

## Towards the sustainable discovery and development of new antibiotics

Marcus Miethke<sup>1,2</sup>, Marco Pieroni<sup>3</sup>, Tilmann Weber<sup>4</sup>, Mark Brönstrup<sup>2,5</sup>, Peter Hammann<sup>6</sup>, Ludovic Halby<sup>7</sup>, Paola B. Arimondo<sup>7</sup>, Philippe Glaser<sup>8</sup>, Bertrand Aigle<sup>9</sup>, Helge B. Bode<sup>10,11</sup>, Rui Moreira<sup>12</sup>, Yanyan Li<sup>13</sup>, Andriy Luzhetskyy<sup>14</sup>, Marnix H. Medema<sup>15</sup>, Jean-Luc Pernodet<sup>16</sup>, Marc Stadler<sup>2,17</sup>, José Rubén Tormo<sup>18</sup>, Olga Genilloud<sup>18</sup>, Andrew W. Truman<sup>19</sup>, Kira J. Weissman<sup>20</sup>, Eriko Takano<sup>21</sup>, Stefano Sabatini<sup>22</sup>, Evi Stegmann<sup>2,23</sup>, Heike Brötz-Oesterhelt<sup>2,23</sup>, Wolfgang Wohlleben<sup>2,24</sup>, Myriam Seemann<sup>25</sup>, Martin Empting<sup>1,2</sup>, Anna K. H. Hirsch<sup>1,2</sup>, Brigitta Loretz<sup>1</sup>, Claus-Michael Lehr<sup>1</sup>, Alexander Titz<sup>1,2</sup>, Jennifer Herrmann<sup>1,2</sup>, Timo Jaeger<sup>2</sup>, Silke Alt<sup>2</sup>, Thomas Hesterkamp<sup>2</sup>, Mathias Winterhalter<sup>26</sup>, Andrea Schiefer<sup>2,27</sup>, Kenneth Pfarr<sup>2,27</sup>, Achim Hoerauf<sup>2,27</sup>, Heather Graz<sup>28</sup>, Michael Graz<sup>29</sup>, Mika Lindvall<sup>30</sup>, Savithri Ramurthy<sup>31</sup>, Anders Karlén<sup>32</sup>, Maarten van Dongen<sup>33</sup>, Hrvoje Petkovic<sup>34</sup>, Andreas Keller<sup>35</sup>, Frédéric Peyrane<sup>36</sup>, Stefano Donadio<sup>37</sup>, Laurent Fraisse<sup>38</sup>, Laura J. V. Piddock<sup>39</sup>, Ian H. Gilbert<sup>40</sup>, Heinz E. Moser<sup>41</sup> ✉ and Rolf Müller<sup>1,2</sup> ✉

**Abstract** | An ever-increasing demand for novel antimicrobials to treat life-threatening infections caused by the global spread of multidrug-resistant bacterial pathogens stands in stark contrast to the current level of investment in their development, particularly in the fields of natural-product-derived and synthetic small molecules. New agents displaying innovative chemistry and modes of action are desperately needed worldwide to tackle the public health menace posed by antimicrobial resistance. Here, our consortium presents a strategic blueprint to substantially improve our ability to discover and develop new antibiotics. We propose both short-term and long-term solutions to overcome the most urgent limitations in the various sectors of research and funding, aiming to bridge the gap between academic, industrial and political stakeholders, and to unite interdisciplinary expertise in order to efficiently fuel the translational pipeline for the benefit of future generations.

This article is conceived as a general roadmap with the central aim of promoting and accelerating translational science in the early stages of novel antibiotic discovery towards lead candidate development. The overuse and misuse of antibiotics in healthcare and agriculture, together with inappropriate waste management and environmental transmission, have led to substantially increased antimicrobial resistance (AMR)<sup>1–5</sup> and associated bacterial persistence<sup>6,7</sup>. This is of major public concern, since most areas of modern medicine are inconceivable without access to effective antimicrobial treatment<sup>8</sup>. It is estimated that at least 700,000 people worldwide die each year as a result of drug-resistant infections, and this could rise to as much as 10 million by 2050 if the problem of AMR is not addressed<sup>9,10</sup>.

The anticipated death toll caused by drug-resistant infections over the next years and decades may be compared with the global fatality rate of the current SARS-CoV-2 (COVID-19) pandemic (<https://coronavirus.jhu.edu/>), which has already led to multibillion-dollar investments in vaccine development, repurposing existing drugs and antiviral discovery. A perhaps overlooked aspect of concern with the COVID-19 pandemic is the high numbers of secondary infections, often associated with multidrug-resistant bacteria, which are observed especially in hospitalized patients and those with already compromised immune systems<sup>11,12</sup>. Associated with this problem is the massive use of antibiotics as a COVID-19 (co)treatment worldwide<sup>13–24</sup>, which is predicted to add to the ongoing emergence of AMR<sup>25–29</sup>. This multiplying effect of COVID-19 on the spread of bacterial resistance

✉e-mail: [heinz.moser@novartis.com](mailto:heinz.moser@novartis.com); [rolf.mueller@helmholtz-hips.de](mailto:rolf.mueller@helmholtz-hips.de)  
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## ESKAPE

Acronym of highly virulent and often (mainly in hospitals) multidrug-resistant bacterial priority pathogens, including *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.

will most likely have further negative clinical, economic and societal consequences in the near future<sup>30,31</sup>.

Unfortunately, the dramatic worldwide rise of bacterial pathogens resistant to antibacterial agents<sup>32</sup> cannot be counteracted by the current low development pace of therapeutics with new mode(s) of action (MoA(s)). While there are nearly 4,000 immuno-oncology agents in development<sup>33</sup>, only about 30–40 new antibacterial compounds are currently in the clinical trial phases of development, and, notably, those candidates targeting World Health Organization (WHO) priority pathogens are derivatives of existing classes<sup>34,35</sup>. Indeed, less than 25% of current drugs in the clinical development pipeline represent a novel class or act through a novel mechanism, and none of these are potentially active against Gram-negative ESKAPE or WHO critical threat pathogens<sup>34,36</sup>. In fact, only a small fraction of the antibiotics approved over the past 40 years represents new compound classes, while the majority were derived from already known chemical structures, and the most recent new class of antibiotics was discovered during the 1980s<sup>37</sup>.

Thus, strategic investment in new therapeutic options to fight AMR is urgently required to address unmet patient need and, additionally, to counterbalance the exponentially increasing financial burden on global health systems<sup>38</sup>. Consequently, the research field should aim to leverage hit identification and hit-to-lead optimization programmes to ensure a sustainable flow of new antibacterial drug candidates into the development pipeline. For this purpose, the initial stages of drug discovery and development need to be strengthened, since they are essential to identify and validate novel therapeutic candidates effective to fight antibacterial resistance. However, for many years, such early-stage projects have been mainly conducted by academia and are generally underfunded, while increased allocation of funding into early-stage and mid-stage research and development (R&D) has been recommended to make the pipeline more robust<sup>39–42</sup>. Our network has identified major funding gaps especially within the academic sector, as well as for small and medium-sized enterprises (SMEs), where research is mainly associated with the

## Author addresses

<sup>1</sup>Helmholtz Institute for Pharmaceutical Research Saarland (HIPS) - Helmholtz Centre for Infection Research (HZI), and Department of Pharmacy, Saarland University Campus E8.1, Saarbrücken, Germany.

<sup>2</sup>German Center for Infection Research (DZIF), Braunschweig, Germany.

<sup>3</sup>Food and Drug Department, University of Parma, Parma, Italy.

<sup>4</sup>The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Lyngby, Denmark.

<sup>5</sup>Department of Chemical Biology (CBIO), Helmholtz Centre for Infection Research (HZI), Braunschweig, Germany.

<sup>6</sup>Infectious Diseases & Natural Product Research at EVOTEC, and Justus Liebig University Giessen, Giessen, Germany.

<sup>7</sup>Epigenetic Chemical Biology, Department of Structural Biology and Chemistry, Institut Pasteur, UMR n°3523, CNRS, Paris, France.

<sup>8</sup>Ecology and Evolution of Antibiotic Resistance Unit, Microbiology Department, Institut Pasteur, CNRS UMR3525, Paris, France.

<sup>9</sup>Université de Lorraine, INRAE, DynAMic, Nancy, France.

<sup>10</sup>Department of Biosciences, Goethe University Frankfurt, Frankfurt, Germany.

<sup>11</sup>Max Planck Institute for Terrestrial Microbiology, Department of Natural Products in Organismic Interactions, Marburg, Germany.

<sup>12</sup>Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal.

<sup>13</sup>Unit MCAM, CNRS, National Museum of Natural History (MNHN), Paris, France.

<sup>14</sup>Pharmaceutical Biotechnology, Saarland University, Saarbrücken, Germany.

<sup>15</sup>Bioinformatics Group, Wageningen University and Research, Wageningen, Netherlands.

<sup>16</sup>Institute for Integrative Biology of the Cell (I2BC) & Microbiology Department, University of Paris-Saclay, Gif-sur-Yvette, France.

<sup>17</sup>Microbial Drugs (MWIS), Helmholtz Centre for Infection Research (HZI), Braunschweig, Germany.

<sup>18</sup>Fundación MEDINA, Granada, Spain.

<sup>19</sup>Department of Molecular Microbiology, John Innes Centre, Norwich, United Kingdom.

<sup>20</sup>Molecular and Structural Enzymology Group, Université de Lorraine, CNRS, IMoPA, Nancy, France.

<sup>21</sup>Manchester Institute of Biotechnology, Department of Chemistry, School of Natural Sciences, Faculty of Science and Engineering, University of Manchester, Manchester, United Kingdom.

<sup>22</sup>Department of Pharmaceutical Sciences, University of Perugia, Perugia, Italy.

<sup>23</sup>Department of Microbial Bioactive Compounds, Interfaculty Institute of Microbiology and Infection Medicine, University of Tübingen, Tübingen, Germany.

<sup>24</sup>Department of Microbiology/Biotechnology, Interfaculty Institute of Microbiology and Infection Medicine, University of Tübingen, Tübingen, Germany.

<sup>25</sup>Institute for Chemistry UMR 7177, University of Strasbourg/CNRS, ITI InnoVec, Strasbourg, France.

<sup>26</sup>Life Sciences & Chemistry, Jacobs University Bremen, Bremen, Germany.

<sup>27</sup>Institute of Medical Microbiology, Immunology and Parasitology (IMMIP), University Hospital Bonn, Bonn, Germany.

<sup>28</sup>Biophys Ltd., Usk, Monmouthshire, United Kingdom.

<sup>29</sup>School of Law, University of Bristol, Bristol, United Kingdom.

<sup>30</sup>Recursion Pharmaceuticals, Salt Lake City, UT, USA.

<sup>31</sup>Drug Discovery/Medicinal Chemistry, HiberCell, New York, NY, USA.

<sup>32</sup>Department of Medicinal Chemistry, Uppsala University, Uppsala, Sweden.

<sup>33</sup>AMR Insights, Amsterdam, Netherlands.

<sup>34</sup>Department of Food Science and Technology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia.

<sup>35</sup>Chair for Clinical Bioinformatics, Saarland University, University Hospital, Saarbrücken, Germany.

<sup>36</sup>BEAM Alliance, Paris, France.

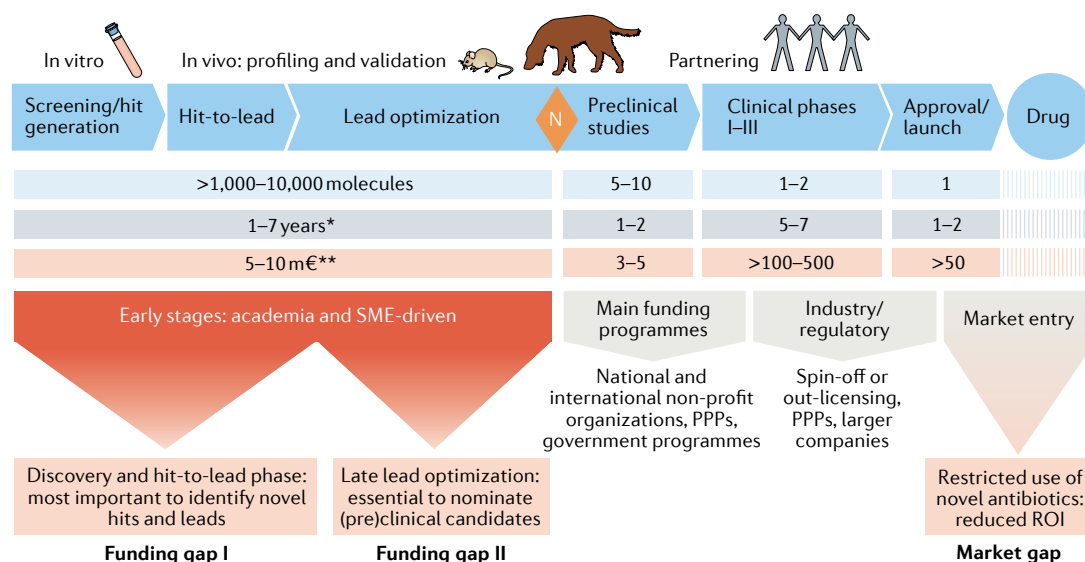
<sup>37</sup>NAICONs Srl, Milan, Italy.

<sup>38</sup>Drugs for Neglected Diseases initiative (DNDi), Geneva, Switzerland.

<sup>39</sup>The Global Antibiotic Research and Development Partnership (GARDP), Geneva, Switzerland.

<sup>40</sup>Division of Biological Chemistry and Drug Discovery, University of Dundee, Dundee, United Kingdom.

<sup>41</sup>Novartis Institutes for BioMedical Research (NIBR), Emeryville, CA, USA.



**Fig. 1 | General scheme of antimicrobial drug development.** Large funding gaps can be seen in the early stages of hit discovery, as well as during hit and lead optimization, which are associated mainly with academic research and small and medium-sized enterprises (SMEs). Indicated figures are representative numbers of typical broad-spectrum antibiotic development programmes leading from several thousands of initial hits to the approval of at least one marketable candidate<sup>72,318–321</sup>. \*Timings are dependent on a number of factors and can vary greatly. A minimum to maximum range for complete development (discovery to market) is 8–18 years (average 13–14 years). \*\*The cost per molecule/candidate (in million euros, m€) does not include extended costs for attrition (failed programmes) and lost opportunities associated with increased cycle time until reaching the next development phase; such extensions can increase the required budget for the early stages up to 50–100 m€ (REFS<sup>39,48,322</sup>). N (orange diamond), nomination of (pre)clinical candidate(s); PPPs, public–private partnerships; ROI, return on investment.

#### Push

Push incentives (for example, grants for the different phases of drug discovery or development) aim to generate and push a product (such as a new antibiotic) into the market.

#### Pull

Pull incentives ensure that a product is established at the market (for example, by defined payments to the manufacturer for a certain time after market release).

#### Subscription models

These models constitute that fixed prices will be paid at regular intervals for a certain period (for example, by governments) to the provider of a product to trigger ('pull') the development of therapeutics, such as novel antibiotics.

#### Delinkage models

These models combine expanded government funding for drug development with cash reward incentives to drug developers in order to delink high innovation costs from high sales prices.

early hit discovery and hit-to-lead phases, as well as with late lead optimization prior to preclinical candidate nomination (FIG. 1). Large pharmaceutical companies across the globe are extremely hesitant to fund early antibiotic R&D and, particularly, new classes of compounds, since the return on investment in this area is generally low or even negative. Further, the costs of developing entirely new scaffolds are much higher than for derivatives of established compound classes, while the attrition rate in antibacterial drug discovery has been particularly high in the recent decades, reflected by the fact that no new class of Gram-negative antibiotics has been launched for more than 50 years<sup>43,44</sup>. In the commercial sector, innovation has, thus, been left to SMEs, which must deal with high attrition associated with the early phases of discovery and optimization<sup>39,43,45–48</sup>, and the huge capital risks<sup>49,50</sup>.

New economic models for development specifically designed for this area are sorely needed to ensure future advancements<sup>51–54</sup>. A recent initiative that supports SMEs in the late-stage development of new antibiotics is the AMR Action Fund, which was launched by more than 20 leading biopharmaceutical companies to push mainly phase II and III trials of advanced candidates<sup>55</sup>. Unfortunately, the fund does not cater for the early stages of research. In addition, several countries are implementing new pull incentive programmes with different priorities. While the Swedish model aims at securing sustained access to relevant antibiotics that have already been approved<sup>56</sup>, plans in the UK<sup>57,58</sup> as well as in the USA (e.g. PASTEUR<sup>59</sup> and DISARM<sup>60</sup> acts) strive to stimulate the development of new antibacterial products

by using subscription models or delinkage models<sup>51</sup>. Such initiatives are promising, as they introduce much-needed market entry rewards, but they might fall short on a global scale if they do not include the 'critical mass' of the world's largest economies.

Innovation in the early stages of antibiotic drug discovery can also be driven by the academic sector. However, from the academic perspective, partnering with external funders such as the pharmaceutical industry is, in many cases, only realistic after the nomination of extensively validated preclinical candidates, and often even requires phase I clinical data. Typically, this cannot be achieved by research-driven funding and infrastructure alone. Several global health organizations and public–private partnerships (PPPs), including the Global Antibiotic Research and Development Partnership (GARDP), Combating Antibiotic-Resistant Bacteria Biopharmaceutical Accelerator (CARB-X), Innovative Medicines Initiative (IMI) and others, started to support, at least partially, the mid-to-late lead optimization through to clinical proof of concept<sup>61–63</sup>, possibly accompanied by stakeholders associated with the Biotech companies in Europe combating AntiMicrobial resistance (BEAM) Alliance or the Replenishing and Enabling the Pipeline for Anti-Infective Resistance (REPAIR) Impact Fund<sup>64,65</sup>. However, even the growing diversity of such push incentives are, in many cases, insufficient and primarily focused on companies. In addition to these approaches, a strategy is required that helps academic researchers to advance their project portfolio to a level that facilitates early interaction and possibly partnering with pharmaceutical companies in the interest of

a successful, cross-sectoral development pipeline<sup>66</sup>. Hence, creating new incentive models in the field is an essential process that can only be moved forward if the public, academic and industrial sectors join forces<sup>39,67–69</sup>.

In this respect, our position paper provides an overview of the early phases of antibacterial drug discovery, including hit and lead identification, optimization and development to the (pre)clinical stages by summarizing current limitations, relevant approaches and future perspectives, as well as by presenting selected case studies. In terms of a principal guidance for researchers in the field, we suggest possible solutions for a number of obstacles to improve both quality and quantity of antibacterial hits and leads. To strengthen and emphasize these early stages as an absolute necessity for a sustained generation of novel antibiotics, we are recommending a new level of interaction between the various stakeholders and academic disciplines in the area of antibiotic drug research. The strengths and opportunities that small-molecule therapeutics offer can help address antibiotic resistance more successfully during the coming years, in the interests of both patients and investors, provided that the multiplicity of hurdles along the translational path will be overcome (TABLE 1). Altogether, our aims are in line with the ‘One Health Action Plan against Antimicrobial Resistance’ introduced by the European Commission<sup>70</sup>, as well as the WHO programme to fight the rising number of bacterial priority pathogens with steadily growing impact on global public health<sup>71</sup>.

### Synthetic hit compounds

Here, we address the development of profitable strategies to identify and prioritize novel antibacterial hit compounds, with a particular focus on synthetic small molecules. As a foundation, we introduce three main pillars that represent core elements of fruitful hit discovery programmes.

**Hit definition, chemical libraries and medicinal chemistry.** The concept of ‘hit compound’<sup>72</sup> as it is widely accepted today needs to be expanded to address the needs imposed by the threat of antibacterial resistance. In this context, a hit compound is a molecule with reproducible activity, with a defined chemical structure (or set of structures), against one or more bacterial target(s). Although the selectivity and cytotoxicity of initial hits are seen as important characteristics, their improvement should remain tasks for the hit-to-lead optimization phase (see below). The activity of hits against (selected) pathogens must be proven in relevant assays, initially in vitro (for example, using exposed/isolated targets or a whole-cell approach), which can be complemented later in the process by the use of animal models of infection to evaluate pharmacokinetic (PK) and pharmacodynamic (PD) properties. In any event, the chemical identity and integrity of a hit must be demonstrated, whereas the actual target and the precise MoA may remain unknown until a later stage. Thus, the initial activity readout for a hit can be on either the molecular or the cellular level (BOX 1).

When considering the definition of valuable hits, it is important to look beyond the simple model of

a single molecule addressing one particular target. Compounds that hit multiple defined targets (known as polypharmacology<sup>73</sup>), or a combination therapy, in which the effects of several molecules are combined, can be equally valuable<sup>74</sup>. Depending on the target(s), hit combinations may act synergistically, preferably with different MoAs, or in an additive fashion. Such combinations can be useful in potentiating the activity of an existing antibiotic, slowing the onset of resistance and restoring the activity of antibiotics that have become inefficient because of resistance.

A major approach to identify novel hit compounds is by high-throughput screening of chemical libraries. It is important to select the correct set of compounds for each screen, for example, a (large) diverse set, a target-focused set or a fragment library. The make-up of a library should be based on specific characteristics or property space requirements, including chemical, structural and physicochemical aspects (BOX 2); these may be tailored to a particular disease area<sup>75,76</sup>. We believe that carefully designed, and possibly even preselected (‘biased’), chemical libraries, which enable screening of a suitable chemical space against the bacterial target(s) of interest, represent an important first step to start a reliable hit identification campaign towards treatment of a specific bacterial infection. The design, assembly and curation of such libraries are costly processes that require the input of highly skilled practitioners. This frequently falls outside the funding range of most academic groups and, indeed, of many small companies. Models need to be found to grant access to the most useful libraries or compound collections for hit discovery, which should be facilitated at least for non-profit research entities.

Interactions and collaborations between academic researchers and pharmaceutical companies can accelerate hit discovery by, for example, using the high-throughput screening infrastructure of companies to interrogate novel targets. At the same time, pharmaceutical partners might search for close analogues of hits initially identified in academic labs, possibly together with existing biological and chemical property profiles. Such analogue series and accompanying data sets can be extremely valuable in enabling early improvement of antibacterial potency, as well as hit series validation. Pharmaceutical partners might also begin building profiles of absorption, distribution, metabolism, excretion and toxicity (ADMET) parameters, thus, accelerating the hit-to-lead transition. Sharing the relevant information will reinforce the efforts of medicinal chemistry and enhance its reliability and robustness. This, in turn, allows programmes to reach Go/No-Go decisions more quickly and can improve the chances of securing external funding or early partnering deals based on the impact of the medical need.

Notably, medicinal chemistry is the key discipline for the subsequent optimization of hits (see case studies in BOXES 1–4). A lack of sufficient funding and expertise to support medicinal chemistry at this early stage is highly detrimental for the entire translational process. Encouragingly, in 2016, a large number of pharmaceutical companies with interests in AMR signed the AMR Industry Declaration<sup>77</sup>,

#### Hit compounds

Molecules that show a desired type of activity in initial screening assay(s).

Table 1 | **SWOT (strength, weaknesses, opportunities and threats) analysis summarizing a path forward in the development of novel antibiotic drugs**

Strengths	Weaknesses (current limitations)	Opportunities (possible solutions)	Threats
Successful clinical proof of concept for antibiotics over ~100 years	Unavoidable resistance development with classic antibiotics	New incentives (push and pull factors) to attract industrial stakeholders:	Rising death toll per year due to AMR
Critical need for intensive care units	Use of antibiotics in agriculture and aquaculture not globally regulated	1. Grants (from governments, health foundations etc.) for innovative programmes, for example, early R&D with academia (PPPs)	Loss of expertise in antibiotic R&D in both academia and industry
Novel antibiotics are last resort against MDR pathogens	Paucity of novel antibiotic classes against Gram-negative priority pathogens	2. Alliances of physicians, patients and politics (WHO, EU etc.) advocating for novel antibiotics	Disconnect between early R&D (left to academia) and clinical stages (industry-dependent)
Knowledge and technologies for antibiotic R&D established and steadily advancing	Lack of innovative assays for hit discovery	3. Market entry rewards like transferable exclusivity extensions/vouchers (for example, prolonged IP protection of new antibiotic classes or extended protection of other products)	Disconnect between researchers and regulatory agencies
High chance of finding new antibiotic classes due to largely underexplored biodiversity	Capacities in academia (MedChem, PK/PD etc.) insufficient for R&D project expansion	4. Delinkage models for novel antibiotics (benchmark-based to ensure innovation-driven development)	Low-cost structure of generic antibiotics impedes the development of novel classes
Large and increasingly diverse chemical libraries available for screening	High attrition rates until market entry, especially in the early stages of discovery and optimization	5. Patent buyouts or payer licenses in return for public control over pricing and distribution	High cost to society (socio-economic burden, increasing patient mortality etc.) over the next decades
Increasingly large and diverse online databases on antibiotic compounds, targets and resistance genes available	High capital risk and negative ROI mainly for industrial sector	6. Long-term benefits through public prestige, advertisement etc.	Risk of empty development pipeline if problems (for example, lack of R&D funding) are ignored
Predictive models available (via integrative chem-bioinformatics, for example PBPK modelling)		Advancing cooperation between academia, health foundations and industry (sharing of libraries, data, discovery and translational know-how, IP etc.)	
Loss of efficacy controllable by antibiotic stewardship		Emerging national or international antibiotics research networks, virtual centres and innovation funds	
Increasing public awareness of AMR demands for and facilitates national and transnational solutions		Academic entrepreneurship (foundation of spin-outs etc.)	
		Multiple innovative concepts for non-traditional antibiotics (virulence inhibitors etc.)	
		Overcoming existing resistances and/or increase efficiency of antibiotics by using molecules with synergistic action or innovative conjugates	
		Hit discovery becoming more efficient by emerging artificial intelligence technologies	
		Innovative concepts can improve: (i) in vivo drug delivery, (ii) antibiotic influx in bacterial cells, (iii) antibiotic efflux inhibition	
		Careful evaluation of regulatory guidelines based on regional discrepancies (high need versus safety)	

AMR, antimicrobial resistance; EU, European Union; IP, intellectual property; MDR, multidrug-resistant; MedChem, medicinal chemistry; PBPK, physiologically based pharmacokinetic; PK/PD, pharmacokinetics/pharmacodynamics; PPPs, public-private partnerships; R&D, research and development; ROI, return on investment; WHO, World Health Organization.

#### ADMET studies

Absorption, distribution, metabolism, excretion and toxicity. A collection of experimental studies that determines the fate of a pharmaceutical compound in an organism.

in which they jointly committed to support antibiotic R&D processes at virtually all stages. This has led to the formation of the AMR Industry Alliance (<https://www.amrindustryalliance.org/>). Additionally, the implementation of new AMR-specific capital resources, for example, through the REPAIR Impact Fund and the AMR Action Fund, and the direct involvement of PPPs like CARB-X in hit-to-lead campaigns during recent years should lead to intensified collaborations between industry and academia as a near-term goal to drive the chemical optimization of hits and leads forward towards new preclinical candidates.

Those academic groups that have already built the capacity to carry out such optimization efforts, including broad know-how in medicinal chemistry, biological assays and ADMET studies, would still benefit greatly from early partnering with biopharmaceutical companies, particularly as their projects will stand a greater chance of attracting external investment. Both not-for-profit initiatives, like the European Research Infrastructure Consortium for Chemical Biology and early Drug Discovery (EU-OPENSREEN; <https://www.eu-openscreen.eu/>), and collaborative PPP models as implemented by the European Lead Factory (ELF)<sup>78,79</sup>,



**Mode of action**

A change in cellular function (referring to the bacterial cell throughout the article) that results from exposure to a drug.

allowing for open drug discovery programmes based on Europe-wide screening resources (for example, the Joint European Compound Library, JECL), could pave the way for such early cross-sectoral interactions and exchanges for the benefit of all involved partners<sup>80</sup>.

**Nature of the target.** We recommend that hit identification against bacteria follows two convergent approaches: (i) identification of molecules active against molecular targets that are vital for all stages of the bacterial life cycle ('essential targets'), thus, directly promoting clearance of the bacteria from the host/patient, and (ii) searching for molecules that inhibit so-called 'non-essential targets'<sup>53,81,82</sup>. The latter can be defined as bacterial

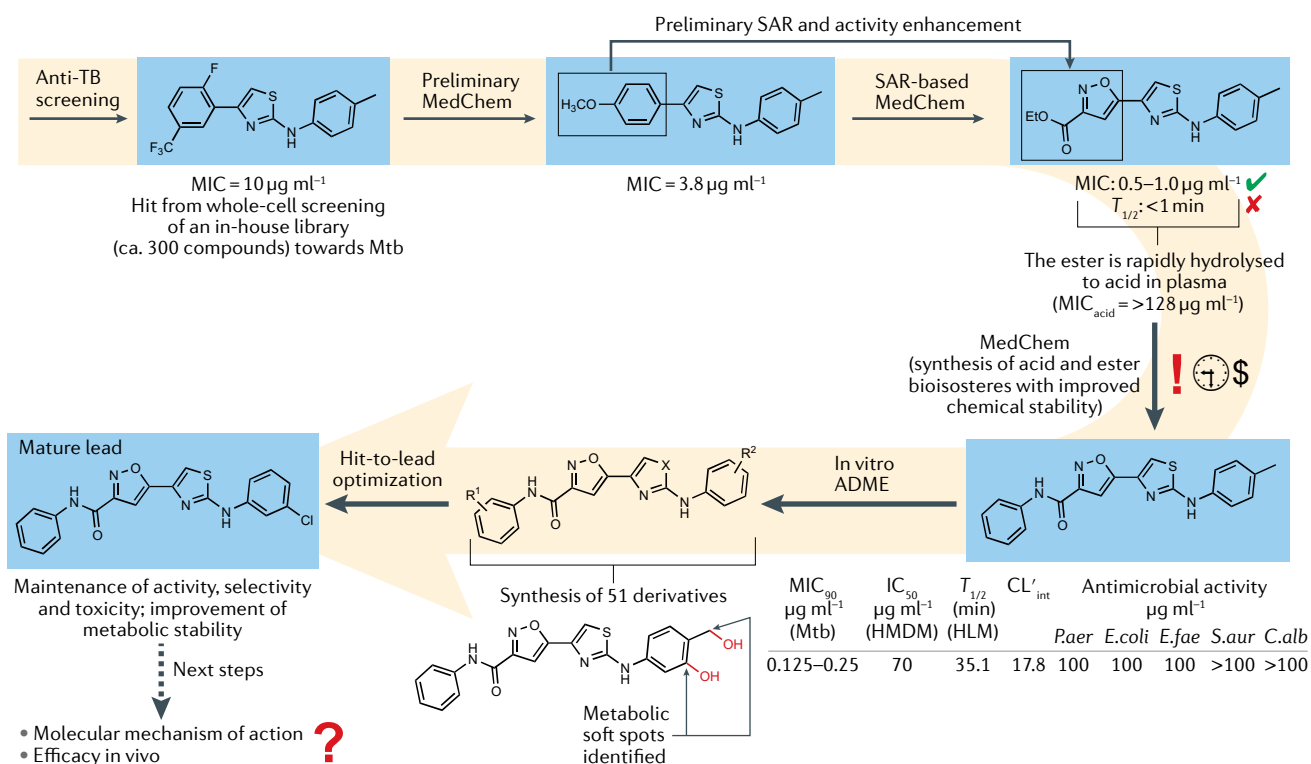
structures that are not vital under standard laboratory growth conditions but become critical during processes of host colonization and infection, for example, by regulating virulence development, by evading host immune response or by triggering bacterial defence mechanisms<sup>83</sup>. Molecules hitting such targets may have weak or even no activity towards bacterial cells under non-infectious (in vitro) screening conditions, but might display highly synergistic or additive effects when tested in relevant in vivo infection models, either alone or in combination with antibacterial agents addressing essential targets. The latter molecules may be found among the current antibacterial arsenal or may be new chemical entities, identified as described above.

**Box 1 | Early-stage development of a synthetic antibiotic against *Mycobacterium tuberculosis***

Historically, the whole-cell assay has generally outclassed target-based methods as the main approach to discover novel antimicrobial drugs. This is particularly true for antitubercular drugs, where the peculiar cellular structure of *Mycobacterium tuberculosis* (Mtb) is responsible for a lack of correspondence between the biochemical and the phenotypic assays. In the case study pictured, a small in-house chemical library was evaluated using phenotypic screening against Mtb to identify novel antitubercular chemotypes. A few 2-aminothiazoles were found to be moderately active, and the initial hit series was expanded to investigate the structure–activity relationship (SAR) by iterative medicinal chemistry (MedChem) efforts<sup>323</sup>, leading to highly potent derivatives (minimum inhibitory concentrations (MICs) in the submicromolar range) towards susceptible Mtb. To further promote the advancement of these compounds, additional biological assays were carried out to investigate the activity against multidrug-resistant and extensively drug-resistant Mtb strains, the selectivity over other bacterial species and eukaryotic cells, and the susceptibility to the action of efflux pumps<sup>324</sup>. The next research step was focused on a hit-to-lead optimization based on the convergent analysis of the SAR and structure–metabolism relationship. Two metabolic soft spots were identified, and these findings were instrumental for the design of

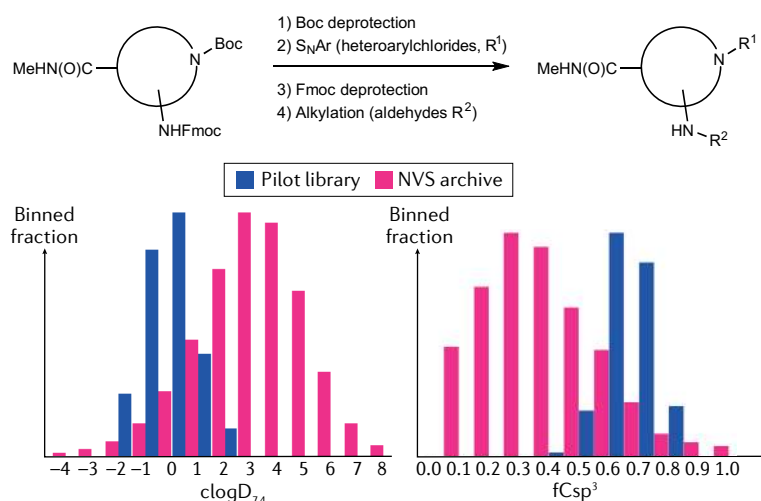
compounds that escaped rapid clearance by human liver microsomes and, at the same time, maintained good antitubercular activity against both drug-susceptible and drug-resistant strains. At this stage, determination of the mode of action at a molecular level and assays in animal model(s) of infection represent the next research progressions. Generally, academic drug discovery can suffer from long timescales and limited resources, which, in turn, make the research process difficult to move forward. For instance, academic chemical libraries are unlikely to yield a significant number of hits from a whole-cell screening, despite the intrinsic chemical novelty that characterizes their creation. Partnership with industrial stakeholders should fill the funding gap and add further expertise, for example, on advanced compound design and in vivo studies, to overcome the limitations mentioned above.

ADME, absorption, distribution, metabolism and excretion; *C.alb*, *Candida albicans*;  $CL'_{int}$ , intrinsic clearance; *E.coli*, *Escherichia coli*; *E.fae*, *Enterococcus faecium*; HLM, human liver microsomes; HMDM, human monocyte-derived macrophages;  $IC_{50}$ , half-maximal inhibitory concentration;  $MIC_{90}$ , minimum concentration at which 90% of isolates were inhibited; *Paer*; *Pseudomonas aeruginosa*; *S.aur*, *Staphylococcus aureus*;  $T_{1/2}$ , half-life; TB, tuberculosis.



## Box 2 | Theoretical example of a focused library design generating new chemical entities within a preferred property space

The chemical drug space has been described as almost infinitely large, with an estimated  $10^{60}$  compounds<sup>325</sup>. To exemplify the ease of accessing novel chemical matter within a desired property space<sup>75,76</sup>, a focused small library could be based on commercially available building blocks. In the example shown, the central building block remains constant and two substituents are added, first by arylation of the Boc-protected secondary amine<sup>326</sup>, followed by alkylation of the Fmoc-protected primary amine following a reductive amination<sup>327</sup>. The in silico design is driven by diversity, clogD (pH 7.4)<sup>328</sup> between -2 and 2, molecular weight below 450 Da and increased  $sp^3$  content (i.e. level of heavy atom saturation)<sup>329</sup>. This hypothetical pilot library represents 15 aldehydes and 15 heteroarylchlorides to provide a hypothetical 225-compound library, which is shown in comparison with the Novartis (NVS) archive based on polarity (clogD<sub>7.4</sub>) and fraction of  $sp^3$  hybridized carbon atoms (fCsp<sup>3</sup>). All 225 compounds are yet unknown in the public domain (Reaxys, <https://www.elsevier.com/solutions/reaxys>; last accessed May 2021) and absent from the Novartis archive (April 2018).



Compounds interacting with non-essential targets are usually classified as antibiotic adjuvants, potentiators or resistance breakers<sup>84,85</sup>. Examples of non-essential target inhibitors are represented by:

- Inhibitors of virulence-conferring factors or pathways (also known as anti-virulence compounds or pathoblockers<sup>86</sup> that target, for example, quorum sensing mechanisms<sup>87</sup>, biofilm formation<sup>88</sup>, bacterial secretion systems<sup>89,90</sup>, enzymes for tissue penetration<sup>91</sup> or intracellular survival<sup>92</sup>).
- Efflux pump inhibitors<sup>93</sup>.
- Suicide substrates such as  $\beta$ -lactamase inhibitors<sup>94,95</sup>.
- Inhibitors of pathways serving as a mechanism of defence, e.g. glutathione biosynthesis<sup>96,97</sup>.
- Modulators and inhibitors of energy metabolism<sup>98,99</sup>.
- Host/pathogen epigenetic modulators<sup>100,101</sup>.

### Pathoblockers

Anti-virulence drugs, i.e. drugs acting against factors (usually non-essential targets) that are involved in the development of bacterial virulence, often combined with a regular antibiotic to provide a synergistic effect.

### Gram-positive bacteria

Bacteria that stain positive with Gram's method by retaining the crystal violet dye in the thick peptidoglycan layer that composes their cell wall, together with the inner cytoplasmic cell membrane; the absence of an outer membrane often makes them more susceptible to cell wall targeting antibiotics and to influx of antibiotics into the cytoplasm.

For some of the mentioned targets, such as efflux pumps, it has been demonstrated that their inhibition can reverse resistance to several antibacterials<sup>102</sup>. Therefore, an attractive therapeutic combination might be composed of a bactericidal agent and an adjuvant molecule, with the aim of potentiating the antibacterial effect(s) and significantly reducing resistance (either intrinsic or evolved)<sup>103</sup>. Since the pathoblocker approach is anticipated to be less susceptible towards resistance development and, in addition, to preserve the commensal bacteria of the microbiome<sup>86</sup>,

it represents a non-traditional strategy for a focused disarming of resistant high-priority pathogens, most likely to be deployed as an adjunctive therapy in addition to antibiotic standard treatment<sup>81</sup> (BOX 3).

**Advanced screening and profiling based on standardized assays.** There is a fundamental need for assays to identify hit compounds (both synthetic and natural-product-based hits, the latter are addressed below) specifically for the clinically most relevant indications. In addition to using focused libraries that cover desirable chemical diversity and property space, innovative screens are essential to increase the chances for identifying potent hits against most prevalent common infections associated with Gram-positive or Gram-negative pathogens, such as hospital-acquired pneumonia, community-acquired pneumonia, complicated urinary tract infection or complicated intra-abdominal infection<sup>104</sup>. To establish a reliable foundation for future development, both academia and industry must use state-of-the-art library screening procedures based on generally accepted rules and basic concepts of standardization.

It is important that a range of relevant assays is used to thoroughly select and profile novel hit compounds. These assays should have a high physiological significance, which may be applicable to biomimetic assays<sup>105</sup>, for example, by using defined culture media such as artificial urine for activity screens with uropathogens<sup>106,107</sup>, iron-depleted media that simulate bacterial growth conditions during bloodstream or wound infections<sup>108,109</sup> or assaying host-bacteria interactions<sup>110</sup>. Such schemes can further include the screening for new MoA(s), new drug sensitizing modes, non-killing mechanisms (e.g. anti-virulence factors like pathoblockers), compounds acting against biofilms and molecules acting synergistically with existing or new antimicrobials to overcome drug resistance<sup>111-114</sup>. Similarly, because hits generated by conventional biochemical assays or screens often fail to become whole-cell active leads, alternative phenotypic assays such as novel target-based whole-cell screening<sup>115</sup> are also a promising foundation for the identification of useful hits. Even known chemical libraries (including proprietary compound archives of pharmaceutical companies), which have failed to deliver antibacterial hits by simple growth inhibition measurement, might bear fruit if reassayed following these approaches. The way in which these innovative screens are envisaged could make them a more appropriate strategy to provide novel hits with a potential therapeutic impact compared with the molecular-target-based drug design approach<sup>116</sup>.

A further aim of the consortium is to design and develop informative assays that can provide information about the desired antibacterial effect, together with further characteristics such as target engagement, bacterial penetration characteristics (for example, kinetics of compound permeation through Gram-negative cell envelope models<sup>117,118</sup>) and potential cytotoxicity.

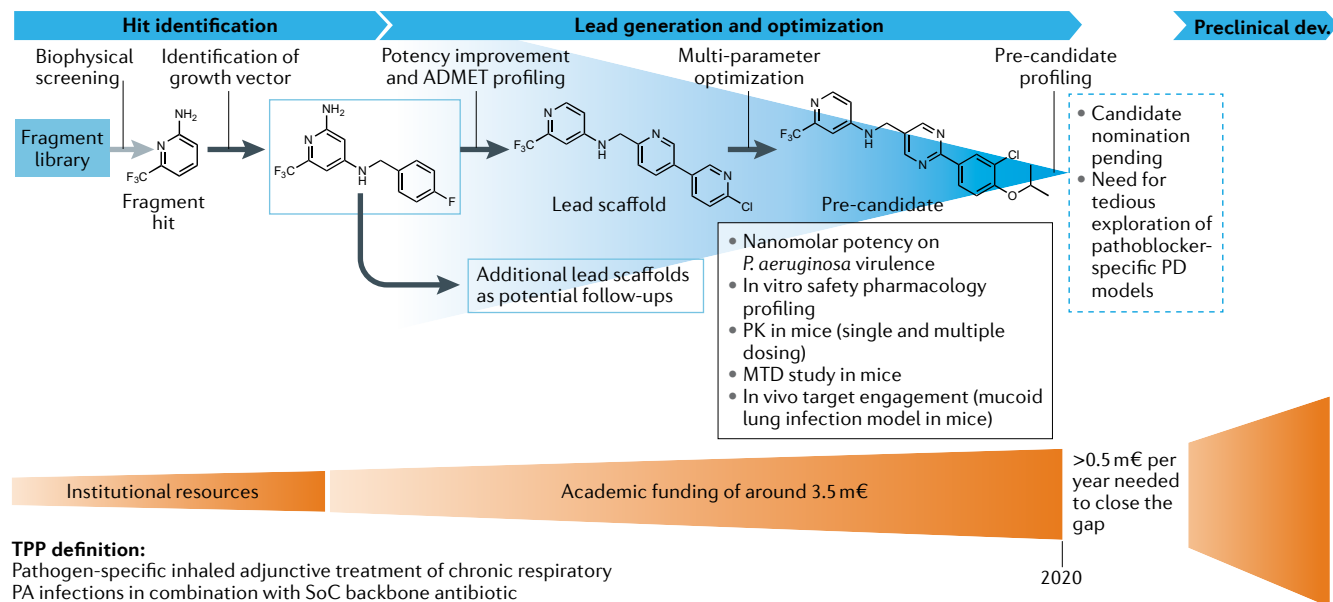
In addition to devising standardized panels of assays according to contemporary technology, developing the respective standard operating procedures (SOPs) is mandatory to meet the requirements for good research

Box 3 | Development of an anti-virulence therapeutic ('pathoblocker') against *Pseudomonas aeruginosa*

The concept of interfering with the *Pseudomonas* quinolone signal quorum sensing system for the discovery of pathoblockers against *Pseudomonas aeruginosa* (PA) has been explored in detail by multiple research groups<sup>87</sup>. Target validation of the bacterial signal molecule receptor PqsR, which functions as a global virulence regulator, has been achieved using mainly acute murine infection models. A target-driven medicinal chemistry campaign tackling this transcriptional regulator has achieved pre-candidate status starting from a fragment-based approach<sup>330–332</sup>. After biophysical screening, initial hit selection was guided by selection of enthalpy-driven binders (as determined by isothermal titration calorimetry). Successful growth vector identification enabled the detection of qualified hits with cellular anti-virulence activity and potential for advancement to the lead generation and optimization stages<sup>331</sup>. Hit identification was achieved with institutional resources. However, cost-intensive medicinal chemistry and compound profiling work towards a preclinical profiling candidate was only possible through non-dilutive joint funding, which amounted to approx.

3.5 million euros (m€). The chosen target product profile (TPP) is defined as a pathogen-specific inhaled adjunctive treatment of chronic respiratory PA infections in combination with a standard-of-care (SoC) backbone antibiotic. Resulting pre-candidates have nanomolar on-target and cellular efficacy, potentiate tobramycin efficacy against PA biofilms, show high exposures in vivo (various routes intratracheal, intravenous, subcutaneous, peroral) and no overt findings in safety pharmacology screens<sup>333</sup>. While demonstration of in vivo target engagement by means of signal molecule quantification was achieved swiftly in a mucoid acute murine lung infection model, assaying in vivo treatment efficacy related to the pathoblocker-specific activities remains a considerable challenge. Candidate nomination is, therefore, pending on tedious and expensive exploration of suitable pharmacodynamic (PD) models. Currently, this milestone is pursued through further public funding.

ADMET, absorption, distribution, metabolism, excretion and toxicity; MTD, maximum tolerated dose; PK, pharmacokinetics.



## Gram-negative bacteria

Bacteria that stain negative (do not retain the crystal violet dye) when using Gram's method for bacterial differentiation; their cell envelopes are composed of an inner cytoplasmic cell membrane and an outer membrane (containing amphiphilic lipopolysaccharides at the outer leaflet), which enclose the periplasmic space containing a thin peptidoglycan layer.

## Minimum inhibitory concentrations

The minimum concentrations, usually expressed in units of mass per litre, that prevent visible growth of bacteria. Complementary to minimum bactericidal concentration.

practice, which facilitates the transfer of compounds with potential to become new drugs from academia to non-profit or private organizations for continued development. By using standardized proof-of-concept assays under predefined SOPs, more robust hit series will emerge, increasing their potential for late-stage development and minimizing reproducibility issues. For example, minimum inhibitory concentrations, and possibly also minimum bactericidal concentrations, should always be evaluated in a screening campaign, for example, by using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (<https://eucast.org/>) or the Clinical and Laboratory Standards Institute (CLSI) (<https://clsi.org/meetings/ast/>) guidelines. In addition, selected hits from standard screening panels should be consequently tested against contemporary clinical isolates to demonstrate that they overcome existing resistance mechanisms.

Owing to the high attrition rates from early hit discovery to advanced hits and leads, it is especially important in the field of antibacterials to diversify and generate multiple hit series, and to characterize them thoroughly regarding all features that appear relevant to the intended

therapeutic use. This includes explorations to expand scaffold diversity in the context of understanding the target-based chemical and physicochemical requirements, as well as potential liabilities, like ADMET.

A summary of early target hit profiles is essential to nominate the most valuable hit series acting against the pathogen(s) or medical indication(s) of interest. The selection of hit series for lead generation follows the target candidate profile (TCP), which is pre-defined at the outset of the development programme according to the desired target product profile (TPP) (FIG. 2). Thus, the optimization of hits should generally be driven by TCPs and compound progression criteria that, in turn, are driven by chosen TPPs. If several TPPs have been selected or outlined for a campaign, for example, based on different indications, together with their corresponding TCPs, it has to be decided which TCP should be used as a base to aim at for a given chemical series or possibly natural-product-based hit that emerges from mining of biological sources (see below).

It is important to implement physicochemical and in vitro ADMET profiling at the start of hit optimization, to make sure that any PK issues are identified early



## Minimum bactericidal concentrations

The minimum concentrations, usually expressed in units of mass per litre, that result in death for  $\geq 99.9\%$  of bacteria. Complementary to minimum inhibitory concentration.

## Target candidate profile

(TCP). Reflects how the chemical matter of an identified compound is optimized towards the target product profile by summarizing the desired chemical, physicochemical and biological characteristics of a preclinical drug candidate.

and can be addressed through the entire chemistry programme. In this respect, a standardized list of essential compound properties is required for successful transfer of hits and early leads into the following discovery and development stages. Depending on the defined TPP, such a dossier on physicochemical and biological properties should comprise a set of minimal criteria for compound progression based on selected, standardized assays or attributes with clear benchmarks for transition to the next stages in the drug discovery pathway and for continued (pre)clinical development according to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines (<https://www.ich.org/page/ich-guidelines>). This will help to ensure developable

compounds of clinical relevance are produced, which are also attractive for potential industrial partners. Relevant parameters (depending on the particular stage of transition) may include:

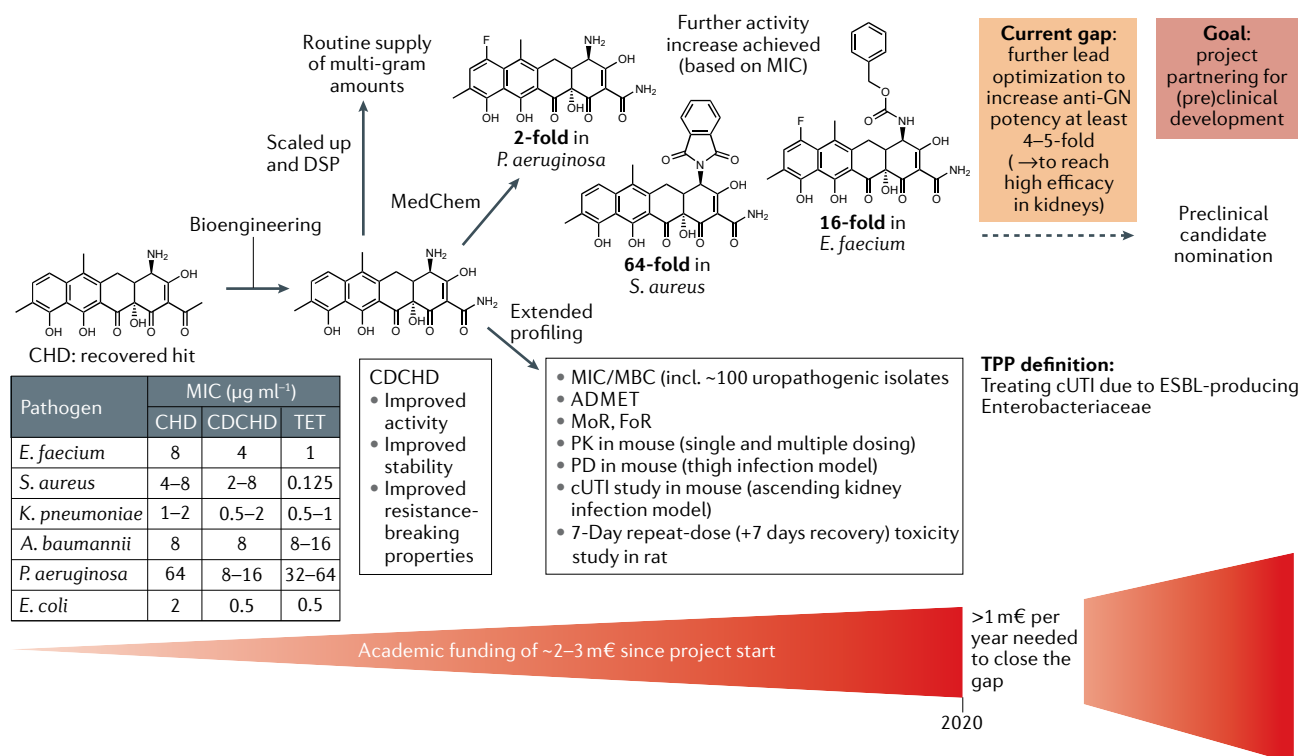
- Potency/cellular activity (e.g. based on minimum inhibitory concentrations and minimum bactericidal concentrations).
- Chemical and metabolic stability, solubility, permeability (e.g. based on logP or, for ionizable compounds, logD, or complex membrane partitioning).
- Distribution, efflux avoidance, selectivity/off-target avoidance (e.g. inhibition assays on receptor panels, hERG etc.).
- Acid/base properties based on  $pK_a$ .
- Cytotoxicity (especially human cell lines).

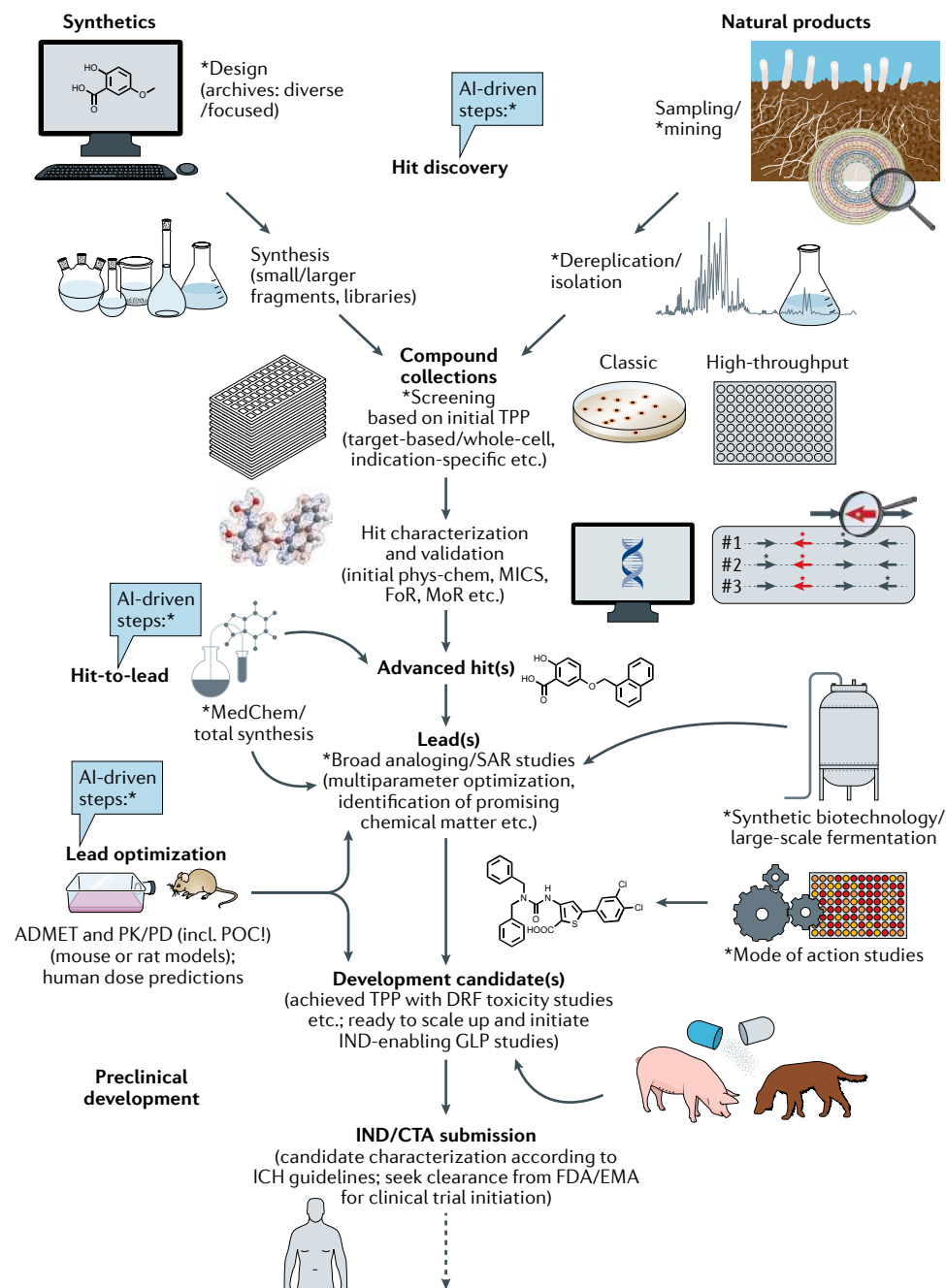
## Box 4 | Reassessing chelocardin for improved lead development towards complicated urinary tract infection therapy

The natural product chelocardin (CHD), a member of the atypical tetracyclines that was first described about 60 years ago<sup>334,335</sup>, has recently been recovered to generate a novel lead scaffold, amidochelocardin (2-carboxamido-2-deacetyl-chelocardin, CDCHD), by rational biosynthetic engineering<sup>336</sup>. For this purpose, the CHD biosynthetic gene cluster in *Amycolatopsis sulphurea*<sup>337</sup> was combined with genes from the oxytetracycline biosynthesis pathway of *Streptomyces rimosus*, and production peak titres of the novel hybrid compound CDCHD up to  $400\text{ mg l}^{-1}$  were achieved<sup>191</sup>. CDCHD represents a new broad-spectrum antibiotic active against pathogens of the ESKAPE panel (including a large number of clinical isolates)<sup>106</sup>, which can be routinely supplied at the multi-gram scale with  $>95\%$  purity by using large-scale in-house fermentation at the Helmholtz Centre for Infection Research (HZI) (~100-l batch cultures) and optimized downstream processing. Due to the lack of cross-resistance to known antibiotics (for example, preserved activity against pathogens carrying multiple tetracycline (TET) resistance determinants), the good production yield and the fact that efficacy for CHD treatment was already shown in a small phase II study<sup>338</sup>, CDCHD was chosen to enter a lead optimization programme (see Acknowledgements).

Optimization of CDCHD includes further bioengineering and medicinal chemistry (MedChem) approaches for extensive structure–activity relationship profiling, which is currently based on  $>70$  generated analogues with modifications achieved at about ten different scaffold positions<sup>192,339</sup>. Extended CDCHD profiling by absorption, distribution, metabolism, excretion and toxicity (ADMET), pharmacokinetics/pharmacodynamics (PK/PD), toxicity studies and validation of therapeutic efficacy in an ascending kidney infection model indicated the use of CDCHD for the treatment of complicated urinary tract infection (cUTI) caused by extended-spectrum  $\beta$ -lactamase (ESBL)-producing Enterobacteriaceae according to the selected target product profile (TPP)<sup>340</sup>. However, further increase in potency is required to achieve higher efficacy in kidneys against clinically most relevant uropathogens, which is essential for preclinical candidate nomination in this project. To achieve this goal, funding limitations in the academic sector shall be overcome by partnering with an industrial stakeholder.

DSP, downstream processing; FoR, frequency of resistance; GN, Gram-negative; m€, million euros; MBC, minimum bactericidal concentration; MIC, minimum inhibitory concentration; MoR, mechanism of resistance.





**Fig. 2 | Summary of major steps and processes in antibacterial drug discovery and development.** Approaches marked with \* can be linked with emerging artificial intelligence (AI)-based technology, for example, for advanced data mining, screening or property predictions, to increase efficiency and outcome. ADMET, absorption, distribution, metabolism, excretion and toxicity; CTA, clinical trial application; DRF, dose range finding; EMA, European Medicines Agency; FDA, U.S. Food and Drug Administration; FoR, frequency of resistance; GLP, good laboratory practice; ICH, International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; IND, investigational new drug; MedChem, medicinal chemistry; MICs, minimal inhibitory concentrations; MoR, mechanism of resistance; phys-chem, physicochemical properties; PK/PD, pharmacokinetics/pharmacodynamics; POC, proof of concept; SAR, structure–activity relationship; TPP, target product profile.

**Target product profile (TPP).** Summarizes the key requirements for a new therapeutic that fulfils a priority medical need; thus, it identifies and outlines the critical attributes and (pre) clinical endpoints of a product as a guidance before development begins.

- Lack of reactive metabolites.
- Phototoxicity.
- Protein binding.
- In vivo efficacy and human dose prediction.
- (Oral) bioavailability.
- Genotoxicity (e.g. based on Ames or mouse micronucleus tests).
- Drug–drug interactions.
- PK linearity.
- Safety (in vivo toxicity).
- Compound access (e.g. synthetic feasibility and scaling up to gram or kilogram).
- Achievable degree of purity.
- Formulation.

Once the hit discovery transitions into the hit-to-lead and lead optimization phases (see below), it is necessary to enlarge the scope of biological studies. These may include bacterial killing kinetics, MoA, frequency of resistance, mechanism of resistance and PK/PD analyses, which will deliver valuable parameters to assess a compound's *in vivo* efficacy (assuming sufficient free drug exposure in a relevant animal model with acceptable tolerability). At this level, it is, once again, important to acquire information on a substantial number of structurally related analogues through extensive medicinal chemistry efforts (perhaps in collaboration with PPPs or the pharmaceutical industry, as suggested above) in order to establish clear and reliable dossiers of structure–activity relationship (SAR) and structure–property relationship. These data are essential to consistently improve all the required parameters as a basis for a continuous advancement of lead structures towards the selection of (pre)clinical candidates. Computational methods based on machine learning techniques like profile-quantitative structure–activity relationship (pQSAR) can help to build predictive models regarding activity, selectivity, toxicity, MoA and further parameters for specific compound classes, hence, providing valuable *in silico* input for more effective hit discovery and lead design<sup>119,120</sup>.

#### Natural-product-based hit compounds

Historically, microbial natural products have been the most important source of antibiotic lead compounds; over the last 40 years, about 60% of all new chemical entities in the field of antibacterials were based on or derived from natural products<sup>121</sup>. Here, to complement the key aspects described above for synthetic hits, we outline the major requirements specific to the identification and prioritization of antibacterial natural product hits. We focus on efficiency and, particularly for the academic sector, achievability in terms of technological and financial demands.

#### Identification of new chemotypes from natural sources.

The known antibiotic activity of natural products has, in general, been identified by phenotypic screening campaigns that determine activity against panels of test organisms in standardized assays. These screens, which constitute the basis for bioactivity-guided isolation of natural products from complex mixtures, efficiently retrieve bioactive compounds when libraries of crude extracts are evaluated. However, their ability to reveal useful novelty is limited by both a high rediscovery rate of already known molecules associated with pre-existing resistance mechanisms, as well as a substantial proportion of hits that show significant cytotoxicity or poor ADMET properties.

We emphasize that there is a general lack of efficient tools and strategies to increase the number of new chemotypes and to reduce the rediscovery rates in antibacterial screening approaches. Even on a global scale, the number of newly discovered chemotypes, especially novel scaffolds acting against Gram-negative bacteria, is consistently low. Several approaches are relevant to improve this situation:

One possibility to enforce the identification of new antibacterial chemistry is to limit screening of already broadly characterized groups of secondary metabolite producers, for example, actinomycetes, and to expand efforts on identifying new types of producers by extensive biodiversity mining. This can be achieved by focusing on the ~99.999% of microbial taxa of the Earth's microbiome that remain undiscovered<sup>122,123</sup>, including the as yet underexplored taxa of human and animal microbiomes<sup>124–127</sup>. Emerging innovative isolation and cultivation techniques such as diffusion bioreactors (also carried out on the microscale as with the iChip<sup>128–130</sup>), microfluidics<sup>131–133</sup>, elicitors<sup>134</sup> and various co-cultivations<sup>135,136</sup> will help to access and understand the rare and less-studied groups of microorganisms from diverse habitats<sup>137–139</sup>. Further, molecular (co-)evolution acting to generate novel metabolites for efficient microbial warfare could be exploited<sup>140,141</sup>, for example, by sampling from environments heavily contaminated with antibiotics (like sewage in Southeast Asia or South America), which are known to contain highly resistant microbes<sup>142,143</sup>. Complementarily, this can be achieved by laboratory exposure of potent producers to sub-inhibitory antibiotic concentrations<sup>144</sup> or by co-culturing them together with drug-resistant (pathogenic) strains<sup>145</sup>. Beyond microbial producers, a great variety of plants<sup>146,147</sup>, macroscopic filamentous fungi (e.g. Basidiomycota)<sup>148</sup> and animals<sup>149</sup> bear the potential to deliver useful compounds as a base for novel antimicrobials. Altogether, the exploration of untapped biological resources, which represent a major reservoir for future therapeutics, should generally be extended within the academic and industrial sector.

After genome mining of novel microbial isolates or metagenome-driven discovery of novel natural products<sup>150–153</sup>, selected biosynthetic gene clusters (BGCs) that potentially produce unknown secondary metabolites should be systematically expressed in specialized heterologous host strains<sup>154–156</sup>. This helps to facilitate a straightforward detection and isolation of the new compounds, particularly if their BGCs are 'silent' (i.e. not expressed under known conditions) in the native host. Such heterologous hosts or chassis strains can be based on microbial species that commonly produce a large variety of natural products, but have been made devoid of their own secondary metabolite BGCs and/or have been further optimized to efficiently express BGCs originating from 'non-common' sources (for example, rare actinomycetes or fungi)<sup>154,157,158</sup>. However, only a limited set of such specialized host strains is available so far, and a much more diverse array of microbial chassis needs to be developed to fit the demands of a growing arsenal of BGCs that potentially produce novel chemistry. BGC expression is often most successful in strains closely related to the native producer, and, thus, it is important to develop methods for standardized heterologous expression in selected host strains with desirable properties that have not yet been domesticated for the use as regular chassis<sup>159</sup>.

Chemical space can also be enlarged by using emerging synthetic biology approaches for medium-to-high-throughput genome editing and pathway engineering.

#### Mechanism of resistance

Native or acquired, this is the route by which a microorganism resists the action of a particular drug. This may include, for example, decreased influx, enhanced efflux, modification of the drug target and modification/inactivation of the drug.

#### Lead compounds

Molecules with validated activity that serve as a basis for the development of a drug candidate.

#### Biosynthetic gene clusters

(BGCs). Sets of genes, typically found close to one another in the genome, that code for the enzymes responsible for the synthesis of a particular secondary metabolite.

**CRISPR/Cas9**

Clustered regularly interspaced short palindromic repeats–CRISPR-associated protein 9. Components of the immunological defence of certain bacteria against viruses and plasmids; used in molecular biology not only for genetic engineering of bacterial genomes.

**Trojan Horse approach**

Describes the addition of another chemical moiety (e.g. a siderophore) to a drug scaffold in order to facilitate the bacterial uptake of this drug conjugate (for example, by exploiting bacterial siderophore transporters).

These approaches, which are primarily based on CRISPR/Cas9 (REFS<sup>160,161</sup>) and diverse recombination, assembly and integrase systems<sup>162–164</sup>, can be followed up with advanced analytics and screening of the potentially modified natural products, which may be produced in only trace quantities. This technology involves the extensive use of information on genome sequences, enzyme activities and compound structures collected by publications, databases and web tools (such as MIBiG<sup>165</sup>, antiSMASH<sup>166</sup> and PRISM<sup>167</sup>) over the past few decades. In many cases, the modularity of the BGC composition, which is found in gene clusters, for example, coding for polyketide synthases or non-ribosomal peptide synthetases, can be used to implement a bioinformatics-supported plug-and-play diversification strategy enabling the exchange and recombination of core units, as well as modifying enzymes<sup>168–171</sup>. A concomitant refactoring of BGCs, especially from rare microbial sources, often allows high-level heterologous production of the antibiotic compounds in suitable hosts<sup>172–175</sup>. However, these methods are still in their infancy and require wider testing with different classes of antimicrobials to define general principles of feasibility and scalability, which, furthermore, necessitates an improved understanding of the complex biosynthetic machineries and their modular evolution.

Advances in analytical chemistry techniques, for example, in mass-spectrometry-based metabolomics and its enhancement by molecular networking and the application of machine learning, support the process of dereplication<sup>176</sup> during (secondary) metabolome mining<sup>177–181</sup>. Known compounds produced in reasonably high yields can be rapidly identified via their high-resolution masses, tandem mass spectrometry fragmentation patterns or structural data in secondary metabolite databases<sup>138,182–187</sup>. However, the remaining bottleneck is to highlight and annotate novel antibiotic compounds, particularly those with low production titres, as early as possible in the discovery process (i.e. from crude extracts if possible, without the need for small-scale fractionation and enrichment). This objective can be supported by innovative extraction methods prior to bioactivity-guided isolation of novel compounds<sup>188</sup>.

In addition, revisiting known potent antibiotics, previously neglected as a result of unacceptable or non-addressable properties such as cytotoxicity or lack of stability, can be a valuable strategy to provide novel leads and candidates. The reassessment of such scaffolds can be based on a variety of efforts, including the improvement of production and purification<sup>189</sup>, reconsideration of application and effective dose for natural derivatives<sup>190</sup>, or advantageous scaffold modification by biosynthetic engineering and semi-synthetic approaches<sup>191,192</sup> (BOX 4).

Further opportunities remain to improve the discovery and development of agents for combination therapy as indicated above, i.e. compounds that act synergistically against multidrug-resistant and/or high-priority pathogens<sup>193,194</sup>. The discrimination of specific synergistic activities from non-specific antibiotic activities remains a challenge during the discovery process.

Improving bacterial target access, enhancing potency and broadening the antimicrobial spectrum of known and novel antibiotic scaffolds can be achieved by using drug-conjugate strategies, for example, linking of pathogen-specific antibodies<sup>195,196</sup>, siderophore moieties<sup>197,198</sup> or positively charged peptides<sup>199,200</sup> to the antibiotic core scaffold. Though these approaches have been proven effective in a number of cases, some of them may also have unintended effects, such as a spontaneously increasing frequency of resistance, which can be problematic, for example, in the case of the Trojan Horse approach<sup>201</sup>.

Overall, a variety of innovative and complementary technologies is required to improve access to novel natural product scaffolds. Computational methods can provide powerful assistance at different levels in many of the areas indicated above, as recent efforts show<sup>202,203</sup>. In this context, artificial intelligence might play a game-changing role in the future. The general power of neural networks for detecting new antimicrobial candidates has already been demonstrated<sup>202</sup>. By using a computational model that screens hundreds of millions of chemical compounds in a few days, potential antibiotics even with new MoA(s) could be proposed rapidly. Given the recent advances in artificial intelligence, these and other models will likely add to the future identification of new candidate drugs.

Interestingly, when looking at compound properties, it appears that there is often more flexibility in the selection of ‘successful’ natural product scaffolds compared with synthetics, for example, regarding Lipinski’s rule of five<sup>204–206</sup>, which natural products frequently ‘disobey’ (such as cyclosporine or macrolides like azithromycin). Thus, antimicrobial drug discovery in ‘beyond rule of five’ chemical space is an opportunity when using natural compound collections or when assembling libraries of de novo designed compounds<sup>207–209</sup>, though the general need for optimizing key pharmacological properties of such hits remains beyond question.

Another major challenge for natural products can be the generation of structurally diverse analogues (particularly if they are not accessible through biosynthesis). Many scaffold positions can be difficult to access by means of semi-synthesis and, thus, broad derivatization of natural-product-based hit and lead compounds is often much more labour-intensive, and establishing synthetic access to these scaffolds with a focus on the ability to systematically diversify their chemical space can require large amounts of resources<sup>210</sup>. Nevertheless, the modification of natural scaffolds with substituents that are often easier to incorporate by (semi-)synthetic or chemoenzymatic approaches, such as halogens that allow the modulation of solubility, permeability, selectivity, target affinity etc.<sup>211,212</sup>, proves that multiple opportunities arise when combining synthetic and biological chemistry.

**Required access to biological and chemical material and data.** Many scientists frequently experience difficulty in accessing and sharing research material from third parties, including microbial strains, cultivation extracts, pure compounds, genome or gene cluster



sequences and further background data (of published or even unpublished results). For example, an interesting BGC is identified in publicly accessible databases, but the strain is not specified or not available from the indicated source. Similarly, access to industrial antibiotic overproducers can be impossible, even when a company no longer has a commercial interest in the resulting molecule. This phenomenon has several origins, including legal restraints (for example, imposed by the Nagoya Protocol<sup>213</sup>) or intellectual property (IP) claims on strains, compounds, biologics or (re)profiling data of already known structures.

In the public interest, standardized procedures are necessary to facilitate access to research materials and to solve IP conflicts, at least within the field of academia, in which it is common practice to share research materials with colleagues by negotiating appropriate cooperation agreements.

Further, the access to in-house compound libraries of pharmaceutical companies (at least subsets of them and especially those that are not intended for antibiotic-related screening) could be very valuable for academic partners who are eager to identify novel antibacterial hits, which could lead to joint drug development programmes. Enabling access to materials can also be extended to strain collections, including clinical isolates representing the diversity of pathogens associated with a certain clinical indication, and advanced compound information based on pre-existing characterization and profiling campaigns. An increased

availability of these resources will be of great benefit to the antimicrobial research community worldwide.

Furthermore, comprehensive databases and data-sharing platforms can provide another valuable resource for present and future antibiotic R&D projects and, hence, should be implemented and maintained with care<sup>214</sup>. There is a growing body of recently initiated and publicly available web-based tools and archives that support accumulation and exchange of data regarding antibacterial compounds in different stages of discovery or therapeutic development, known or predicted antibiotic targets and the diversity of antimicrobial resistance determinants (BOX 5). Further connection and integration of such databases is desirable to optimize the output for a specific search request. In addition, initiatives comparable with the European Commission's manifesto to maximize the public accessibility of research results in the fight against COVID-19 (REF.<sup>215</sup>) are also highly recommended to support AMR-related scientific research at all levels, including facilitated access to online resources.

**Prediction of antimicrobial structure and function from genome sequence data.** Driven by breakthroughs in sequencing technologies and genome mining, the identification of BGCs encoding the biosynthesis of natural products has matured to complement the chemistry-driven and bioactivity-driven screening processes for natural product hits. Computational methods are established and continuously improved to identify novel biosynthetic pathways in (meta)genomic sequence data<sup>150,151</sup>. Recently, third-generation genome sequencing techniques such as PacBio and Oxford Nanopore have been developed that provide high-quality full genome data even for complex microorganisms like filamentous fungi at reasonable cost, which is an ideal prerequisite for large-scale genome mining approaches<sup>216</sup>.

However, linking the obtained sequence information to possible structural or functional features of the encoded molecules remains a great challenge. Prediction of chemical structures directly from genome data would help to distinguish known from potentially novel scaffolds during a very early stage of dereplication; the training of machine learning algorithms with sufficient quantity of genome data from microbial producers could ultimately lead to fairly accurate predictions of chemical structures linked to specific BGCs and possibly even their biological activities<sup>167</sup>.

A successful strategy to decipher antibacterial targets of new natural products, without the need to isolate them, is a directed search for known resistance factors in the genomes of antibiotic-producing microbes<sup>217,218</sup>. These producers may code for resistant variants of the molecular target(s) that interact with the intrinsic antibiotic(s) without damaging the host or conserved class-specific transporters that release the compound(s) into the environment. This approach recently led to the discovery of novel antibiotic scaffolds<sup>219</sup>. However, most BGCs do not contain apparent or specific drug-resistance genes that could straightforwardly indicate a compound's function. In the majority of cases, very limited predictions based on genomic data

#### Box 5 | Examples of public databases and tools related to antimicrobial compounds, targets and resistance

##### • **Discovery of antibacterial compounds and development into (potential) therapeutics:**

<https://db.co-add.org/downloads/>  
<https://globalamrhub.org/dynamic-dashboard/>  
<https://chemdb.niaid.nih.gov/DrugDevelopmentTB.aspx>  
<https://coconut.naturalproducts.net/>  
<https://zinc.docking.org/>  
<https://revive.gardp.org/resources/>  
<https://go.drugbank.com/>  
<https://www.antibioticdb.com/>  
<https://www.pewtrusts.org/en/research-and-analysis/articles/2018/09/21/the-shared-platform-for-antibiotic-research-and-knowledge>  
<https://www.pewtrusts.org/en/research-and-analysis/data-visualizations/2014/antibiotics-currently-in-clinical-development>  
<https://www.pewtrusts.org/en/research-and-analysis/data-visualizations/2017/nontraditional-products-for-bacterial-infections-in-clinical-development>  
<https://www.who.int/observatories/global-observatory-on-health-research-and-development/monitoring/who-antibacterial-preclinical-pipeline-review>  
<https://www.who.int/observatories/global-observatory-on-health-research-and-development/monitoring/antibacterial-products-in-clinical-development-for-priority-pathogens>

##### • **Antimicrobial target search and prediction:**

<https://pypi.org/project/targetDB/>  
<https://platform.opentargets.org/>  
<https://arts.ziemertlab.com/>

##### • **Antimicrobial resistance:**

<https://card.mcmaster.ca/>  
<https://www.ncbi.nlm.nih.gov/pathogens/antimicrobial-resistance/>  
<https://bench.cs.vt.edu/deeparg>  
<https://github.com/abu034004/PARGT>

concerning function and potential target(s) of a natural product are currently possible, although advanced automated tools for target-directed genome mining are available<sup>220</sup>. Thus, there is a high demand for innovative methods to predict the molecular function or target of a natural compound based on genomic data. Such data would be extremely valuable in order to prioritize BGCs for experimental characterization. In the future, artificial intelligence approaches, based on either classical machine learning methods (extracting new knowledge from preprocessed data sets) or on deep learning (drawing conclusions from raw data such as representative examples, often by using multilayer neural networks), may deliver such predictions with increasing accuracy<sup>221</sup>. However, existing algorithms need to be improved, and new ones have to be developed to specifically address the question of how to assign target-based functions to natural products with confidence during the early stages of discovery and prioritization. These approaches also require a huge amount of validated training data<sup>222</sup>.

### Advancing hits to (pre)clinical status

Regardless of whether antibacterial hits emerge from rationally designed synthetic molecules or from the pool of natural products, the subsequent hit-to-lead and lead-to-candidate optimization phases are very similar for compounds irrespective of origin ('Y model', see FIG. 2). We now discuss the most critical obstacles and requirements for delivering those advanced leads that may eventually become the next generation of (pre) clinical candidates.

**Drug–target interaction studies as a base for hit development.** For hits arising from phenotypic assays, cellular MoA(s) or specific molecular target(s) may not be known at the hit-to-lead stage, and, sometimes, the precise MoA is elucidated years after the approval of a drug, as in the case of daptomycin<sup>223</sup>. However, detailed insight into the mechanism(s) by which compounds exert their pharmacological activity is highly desirable for further rational optimization of chemical scaffolds, particularly when structurally enabled approaches can be used, for a convincing presentation of preclinical candidate dossiers and for regulatory requirements. Since universally applicable methods for characterizing the MoA(s) of antibiotics do not exist, a full suite of expertise in genetics, genomics, microbiology, chemical biology and biophysics is required. Identification of the molecular target can be achieved by targeted screens of indicator or mutant strains, whole-genome sequencing upon focused resistance development<sup>224,225</sup>, pattern recognition techniques based on transcriptomics<sup>226</sup>, imaging<sup>227,228</sup>, metabolomics<sup>229</sup>, macromolecular synthesis<sup>230,231</sup> or mutant fitness profiles<sup>232,233</sup>, which can be coupled with machine learning approaches for directed predictions<sup>225,233</sup>, or chemoproteomics<sup>234,235</sup>. The latter is specifically useful in the case of non-essential target inhibitors like pathoblockers, since these may not generate resistant mutants (at least under standard laboratory conditions). Additional techniques for MoA studies may include crystallography, a diverse set of spectroscopic and calorimetric analyses<sup>236–240</sup>,

as well as the use of functionalized derivatives ('tool compounds')<sup>241,242</sup>, which can support both target identification and validation and may provide in-depth information of drug–target interactions to drive the rational hit-to-lead optimization process forward. Alternatively, identification of drug–target (or ligand–protein) interactions formed under native (unbiased) conditions by using specialized proteomic approaches is becoming increasingly successful<sup>243–246</sup>. Current bioinformatic tools can also combine genome-mining approaches with the prediction of potentially innovative MoA(s) based on the presence of resistant target genes in BGCs encoding novel antibiotics<sup>220</sup>. These and other examples illustrate how a diverse set of emerging learning methods is steadily enhancing the predictability of drug–target interactions<sup>247,248</sup>.

In addition to the specific molecular target(s), it is important to understand the impact of the antibiotic compound on the general physiology of the bacterial cell. This includes the sequence of events leading to bacterial death, the time point when killing occurs (based on either individual bacterial cells or their population/colonization level) and the conditions that might enhance or preclude it. Such characterizations may require the application or development of a range of secondary assays. For compounds acting on intracellular bacterial targets (i.e. targets located in the cytoplasm), the processes of compound influx and prevention of efflux (especially so for Gram-negative bacteria as a result of their complex cell envelope and presence of numerous multidrug efflux pumps) are both critical optimization parameters to ensure sufficient target engagement<sup>249–253</sup>. These factors can be addressed by suitable compound design, which generally remains rather empirical and challenging<sup>254–257</sup>. Other possibilities to address this key area would be to use these compounds in combination with outer membrane permeabilizing agents<sup>258,259</sup> or efflux inhibitors<sup>93,260</sup>. Alternative approaches targeting extracellular virulence factors, for example, extracellular lectins required for attachment and biofilm formation or secreted proteolytic enzymes, do not suffer from a possible lack of bacterial uptake<sup>261</sup>. Often, antibiotics, and particularly natural products, have more than one target and disturb bacterial physiology in several different pathways, a phenomenon referred to as polypharmacology<sup>73,262,263</sup>, which is beneficial for inflicting severe damage on the bacterial cell and slowing down target-mediated resistance development. Information related to such effects should be acquired for all bacterial species within the spectrum of activity of the potential drug, and it may diverge significantly across phylogenetically distant species.

Apart from the desired biological effects on bacterial pathogens, knowledge about undesired adverse effects on eukaryotic cells ('off-target effects'<sup>264–269</sup>) should be acquired early on, since toxicity is a major contributor to attrition in the drug development process. However, whilst *in vitro* cytotoxicity screens are useful during the early discovery process, they are often not predictive of toxicological effects that can become most significant during *in vivo* studies. Furthermore, collateral damage to the microbiome needs to be considered<sup>270–273</sup> and can be modulated by selective drug design<sup>274</sup>. For compounds

with a novel or particularly complex MoA, it often takes several years to achieve a detailed molecular understanding and the cellular consequences of exposure. Therefore, acquiring this knowledge as early as possible is a key aspect for further rational drug optimization, including SAR studies and structure-guided hit/lead optimization. We recommend investing resources into expanded MoA studies already during the initial stages of the drug development process and, furthermore, building a network of experts who can provide MoA analyses that fulfil the requirements of a preclinical candidate dossier. While these aspects are standard for drug development projects in the pharmaceutical industry, academia usually suffers from insufficient funding to appropriately address such requirements, and, therefore, additional resources need to be secured.

**Limited resources to move from hit into lead stage.** Once a hit validation has been accomplished, the resources needed to advance the selected compound series into hit-to-lead and lead optimization greatly increase. These stages require a diverse scientific team covering analytical, computational and medicinal chemistry, biochemistry, microbiology, bioinformatics (ideally including machine learning and artificial intelligence methods), drug metabolism and pharmacokinetics, as well as, specifically for natural-product-based compounds, biotechnology and genetic engineering. In industrial projects, typically 5–15 medicinal chemists work on the optimization of a hit (depending on how complex the chemistry of a certain compound is) to create promising leads or preclinical candidates, essentially by generating, testing and advancing SAR-based analogue series in an iterative manner. The challenge is to simultaneously optimize all properties necessary for the drug to be most effective and least toxic. This includes potency, selectivity, physicochemical parameters and cytotoxicity, as well as pharmacokinetics and pharmacodynamics (FIG. 2). The multi-parameter optimization can usually be achieved within a time frame of about 2–4 years, but remains dependent on the human, technological and financial capacities, as well as the particular challenges represented by the chemical series. Such resources are difficult to acquire through classical academic funding schemes, which usually reward new discoveries in fundamental science, rather than subsequent steps of time-consuming and resource-consuming optimization, where there is no guarantee of success.

Academia must, therefore, find new ways to provide suitable resources for early-stage translational research. Since few academic institutions possess the relevant expertise and facilities to carry out lead optimization, they usually require access to high-quality expertise and/or capacities in cooperation with pharmaceutical companies/SMEs or through contract research organizations (CROs), which can only be achieved through additional funding or partnerships. One possible strategy to acquire appropriate resources in future could be the application of alternative reward schemes for evaluation of academic project funding, which might not only be based on high-impact publications but also on verifiable commitment to health research, such as

making dedicated contributions to a global antibacterial portfolio. The emergence of centres for translational science in many countries (for example, the German Center for Infection Research; <https://www.dzif.de/en>) could be an opportunity to develop and implement such measures, possibly at an international level.

**The bottleneck of compound supply.** The enhanced biological profiling that is mandatory in hit and lead optimization programmes requires a considerable amount of sufficiently pure compounds to be tested. While this can be a problem for chemists in general even with respect to synthetic hits and leads (especially the massive scale-up of typical laboratory test reactions)<sup>275,276</sup>, the problem of supplying increasingly large quantities of natural products originating from bacteria, fungi or plants is particularly challenging. Indeed, academic projects are often concluded when natural compounds or biotechnologically generated variants thereof are identified at small scale (often <10 mg), with only rudimentary profiling. In many laboratories, there are no additional resources to increase the yields of natural product hits or initial leads, or to scale up production in a pre-pilot plant environment that is capable of carrying out the fermentation (possibly by using heterologous production hosts to achieve attractive yields<sup>277,278</sup>). In addition, downstream processing has to be established and optimized for every new compound to ensure satisfactory purity at a sufficient quantity for the following stages, including scaffold optimization by medicinal chemistry or extended biological profiling. The fact that sufficient amounts of compounds (multigram-to-kilogram scale) cannot be produced in many cases severely decreases the chances of developing novel therapeutics from natural products. This is particularly unfortunate in the antibiotics field, because about two-thirds of all antibiotic drugs in therapeutic use are derived from natural products<sup>44,121</sup>. Regrettably, fermentation-independent supply, for example, through the total synthesis of complex natural compounds, can only be achieved for a low percentage of novel hits and leads and requires a tremendous amount of additional capacity and resources<sup>279–282</sup>.

Thus, suitable funding instruments are needed to cover the essential processes of natural compound scale-up and supply based on biotechnological methods, including large-scale fermentation and efficient downstream processing<sup>283–285</sup>, towards obtaining high-quality source material for semi-synthesis and further studies. In addition, a robust method for large-scale production and downstream processing of the candidate molecule is a prerequisite for process transfer to good manufacturing practice (GMP) production before entering (pre)clinical stages. Generally, further scientific and technological development is required to make the provision of compound material from various sources a more routine and affordable task, particularly in the non-industrial research environment.

**Requirements for in vivo studies and project transfer.** The primary assays in most discovery programmes usually address biochemical, biophysical and/or microbiological functionality of newly generated compounds.

Good manufacturing practice (GMP). The set of minimum requirements that must be followed in manufacturing in order to satisfy the agencies responsible for licensing.

In order to convert a molecule with in vitro activity into a drug, sufficient exposure at the infection site in vivo must be achieved. To analyse this, a full suite of ADMET assays is required<sup>286,287</sup>, followed by pharmacokinetics experiments in animals (usually starting with rodent models)<sup>288,289</sup>, which can be combined with physiologically based PK modelling and in silico ADMET prediction<sup>290,291</sup>.

A sufficient correlation between in vitro and in vivo data, which is not always achievable for all antimicrobial compounds, should generally be pursued as early as possible in the programme, otherwise, continued lead design might be based on irrelevant or misleading data points (for example, see some case studies with LpxC inhibitors<sup>292,293</sup>). Furthermore, the availability of PD models<sup>294,295</sup> of high translational relevance, i.e. reliably predicting a minimal efficacious dose in humans, is a critical factor of success in order to generate the optimal drug candidate during lead and lead-to-candidate optimization. In the field of antibiotics, in particular, preclinical PK/PD relationships are generally predictive and have a high relevance for regulatory dossiers<sup>296,297</sup>, for example, for human PK/PD target attainment at therapeutic doses and drug formulation development, and, as such, they have to be evaluated carefully at the earliest possible stages<sup>298–300</sup>. Typically, PK/PD target attainments for antibiotics require relatively high doses compared with other drug classes (particularly to achieve sufficient exposure at the site of infection), limiting the successful application of existing formulation and delivery technologies. This constraint is especially true for oral medications that may present further challenges, for example, to reach an adequate bioavailability of the drug. Hence, a broader array of potential delivery systems should be tested systematically, which may include conventional permeation enhancers<sup>301</sup>, as well as sophisticated nanoformulations, for example, liposome-based drug delivery systems<sup>302–305</sup>. The latter, however, can only be produced based on expert knowledge and infrastructure, which is, once again, not often available in academia, and, thus, specialized CROs or SMEs may be approached based on available funding.

A further obstacle is the need to perform (initially) rather extensive studies in laboratory animals to understand the PK/PD relationship of a novel compound, which, at subsequent stages, allows the number of animal experiments to be minimized according to the 3Rs principle<sup>306</sup>. However, these studies are generally associated with ethical concerns, high costs and administrative burden. Likewise, these matters are relevant for the in vivo evaluation of toxicology, toxicokinetics and safety pharmacology to cover safety aspects before entering clinical trials<sup>307,308</sup>. Here, exploratory or early-stage predictive assays using computational models, as well as in vivo systems with minimal ethical concerns, for example, in vertebrates like *Danio rerio* (zebrafish), insects like *Galleria mellonella* (the greater wax moth) or worms like *Caenorhabditis elegans* (a soil-dwelling nematode), are an opportunity to estimate both efficacy and potential toxicity risks before considering standard in vivo experiments in rodents and other mammals<sup>309–311</sup>.

Ultimately, the demonstration of efficacy in a relevant animal model, associated with convincing exposure at the site of infection and a rough estimation of a reasonable safety margin, is often a prerequisite to attract an investor's interest; typical minimum requirements are a tolerance/dose range finding study in one or two animal species, as well as human dose prediction based on a solid set of PK/PD data, for example, by testing efficacy in the neutropenic thigh infection model in mice<sup>312</sup>.

Generally, TPPs and the corresponding TCPs should continue to be the base for all further optimization attempts, especially when including in vivo studies, and, hence, should be thoroughly compiled before the development programme starts, with the help of subject matter experts. These data will guide the strategies and decisions for all chemical and biological development processes during the optimization phases, mainly with respect to one (or more) clinical indication(s). In order to specify robust finishing lines, these documents should outline sets of minimum acceptable criteria for each phase, for example, for biochemical assays during early stages and (pre)clinical endpoints at later stages. Such compound progression criteria should be defined for a validated hit, entry into lead optimization, a late lead and a preclinical candidate. There are different TPPs for different bacterial infections. As projects evolve, they may encounter serendipitous discoveries, unsurmountable hurdles or important findings from other groups or competitors, which may affect the TPP that they target. Therefore, the TPP can be critically reviewed and possibly refined or adapted throughout the project, for example, at each transition into the next development stage. Ideally, a pool of commonly accepted TPPs (i.e. approved by the pharmaceutical industry as well as the public health sector) should be available for the multitude of clinical indications to serve as a base for each discovery and development programme of novel therapeutics. The WHO and the GARDP have already started to produce such TPPs for public health concerns, for example, in the field of sexually transmitted infections<sup>313</sup>. These TPPs need to be regularly reviewed, and, where necessary, updated, to make sure that they reflect the current clinical situation; for example, TPPs addressing indications caused by bacterial infections may be affected by the latest emerging (or anticipated) drug-resistant pathogens of critical relevance. It is important to note that only convincing TPPs together with comprehensive preclinical candidate dossiers (highly informative TCPs) and reliable SOPs for scalable compound supply will allow early partnering and a smooth transfer of the project to an industrial stakeholder to move into (pre)clinical development (BOX 6).

**The management challenge in hit and lead optimization programmes.** As the development of antibacterials requires a multidisciplinary approach, knowledge of a diverse set of techniques and domains (for example, assay development, high-throughput screening, medicinal and computational chemistry, ADMET, PK/PD, drug delivery, clinical background of disease processes etc.) is required in order to develop a compound to the level

#### LpxC inhibitors

Inhibitors targeting the enzyme LpxC that catalyses the initial step in the biosynthesis of lipid A — a structural component of the lipopolysaccharide molecules in Gram-negative bacteria.

#### The 3Rs principle

'Replacement, reduction and refinement'; guiding principles defined for a more ethical approach to animal research.

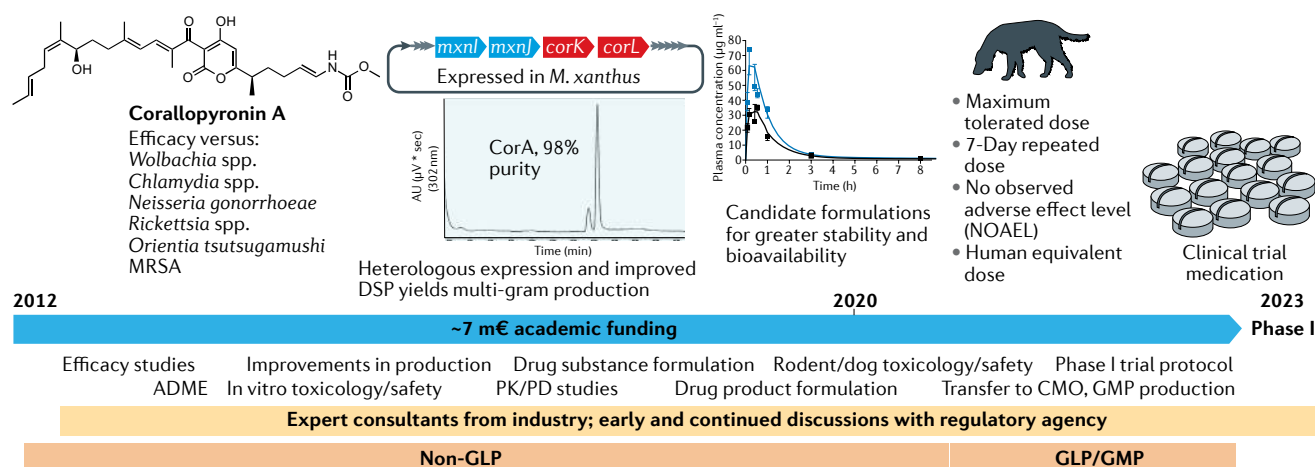


## Box 6 | Late preclinical development of corallopyronin A to first-in-human trial

The bacterial DNA-dependent RNA polymerase inhibitor corallopyronin A (CorA), produced by the soil-dwelling myxobacterium *Coralloccoccus coralloides*, is active against Gram-negative and Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA)<sup>341,342</sup>, by targeting the hinge (switch) region of the holoenzyme<sup>343</sup>. Structure–activity relationships demonstrated that the initial natural product hit was the most effective compound<sup>344</sup>, allowing for its development without extensive medicinal chemistry. The essential intracellular *Wolbachia* symbionts (Gram-negative) of human filarial nematodes, which cause the neglected tropical diseases lymphatic filariasis and onchocerciasis (river blindness), are also targets of CorA<sup>345</sup>. Currently, the compound is being developed to clinical phase I to support elimination of these nematode infections<sup>346–348</sup>, an aim of the United Nations' Sustainable Development Goal 3 (UN-SDG)<sup>349</sup>. CorA also has activity against the human pathogenic bacteria *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Rickettsia* spp. and *Orientia tsutsugamushi*<sup>350–352</sup>, which are included in the UN-SDG or World Health Organization/Centers for Disease Control and Prevention (WHO/CDC) lists for priority antibiotic discovery and development. Heterologous expression of the CorA biosynthetic cluster in *Myxococcus xanthus*, yielding >100 mg l<sup>-1</sup> (REFS<sup>277,278,353</sup>), allow consistent multi-gram-scale production with a purity of 90–95%. This preliminary product specification has been formally accepted by the German Federal Institute for Drugs and Medical Devices (BfArM). Most standard in vitro and in vivo non-GLP (good laboratory practice) absorption, distribution, metabolism, excretion and

toxicity studies have been successfully completed. Compared with rifampicin, the expression of CYP450s is not altered and CYP3A4 induction is eightfold lower. CorA is stable in plasma >240 min. Its metabolism in human and dog microsomes is  $T_{1/2}$  > 45 min, resulting in oxidation metabolites and minimal glucuronidation. Off-target profiling resulted in three hits (inhibition/activation), but the half maximal effective concentration (EC<sub>50</sub>) are 170–1,500-fold higher than the in vitro EC<sub>50</sub> against *Wolbachia*. CorA does not inhibit hERG, and no genotoxicity was observed. These results indicate that CorA is non-toxic and pharmacologically safe (awaiting further in vivo validation). The project is publicly funded to finalize preclinical studies, including formulation development and in vivo toxicity in rodents and dogs (see Acknowledgements). In parallel, the manufacturing protocol for heterologous production and optimized, upscalable downstream processing (DSP) has been recently transferred to a good manufacturing practice (GMP)-certified contract manufacturing organization (CMO) to produce cGMP-grade material for the GLP and phase I studies. Completing the clinical studies will require a product development partnership. Provision of CorA to countries endemic for filarial infections is envisaged via public–private partnerships to achieve the United Nations' Sustainable Development Goal 3. After regulatory approval, provision of CorA for treating sexually transmitted infections and as a reserve antibiotic for MRSA shall be achieved through licensing.

ADME, absorption, distribution, metabolism and excretion; m€, million euros; PK/PD, pharmacokinetics/pharmacodynamics.



of a preclinical candidate. Single principal investigators (PIs) will usually not possess the broad base of expertise that is necessary, since academia largely focuses on early-stage discovery and compound optimization at the laboratory scale. Hence, research groups that do not possess the extensive skill set for drug development in its various stages should pursue a team approach by collaborating with organizations that have the relevant experience, be it within the academic or the industrial sector. There is also the possibility of calling on specialized consultancy or outsourcing packages of work (for example, ADMET) to CROs that possess relevant expertise and experimental capabilities. However, in addition to the relatively high costs of such services, PIs often struggle with remaining questions once a CRO assignment ends, and sufficient resources for tailor-made optimizations are often lacking. Moreover, the need to interpret results and devise a clear path forward towards the TPP from multiple data packages remains with the project teams. Hence, partnerships and collaborations are essential if relevant in-house expertise or infrastructure is

missing. Therefore, we propose the following solutions for efficient translational project management:

Aligning and collaborating with suitable partners from various sectors or disciplines is crucial for groups with limited know-how in drug discovery and development. Generally, larger project teams can provide or identify expertise much more quickly to sufficiently resolve emerging knowledge gaps. Additionally, project consultants or CROs can be approached at different levels to fulfil remaining tasks, for example, data evaluation or processing defined and highly specific work packages, for example in PK and toxicological studies.

Databases of experts should be available for relevant research areas or services, and the various technical and IP-related aspects need to be elaborated on a case-by-case basis. Unbiased partners have to be identified to host and curate such databases on a regular basis, which could fall into the remit of non-profit health organizations such as the Joint Programming Initiative on Antimicrobial Resistance (JPIAMR) or the GARDP.

Training of PIs on a frequent basis is required to broaden their knowledge and to ensure a high-level understanding of potential barriers and pitfalls at least until projects reach the (pre)clinical stages. A number of renowned institutions already offer regular workshops and seminars (often as interactive webinars, for example, GARDP REVIVE; <https://revive.gardp.org/>), as well as extended training programmes (for example, the Interdisciplinary Course on Antibiotics and Resistance; <https://www.icarecourse.org/>, hosted by Institut Pasteur, France), and these are increasingly popular. In addition to PIs from academia, non-academic experts from industry, health and political sectors should share their perspectives on current research and funding aspects more regularly within interdisciplinary settings.

Overall, it is important that the necessary financial and legal frameworks for efficiency-oriented project management are established as early as possible during the course of a development programme to avoid loss of time and resources. The required settings can be implemented either individually at a particular project level or existing management models (for example, available in the industrial sector or in translational research centres) could be used or adapted for the specific purpose.

### Conclusions and outlook

To ensure a healthy and vibrant antimicrobial pipeline, considerable efforts are needed not only to develop the next generation of antibacterial drugs but also to safeguard and foster profound expertise in antibiotic drug discovery and development. In the short and medium term, such capacity-building must be performed as a collaborative and iterative process between academia and industry to ensure that the necessary skills are available to translate validated hits into potential drug products. The development of joint initiatives for education in translational sciences will require specific funding, as they are not part of most universities' standard curricula. Many experienced scientists in the pharmaceutical industry are eager to share their translational and regulatory knowledge, often after retirement or due to change of operations. Thus, pharmaceutical companies could serve as a valuable training ground for acquiring and developing specific skills in the antimicrobial sector. However, limited funding (especially for SMEs) and economic uncertainties negatively affect this premise because it leads to business closures, high employee turnover rates, prevents the recruitment and training of inexperienced staff and deters scientists from embarking on a career in SMEs. In order to achieve transfer of vital expertise, workshops, symposiums and exchanges that foster academic–industrial interactions between students and advanced researchers are required and need financial support. Unfortunately, there are very few market-driven initiatives for such events and, therefore, a connection to already existing education and training programmes, for example, those supported by IMI, European Society of Clinical Microbiology and Infectious Diseases (ESCMID) or British Society for Antimicrobial Chemotherapy (BSAC), can be a valuable option as long as the transition into an era of mutually sustained knowledge transfer between industry and academia continues.

Another critical aspect for all future antibiotic R&D projects is the implementation of a legal framework for IP ownership at project commencement. The multidisciplinary and collaborative nature of antibiotic drug discovery often results in collaborations between different institutions on a national or international level. This creates challenging ownership structures with increasing complexity of such consortia, especially when an antibacterial programme is out-licensed, for example, to an SME. Negotiating ownership agreements among inventor institutions can be lengthy and may discourage industry from in-licensing valuable assets for further development. The increased collaboration between academia and industry requires fair and justifiable guidelines for knowledge and compound transfer outlined in appropriate agreements. The creation of such guidelines should be supported, for example, in the form of templates to settle ownership agreements between project partners or third parties, to facilitate processes for the benefit of researchers with limited experience in these matters. Such a framework will accelerate potential technology and compound transfer towards industrial drug developers, will make the relative commitment for each participant clearer and, thus, their gains more attractive.

Finally, we believe that AMR research requires diligent lobbying at the national and international levels to create entry points for large funders. Many scientists working on antimicrobials in either academia or SMEs are outside the few existing networks that involve decision makers within commercial funding sources, such as venture capitalists, including the newly announced AMR Action Fund, philanthropic organizations, national or regional governments or international bodies. This situation has resulted in an environment in which the challenges of antimicrobial drug developers are either not heard or are even ignored, even as public awareness of AMR steadily increases. It is evident that a strong lobbying position will lead to changes, which has recently been shown by the BEAM Alliance and their interaction and negotiations with diverse political bodies in Europe, leading to increased recognition of the challenges for antibacterial drug developers by the European Commission and Europe's national governments<sup>314–316</sup>.

For the above reasons, we recommend that an international group of experienced AMR lobbyists should be formed that, together, can campaign for funding of early antibacterial drug discovery research along the principles set out in this article. Such a group should include national, regional and global scientific and industry associations that have practice in interacting with relevant stakeholders connected with national parliaments, EC, G7, G20 and further decision-making entities<sup>317</sup>. The Global AMR R&D Hub (<https://globalamrhub.org/>) could be a crystallization point to pioneer such developments, which can be supported by various consortia, including the authors of this article: The International Research Alliance for Antibiotic Discovery and Development (IRAADD; <https://www.iraadd.eu/>), which we have recently established with the support of the JPIAMR Virtual Research Institute (JPIAMR-VRI; <https://www.jpiamr.eu/jpiamr-vri/>), identifies itself as a part of the mission that is addressed by the current

roadmap. The IRAADD aims to improve the situation of novel antibiotic discovery and development by bringing together experts for early drug research from the academic and industrial sectors, who can provide knowledge and advice for diverse projects in the field. A recent example of our activities is the support of the JPIAMR-VRI to create a new online resource (the JPIAMR-VRI Digital Platform 'DISCOVER'; <https://www.jpiaamr.eu/activities/jpiaamr-vri/digital-platform/>), serving as a comprehensive and interlinked database for AMR-related research at multiple levels. Although the IRAADD currently has only a short-term funding perspective, it is one of our main goals to help define and implement interdisciplinary innovative antibiotic development programmes based on sustainable research funding, in order to refill the translational pipeline with new drug candidates in the foreseeable future. In this respect, and as a possible long-term vision, the creation of internationally operating antibiotic research hubs,

which may emerge from already existing pre-stage platforms such as the IRAADD, can be a major step forward to engage as many members as possible from academia, industry and public health organizations in antimicrobial R&D collaborations, and to create a strong and path-breaking position that cannot be overlooked. Only a responsible connection of thought leaders and dedicated experts from all relevant sectors of society, joining together now and for the future, will allow suitable rapid responses to globally emerging pathogens. Therefore, taking corrective and preventive action now through concerted and innovative approaches in the field of novel antibiotic drug discovery and development is the essential path forward to be prepared for future pandemics caused by multi-to-pan drug-resistant (so-called superbug) bacteria, which is an aim that deserves our undivided attention.

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#### Author contributions

All authors contributed to researching data, discussion of content, writing of the article, as well as reviewing and editing the article.

#### Competing interests

M. H. Medema is a co-founder of Design Pharmaceuticals and a member of the scientific advisory board of Hexagon Bio, and S. Donadio is a co-founder and shareholder of NAICONS, owning intellectual property on antibacterial compounds. The remaining authors do not declare any competing interests.

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