- Multiple compound collections & Natural Products
- Phenotypic and target based screening
- Readouts: fluorescence, luminescence, optical density, SPR, HCS

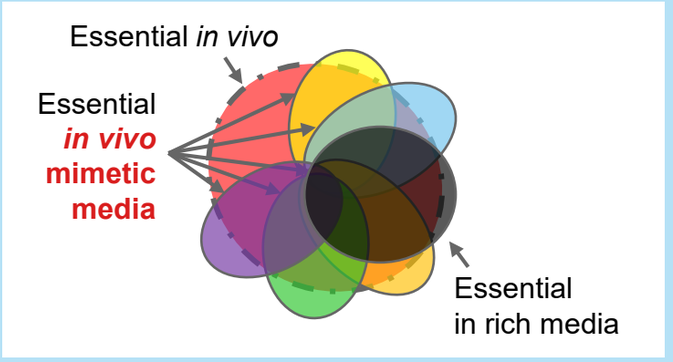
Vivo Mimetic Media (VMM)

A novel paradigm for discovery of novel Gram (-) antibacterials



Addresses limitations in Classical Phenotypic & Target-Based Screenings

- Screen in several alternative culture media that better mimic the conditions bacteria are facing during infection, i.e. limited access to nutrients, iron deprivation, stresses, *etc*.
- Growing bacteria in VMM conditions will affect their physiology, leading to:
  - altering the bacterial **permeability** (regulation of porins, efflux pumps, modification of outer membrane, …)
  - unveiling different overlooked cellular **targets** (i.e. functions dispensable in rich media becoming essential in VMMs and *in vivo*)
- This strategy will be important in identifying:
  - **New MoAs**
  - **New chemical space**

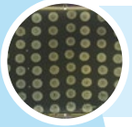


The Evotec VMM platform

Wholistic approach

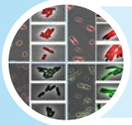
- **Validation of 5 selected Vivo Mimetic Media**, including human biological fluids and chemically-defined media
  - Investigated by genotype-phenotype studies
  - Proof-of-concept study performed with a proprietary compound library
  - Media-dependent activity exemplified using a phenotypic microarray (Biolog)
- **A unique set of assays developed in VMM** to assess *in vitro* potency, resistance development, MoA and *in vitro* / *in vivo* translation
  - Database generated in VMM with reference antibiotics and toxic compounds

High-Throughput Screening and counterscreening in key Gram-negative pathogens

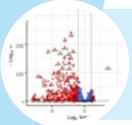


Potency on panels of strains

Resistance studies



MoA by fluorescent microscopy



In vitro / *in vivo* translation by TnSeq

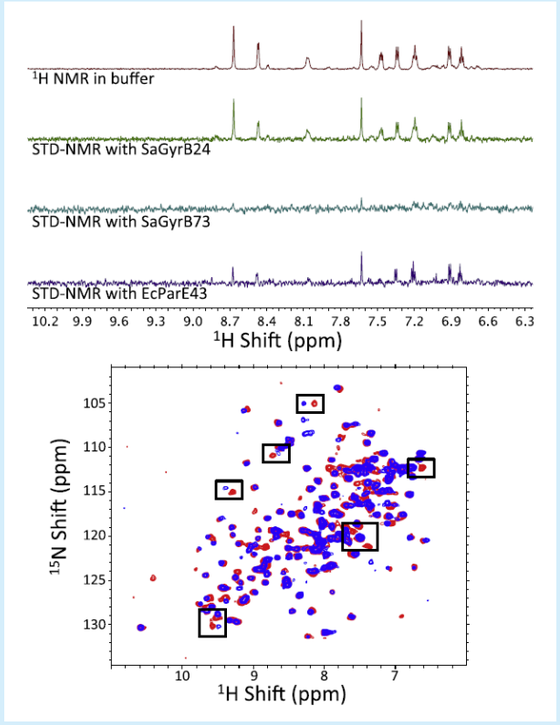
Accumulation studies (Mass Spectrometry)[1)]

[1)] under development

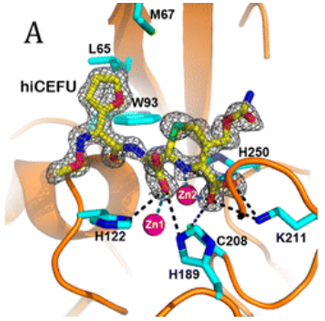
Utilizing all information: Structure- and ligand-guided approaches

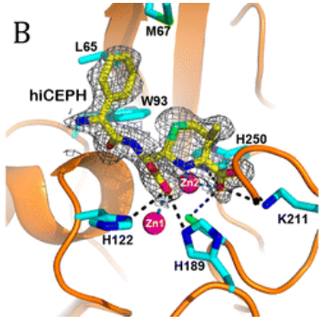
Fragment-based screening and computationally-guided DD to identify new antibacterials

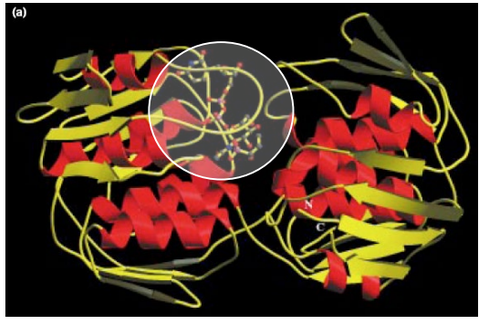
NMR-based screening

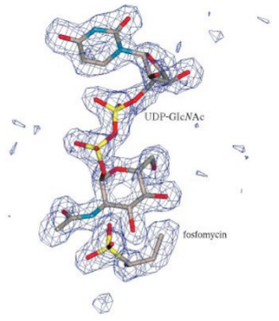


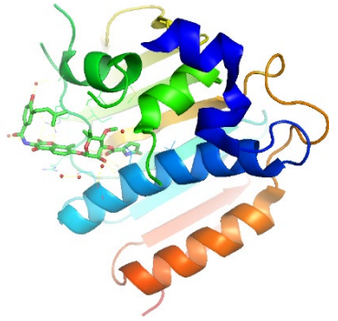
- HT docking using either X-ray or protein homology models
- Developed docking protocols with different software platforms
- Structure- and ligand-based compound/scaffold hopping and *de novo design*
- Concept of “antibacterial-like” *via* analysis of property space, 2D (topological) & 3D (pharmacophoric) "diversity"











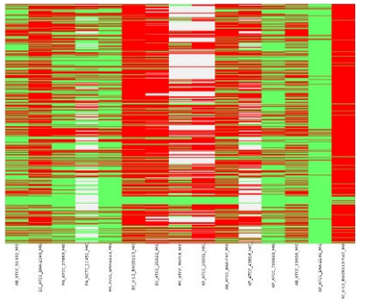
Machine learning in antibacterial discovery

Big Data approaches – cross program learning and knowledge building

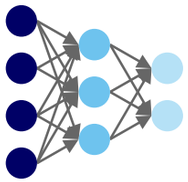
Biological target data

Integrated Database

Efficient data integration

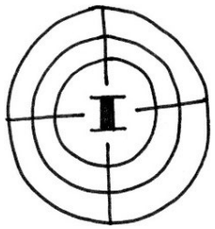


Deep learning



- Multitask Neural Network Model
- Prediction of antibacterial activities against 15 bacterial strains
- 76% of accurate predictions on newly designed compounds

Synthesis and Test



Effective idea prioritisation with AI based approach

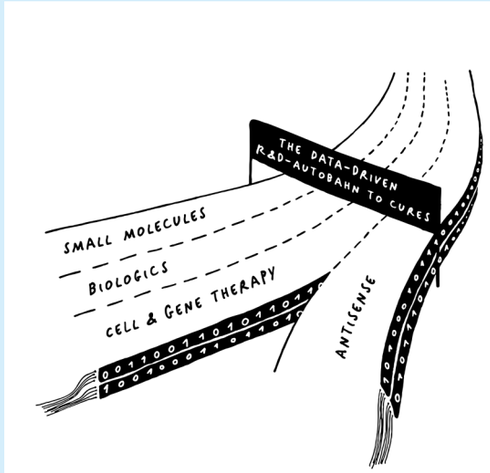
Augmented Idea Generation

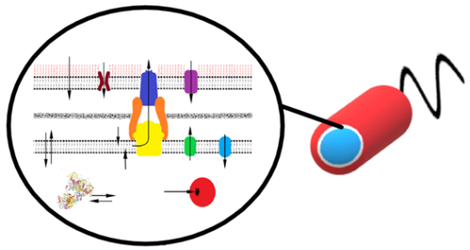
Medicinal Chemistry Expertise

AI for molecular *de-novo* design

Discovery and optimization of novel antibiotics

Antibacterial medicinal chemistry at Evotec





Expert chemistry resource

- Global capacity of ~350 chemists
- Strong synthetic capabilities, multiple classes, including process chemistry

Proven anti(myco)bacterial Med Chem Experience and Leadership

- Multiple integrated projects in antibacterial research (Hit ID, Hit-to-Lead and Lead-to-Candidate)
- Experienced pool of recognised expert project leaders[1)] with a proven track record of delivery and able to manage complex/multi-cultural projects

Addressing factors particular to antibacterial discovery

- Defining and targeting appropriate physicochemical space
  - According to bacterial species & target location
- Optimising accumulation at the target site
  - Penetration / Efflux
  - Using data from appropriate compromised strains, addressing liabilities

[1)] Peer reviewed publications and regular presentations at Antibacterial-focussed conferences

The anti-infective Autobahn: from discovery biology to the clinic

Seamless program progression from discovery to development

Discovery biology

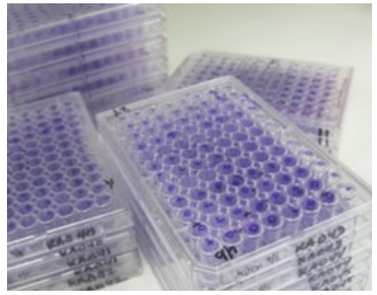
- Standard microbiology on a large strain collection (MIC, MIC_{90}, TKC, FoR, PAE, resistant mutants characterization, etc.)
- Target identification
- MoA/MoR determination and molecular profiling
- Omics and sequencing technologies
- Generation of engineered bacteria
- Target or Whole-cell based assay development

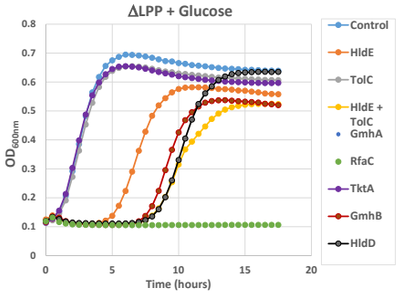
Integrated Drug Discovery

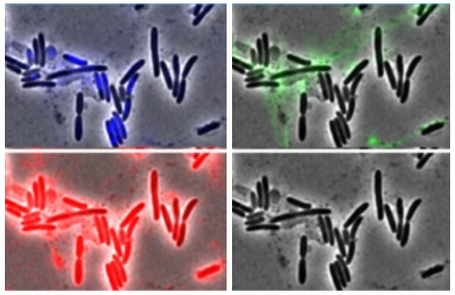
- Label-free bacterial intracellular compound accumulation assay
- Phenotypic screening (Biolog)
- Vivo-mimetic screening
- Target-based screening including fragment approaches
- Medicinal chemistry
- Computational chemistry and structure based drug design
- Highly efficient DMTA cycles

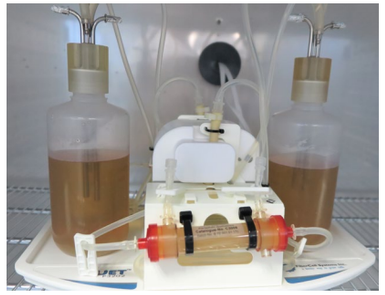
Translational microbiology and PK/PD

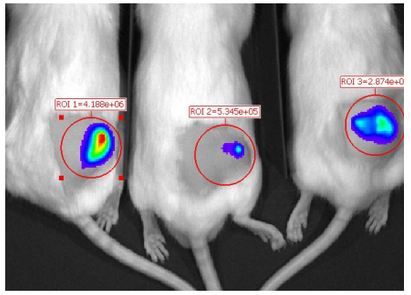
- State of the art *in vivo* DMPK
- *In vivo* microbiology for efficacy profiling
- *In vivo* and *in vitro* PK/PD platforms including Hollow fibre systems
- Mathematical modelling and simulations
- Translation of discovery data to the clinical setting

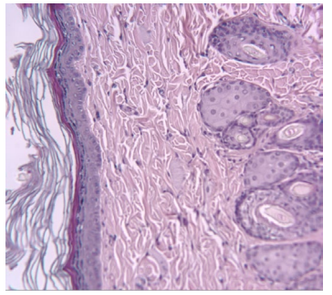






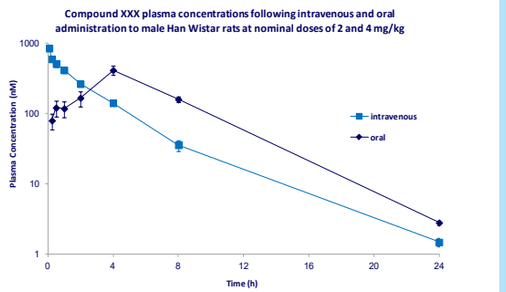






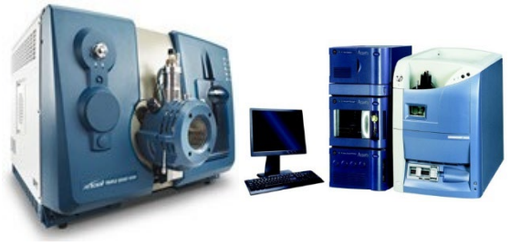
Pharmacokinetic profiling in anti-infective drug discovery

From *in vivo* characterisation to bioanalysis and biomarker assessment



PK Parameter	Intravenous	Oral
Dose (mg/Kg)	1.83 ± 0.04	4.07 ± 0.02
C_0 / C_{max} (ng/mL)	492 ± 25	206 ± 80
T_{max} (h)	-	4
V_{dss} (L/Kg)	6.9 ± 0.3	-
CL (mL/min/Kg)	34.6 ± 0.3	-
Liver Blood Flow (%)	48.1 ± 0.5	-
AUC_{inf} (ng.hr/mL)	880 ± 28	1248 ± 380
Bioavailability (%)	-	64 ± 0.2

- **Standard and specialised PK studies in multiple rodent species:**
  - Administration routes; intravenous (prolonged infusion), per oral, intraperitoneal, subcutaneous, intra cerebrospinal, intramuscular, pulmonary (nebulized, aerosolized), iPrecio & Alzet pumps
- **Sampling types:** jugular vein cannulation, cardiac puncture, tail vein microsampling
- **Matrices:** blood, plasma, CSF, BALF, whole tissues, bile, urine and faeces
- PK in infected animals assessing impact of disease state on drug exposure
- Data directly translated into efficacy studies to optimise outcomes
- PK experiments designed to accompany PK/PD profiling programmes

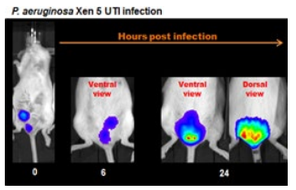


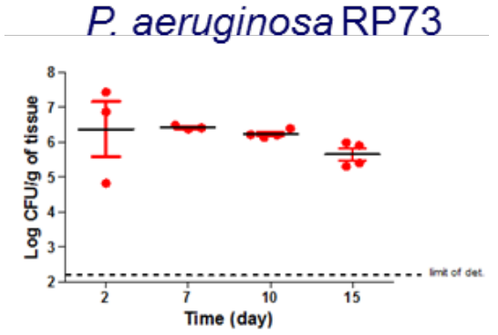
- **State-of-the-art bioanalytics**
  - Highly sensitive biophysical methods including LC-MSMS
  - Bioassay techniques: tracking biological activity
- **Biomarker quantification:** pathogen/infection specific and host response

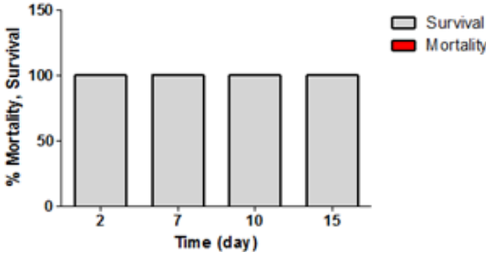
Comprehensive translational microbiology platform

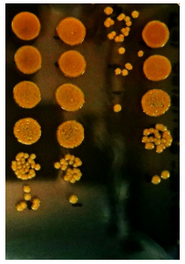
Integrating *in vivo* pharmacology and DMPK

- State-of-the-art animal facilities (**BSL 2 & 3**) to house rodents, immunocompromised animals, and multiple backgrounds (incl. dogs and monkeys AAALAC accredited)
- Target validation, tolerability studies, DMPK studies, PK/PD studies, *in vivo* MoA studies, efficacy screening
- Multiple hosts and fully validated models of infection: Rat, mouse, guinea pig, hamster, cotton rat, rabbit
- Multiple routes of infection include: lung, thigh, blood (sepsis), skin, urinary tract, GI tract, vagina, bone
- Full range of endpoints: pathogen burden (culture, qPCR, biomarkers), host response
- Real time imaging of microbes during infection: IVIS, MRI, CAT, PET
- New model development program
- Invertebrate screening model for bacteria & fungi (wax moth larva rapid screening models)









In vivo technical expertise and model endpoints

Overview

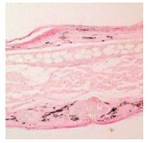
Technical expertise	
Infection route	IV, IP, IM, SC, ID, IN, IT, OP
	Aerosol
	Intra-ocular
	Intra-tissue cage / catheter
Treatment route	IV, IP, IM, SC, ID, IN, IT, OP
	Aerosol of solution/suspension/dry powder
	SC/IV Infusion with infusion pump/osmotic/iPrecio-programmable pump
Surgical capabilities	Vessels cannulation (rat/mouse)
	Tissue cages/catheter/pump SC implantation
PK/PD	Bioanalysis of small and large molecules
	Bioluminescence / fluorescence for labelled molecule (IVIS)
	ELISA quantification of therapeutic protein or antibody
	PK/PD Modelling

Model endpoints	
Pathogen burden	Microbial culture (aerobic and anaerobic)
	qPCR quantification
	Imaging/quantification (IVIS)
	Plaque assay (virus or bacteriophage)
Survival	Humane endpoint/clinical scoring
Phys. parameters	Body weight, body temperature (chips)
Immune response	Cytokine, chemokine quantification (ELISA, multiplex)
	Whole blood cells quantification (IDEXX blood analyser)
	Immune cells quantification (FACS, DASIT)
Clinical pathology	Haematology and clinical chemistry
Survival	Histopathology / immunochemistry / histo-cytology
Biofilm detection	Scanning electron microscopy (SEM)
Exposure	Tissues collection/analysis for PK purposes (blood, plasma, serum, bile, urine and faeces, lung, BALF, spleen, kidneys, liver, …)

Clinically relevant readouts for *in vivo* studies

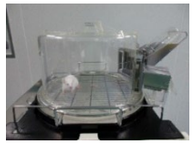
Translating discovery data, human dose prediction and clinical studies

Organ and fluid collection


Mouse ear histology

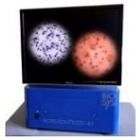

Rat caecum

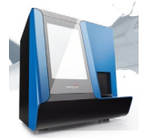

Blood, plasma, tissues


Metabolic cages

Flow cytometry, haematology and clinical chemistry


Flow cytometry (FACS Canto II, Aria)

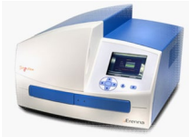

ELISpot


HM5


Biochemistry analyzer

Gene/mRNA/protein analysis


Viia7, QuantStudio


Singulex


MSD technology ELISA

Histology/IHC (FFPE/frozen tissues)


Ventana
Automated
histoembedder


Microtome

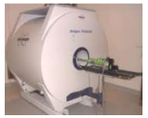

Digital slides scanner (Nanozoomer)

In vivo imaging systems


Bioluminescence
Fluorescence
3D tomography
(IVIS Spectrum)


X-ray imager
(Faxitron MX20)

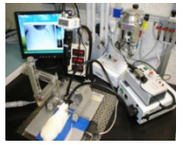

Laser Doppler
(MoorLDI2)


Bruker 7T
small bore MRI

Mass spectrometry


Bioanalysis


Proteomics


Metabolomics / Microdialysis

Overview of bacterial infection models

Gram-positive and Gram-negative sensitive or resistant strains (a growing list)

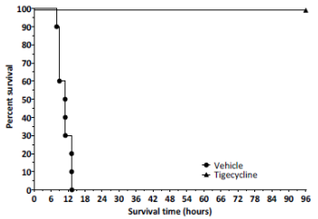
Model	**Pathogen**	**Immune System** (Rodent species, gender)	**Number of strains validated/species**
IP Sepsis	*A. baumannii, E. coli, K. pneumoniae, P. aeruginosa, E. cloacae, S. aureus, S. pneumoniae, E. faecalis*	Competent & Neutropenic (some *E. coli* strains)	1 to 27
IV Sepsis	*A. baumannii, E. coli, P. aeruginosa, S. aureus, S. pneumoniae*	Competent or Neutropenic	1 to 6
Thigh infection	*A. baumannii, E. coli, K. pneumoniae, P. aeruginosa, P. mirabilis, P. stuartii, E. cloacae, C. freundii, S. aureus, S. pneumoniae, E. faecalis, E. faecium, S. pyogenes*	Neutropenic (mouse or rat)	2 to 32
Lung infection	*A. baumannii, K. pneumoniae, P. aeruginosa (acute or chronic), H. influenza, S. pneumoniae, S. aureus (nasal colonisation in cotton rat)*	Competent or Neutropenic	2 to 26
UTI	*A. baumannii, E. coli, K. pneumoniae, P. aeruginosa, P. mirabilis*	Competent (mouse or rat, male or female)	1 to 9
Skin/foreign body/endo-carditis/Osteomyelitis	*S. aureus, P. aeruginosa, P. acnes, S. epidermidis*	Competent (mouse, rat guinea pig, rabbit)	1 to 5
Gastrointestinal infection	*C. difficile, V. cholerae, S. enterica, E. coli, K. pneumoniae*	Competent (mouse or hamster)	1 to 4

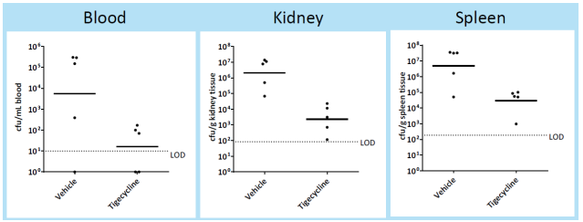
Comprehensive and growing portfolio of disease models to support AMR programs

In vivo models of ***Enterobacterales*** infection

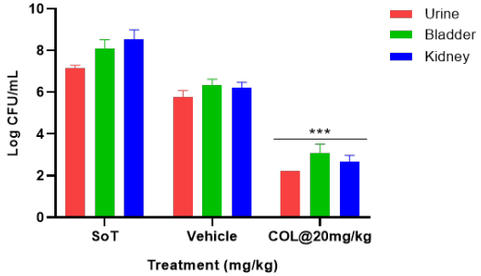
Model	Pathogen	Animal Species	Immune System
Acute Sepsis	*E. cloacae*	Mouse	Competent
	E. coli	Mouse	Competent
	K. pneumoniae	Mouse	Neutropenic
Thigh	*E. cloacae*	Mouse	Competent
	E. coli	Mouse	Neutropenic
	K. pneumoniae	Mouse	Neutropenic
Lung	*E. cloacae*	Mouse	Neutropenic
	E. coli	Mouse	Neutropenic
	K. pneumoniae	Mouse	Neutropenic & Competent
UTI	*E. cloacae*	Mouse	Competent
	E. coli	Mouse	Competent
	K. pneumoniae	Mouse	Competent

***E. coli* IR45 (bla_{NDM1})**
Sepsis
Survival





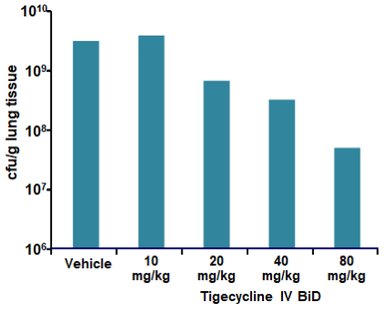
***K. pneumoniae* BA454**
(ESBL+, $bla_{TEM\ 1}$, bla_{CTXM15}, $bla_{SHV\ 11}$)
UTI infection

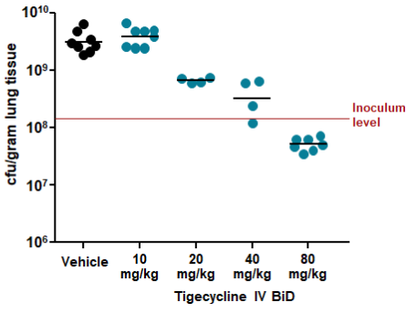


Survival

Treatment	Time (hours)			
	0	24	48	72
Vehicle	8	5	3	3
COL	8	6	6	6

K. pneumoniae
Lung infection



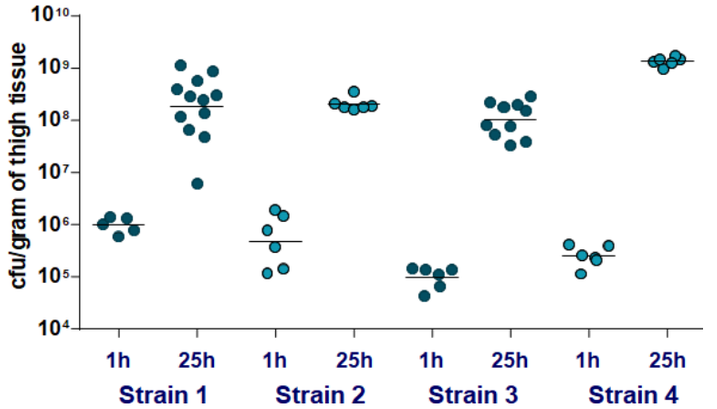


Comprehensive and growing portfolio of disease models to support AMR programs

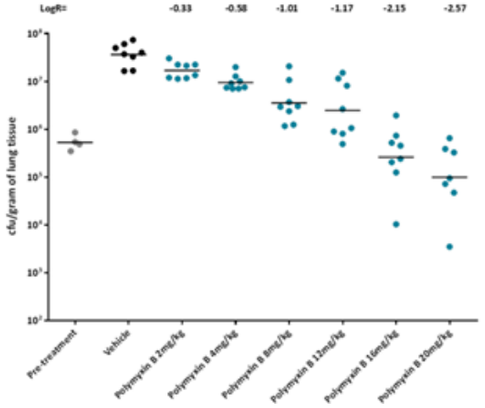
In vivo models of ***P. aeruginosa*** infection

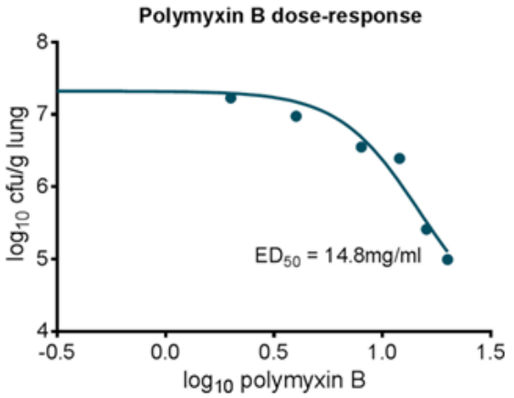
Model	Pathogen	Animal Species	Immune System
Acute Sepsis	*P. aeruginosa*	Mouse	Neutropenic & Competent
Acute Lung	*P. aeruginosa*	Mouse	Neutropenic
Chronic Lung	*P. aeruginosa* (from CF)	Mouse & Rat	Competent
Foreign Body	*P. aeruginosa*	Mouse	Competent
Thigh	*P. aeruginosa*	Mouse	Neutropenic & Competent
UTI	*P. aeruginosa* (bioluminescent)	Mouse	Competent

THIGH INFECTION
P. aeruginosa MDR
several strains



LUNG INFECTION
P. aeruginosa MDR



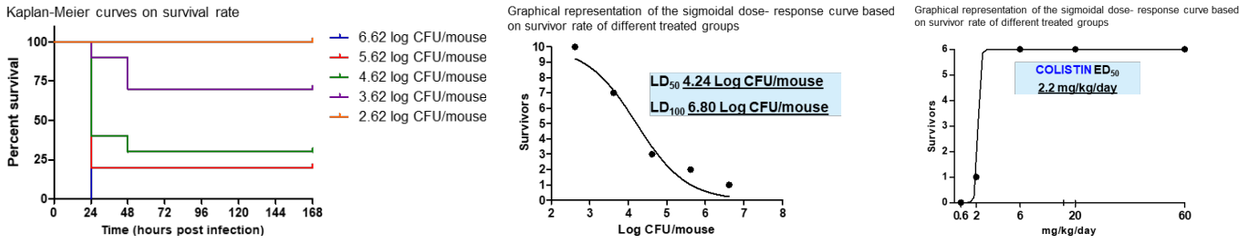


Comprehensive and growing portfolio of disease models to support AMR programs

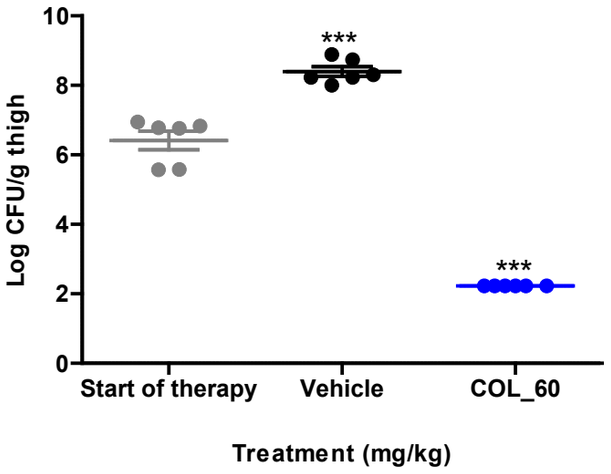
In vivo models of ***A. baumannii*** infection

Strain	Sepsis	Thigh	Lung	Pyelonephritis
A. baumannii ATCC 17978	■	■	■	
A. baumannii ATCC 19606	■	■		■
A. baumannii NCTC 13420	■	■		
A. baumannii ATCC BAA-747			■	
A. baumannii ACC 00535	■	■	■	
A. baumannii ACC 00445	■	■	■	
A. baumannii ACC 00934		■		
A. baumannii ACC 00935		■	■	
A. baumannii ACC 01073			■	
A. baumannii ACC 01077			■	
A. baumannii ACC 01085			■	
A. baumannii ACC 01094			■	
A. baumannii ACC 01097			■	
A. baumannii ACC 01099			■	
A. baumannii ACC 01083			■	
A. baumannii ACC 01087			■	
A. baumannii ACC 00727				■

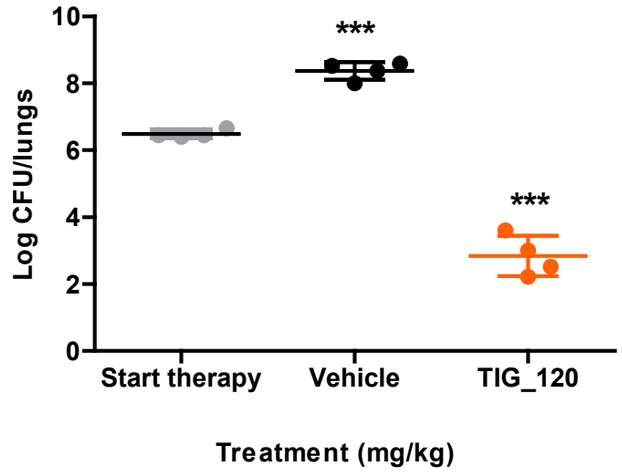
SEPTICAEMIA: *A. baumannii* ACC00445



THIGH: *A. baumannii* ACC00535



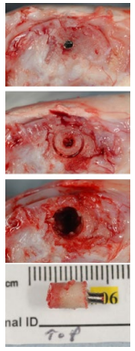
LUNG: *A. baumannii* ACC00535



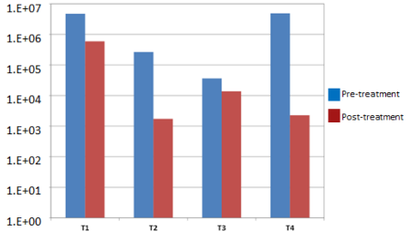
Comprehensive and growing portfolio of disease models to support AMR programs

In vivo models of **Gram-positive-driven** infection

Model	Pathogen	Animal Species	Immune System
Sepsis (IV/IP)	*S. aureus*	Mouse	Competent
	S. pneumoniae	Mouse	–
	E. faecalis	Mouse	–
Thigh infection	*E. faecalis*	Mouse	Competent
	E. faecium	Mouse	–
	S. aureus	Mouse	–
	S. pyogenes	Mouse	–
Acute Lung	*S. pneumoniae*	Cotton Rat	–
Nasal Colonisation	*S. aureus*	Mouse & Guinea pig	Neutropenic
Tissue Cage model	*S. aureus*	Mouse & Rat	Competent
Human Foreign Body	*S. aureus*	Mouse & Rat	Neutropenic
	S. epidermidis	Mouse & Rat	Neutropenic
Osteomyelitis	*S. aureus*	Rabbit	Neutropenic
Wound infection	*S. aureus*	Mouse	Neutropenic
Endocarditis	*S. aureus*	Rat	Neutropenic

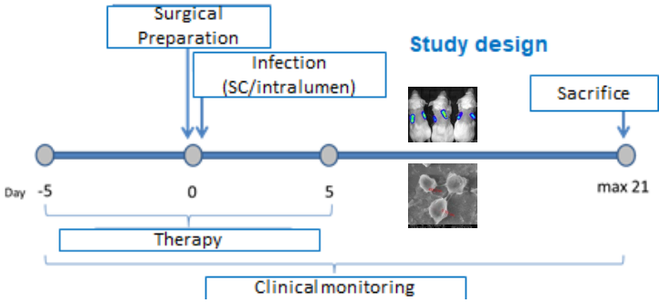


RABBIT OSTEOMYELITIS
S. aureus ATCC33591

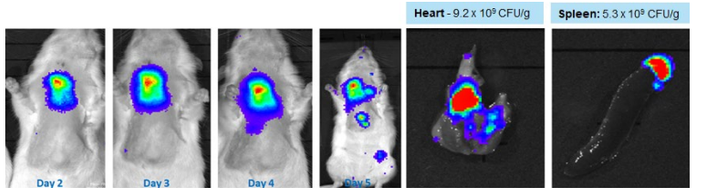


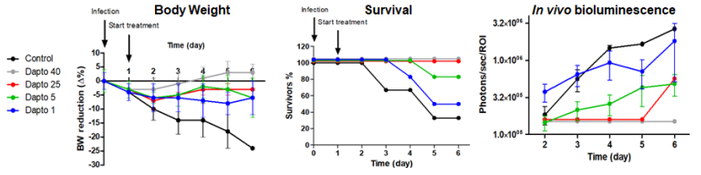
Bar chart showing burdens of bone tissue collected at the time of revision surgery and terminal samples following four different therapies

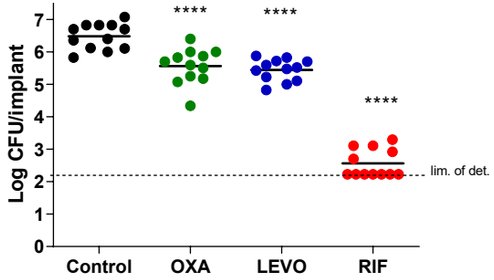
MOUSE HUMAN FOREIGN BODY MODEL
S. aureus Xen29



RAT AORTIC VALVE ENDOCARDITIS
S. aureus Xen29







IP treatment with oxacillin (MIC 0.25 µg/mL), levofloxacin (MIC 0.125 µg/mL) and rifampicin (MIC≤0.04 µg/mL) at 50 mg/kg BID - 4 days of therapy

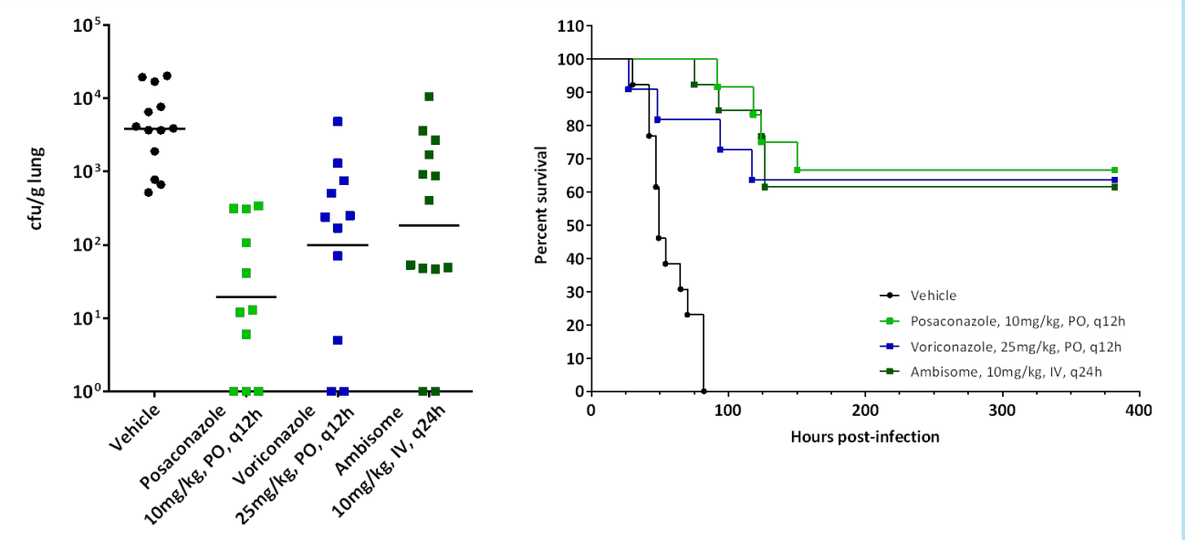
Statistical analysis: one-way Anova followed by Dunnet's post test (**** P<0.0001)

Breadth of *in vivo* models of fungal infection

Summary and ***Aspergillus*** lung infection as example

Model	Pathogen	Animal Species	Immune System
Acute Sepsis	*A. fumigatus, A. flavus, A. terreus*	Mouse	Neutropenic
	C. albicans, C. glabrata, C. tropicalis	Mouse	Neutropenic
	C. auris	Mouse	Neutropenic
	C. neoformans	Mouse	Neutropenic
	C. albicans	Mouse	Competent
Chronic Sepsis	*C. albicans*	Mouse	Neutropenic
Brain	*C. neoformans*	Mouse	Neutropenic
	C. albicans	Mouse	Competent
GI Tract	*C. albicans*	Rat	Competent
	C. albicans	Guinea pig	Competent
Skin	*M. pachydermatis*	Guinea pig	Competent
	T. mentagrophytes	Mouse	Competent
Vaginal	*C. albicans*	Rat	Competent
	C. albicans	Mouse	Neutropenic
Lung	*A. fumigatus, A. flavus, A. terreus*	Mouse	Neutropenic
Lung aerosolized	*A. fumigatus*	Mouse	Neutropenic
Oropharyngeal	*C. albicans*	Mouse	Neutropenic

Neutropenic murine model of *A. fumigatus* lung infection. Survival and lung burden as endpoints:

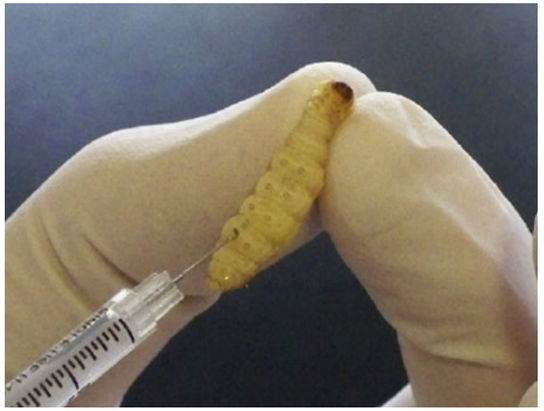


Additional endpoints: cytokines, cellular response, galactomannan, β glucan, histopathology

Alternative models for predictivity & 3Rs

Galleria melonella

Model with different pathogens



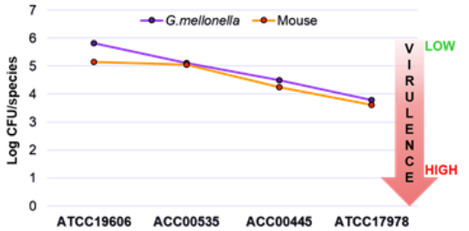
Model	Pathogen
Invertebrate Model Wax Moth	*A. baumannii*
	P. aeruginosa
	A. fumigatus
	A. terreus
	C. albicans

Read out

- Pathogen burden
- Survival models

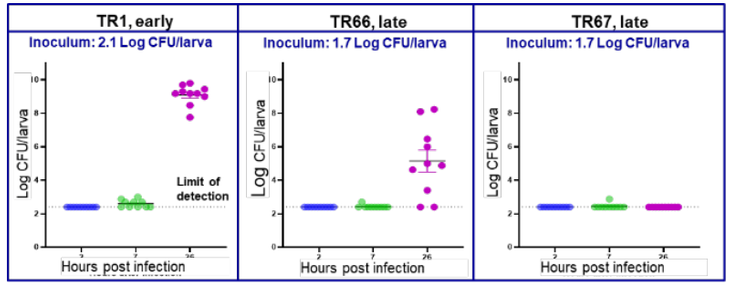
LD_{50} Log CFU/species

Bacterial strain	Resistance Phenotype	*G. mellonella*		Mouse model (septicaemia)
A. baumannii ATCC19606	Sensitive Reference Strain	5.83 ± 0.01	⟷	5.16 ± 0.36
A. baumannii ATCC17978	Sensitive Reference Strain	3.79 ± 0.01	⟷	3.62 ± 0.18
A. baumannii ACC00445	MDR Clinical Strain	4.49 ± 0.30	⟷	4.24 ± 0.18
A. baumannii ACC00535	MDR Clinical Strain	5.12 ± 0.17	⟷	5.05 ± 0.01



Galleria* is a predictive model for virulence assessment in *A. baumannii

CF *P. aeruginosa* TR-family from same patient



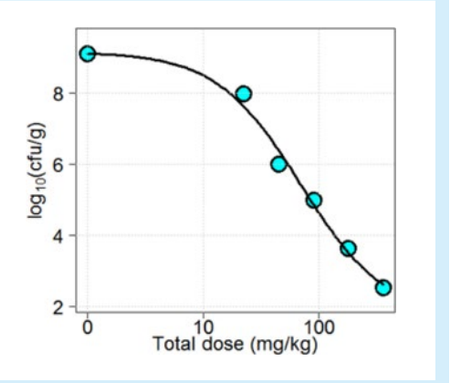
Growth profiles analysis in Galleria could help in identifying strains inducing a chronic infection in rodents

Translation into the clinic: *in vivo* and *in vitro* PK/PD strategies

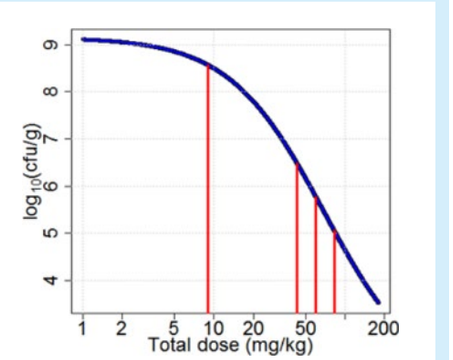
From discovery to clinical trial design

- **Utilising the Evotec PK/PD profiling platform and expertise**
  - Correlation of exposure with efficacy and drug response
  - Magnitude of effect
  - Understanding PD drivers and what is important to achieve efficacy
  - Characterisation of post-antibiotic effect (PAE)
  - *In vitro* **and** *In vivo* PK/PD
- **Humanised dosing using infusion or dose fractionation**
  - Translation of efficacy in pre-clinical models and microbiological assays to predict efficacy in humans
  - Use and analysis of PK parameters determined in the alternative host system to predict exposure in humans

Dose response study



Modelled dose fractionation design



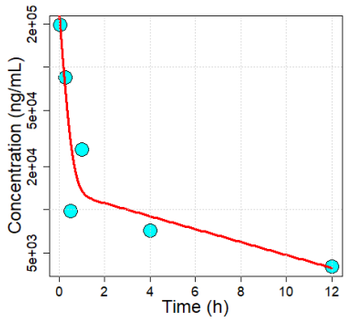
Determination of the PD driver in a novel antibacterial class

Use of PK/PD in an early discovery program

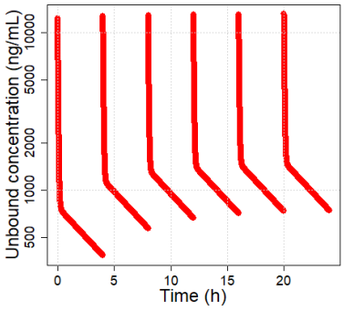
- PK/PD system to study the main PK/PD driver of anti-infective drugs following multiple dose fractionation studies for different pathogens
- Dose fractionation studies to determine the PK/PD driver, dose level and/or interval to inform clinical regimens
- The PK of the antibiotic was evaluated in mice where the compound was administered IV
- Two-compartment model to fit the PK data, mathematical model for each of the hybrid constants to simulate the dosing regimens used in the dose fractionation study
- PK model used to calculate key PK/PD metrics including AUC/MIC and %T>MIC

→ Efficacy of the antibacterial in *E. coli* is time- or AUC-driven

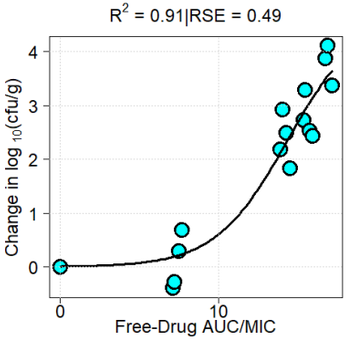
Bi-exponential fitting of PK data



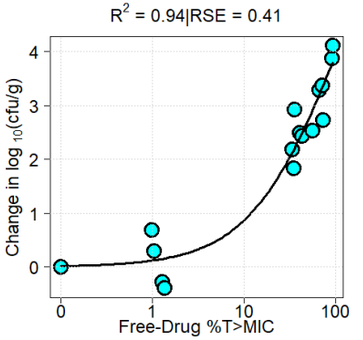
PK simulations



AUC/MIC

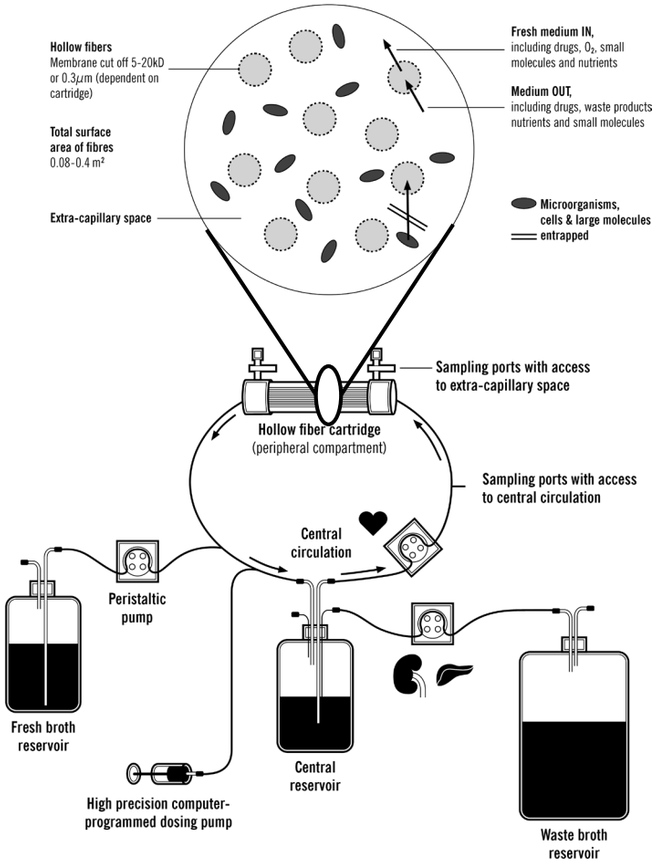


%T>MIC



Hollow fibre infection model

- The Hollow fibre infection model provides a dynamic *in vitro* method of assessing the impact of a time course of drug exposure(s) on a cell or combination of cells
- The most capable *in vitro* model for evaluating PK/PD indices and optimising dosing regimens for bacterial killing and suppressing the amplification of drug resistant mutant subpopulations
- Two principal compartments:
  - Central reservoir and associated tubing which constitutes a circulating system
  - A hollow fibre cartridge containing thousands of permeable capillaries, sealed at both ends within a tubular polycarbonate shell. The extracapillary space (ECS) is defined as the area outside the fibres but within the cartridge housing where the target organism is contained.
- Drug-infused growth medium in the central reservoir is continuously pumped to the hollow fibre cartridge
- Rapidly passes through the capillaries and equilibrates with medium in ECS
  - Nutrients and oxygen continuously refreshed
  - Waste products are removed
- To simulate drug clearance, fresh medium from an external supply is pumped into the central reservoir effectively diluting the drug from the system which is eliminated to a waste reservoir

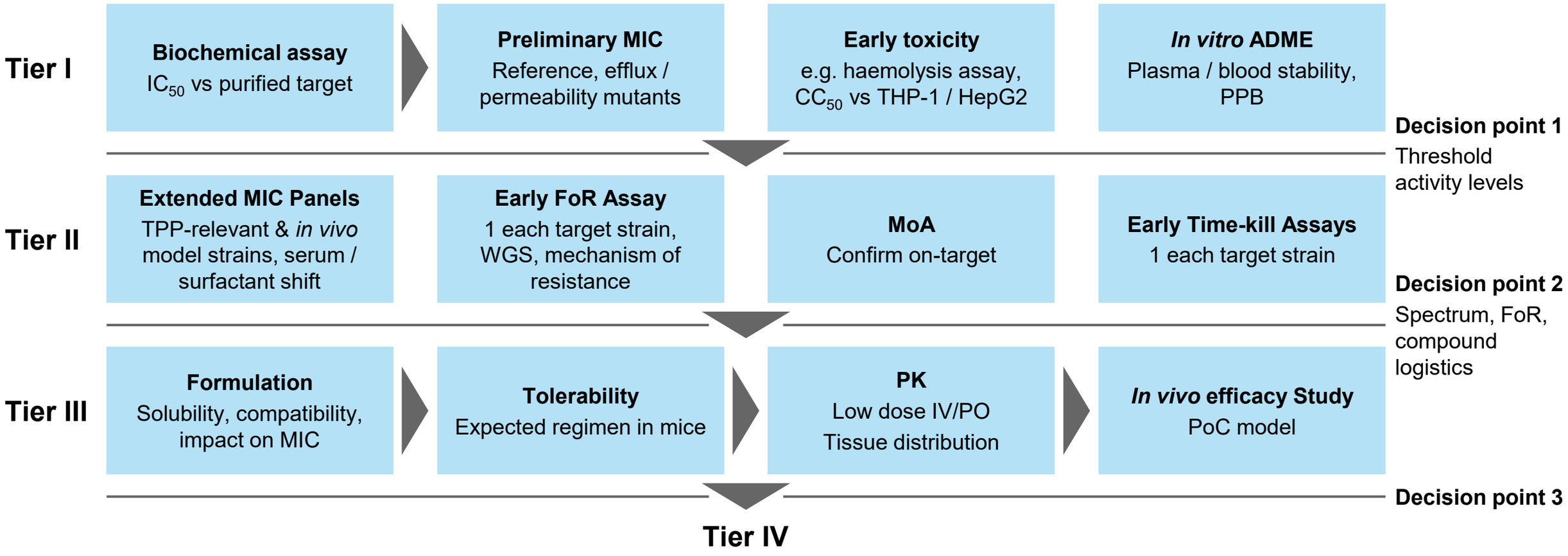


Hollow fibre infection model (HFIM)

- Current set up
  - Dedicated HFIM laboratory space at CL2
  - A dedicated team of scientists trained in setting up and running the system
  - Full microbiology support
  - Bioanalysis facilities for LC-MS analysis of PK samples
  - A dedicated PK/PD modelling team
- Up to 34 cartridges (depending on model type and duration) can be run in parallel for different organisms, variable drug infusion and clearance rates with study duration from hours to 6 weeks
- Experience with:
  - *Mycobacterium tuberculosis* H37Ra
  - *Acinetobacter baumannii*
  - *Klebsiella pneumoniae*
  - *Escherichia coli*
  - *Pseudomonas aeruginosa*
  - *Aspergillus fumigatus*

Example screening cascade up to first *in vivo* efficacy

Flexible tiered approach, generated based on TPP



Creating the next major classes of antibacterial drugs: ‘TriBE’

Partnership between Evotec, Resolute Therapeutics, and CARB-X

- Global antibiotics market: $ 45 bn in 2018 and expected to reach $ 62 by 2026[1)]
- Huge imbalance between demand-supply of antibiotics
- WHO lists antimicrobial resistance among Top-10 threats to global health[2)]

- Evotec received an award of up to $ 8.4 m for development of novel broad-spectrum antibiotic
- Gram-positive and Gram-negative coverage, engaging well-validated antibacterial targets through novel MoA
- Programme was in-licensed to Evotec from Resolute Therapeutics in exchange for an upfront and success-based milestone payments
- Evotec retains the right to take over the project and develop with other potential clinical and marketing partners

RESOLUTE THERAPEUTICS | CARB-X | evotec

[1)] According to a report by Grand View Research from February 2019

[2)] https://www.who.int/news-room/feature-stories/ten-threats-to-global-health-in-2019

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