IMI1 Final Project Report
Public Summary

Project Acronym: COMBACTE-MAGNET

Project Title: Combatting Bacterial Resistance in Europe – Molecules Against Gram - Negative Infections

Grant Agreement: 115737-4
Project Duration: 01/2015 - 12/2022

1. Executive summary

1.1. Project rationale and overall objectives of the project

Antimicrobial resistance (AMR) is a major global public health threat, and most troublesome is the rapid emergence and dissemination of multidrug resistant (MDR) Enterobacteriaceae, *Acinetobacter* species and *Pseudomonas aeruginosa*. There is an unmet medical need to prevent *P. aeruginosa* infection in critically ill patients and to develop new antibiotics for infections caused by Gram-negative bacteria (GNB), and maximise epidemiological strategies. The **Combatting Bacterial Resistance in Europe – Molecules against Gram Negative Infections** (COMBACTE-MAGNET) consortium brings together 39 partners from pharmaceutical industries (5 partners) and academic (34 public partners). As such, it is a nexus of world-class researchers from 10 European countries with expertise in (i) designing and executing observational and interventional studies related to antibiotic-resistant bacteria; (ii) conducting high-quality research in the epidemiology, prevention, and treatment of infections caused by multidrug-resistant gram-negative bacteria (MDR-GNB); (iii) identifying novel biomarkers in critically ill patients; (iv) performing Phase I Pharmacokinetic (PK)/Pharmacodynamic (PD) studies; (v) performing Phase 2 studies enriched for PK-PD endpoints; and (vi) performance of large definitive Phase 3 randomized clinical trial (RCT) for regulatory purposes. Methodological expertise includes clinical epidemiology, consensus building and agenda setting (for health research prioritization), statistics, health economics, PK/PD, and (clinical) microbiology. Together, the consortium's researchers possess expertise in the execution of clinical trials and studies evaluating new (and established) antibacterials, preventive measures against Hospital-acquired infection (HAI), guiding antibiotic and infection prevention policies, and many already participate in *7th Framework Programme for Research and Technological Development* (FP-7) and Innovative Medicines Initiative (IMI)-funded antibiotic-resistant bacteria (ARB) research consortia. Furthermore, the consortium has extensive experience in pharmaceutical medicine including regulatory processes and public and patient involvement (PPI) in infection research.

1.2. Overall deliverables of the project

The overall deliverables of the COMBACTE-MAGNET Project Topic 6 are:

1) To map sources of epidemiology data both within and outside the New Drugs 4 Bad Bugs (ND4BB) programme and to define ways of optimal utilization of available data to inform clinical study designs for this project as well as future efforts, and to drive public health actions and improve patient outcomes
2) To assess risk factors for and costs of Hospital-acquired Infections (HAI) attributed to *P. aeruginosa* among Intensive Care Unit (ICU) patients in Europe to identify the target population for the development of MEDI3902 for the prevention of serious disease caused by *P. aeruginosa*
3) To assess the potential impact of antimicrobial interventions and improved treatment regimens on *P. aeruginosa* attributed disease burden among ICU patients
4) To characterize clinically relevant strains of *P. aeruginosa* to determine the prevalence of expression of the MEDI3902 targets Psl and PcrV and to create a bank of organisms linked to patient-specific outcomes for future use

5) To assess safety, efficacy, and PK of MEDI3902 and antidrug antibodies (ADA) developed in response to MEDI3902 treatment in a Phase 2 proof of concept (POC) study in an enriched population

6) To assess the safety and PK of MEDI3902 in paediatric patients

7) To provide recent information about the clinical management and outcomes of patients with complicated urinary tract infection (cUTI) (including pyelonephritis) in Europe with known or suspected increased resistance rates in clinically relevant Gram-negative pathogens, including *P. aeruginosa*

1.3. Summary of progress versus plan since last period

Work Package	Planned Achievements Completed 2022	Missed Planned Objectives in 2022
WP1 - PMO & Governance	• The annual General Assembly meeting was held in a hybrid format in Lisbon, Portugal on 30-31 May 2022 • Annual Report for Jan/2021 – Dec/2021 completed and approved by IHI. • Organisation of regular SCB meetings, management board meetings and PMO meetings • IHI instructed the Managing Entity that the 2021 accepted costs are to be set against the pre-financing payment with almost all partners to cover their expenditures in 2021. • EFPIA payments for 2021 costs are being processed.	Annual Report was submitted with all Deliverable and Scientific Reports on time, on 1 March 2022, although not all financial reports were submitted with the initial report, they were submitted with a delay.
WP2 – Develop of Capabilities in Epidemiology	• Data collection and consolidation of the EPI-Net Excellence Centers Network - a network of healthcare centers sharing surveillance data for epidemiological research, with a geographical outreach covering at least all European countries (https://epi-net.eu/excellence-centers/overview/) • Biannual update of European national/international, voluntary/mandatory surveillance data for target antibiotic-resistant bacteria and healthcare-associated infections from multiple external sources and online release (https://epi-net.eu/)	

Work Package	Planned Achievements Completed 2022	Missed Planned Objectives in 2022
	• Elaboration of a sub-study to evaluate the ability of current surveillance to monitor and inform on emerging bacterial resistances (https://epi-net.eu/studies/) • Implementation, data collection, and analysis for the ENSURE (Enforcing surveillance of antimicrobial resistance and antibiotic use to drive Stewardship) study (https://epi-net.eu/studies/) • Organization of the final annual EPI-Net meeting 1st- 2nd December 2022, hybrid format • Publication of the manuscript describing the work done to build the EPI-Net AMR travel tool (https://epi-net.eu/travel-tool/overview/) (Journal of Travel Med 2022) • Publication of the manuscript evaluating European surveillance capacity for antifungal resistance (Journal of Fungi, 2022) • Conclusion and publication of the EPI-Net One Health consensus document (Lancet Regional Health - Europe, 2022) • Finalization of manuscript, "Modelling antimicrobial resistance transmission to guide personalized antimicrobial stewardship interventions and infection control policies in healthcare setting: a pilot study", which will be published soon in a peer-reviewed journal	
WP3A Central lab - UA and UMCU	• Culture of study samples and strain whole genome sequencing has been completed	• Metagenomics analysis ongoing • Final comprehensive analysis ongoing
WP3A – Research labs	• Study on strain and sample analysis is ongoing/finishing as well as multiple publications prepared, submitted and published.	• Completion of expanded biomarker studies

Work Package	Planned Achievements Completed 2022	Missed Planned Objectives in 2022
WP3B – Mathematical modelling	• Results dissemination and scientific publications	
WP4A Phase 2 Proof-of-Concept Trial of MEDI3902 Safety, Efficacy, Pharmacokinetics, Pharmacodynamics, and Antidrug Antibodies	• Report on 16S/ITS sequencing on respiratory microbiome • Publication of the study results	

1.4. Significant achievements since last report

The COVID-19 pandemic exerted significant burden on the activities performed in COMBACTE-MAGNET, including clinical priorities of principle investigators and the closure of labs for a significant period of time. However, significant achievements were still made.

An overview of achievements per work package can be found below:

WP1: Project management, collaboration, and dissemination

The coordinator of the COMBACTE-MAGNET Consortium remains AZ and the lead of the Project Management Office (PMO) remains UMCU. Combined meetings were organised, for the Management Board (MB) and for the PMO.

The annual General Assembly was held in a hybrid format, along with the COMBACTE-NET and COMBACTE-CARE projects, on 30-31 May 2022 in Lisbon, Portugal. Seventy-four attendees from 20 EFPIA and academic partners attended the event and presented progress and results from COMBACTE-NET, COMBACTE-MAGNET and COMBACTE-CARE projects. The General Assembly included presentations and discussions on the workpackages and studies from the COMBACTE projects and consortia.

The Annual Report for Jan/2021 – Dec/2021 was completed by WP1 and approved by IHI. IHI instructed the Managing Entity that the 2021 accepted costs are to be set against the pre-financing payment with almost all partners to cover their expenditures in 2021. EFPIA payments to the relevant academic partners for 2021 costs related to the clinical trial are being transferred.
WP1 submitted a Description of Work (DoW) Amendment no. 6 to IHI. This amendment included an update of the project budget. The amendment was approved by IHI.

The meeting of the COMBACTE-MAGNET Scientific Coordination Board was held virtually on 14 December 2022. It included presentations and discussions of workpackage updates by workpackage leads, and a reflection on the ending of the project.

In addition, the communications office continued to stimulate communication through the website, newsletters social media channels, etc. to update on progress made and the multiple publications that were submitted to and accepted by peer-reviewed journals.

WP2: Development of capabilities in epidemiology

The WP2's EPI-Net Excellence Centers Network initiative aims to connect healthcare centers across Europe to facilitate high level international projects and mobilise site selection for clinical research on antimicrobial resistance (AMR). Interested centers must submit anonymised, routine surveillance data to be linked with potential research studies. This initiative was launched in 2021 at the peak of the COVID-19 pandemic, delaying recruitment and data collection. Due to the extended time frame offered by a cost-neutral extension of the project duration, 16 healthcare centers from 9 European countries were successfully recruited for the EPI-Net Excellence Centers Network, of which 8 Centers (from 7 different countries) completed the data submission as of December 2022. The number of centers recruited and providing data was still low, reflecting on continued burden of COVID-19 on healthcare staff and facilities. However, the initial pool of Excellence Centers were incentivized for data submission through dissemination of their clinical research portfolio in the EPI-Net newsletter (Issue no. 15, August 2022), their link to the IMI PrIMAVeRa project's work package 3 (https://www.primavera-amr.eu/wp3-data-gathering), and their inclusion in an evidence-based study to evaluate the ability of current surveillance to monitor and inform on emerging bacterial resistances. This pilot effort will serve as an example for further recruitment and data collection from European healthcare centers– the EPI-Net Excellence Centers Network initiative will be continued within Horizon 2020 ECRAID-Base project (https://www.ecraid.eu/ecraid-base), starting 2023.

The cost-neutral extension of the project duration was also of significant help in overcoming the impact of COVID-19 pandemic on the ENSURE study, which aimed to translate the WP2's consensus recommendations (JAC, 2020) into practice to guide an educational antimicrobial stewardship (AS) programme in high-risk settings where antimicrobial surveillance, reporting, and AS implementation are based on a lower level of evidence. The study was carried out in tertiary hospitals in the Veneto region of Italy, and comprised of two separate activities dedicated to the paediatric and adult patient populations. An abstract describing the results of this study have been submitted to 33rd European Congress of Clinical Microbiology & Infectious Diseases, Copenhagen, Denmark on 15 - 18 April 2023.

WP2's study on emerging resistances aims at providing a clear picture of the current ability to efficiently monitor resistance to recently approved antibiotics in Europe. This evidence-based study is based on a literature review carried out annually since 2017 to track the reporting of AMR to antibacterial agents for which market authorisation has been granted since 2012 by the EMA. So far, 12 antibiotics have been identified for the treatment of different bacterial infections. For each antibiotic agent, data have been obtained from two main sources: i) journal publications and; ii) national surveillance reports and online repositories. Analysis of these data indicated that although resistance is consistently reported for new antibiotics and journal publications reporting resistance has steadily increased in the last 5 years, only a limited number of European countries include at least one "new" antibiotic as a surveillance target for their national surveillance programmes on antimicrobial resistance and consumption. Strengthened by these preliminary results, the study will

be continued within the Horizon 2020 ECRAID-Base project, starting January 2023, to inform policy priority decisions and timely actions at European level.

Furthermore, a paper describing the results of WP2's One Health consensus initiative, conducted between March 2021-January 2022, was completed and published with 'The Lancet Regional Health-Europe' journal (https://doi.org/10.1016/j.lanepe.2022.100563). This paper outlines recommendations from a panel of 56 experts from 20 countries to guide strategic reporting of AMR and antimicrobial consumption surveillance data from the human, animal, and environmental sector.

Lastly, a closing project meeting was organized by WP2 on the 1st and 2nd December 2022. The meeting was held in a hybrid format, with the face-to-face format taking place in Verona, Italy (Appendix 2). The meeting brought together 48 participants from 16 countries and served as a platform to disseminate significant scientific outputs from the WP2 among representatives from other international projects, public health agencies, and organizations working towards the mitigation of the global issue of antimicrobial resistance (AMR).

WP3A: Epidemiology of hospital-acquired infections in the intensive care unit (ICU)

Within WP3A, 2022 was focused on completing multiple laboratory analyses and working towards manuscripts, some of which combine WP3A and WP4A (Phase 2 Proof-of-Concept Trial of MEDI3902 Safety, Efficacy, Pharmacokinetics, Pharmacodynamics, and Antidrug Antibodies) data. One published study showed a high prevalence (33.6%), of multidrug resistant *P. aeruginosa* within European ICUs as well as wide intercountry variability determined by the dissemination of XDR high-risk clones, thus arguing for the need to reinforce infection control measures (Journal of Antimicrobial Chemotherapy 2022). Another published study showed that the gut can act as a reservoir for resistant *P. aeruginosa* with potential for translocation to the lung. These findings suggest that reducing intestinal colonization of *Pseudomonas* may be an effective way to prevent lung infections in critically ill patients. Nonetheless, resistance was primarily shown to be driven by parallel evolution in the gut and lung coupled with organ specific selective pressures (Nature Communications 2022). Another study aimed for the rapid identification of *P. aeruginosa*-derived host markers to enable an early detection of *P. aeruginosa* VAP (VAP-PA) in easily accessible patients' samples such as urine. Using metabolomics, the authors identified 58 metabolites that were significantly elevated or uniquely present in VAP-PA compared to the VAP–non-PA and pre-infection groups (Biomarker Insights 2022). These, if further validated, could serve as highly specific diagnostic biomarkers of VAP-PA, thereby stewarding antibiotic use and improving clinical outcomes. There are currently two new manuscripts published in 2023, one showing that pulmonary populations of *P. aeruginosa* are often polyclonal, and resistance emergence is through selection for pre-existing resistant strains. However, strong trade-offs between resistance and fitness occur in polyclonal populations that can drive the loss of resistant strains when antibiotic pressure is weak. These data show that the within-host diversity of pathogens plays a key role in shaping the emergence of resistance in response to treatment (Nature Communications 2023)[4]. The other recently published study explores predictive biomarkers of *P. aeruginosa* ventilator-associated pneumonia in blood/plasma due to dysregulation of pro-inflammatory and endothelial factors at time of admission (Critical Care 2023)[5]. Ongoing work includes studies on the prevalence and risk factors of *P. aeruginosa* and the relation to ICU pneumonia, host and microbial biomarkers in respiratory and blood samples as novel (predictive) diagnostic aids, genomic population analysis of the *P. aeruginosa* isolates as well as changes in the

respiratory microbiome of patients developing VAP-PA. These are currently being finalized into different manuscripts. All of this work has created very novel and useful insights into earlier detection and prevention of *P. aeruginosa* ICU pneumonia as well as adding considerations to treatment options and even potential vaccine or new treatment targets.

Publications:

1. Gabriel Torrens and others, Susceptibility profiles and resistance genomics of Pseudomonas aeruginosa isolates from European ICUs participating in the ASPIRE-ICU trial, Journal of Antimicrobial Chemotherapy, Volume 77, Issue 7, July 2022, Pages 1862–1872, https://doi.org/10.1093/jac/dkac122
2. Wheatley, R.M., Caballero, J.D., van der Schalk, T.E. et al. Gut to lung translocation and antibiotic mediated selection shape the dynamics of Pseudomonas aeruginosa in an ICU patient. Nat Commun 13, 6523 (2022). https://doi.org/10.1038/s41467-022-34101-2
3. Jongers B, Hotterbeekx A, Bielen K, et al. Identification of Potential Urinary Metabolite Biomarkers of Pseudomonas aeruginosa Ventilator-Associated Pneumonia. Biomarker Insights. 2022;17. doi:10.1177/11772719221099131
4. Diaz Caballero, J., Wheatley, R.M., Kapel, N. et al. Mixed strain pathogen populations accelerate the evolution of antibiotic resistance in patients. Nat Commun 14, 4083 (2023). https://doi.org/10.1038/s41467-023-39416-2
5. van Engelen, T.S.R., Reijnders, T.D.Y., Paling, F.P. et al. Plasma protein biomarkers reflective of the host response in patients developing Intensive Care Unit-acquired pneumonia. Crit Care 27, 269 (2023). https://doi.org/10.1186/s13054-023-04536-0

WP3B: Impact of different Interventions on *P. aeruginosa*-attributed disease burden among patients in the ICU

In 2022, we published following article:

- Staus, P, von Cube, M, Hazard, D, Doerken, S, Ershova, K, Balmford, J, Wolkewitz, M (2022). Inverse Probability Weighting Enhances Absolute Risk Estimation in Three Common Study Designs of Nosocomial Infections. Clin Epidemiol, 14:1053-1064.

In 2022, we wrote following manuscripts:

- Grodd M, Weber S. and Wolkewitz M. Stacked probability plots of the extended illness-death model using constant transition hazards – An easy to use shiny app

- Paulina Staus, Maja von Cube, Tobias Bluhmki, Klaus Kaier, Frangiscos Sifakis, Omar Ali, Jafri Hasan, Surbhi Malhotra, Fleur Paling, Jan Kluytmans, Jan Beyersmann, Martin Wolkewitz and the ASPIRE-ICU Study Team, The burden of hospital acquired S. aureus and P. aeruginosa pneumonia: Results from a European multi-centered study

In 2022, we developed a statistical tool (RShiny app):

- https://eidm.imbi.uni-freiburg.de/

Illness-death models can help analyse situations such as infections acquired in hospital, pneumonia caused by ventilation, or transfers between hospitals. Hazard rates and transition probabilities are the key components of these models. These measures can be difficult to calculate and interpret because of their complexity. Assuming time-constant hazards makes it easier to manage the complexity of these models, and it is possible to obtain closed mathematical forms for transition probabilities. We

have created a tool in R (RShiny app) that uses stacked probability plots to visualise transition probabilities. Our goal was to create a tool that can aid in obtaining a deeper understanding of complex multistate settings. Although multistate models can be highly complex, this tool can help understand the assumptions made while planning studies or take the first step in analyzing complex data structures from such studies. The statistical tool (RShiny app) has several purposes. First, it helps regulatory authorities understand hidden effects in published randomized trials. Second, it assists scientists in planning randomized trials with complex time-dependent outcomes. Finally, it improves the understanding of observational data analysis using multistate models.

WP4A: A phase 2 study to evaluate the efficacy of MEDI3902 in mechanically ventilated subjects

- The results of the EVADE study were published in the Critical Care review in October 2022 / Month 94 (Impact Factor 19.334)
- Microbiome study: bioinformatics analysis finalised; final report pending

1.5. Scientific and technical results/foregrounds of the project

WP2: Development of capabilities in epidemiology

Within the COMBACTE-MAGNET project, WP2 successfully established a core epidemiology structure, the epidemiology network (EPI-Net), to develop coherent epidemiology strategies and organise pertinent expertise and available data sources in Europe to lower the burden of antimicrobial resistance (AMR) and healthcare-associated infections (HAI). EPI-Net has served as a forum for collaboration of not only project partners but also for experts in the subject matter from other international projects, public health agencies, and research networks. Through collaborative efforts, EPI-Net has in total published 21 peer-reviewed journal articles with a total impact factor of 128 and an average 6.4. The scientific and technical outputs delivered between 2015 and 2022 include: a) four systematic reviews critically appraising surveillance capacity and/or surveillance definitions in Europe (https://epi-net.eu/publications/); b) a central data repository/surveillance-dedicated online platform, providing timely access to geographical and temporal updates on the emergence, spread, and frequency of occurrence of AMR and HAI in Europe (https://epi-net.eu/); c) a public service tool to raise awareness on the risk of acquiring AMR and prevention measures related to international travel (https://epi-net.eu/travel-tool/overview/) which -before its launch- was evaluated by a group of external experts in antimicrobial resistance and by the COMBACTE-MAGNET public and patient involvement (PPI) group; d) a framework for semi-automated surveillance of surgical site infections in hospitals (Infection Control & Hospital Epidemiology, 2019); e) expert consensus for best practices in surveillance and practical tools for surveillance implementation, addressing key limitations and knowledge gaps (nine consensus documents developed - 1 on harmonization of public health surveillance systems for AMR/HAI; 2 on automation of HAI surveillance; 5 on linking AMR and antimicrobial consumption [AMC] surveillance data to antimicrobial stewardship [AMS] activities in hospital, long-term care facility, outpatient, or veterinary settings; 1 on reporting AMR and AMC data from the human, animal, environmental sectors) (https://epi-net.eu/publications/); f) proof of principle – a large-scale implementation of EPI-Net consensus recommendations for linking AMR and AMC to AMS in high-risk hospital settings (ENSURE study) ; g) EPI-Net Excellence Centers Network – a

network of healthcare centers sharing data to facilitate international projects and expedite epidemiological research on AMR (https://epi-net.eu/excellence-centers/overview/).

WP3A: Epidemiology of hospital-acquired infections in the intensive care unit (ICU)

The goal of WP3A was to assess the impact of host- and pathogen-related factors on the incidence of *P. aeruginosa* ICU pneumonia as well as the identification as well as identify population characteristics for the clinical trial patient selection of WP4A (EVADE). Additional goals were focussed on the improvement of diagnostics, patient prognosis and *P. aeruginosa* ICU pneumonia control.

1. To estimate the incidence of ICU pneumonia caused by *P. aeruginosa* (PAIP).
    a. The overall weighted incidence of *P. aeruginosa* caused ICU pneumonia was 1.8%. Incidence was higher in patients colonised prior to the 3-day window around infection whereas respiratory colonization was found to be to result in a higher incidence rate compared to peri-anal colonization. In comparison to the total study population of 1971 patients, 214 (10.8%) were colonized within the first 72 hours after ICU admission. In total, 391 patient became colonized with *P. aeruginosa* during ICU stay.
2. To determine factors that are independently associated with PAIP. As above mentioned, colonization both peri-anal as well as respiratory are increasing the chances of developing PAIP.
    a. When comparing patient *P. aeruginosa* positivity in lower respiratory tract samples, the majority of the patients who did not develop any infection during ICU stay (controls) compared to patients that developed PAIP was shorter (on average 4.3 compared to 15.4 days). When investigating the *P. aeruginosa* load in comparison to total bacterial growth there were a few slight significant differences between the controls and PAIP patients. One of the main differences is that at colonization with *P. aeruginosa*, more control patients had a light load of *P. aeruginosa* and while more PAIP patients had a moderate load. However, both patient groups showed an equal percentage with a heavy load of *P. aeruginosa*. When investigating the *P. aeruginosa* loads overtime there was in the controls an average decrease overtime while the PAIP patients showed on average an increase in *P. aeruginosa* load overtime.
    b. When looking into any genomic factors potentially associated with PAIP there were a few interesting discoveries. Overall, the *P. aeruginosa* population across the European ICUs is highly diverse, most clinical sites showed a diversity in sequence types. There were only a few hospitals were from multiple patients the same sequence type was isolated. When looking into any predictive genomic aspects within the first available *P. aeruginosa* isolates there was one significant gene found to show an association towards the PAIP development in comparison to controls. This gene, PA0631, encodes a hypothetical protein that is associated with genome plasticity and showed increased expression during oxidative stress. Longitudinal genomic analysis showed several interestingly altered pathways and genes overtime. One of these genes was the *lasR* gene associated in chronic infections with increased inflammation and increases fitness in low oxygen environments. The *lasR* gene was found to be altered more often in PAIP patients than controls. Other pathways and genes affected

longitudinally were the LPS biosynthesis, motility/attachment type 4 pili, the antibiotic resistance–associated porin *oprD*, multiple efflux systems, cell wall biosynthesis and *ampD*, a β-lactamase regulator.

3. A description of the antimicrobial susceptibility and the prevalence of specific antigens and their expression among the *P. aeruginosa* isolates from European ICU patients.
    a. A study of 723 *P. aeruginosa* isolates from respiratory and peri-anal swabs into antimicrobial susceptibilities from 402 patients looking into the susceptibilities against 12 antibiotics and genetic and phenotypic carriage of transferable resistance genes. Investigation into the first available isolate per patient showed that more than a third of the isolates was highly resistant (33.6%, (multidrug resistant 8%, extremely drug resistant 24.9% and pan drug resistant 0.7%)). Most of these high resistance isolates were from specific sites/countries. Resistance towards β-lactam antibiotics was found in almost a third of the first available isolates were 21.4% carried genes that are transferable between bacteria. Analysis into carriage of the PcrV antigen (the tip of the type 3 secretion system needle-like structure) and showed that of 114 analysed isolates there were seven that were negative. Another analysis into the expression of psl (an important polysaccharide for biofilm formation) showed that out of the 114 isolates there were 11 isolates that had undetectable amounts of psl.
        i. Torrens G, van der Schalk TE, Cortes-Lara S, Timbermont L, Del Barrio-Tofiño E, Xavier BB, Zamorano L, Lammens C, Ali O, Ruzin A, Goossens H, Kumar-Singh S, Kluytmans J, Paling F, MacLean RC, Köhler T, López-Causapé C, Malhotra-Kumar S, Oliver A; ASPIRE-ICU study team. Susceptibility profiles and resistance genomics of Pseudomonas aeruginosa isolates from European ICUs participating in the ASPIRE-ICU trial. J Antimicrob Chemother. 2022 Jun 29;77(7):1862-1872. doi: 10.1093/jac/dkac12
4. To investigate host biomarkers and association with ICU infections and disease severity. There were two different partners working towards host biomarkers, one focusing on respiratory samples (a.) and the other on blood and serum samples (b.)
    a. A trial study into metabolomics differentiation using urine as samples showed that *P. aeruginosa* caused ventilator-associated pneumonia could be distinguished from other infections. A follow-up is planned to use respiratory samples which are a difficult matrix to perform these types of analyses. Within respiratory samples a difference was detected with a proteomic analysis between *P. aeruginosa* and *S. aureus* ventilator-associated pneumonia as well as control patients. Analysis into cytokines, chemokines and growth factors showed significant differences overtime within *P. aeruginosa* pneumonia as well as with controls. These results showed a proof of concept within respiratory samples to contain predictive biomarkers for both the general ventilator-associated pneumonia and specific agents as causes of the pneumonia.
        i. Jongers B, Hotterbeekx A, Bielen K, et al. Identification of Potential Urinary Metabolite Biomarkers of Pseudomonas aeruginosa Ventilator-Associated Pneumonia. Biomarker Insights. 2022;17. doi:10.1177/11772719221099131

b. Within blood plasma samples, a comparison between control patients and patient who develop pneumonia showed multiple interesting results. All of the 316 patients that developed pneumonia and 632 matched controls were analysed for blood plasma biomarkers. Measurement of 19 different biomarkers showed that within pneumonia patients there was a dysregulation of pro-inflammatory response even at ICU admission. A follow-up study into the RNA profiles within whole blood is currently being performed.
    i. van Engelen, T.S.R., Reijnders, T.D.Y., Paling, F.P. et al. Plasma protein biomarkers reflective of the host response in patients developing Intensive Care Unit-acquired pneumonia. Crit Care 27, 269 (2023). https://doi.org/10.1186/s13054-023-04536-0

5. Intra-patient population biology of *P. aeruginosa* from both respiratory and peri-anal samples focussing on antibiotic resistance selection.
    a. Two case studies concerning the *P. aeruginosa* intra-patient population during their ICU stay have been published. Both focussing on the evolution and selection of *P. aeruginosa* within a patient showing how antibiotic treatment and immunity can select a resistant sub-population and its rise as well as how the gut can function as a reservoir for *P. aeruginosa* when antibiotics are utilised that are less effective in the gut than in the lung.
        i. Wheatley, R., Diaz Caballero, J., Kapel, N. et al. Rapid evolution and host immunity drive the rise and fall of carbapenem resistance during an acute Pseudomonas aeruginosa infection. Nat Commun 12, 2460 (2021). https://doi.org/10.1038/s41467-021-22814-9
        ii. Wheatley, R.M., Caballero, J.D., van der Schalk, T.E. et al. Gut to lung translocation and antibiotic mediated selection shape the dynamics of Pseudomonas aeruginosa in an ICU patient. Nat Commun 13, 6523 (2022). https://doi.org/10.1038/s41467-022-34101-2
    b. Both above-mentioned cases consist of initial *P. aeruginosa* populations were the population consist of a single sequence type and has a high relatedness. However, there were patients with a mixed population, meaning multiple sequence types or in some cases within the sequence type a high diversity. Comparison between mixed population and non-mixed populations showed a more rapid emergence of resistance due to selection of existing resistance and not the development of new resistance.
        i. Diaz Caballero, J., Wheatley, R.M., Kapel, N. et al. Mixed strain pathogen populations accelerate the evolution of antibiotic resistance in patients. Nat Commun 14, 4083 (2023). https://doi.org/10.1038/s41467-023-39416-2

WP3B: Impact of different Interventions on *P. aeruginosa*-attributed disease burden among patients in the ICU

In the entire project time of WP3B, we published 27 original articles in methodological, as well as applied journals; and in addition 9 letters. As a main result, we developed and extended statistical methods and designs to the specific issues regarding the data complexity in clinical COMBACTE research.

1. Staus, P, von Cube, M, Hazard, D, Doerken, S, Ershova, K, Balmford, J, Wolkewitz, M (2022). Inverse Probability Weighting Enhances Absolute Risk Estimation in Three Common Study Designs of Nosocomial Infections. Clin Epidemiol, 14:1053-1064.

2. Hazard, D, von Cube, M, Kaier, K, Wolkewitz, M **(2021)**. Predicting Potential Prevention Effects on Hospital Burden of Nosocomial Infections: A Multistate Modeling Approach. *Value Health*, 24, 6:830-838.

3. Weber, S, Wolkewitz, M, on behalf of COMBACTE-MAGNET Consortium. Accounting for length of hospital stay in regression models in clinical epidemiology. Statistica Neerlandica. 2020; 74: 24– 37

4. Kaier, K, Heister, T, Götting, T, Wolkewitz, M, Mutters, NT **(2019)**. Measuring the in-hospital costs of Pseudomonas aeruginosa pneumonia: methodology and results from a German teaching hospital. *BMC Infect. Dis.*, 19, 1:1028.

5. Heyard R, Held L, The quantile probability model, Computational Statistics & Data Analysis, Volume 132, 2019, Pages 84-99, https://doi.org/10.1016/j.csda.2018.08.022.

6. Kaier, K., Heister, T., Motschall, E., Hehn, P., Bluhmki, T., & Wolkewitz, M. (2019). Impact of mechanical ventilation on the daily costs of ICU care: A systematic review and meta regression. *Epidemiology and Infection, 147*, E314. doi:10.1017/S0950268819001900

7. Bluhmki, T, Putter, H, Allignol, A, Beyersmann, J **(2019)**. Bootstrapping complex time-to-event data without individual patient data, with a view toward time-dependent exposures. *Stat Med*, 38, 20:3747-3763.

8. Heyard, R, Timsit, JF, Held, L **(2019)**. Validation of discrete time-to-event prediction models in the presence of competing risks. *Biom J*, https://doi.org/10.1002/bimj.201800293

9. Pham, TM, Kretzschmar, M, Bertrand, X, Bootsma, M **(2019)**. Tracking Pseudomonas aeruginosa transmissions due to environmental contamination after discharge in ICUs using mathematical models. *PLoS Comput. Biol.*, 15, 8:e1006697.

10. von Cube M, Schumacher M, Wolkewitz M: Causal inference with multistate models—estimands and estimators of the population attributable fraction. *J R Stat Soc A Stat*, 2019. : (in press)

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WP4A: A phase 2 study to evaluate the efficacy of MEDI3902 in mechanically ventilated subjects

EVADE study:

Pseudomonas aeruginosa (PA) is a common cause of ventilator-associated pneumonia (VAP) in patients admitted to ICU, associated with mortality rates >30% in patients with antibiotic-susceptible strains and >44% with multidrug-resistant (MDR) strains. More effective preventive measures are therefore urgently needed. Nevertheless, no systemic agents are currently approved for pre-empting PA pneumonia in ventilated patients with *Pseudomonas* airway colonisation, highlighting an unmet need for effective, targeted prevention. Although monoclonal antibodies blocking the virulence factors used by the bacteria to invade and destroy lung tissue are an attractive alternative to systemic antibiotics for the prevention of PA infections, no controlled studies were available showing their efficacy in intensive care patients when our study was planned and executed, underscoring its very timely clinical importance in clarifying whether such antibodies might be able to decrease the rate of *P. aeruginosa* pneumonia. Obviously, only data from rigorous clinical trials can determine whether the positive results that have been observed with various monoclonal antibodies in experimental models of pneumonia can be translated into positive outcomes in ICU hospitalized patients. Among the several antibodies targeting specific pathogens commonly responsible for nosocomial infections in ICU patients currently in development, MEDI3902 (gremubamab) was one of the most attractive. Indeed, it is a bispecific human monoclonal antibody developed by MEDIMMUNE/ASTRA-ZENECA that selectively targets two major virulence factors used by *P. aeruginosa* for colonizing and invading tissue: the PcrV protein − a major component of the type-3 secretion system − and Psl exopolysaccharide critical for biofilm formation and tissue adherence. This is why we decided in line with the general objectives of IMI to conduct the EVADE trial, hoping that such a study could clarify the potential efficacy, PK and safety of MEDI3902 when assessed in a rigorous, double blind, randomized study.

EVADE (Clinicaltrials.gov NCT02696902; EudraCT 2015-001706-34) was a phase 2, proof-of-concept, double-blind, placebo-controlled study conducted across 48 sites in 13 countries in Europe, Israel, and the USA within the European public–private partnership Combatting Bacterial Resistance In Europe – Molecules Against Gram-Negative Infections (COMBACTE-MAGNET) consortium in mechanically ventilated patients with PA lower respiratory tract colonisation confirmed by polymerase chain reaction (PCR) on tracheal aspirates.

Even if the initial sample size was not reached because of low recruitment, 188 subjects were eventually randomised (MEDI3902 500/1500 mg: n=16/87; placebo: n=85) between 13 April 2016 and 17 October 2019, making this the PA prevention study that recruited by far the largest number of patients. Although 81% of subjects who received the high dose of the antibody achieved serum concentrations well above levels associated with improved outcomes in animal models, MEDI3902 undisputedly did not reduce the incidence of PA pneumonia in our study population (22% and 18% at day 21 in MEDI3902 1500mg and placebo recipients, respectively), highlighting that many factors that are not influenced by monoclonal antibodies may also contribute to the development of pneumonia, including disease severity, underlying immune function, and concomitant medications..

However, a positive exposure-response relationship was observed for the MEDI3902 1500 mg group, with a greater MEDI3902 area under the concentration–time curve from time zero to 21 days post-dose associated with a lower probability of PA pneumonia. Exploratory post-hoc analyses also demonstrated a greater response to MEDI3902 treatment compared to placebo for PA pneumonia in

the subgroup of subjects with a lower baseline Procalcitonin (PCT) plasma concentration or absolute neutrophil count.

These results provide important lessons on pathogen-specific PA pneumonia complexity in ICU trials with potential implications for future study design. Whereas APACHE-II scores have been used in many previous ICU studies as eligibility criteria to avoid enrolling the sickest patients, clinicians might also consider baseline levels of certain biomarkers as exclusion criteria and/or for stratifying patients by severity at randomisation, particularly in pre-emptive treatment studies of colonised patients. While PCT and neutrophil counts levels may not correlate directly with PA pneumonia, these markers may identify patients with higher bacterial load and higher inflammatory status, and therefore a higher risk of pneumonia. Conversely, patients with higher levels of these biomarkers (regardless of apparent pathogen levels) may be too sick to benefit from treatment, or may have progressed too far in the development of symptomatic pneumonia. For mechanically ventilated critically ill subjects, high baseline inflammatory status or cachexia increases protein catabolism and volume distribution of many drugs, potentially lowering MEDI3902 exposure and increasing PA pneumonia susceptibility simultaneously. A higher MEDI3902 dose and/or direct administration into the tracheobronchial tree by aerosolization may be considered for the most seriously ill subjects to achieve protection, especially since MEDI3902 ETA/serum PK ratio was low (0.1% on day 4).

The scientific production delivered by the Consortium included the publication of the main results of the EVADE trial in the journal Critical Care (IF 19.34) (Chastre J, et al. Safety, efficacy, and pharmacokinetics of gremubamab (MEDI3902), an anti-*Pseudomonas* aeruginosa bispecific human monoclonal antibody, in *P. aeruginosa* colonised, mechanically ventilated intensive care unit patients: a randomised controlled trial. Crit Care. 2022 Nov 15;26(1):355. doi: 10.1186/s13054-022-04204-9) and several presentations at important scientific meetings/conferences, including the 2022 ECCMID meeting, the 2022 IDWEEK, the 6th Immunotherapies & Innovations for Infectious Diseases, and the 11th Sepsis Update that will take place from September 6-8, 2023 in Weimar. In addition, several articles are being prepared and will contribute to the dissemination of the results of the project.

<u>Microbiome study:</u>

The purpose of the study was to perform the whole genome sequencing on ETA samples of subjects enrolled in the EVADE study and that consented to future use of their samples, to study the dynamic behaviour of P. aeruginosa in the in the respiratory microbiome and to explore the relationships between the respiratory microbiome and outcome of ventilator associated pneumonia (VAP) and all-cause mortality in relation to efficacy of MEDI3902.

The dynamic composition of the respiratory microbiome over the course of study follow-up, with or without development of VAP was investigated, as well as any effect of MEDI3902 on this composition. Not only will this provide insight on the effect of MEDI3902, it will also generate deeper understanding on the independent association between *P. aeruginosa* colonization and risk of *P. aeruginosa* infection.

As a conclusion:

- Alpha and Beta- diversity metrics are comparable by VAP status at baseline and longitudinally within each arm.
- Firmicutes and Proteobacteria are commonly identified in the respiratory microbiome of patients in the EVADE trial.
- Specifically, Staphylococcus species were significantly more abundant at baseline in the MEDI3902 arm, whereas Micrococcus and Burkholderia species were more abundant at baseline in the placebo arm.
- There were greater differentially enriched bacteria at baseline in the placebo group in pneumonia patients.
- Specifically, Staphylococcus species were significantly more abundant at baseline in the MEDI3902 arm, whereas Micrococcus and Burkholderia species were more abundant at baseline in the placebo arm.
- MEDI3902 seems to have minimal impact on the respiratory microbiome longitudinally.

1.6. Potential impact and main dissemination activities and exploitation of results

WP2: Development of capabilities in epidemiology

The EPI-Net surveillance-dedicated platform (https://epi-net.eu/) comprises data gathered from 15 national mandatory antimicrobial resistance (AMR) surveillance systems, 34 national voluntary AMR surveillance systems, 4 international AMR surveillance systems, 30 national healthcare-associated infections (HAI) surveillance systems, 1 international HAI surveillance systems in Europe (corresponding to data on 39.198.414 strains from humans; 932.140 strains from animals; 2.943.702 HAI); outbreak reports from 806 journal publications and 4 national mandatory AMR outbreaks database; emerging resistances reports from 550 journal publications for 12 “new” antibiotics. Such a large central data repository (CDR) for epidemiology data derived from multiple European public health surveillance activities to our knowledge does not exist elsewhere. Thus, the EPI-Net CDR is a unique, resourceful asset for epidemiological research on AMR and HAI. The health dataset at the core of EPI-Net CDR can be analysed by interested parties to inform among others European public health actions, biopharmaceutical R&D on AMR and HAI, and country site selection for clinical trials on AMR and HAI.

The EPI-Net AMR travel tool (https://epi-net.eu/travel-tool/overview/) includes data on: AMR surveillance for 2.018.241 isolates from 86 European and non-European countries; antibiotic-resistant bacteria prevalence of carriage from 11.679 international travellers collected from 34 studies; and indications from 15 guidance documents published by major public health agencies. The different sections of the tool once revised and approved by a panel of experts on AMR surveillance and by members of the public and patient involvement (PPI) panel, was made available online. The tool provides a valuable resource for teaching and a repository that facilitates a stepwise assessment of the risk of AMR spread and implementation of optimized infection control measures. It also provides a source of knowledge of global AMR epidemiological data, making information available for decision-making on personalized antibiotic therapies, screening activities and guidelines development.

The EPI-Net consensus programmes have resulted in significant educational tools that address key knowledge gaps and are aimed to assist healthcare professionals and other stakeholders in AMR research and policy, including members of the veterinary and environmental sectors (https://epi-net.eu/publications/). In collaboration with the JPI-AMR ARCH network, for example, the team brought together experts from different sectors and networks in the field of animal and human surveillance to bridge the gap between surveillance data and antibiotic stewardship in both sectors. Following up this approach, the EPI-Net team has taken a further initiative whereby an expanded multidisciplinary expert panel representing the human, animal, and environmental sectors, elaborated proposals for structuring and reporting AMR and antimicrobial consumption (AMC)/antimicrobial residues (AR) surveillance data across the three sectors. The evidence-supported, modified Delphi approach was used to reach consensus among the experts for different aspects including i) dissemination frequency, language and overall structure of reporting; ii) core elements and metrics for AMC/AR data; iii) core elements and metrics for AMR data. The indications proposed can support multisectoral policy and decision-making to reduce AMR rates applying a One Health approach.

WP3A: Epidemiology of hospital-acquired infections in the intensive care unit (ICU)

WP3A partners are taking their work forward through several other project avenues including a recently granted JPIAMR " Improving surveillance of antibiotic-resistant *Pseudomonas aeruginosa* in Europe (ISARPAE)".

WP3B: Impact of different Interventions on *P. aeruginosa*-attributed disease burden among patients in the ICU

The statistical methodology improved the understanding of the data analysis and provided additional insights. One way to advance the field of study would be to incorporate artificial intelligence algorithms in the multistate analysis. Another promising research area is to combine or expand on new approaches from a causal perspective, such as emulating target trial design or g-computation. As time zero is typically fixed (the time from treatment initiation) and medical professionals frequently require dynamic treatment regimes, there is potential to extend the methodology to incorporate 'flexible and dynamic' time zeros.

WP4A: A phase 2 study to evaluate the efficacy of MEDI3902 in mechanically ventilated subjects

Overall, the EVADE study was essential in helping to build the COMBACTE network as the first efficacy trial initiated by the COMBACTE-MAGNET consortium. This required a highly productive collaboration between academia and industry. EVADE also greatly contributed to site capacity building through several investigator and laboratory trainings, site visits, site calls, providing sites with state-of-the-art molecular diagnostic tools and the opportunity for less experienced site personnel to visit top performing sites for on-site training.

Several innovative methods were developed during the conduct of EVADE. First, only patients colonized by *P. aeruginosa* in their airways were eligible for inclusion in the study. Restricting inclusion

to these patients was expected to enrich the population likely to develop *P. aeruginosa* pneumonia, since tracheobronchial colonization by this bacterium increases the risk of developing VAP by approximately 8-fold. Accordingly, such a "prognostic enrichment" increases the likelihood of identifying a signal of benefit or harm in a less heterogeneous population of ventilated patients and increases efficiency by reducing the sample size needed to observe an effect. Such a strategy is now also used by ARSANIS, a biotech company interested in developing pathogen-specific monoclonal antibodies.

Second, airway colonization was assessed by rapid polymerase chain reaction assays specifically designed for this study by Cepheid GeneXpert, hoping that such a rapid diagnostic testing would bypass the time required to obtain results from conventional microbiological cultures which can take 48-72 hours. Such tests would certainly greatly simplify the screening process in future studies and allow patients to be recruited before the bacterial load present in the airways spirals out of control. Several assays were also developed thanks to the samples of the patients enrolled in EVADE, such as the PA Luminex multiplex-20 assay to detect antibodies against 20 P. aeruginosa antigens in serum, or to validate GeneXpert PA assay on endotracheal aspirates (cf. to WP3A results).

Third, study endpoints were developed in accordance with the US Food and Drug Administration and the European Medicines Agency, opening the possibility of speeding the administrative review of the results in case of positive results. Finally, an interim pharmacokinetic analysis was done to assess the pharmacokinetic profile of the monoclonal antibody in mechanically ventilated critically ill patients, which was pivotal for discontinuing the MEDI3902 low dose and understanding why the results of the study were negative.

Monoclonal antibodies represent a potential alternative to systemic antibiotics for the prevention of infections because they do not influence antibiotic sensitivity. Unfortunately, our study was not powered enough to confirm this potential benefit. However, additional studies are underway to see if their use could modify the microbiota in a favorable way.

Although the primary efficacy endpoint of reduction in PA pneumonia incidence was not achieved in the present study, our results provide important lessons on pathogen-specific PA pneumonia complexity in ICU trials, with potential implications for future study design. For instance, our trial did not achieve its planned sample size, with the recruitment being stopped early due to low enrolment and thus was underpowered to detect small but clinically important treatment effects in the entire study population, as well as in specific subgroups of patients. The main contributing factor of low recruitment was that the proportion of patients meeting all the eligibility criteria was lower than expected, which resulted in an average of 4 patients randomised per site. Other major contributing factors included the unusually complex screening process and the difficulty for the attending clinicians to distinguish patients who were actually infected with PA from those only colonised, often resulting in the immediate administration of new antibiotics and making patients ineligible for randomisation. Future studies should certainly take these difficulties into account and deploy appropriate strategies to counteract as much as possible the difficulties in recruitment of appropriate subjects that are inherent in such a trial.

In summary, designing clinical trials to assess the safety and efficacy of anti-virulence agents is challenging from several standpoints and cannot be done efficiently without combining the forces and expertise of academia and pharma companies. The Innovative Medicines Initiative–funded COMBACTE consortium was able to foster such an academic-industry partnership between GHPS/CHU Limoges and AstraZeneca. This allows to design a very relevant trial in accordance with the actual clinical scenario of pneumonia in ICU patients requiring mechanical ventilation at high risk of developing pneumonia caused by *P. aeruginosa*. Other advantages of this collaboration were access to and proper use of rapid diagnostic platforms at clinical sites, specimen collection and analysis, statistical considerations for determining population size, and identification of the most important and critical primary clinical end points, according to guidance from the U.S. Food and Drug Administration and the European Medicines Agency.

Microbiome study:

Low sample availability and unbalanced sample distribution by outcome categories are major limitations of this study. Due to the post-hoc nature of this study and to ensure data quality, only aliquots never exposed to a freeze/thaw were used in this analysis. To avoid such limitations microbiome analysis should be included as an exploratory endpoint and samples should be collected specifically for microbiome analysis using the best practices established by this study.

WP5: Epidemiology study to inform WP6 clinical studies

Complicated urinary tract infections (cUTI) are among the most common health-care associated infections and nosocomial bacteremia. A recent publication acknowledges that cUTI accounted for over 625,000 hospitalizations yearly in the US, comprising 1.8% of all admissions (doi:10.1093/ofid/ofw2813). The biggest challenges in treating cUTIs are the wide variety of presentations and severity of the disease, and the sustained rise of antimicrobial resistance.

According to this data, the impact of cUTI in the healthcare system is substantial. Our research was focused in the epidemiology, antimicrobial resistance and outcomes of cUTI in south European countries and Israel, which have a high prevalence of MDR Gram-negative bacteria. We have studied in detail more than 1,000 patients with cUTI. The results of the clinical epidemiology, multidrug resistance and outcomes have been published in eight articles with the following citations according to WEB of SCIENCES (date March 13, 2023):

1. Shaw E, Addy I, Stoddart M, Vank C, Grier S, Wiegand I, Leibovici L, Eliakim-Raz N, Vallejo-Torres L, Morris S, MacGowan A, Carratalà J, Pujol M; COMBACTE-MAGNET Consortium. Retrospective observational study to assess the clinical management and outcomes of hospitalised patients with complicated urinary tract infection in countries with high prevalence of multidrug resistant Gram-negative bacteria (RESCUING). BMJ Open. 2016;6:e011500. doi: 10.1136/bmjopen-2016-011500. Citations: 10

2. Gomila A, Shaw E, Carratalà J, Leibovici L, Tebé C, Wiegand I, Vallejo-Torres L, Vigo JM, Morris S, Stoddart M, Grier S, Vank C, Cuperus N, Van den Heuvel L, Eliakim-Raz N, Vuong C, MacGowan A, Addy I, Pujol M; COMBACTE-MAGNET WP5- RESCUING Study. Predictive factors for multidrug-resistant

gram-negative bacteria among hospitalised patients with complicated urinary tract infections. Antimicrob Resist Infect Control. 2018;7:111. doi: 10.1186/s13756-018-0401-6. eCollection 2018. Citations: 25

3. Gomila A, Carratalà J, Eliakim-Raz N, Shaw E, Wiegand I, Vallejo-Torres L, Gorostiza A, Vigo JM, Morris S, Stoddart M, Grier S, Vank C, Cuperus N, Van den Heuvel L, Vuong C, MacGowan A, Leibovici L, Addy I, Pujol M; COMBACTE MAGNET WP5 RESCUING Study Group and Study Sites. Risk factors and prognosis of complicated urinary tract infections caused by Pseudomonas aeruginosa in hospitalized patients: a retrospective multicenter cohort study. Infect Drug Resist. 2018;11:2571-2581. doi:10.2147/IDR.S185753. eCollection 2018. Citations: 19

4. Vallejo-Torres L, Pujol M, Shaw E, Wiegand I, Vigo JM, Stoddart M, Grier S, Gibbs J, Vank C, Cuperus N, van den Heuvel L, Eliakim-Raz N, Carratala J, Vuong C, MacGowan A, Babich T, Leibovici L, Addy I, Morris S; RESCUING Study Group and Study Sites. Cost of hospitalised patients due to complicated urinary tract infections: a retrospective observational study in countries with high prevalence of multidrug-resistant Gram-negative bacteria: the COMBACTE-MAGNET, RESCUING study. BMJ Open. 2018 Apr 12;8(4):e020251. doi: 10.1136/bmjopen-2017-020251. Citations: 24

5. Eliakim-Raz N, Babitch T, Shaw E, Addy I, Wiegand I, Vank C, Torre-Vallejo L, Joan-Miquel V, Steve M, Grier S, Stoddart M, Nienke C, Leo VDH, Vuong C, MacGowan A, Carratalà J, Leibovici L, Pujol M; RESCUING Study Group. Risk Factors for Treatment Failure and Mortality Among Hospitalized Patients With Complicated Urinary Tract Infection: A Multicenter Retrospective Cohort Study (RESCUING Study Group). Clin Infect Dis. 2019;68:29-36. doi: 10.1093/cid/ciy418. Citations: 34

6. Gomila A, Carratalà J, Eliakim-Raz N, Shaw E, Tebé C, Wolkewitz M, Wiegand I, Grier S, Vank C, Cuperus N, Van den Heuvel L, Vuong C, MacGowan A, Leibovici L, Addy I, Pujol M; RESCUING Study Group and Study Sites. Clinical outcomes of hospitalised patients with catheter-associated urinary tract infection in countries with a high rate of multidrug-resistance: the COMBACTE-MAGNET RESCUING study. Antimicrob Resist Infect Control. 2019;8:198. doi: 10.1186/s13756-019-0656-6. eCollection 2019. Citations: 18

7. Turjeman A, Babich T, Pujol M, Carratalà J, Shaw E, Gomila-Grange A, Vuong C, Addy I, Wiegand I, Grier S, MacGowan A, Vank C, Cuperus N, van den Heuvel L, Leibovici L, Eliakim-Raz N; COMBACTE MAGNET WP5 RESCUING Study Group and Study Sites. Risk factors for enterococcal urinary tract infections: a multinational, retrospective cohort study. Eur J Clin Microbiol Infect Dis. 2021;40:2005-2010. doi: 10.1007/s10096-021-04207-4. Citations: 3

8. Babich T, Eliakim-Raz N, Turjeman A, Pujol M, Carratalà J, Shaw E, Gomila Grange A, Vuong C, Addy I, Wiegand I, Grier S, MacGowan A, Vank C, van den Heuvel L, Leibovici L. Risk factors for hospital readmission following complicated urinary tract infection. Sci Rep. 2021;11:6926. doi: 10.1038/s41598-021-86246-7. Citations: 1

The impact that our studies can have is remarkable. We have clarified the frequency of the different types of cUTI, particularly urinary catheter associated UTI and the impact on antimicrobial resistance. In addition, a major potential impact is that in patients with cUTI we found no benefit of early appropriate empirical treatment on survival rates or other outcomes. Thus, physicians might consider

supportive treatment and watchful waiting in stable patients until the cause of sepsis is clear and the causative pathogen is defined. This can avoid unnecessary antibiotic treatments and reduce selective antibiotic pressure on multidrug-resistant bacteria.

1.7. Lessons learned and further opportunities for research

Public private partnership (PPP)

The interdisciplinary interplay between clinicians, epidemiologists, statisticians and our industry partners was very constructive and fruitful. More specifically, the collaboration in a public private partnership helped to identify areas of interest and specific objectives both for academic and industry partners in areas related to surveillance, and provided each partner with a broader scope. Also, the exchange of information about methods for data detection, collection and analysis for surveillance of resistant pathogens were helpful.

The collaboration with **AstraZeneca** was very useful and added multiple benefits to the work done in the project. The expertise and knowledge on VAP and on setting up clinical trials in Europe and beyond was helped to facilitate a relatively quick set-up of the ASPIRE-ICU and EVADE trials. Other key aspects of added value were in the support in setting up assays and in tech transfer from AstraZeneca to biomarker partners. There were several immunological/serological assays that were transferred to UMCU. UA aimed to perform immunological assays in respiratory samples which was a first-of-its-kind analysis. The expertise and interest of AstraZeneca was very valuable in taking these forward successfully. Also, collaboration on bioinformatics analysis of *P. aeruginosa* whole genome sequencing data helped with performing rapid analyses on large numbers of isolates/samples as well as disseminating the data quickly towards all the partners for downstream analyses. The capacity to perform Big Data analysis on sequenced genomes was specifically useful. UA also collaborated with AstraZeneca by performing validation of the GeneXpert PA assay with fresh respiratory samples collected from the Antwerp University Hospital to support its utilization of this assay.

The collaboration with **AiCuris** was also very useful and added multiple benefits to the work in COMBACTE-CARE. In the retrospective epidemiological study in which more than 1,000 patients with cUTI have been included, public-private collaboration has provided a very important benefit. Under public collaboration, based on infectious disease clinicians, the most relevant epidemiological, clinical and microbiological aspects of cUTI were discussed and established. Complicated urinary infection includes a wide range of different infections. This perspective has been very important to carry out the study and focus on some outstanding aspects such as urinary catheter-associated infections and multidrug-resistance. Despite the fact that the urinary catheter is an essential device in healthcare, there is still a great lack of knowledge about infectious complications in the modern time under severe antibiotic pressure. On the other hand, the collaboration of the pharmaceutical industry has been very relevant. The pharmaceutical companies have extended experience in clinical research. They have been involved from the beginning in the development of the study, from the elaboration of the study protocol, investigator meetings, development of the study, contingency plans, study reports and study publications. After this experience, we can emphasize that this public private partnership have had synergistic effects.

A key infrastructure established in COMBACTE-CARE is the publicly-accessible **EPI-Net surveillance-dedicated platform** (epi-net.eu), which provides access to public health surveillance data on antimicrobial resistance, healthcare-associated infections, outbreaks, and emerging resistances from multiple data sources. It also includes educational tools and interactive visualizations, such as the AMR travel tool, that provide an example on how to facilitate a straightforward access to surveillance data and how these data can be made timely available for different users. The interest, support, and feedback from the industry leads and partners for the EPI-Net website have been crucial in driving its development and sustainability. In this regard, the industry is also showing an increased interest in sharing its surveillance data (example, the Vivli platform). Therefore, future efforts could focus on combining public and private data sharing platforms to enhance accessibility of data for future epidemiological/clinical research.

Furthermore, one of the main objectives of the COMBACTE-CARE project was to develop consensus documents to improve European surveillance capacity. All consensus efforts have encompassed representatives from different sectors including the industry, thereby providing a comprehensive public-private perspective on limitations, solutions, and agenda for future research. Inclusion of both industry and public partners in our experience enriches the debate and the momentum for innovation, particularly if the research outputs from European projects are to be beneficial on a global scale and for both high and low- and middle-income countries.

Recommendations from the public-private partnership (PPP)

The combined knowledge of a PPP for the validation and implementation of assays beyond standardised tests should be more utilized in performing in-depth studies while being capable of both validating novel diagnostic tools or markers as well as potential therapeutics.

Potential new research to further advance the field

Many of the biomarker partners are finalizing their current manuscripts on the analyses and are planning individual follow-up studies with the large sample/isolate collections available. The biomarker partner collaboration formed within the consortium is already aiming to collaborate in follow-up studies. One of these studies is investigating and surveillance of antibiotic-resistant *Pseudomonas aeruginosa* in Europe, which aims to provide training and manuals in order to decrease treatment failures and stop the spread of antibiotic-resistant *P. aeruginosa*. There currently is also a large collection of *P. aeruginosa* isolates at the central lab and that will be utilized in future research. There was already a request from the biomarker partners to create a reference panel with these isolates for potential phage treatments. Further we plan to validate the robust biomarkers emerging from the respiratory analyses. These will be utilized to predict development of PA or SA VAP.

Additionally, there is a wide range of unmet needs in cUTI. The major needs to further advance in this field should be focus in the prevention as well as in the assessment of effective antimicrobial therapy. Similar to how it has been carried out in the prevention of vascular catheter-related bacteremia, it is necessary to establish which measures are effective and must be included in the bundles for the prevention of complicated urinary tract infection. Likewise, it is necessary to investigate innovative technologies and materials that allow the reduction or delay of infectious complications related to the

urinary catheter. The prevention of infections leads to a lower use of antibiotics and should be a preferred way to avoid antibiotic resistance.

On the other hand, the field of clinical trials and antibiotic therapy is very important. It is necessary to establish the role that certain antibiotics must play, for example fosfomycin, plazomicin, or new cephalosporins or carbapenems in the treatment of cUTI. Other relevant aspects are related to the duration of antibiotic treatment. In general, antibiotic treatment for urinary catheter-associated infection is usually prolonged, longer than necessary, due to the comorbidities that these patients usually present. It is necessary to delve into studies that confirm our preliminary data in relation to which patients in a stable situation can wait and receive targeted antibiotic treatment. On the other hand, since complicated UTI is one of the most prevalent health-care related infections associated with antibiotic resistance, the patient public involvement (PPI) could be an important tool in the development of new strategies that allow us to prevent or control antibiotic resistance.