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FULL REVIEW DRAFT

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Abstract
For 70 years antibiotics have saved countless lives and enabled the development of modern medicine, but it is becoming clear that the success of antibiotics may have only been temporary and we now anticipate a long-term, generational and perhaps never-ending challenge to find new therapies to combat antibiotic resistant bacteria. As the search for new conventional antibiotics has become less productive and there are no clear strategies to improve success, a broader approach to address bacterial infection is needed. This review of potential alternatives to antibiotics (A2As) was commissioned by the Wellcome Trust and jointly funded by the Department of Health, involving scientists and physicians from academia and industry, has identified 20 A2A approaches now being actively explored. The feasibility and potential clinical impact of each approach was considered. The most advanced approaches (and the only ones likely to deliver new treatments by 2025) are antibodies, probiotics, and vaccines now in Phase II and Phase III trials. These new agents will likely target *P. aeruginosa*, *C. difficile* and *S. aureus*. However, other than probiotics for *C. difficile*, this first wave will likely serve best as adjunctive or preventive therapies. This means that conventional antibiotics will still be needed. The economics of pathogen-specific therapies must improve soon to encourage innovation and greater investment into A2As with broad-spectrum activity (e.g. antimicrobial-, host defense- and, anti-biofilm peptides). Increased funding, estimated at >£1.5 bn over 10 years is required to validate, develop and exploit these A2As. Investment needs to be partnered with translational expertise and targeted support to validate these approaches to Phase II proof of concept. Such an approach could transform our understanding of A2As as effective new therapies and should provide the catalyst required for both active engagement and investment by the pharma/biotech industry. Only a sustained, concerted and coordinated international effort with funding akin to space exploration will provide the answers needed for the next decade.

Introduction

Given the rapidly rising tide of antibacterial resistance and the very limited pipeline of conventional antibacterial agents, a review, delivered by a working party of 24 scientists from academia and industry was commissioned by the Wellcome Trust and jointly funded by the Department of Health to consider the prospects for alternatives to antibiotics (A2As). While there have been technical reviews of individual alternative approaches,1 this review seeks to define the current state of A2As at the portfolio level, prioritise approaches, and provide evidence-based expectations for their delivery in order to inform funding decisions and policy in this crucial area of healthcare.

Search strategy and selection criteria

A2As were defined as non-compound approaches that target bacteria or approaches that target the host. Thus an antibody targeting a virulence factor or quorum sensing would be included but a compound targeting these processes would not.[2,3] Biologicals or compounds targeting the host were included. This review focused on therapies that could be developed to treat systemic/invasive rather than superficial infections and is therefore limited to therapies that are administered orally, by inhalation or by injection. External topical administration was beyond its scope. The primary objective was to identify and review prospective therapeutic replacements for antibiotics. Alternatives that could be used in combination with conventional antibiotics and prophylactic approaches were also considered.

The review benefited from non-confidential information on projects provided by its members and their knowledge base which was used to identify projects and companies, key publications and provision of expert project summaries to inform the working party. Additional preclinical and clinical projects were identified through a series of searches of PubMed, the internet using Google and ClinTrials.gov up to 27 February 2015, by use of key terms such as “antibody”, “probiotic”, “lysin”, “bacteriophage”, “vaccines”, “antimicrobial peptide”, “lantibiotic”, “host defense peptide”, “innate defense peptide”, “antibiofilm peptide”, “immunomodulation”, immune stimulation”, “immune suppression”, “vaccine”, “liposome”, “chelation” and, if necessary, their use with “*E. coli* OR *P. aeruginosa* OR *K. pneumoniae* OR *A. baumanii* OR *C. difficile* OR *S. aureus* OR infection OR bacteria” followed by inspection of the papers and top 30 websites listed. Once proteins or compounds (Tables 1 and 2) and the organization developing them has been identified, their names were used for additional searches e.g. “Merck”. “MedImmune”, “Aridis”, “Seres”, “Rebiotix”, “Shire”, “Viropharma”, “Intron Biotechnology”, “Contrafect”, Ampliphi”, “Phico”, “Akthelia”, “Sanofi Pasteur”, “Valneva”, “Pfizer”, “Roche”, “Novacta”, “Adenium” and the associated company website overview, pipeline and news pages. The state of alternative project pharmacology was assessed by searches of PubMed for articles to 27 February 2015 by use of terms “pharmacokinetic OR safety” with “human OR mouse OR rat” in combination with “host defense peptide”; Antibiofilm peptide”; “lantibiotic”; bacteriophage”; “lysin” and in the case of antimicrobial peptides “antimicrobial peptide” with “pharmacokinetic OR safety” and “*E. coli* OR *P. aeruginosa* OR *C. difficile* OR *S. aureus*” followed by inspection of the 238 papers listed. We also reviewed studies cited in articles identified by this search and included them when relevant.

The review considered 1. Feasibility of informative trials; 2. Magnitude of medical potential; 3. Likelihood and consequences of resistance; 4. Level of current research activity; 5. Likely timeline to registration, and 6. Activities that might enable validation and progression. The review process comprised (a) preparation of a ~50-page working document summarising 20 current A2A, (b) a meeting to review and prioritise approaches, and (c) collective preparation of a report for the funders which is summarised in this review. This allowed the group to compile and share broad and well-informed views on the state-of-the-art for A2As with a wider community. Editorial control of the report was the responsibility of a sub-group without conflicts of interest (LC, BFG, IRH, BVJ, AK, H-HK, SO, SS, TT and JHR).

The portfolio of alternative approaches

The working party identified 20 approaches for consideration and recognised that the list may be incomplete (Tables 1 and 2). Projects were not reviewed in sufficient detail to make individual funding recommendations. The technical feasibility and clinical potential of the approaches were considered but the commercial attractiveness, potential return on investment or potential for reimbursement were not. Given the wide range of views within the group, this review does not represent a unanimous consensus. We recognise perspectives differ, that there are gaps in available data, and that science will continue to advance. This means that this

review should be taken as a snapshot of A2As and their perceived potential. Ten alternatives were prioritised and considered in more detail (Table 1). The other 10 approaches were not prioritised at this time because (i) other projects were considered more advanced in the translational pipeline; (ii) there was insufficient peer-reviewed information to assess their potential clinical impact, feasibility, or safety (Table 2). Regular review of the portfolio at 2-year intervals is recommended.

The potential of the top 10 approaches, with the exception of the recently discovered antibiofilm peptides, has been known for more than a decade but has not led to therapeutic products.[90] This is an emerging field. For instance, there appear to be only 5 published pharmacology studies (two for Plectasin, two for lantibiotics and one for cyclic peptides) in the antimicrobial peptide field, and one published preclinical safety study across the field of Lysin, Bacteriophage, Antimicrobial-, Host Defense- and, Antibiofilm- Peptides.[91-96] While there have been A2As clinical studies, the associated preclinical characterisation remains proprietary with insufficient published peer-reviewed evidence to understand the pharmacokinetic, pharmacodynamic, toxicology and safety strengths and liabilities of these approaches. This increases risk and averts funding. However, failure of the first clinical studies should not be allowed to block future exploitation. Indeed, many of the barriers to the development of these alternative strategies also exist for the development of novel small molecule antibiotics.

The top 10 approaches, which the working party considered merited attention now were placed into two tiers. The focus within Tier 1 was on clinical development and in Tier 2 on preclinical development over the next 5 years. Success of Tier 1 projects in Phase II and Phase III studies could transform the perception of the A2As portfolio. Access to funding through key preclinical and clinical development steps (e.g. production and characterisation, formulation, pharmacokinetics and pharmacodynamics, toxicology and safety pharmacology) with subsequent publications showing how these data support continued drug development was considered to be critical to progress towards clinical validation and to build confidence in the field. Supported studies should define and test clear go/no-go decision points for product progression. Primarily *in vitro* programmes of work or those focused entirely on surrogate endpoints e.g. characterising cytokines rather than pathology or microbiology may not be competitive. Application of "major pharma" development resources and expertise will be critical to validation and progression of A2As in a timely manner. Reliance on the academic and biotech communities alone may not not be sufficient to provide new products within a decade.

Based on a combination of high clinical impact and high technical feasibility, the approaches anticipated to have the greatest potential to provide A2As were: (a) Phage Lysins as replacements; (b) Vaccines as prophylactics; (c) Antibodies as prophylactics, and (d) Probiotics as treatments or prophylactics for *Clostridium difficile*- and antibiotic- associated diarrhoea (CDAD/AAD). Bacteriophages (Wild-type and Engineered) were also considered to have potentially high impact as replacements but the feasibility of their entry into the market was unclear. Selected Immune Stimulation approaches were considered feasible as prophylactics or adjuncts to conventional treatments but their clinical impact was also unclear at this time.

A2As portfolio analysis

To enable an evidence-based review of the current state of development and likelihood of success of the prioritised alternative approaches, extensive internet searching and the knowledge base within the working party were used to define the breadth (number of projects and targets) and depth (current phase of development) of the A2As portfolio. In particular, company websites and news releases were used to identify projects currently (Q1 2015) being actively progressed (Table 3).

Industry standard timelines for clinical development phases, Phase I - 1 year, Phase II - 2 years, Phase III - 3 years, and Registration - 1 year were used to estimate the earliest likely date of product registration.[97–100] The estimated year of registration will likely differ from a particular sponsor's estimates or project timelines. Host Defence Peptides and Antibiofilm Peptides were excluded because they were too early for this analysis.

Similarly, industry-standard probabilities of success across projects in different phases of development; preclinical to Phase I – 23%, Phase 1 – 45%, Phase II – 47%, Phase III – 71%, and Registration – 90%, were applied.[97–100] The estimates of the probability of success for individual projects within the class were summed. Values >100% indicate that there are sufficient project numbers and/or project maturity to anticipate that at least one product could be registered if access to sufficient funding and skilled development resources is provided.

Industry standard costs for clinical development phases: Phase I - £6m, Phase II - £10m, Phase III - £45m, and Registration – £1.3m were used to estimate the cost of portfolio projects. [97–100] These estimated costs will likely differ from a particular sponsor's estimates.

This uniform approach was taken because (a) similar levels of project planning data are not available for all projects, (b) when available, project-specific timelines developed by sponsors often shift, and (c) use of standard timelines allows uniform (re)calculation of the data as required.

The strength of this type of analysis is that it removes any personal bias but its weakness is that it is almost always incorrect in the specifics of its details.

Analysed by approach, the pipeline for Antibodies, Probiotics and Vaccines was sufficient to anticipate success as the probability of registration is >100%. However, for other alternative areas there are too few projects ongoing and/or they are currently too early to allow for anticipated project attrition. For instance, on the basis of the current portfolio, we cannot assume that Lysins, Bacteriophages or Antimicrobial Peptides will contribute to new therapies.

It is important to note that most of the current novel activity is focused on C. *difficile*, *P. aeruginosa* and *S. aureus* only. The timeline analysis suggests that if successful, registrations might be: Antibodies – 2017; Probiotics – 2018; Vaccines – 2019; Immune Stimulants 2021; Lysins and Antimicrobial Peptides – 2022; Wild-type and Engineered Bacteriophages – 2023; and Host Defense and Antibiofilm peptides from 2027 onwards.

When analysed by pathogen, the 'Probability of Success' analysis indicates that if the alternative portfolio is adequately funded, we could expect two new products (Antibody, Probiotic or Vaccine) for CDAD/AAD by 2019; one for *P. aeruginosa* (Antibody or Vaccine) by 2021, and one for *S. aureus* (Antibody, Lysin, or Vaccine) by 2022. The current portfolio lacks sufficient breadth and depth to anticipate multiple new products for these pathogens during this time frame. It is a matter of concern that there is little activity on the other ESKAPE pathogens (e.g., *Enterococcus, Klebsiella, Acinetobacter* or *Enterobacter*) or directed towards other *Enterobacteriaceae*. This means that it is most unlikely that A2As for these life threatening pathogens will emerge in the next 10 years.

As the portfolio advances through the later development phases, costs will increase and innovative funding arrangements will be required to maintain momentum given that most pharma companies have withdrawn from the "antibiotic area".

The working party recognised that by 2018/2019 we could anticipate observing success in multiple projects at Phase II and that this could encourage greater investment in the sector. New projects starting in 2018/2019 might be expected to reach registration by 2030.

The working party found that A2As have the potential to deliver clinical benefit but the scale of current activity and availability of funding will have to increase substantially to achieve that benefit.

What will the portfolio cost?

To estimate funding requirements, the named projects from Table 3 were budgeted to 2025 using industry standard costs for clinical development phases. Although some organisations may currently aim to deliver with smaller budgets, the application of standard costs serves to reflect prior reality for delivery and to remove bias.

The funds for the current phase of the project are assumed to be in place and committed. By adding the cost of each subsequent stage that each project has to pass up to registration and application of the risk estimates at each stage of development, as described above, the funds required to registration were calculated.[73]

Where the alternative project portfolio is too thin or early to anticipate success, new funding is required to strengthen the portfolio. A key objective should be to test A2As at Phase II to validate the approach and to adequately understand the clinical potential of the approach it may be necessary to take several projects to Phase II. The Lysins, Bacteriophage and Antimicrobial Peptides approaches have advancing projects but too few projects to anticipate adequate testing of the concepts. They also require additional investment to build capacity and translational expertise to exploit their full potential. Allowing for anticipated project attrition, a pipeline to support the evaluation of a single project at Phase II would require 9 preclinical projects at £12.5 m/project over

5 years, leading to two Phase I and potentially one Phase II study at £135m. This funding recommendation is contingent on the results being peer reviewed and open access publication to provide the requisite evidence-base for informing future R&D.

The Host Defence and Antibiofilm peptide approaches are innovative and have broad spectrum potential. It may be necessary to advance the first wave of these innovative projects beyond Phase II to validate the approaches and to convince pharma, investors and clinicians. A greater level of investment (£575m) would be required to build a pipeline of Host Defence Peptide and Antibiofilm Peptide projects because they are currently in an early stage of development. An estimated 34 preclinical projects are required to provide 8 Phase I and 4 Phase II studies to get at least one project through to Phase III and product registration (Table 4). There are several natural and synthetic Host Defense Peptides and Antibiofilm peptides as potential starting points. Chemical modifications, hybrid peptides and chemical mimetics could be explored. Project creation and translational research and in this area could be accelerated by committing £85m/year for 5 years. This would provide a powerful incentive to build capacity and to progress towards clinical validation of these peptide based approaches.

Our analysis assumes that funding for named projects of £221m is available to complete their progression through their current project phase. Additional risk adjusted funding of £469m will be required for subsequent phases and should support development of one new product for each of *P. aeruginosa*, *C. difficile* and *S. aureus* by 2022. This level of investment would enable validation of antibodies, probiotics, and novel vaccines as A2As.

The Lysin, Bacteriophage and Antimicrobial Peptides portfolios needs to be increased in order to adequately test these approaches in a timely manner. This could be achieved with risk adjusted investment of £405m. Building an adequate Host Defense Peptide and Antibiofilm peptide portfolio will require £575m.

The working party therefore identified ~£1.5 billion of risk-adjusted funding that will be required to validate and exploit the current 10 high priority A2As in a timely manner. We did not forecast the funding requirements for the remaining 10 approaches or for additional blue-sky activity to add to the pipeline in the future.

Challenges to developing and deploying A2As

1. Innovation must be linked to translation expertise.
The innovators in this space (largely academics and biotechs) often lack industry-level development skills. Hence, increased funding needs to be partnered with investment in translational skills development. Alternative programmes may benefit from greater access to PK/PD, formulation, toxicology and manufacturing expertise. Provision of adequate funding for the multi-disciplinary teams and costs associated with the preclinical characterization of a lead candidate for clinical development will be a critical factor for success. Pre-competitive partnerships and the creation of development hubs might be one way to support this area. Implementation of US SBIR (Small Business Innovation Research)-like awards and calls to tender for and purchase desired research activities from biotech and pharma on behalf of the academic and SME/biotech alternative community is another innovative way to support this area. Such activities might encourage industry to become involved in a manner that develops both critical mass and sustainability.

2. Clinical Trials
Careful clinical trial design will be critical. Projects need to ensure that endpoints are relevant to both the patient and the physician, often but not necessarily exclusively based on endpoints grounded in how patients feel, function and survive. Unless the clinical signal is strong, there is a risk that the size and cost of the clinical trials required to demonstrate an incremental benefit will be too large for industry to support. Thus, it will be important to be willing to terminate projects if clinical success is either low probability or likely to have low impact. As with trials of new antibiotics, surrogate endpoints that are predictive of clinical efficacy should be included as secondary endpoints (e.g., changes in cytokine levels or changes in imaging of infections) but are unlikely to be acceptable as the basis for registration for high mortality life threatening infections.

3. **Economic models in this therapy area must be improved.**

In addition to adequate funding and expertise, development and deployment of alternative antibacterial medicines is dependent on a return on investment. The working party did not consider the economics of A2As but noted that replacing antibiotics will be a major challenge. At present, many of the A2As are pathogen- or

strain-specific. By comparison, most modern antibiotics have a broad spectrum of activity. For example, the recently approved combination of ceftolozane and tazobactam, for cIAI, cUTI and pyelonephritis, has clinical efficacy data for 10 pathogens including *Klebsiella spp*, *Escherichia coli* and *Pseudomonas aeruginosa* with clinical microbiology suggesting potential against another 20 pathogens.[101,102] Multiple alternative therapies would be required to provide similar spectrum of coverage. We did not address the additional costs for the diagnostics that will also be required. In the first instance, A2As are likely to focus on the most prevalent infections and may provide sufficient clinical benefit to ensure a return on investment. At best, they will be a partial replacement for antibiotics.

Innovative regulation:

Innovative therapies may require innovative regulation but there is a risk that inexperienced developers may increase barriers to commercialisation by unnecessarily raising regulatory and clinical hurdles. Bacteriophage therapies currently in development are an example of the kind of product driving the evolution of regulatory approaches. Broad conversations about options for the unique challenges of each alternative are required: a recent workshop hosted by EMA on bacteriophage is an example of how this work needs to be progressed.

3. Flexible delivery models:

Some A2As could be delivered by different mechanisms to those for traditional antibiotics. Instead of a single global manufacturing pipeline, the development of localised services akin to blood transfusion or stem cell harvesting and transplantation could facilitate patient benefit and should be considered. For example, localised bacteriophage therapy attuned to patient need within a hospital might be an appropriate model for some products.

4. One Health: the potential for alternatives in animal health

All of the A2As have potential uses in animal health and demonstration of efficacy in companion and agricultural animals could be important steps in derisking an approach before its clinical development in humans. The anticipated costs for many of the approaches may however, be prohibitive for animal use. Commitment to substantial subsidies may be required to incentivise alternative development for animal health.

5. Conventional antibiotics will still be needed.

At least initially, many of the A2As are likely to be trialled and used as adjuncts to antibiotics because their activities may be insufficient to provide sufficient therapeutic benefit on their own. While effective antibiotics are still available it may prove difficult to demonstrate superiority of standard of care when comparing an antibiotic with an antibiotic plus an A2A adjunct treatment. If resistance to the antibiotic develops, then its use in combination therapies will be compromised. In the longer term, it may be possible to demonstrate that combinations of A2A therapies could be used without antibiotics.

6. We anticipate that deployment of Alternatives will:

I. Entail reliance on improved and faster diagnostic technology to enable targeting of individual bacterial species, or even strains of species, rather than clinical indications
II. Be more often used for prophylaxis than for treatment
III. Require of multiple products to replace a single antibiotic
IV. Substantially higher costs than for traditional antibiotics
V. Require access to sufficient and sustained funding to enable timely R&D and prompt clinical evaluation.

Future outlook

The working party recognised that academic researchers and industry have successfully generated a diverse portfolio of potential A2As comprising projects from preclinical optimisation to Phase III studies and prioritised 10 approaches for more detailed review. The field is still emerging and holds promise provided that adequate funding is available to build capacity, a preclinical evidence-base is created to enable prioritisation and to progress optimised drugs to critical Phase II validation of innovative approaches.

There was little doubt that the field might deliver new medicines for *P. aeruginosa*, *S. aureus* and *C. difficile*. However, other than probiotics for *C. difficile*, this first wave of new agents is likely to serve best as adjunctive or preventive therapy. Therefore, traditional antibiotics will still be required.

If we have to depend on A2As in the future, we need to build capacity now and increase substantially the throughput of projects[103]. The working party estimated that the priority approaches alone require an investment of at least £1.5bn, committed in the next 5 years and spent within 10 years to initiate a pipeline of translational projects that would deliver these new therapies. Longer term significant and sustainable funding will be required to advance and exploit the wider A2A portfolio. Policy and funding must now be linked. Without adequate funding we must assume that new treatments to replace and/or supplement antibiotics will not be available for more than a decade if at all. Our analysis of just a subset of all of the activities that could contribute towards the fight against antimicrobial resistance suggests that funding is now the key limiting factor that is stalling a global response. Antimicrobial resistance has to become a major international science programme in order to deliver the solutions that society needs now. By comparison the Large Hadron Collider project cost ~£6bn and the International Space Station £96bn. Antimicrobial research probably requires an effort somewhere between the two.

Key Messages (as a text box)

- Alternatives to antibiotics: Non-compound approaches that target bacteria or approaches that target the host to treat infection
- Academics and industry have created at least 20 approaches that need to be further evaluated
- Understanding the potential of A2A will require experimental clinical medicine and not just drug discovery
- Enhanced translational expertise must be deployed to aid validation and progression of these A2As
- Exemplar projects must be advanced to II to enable clinical validation of approaches
- Antimicrobial resistance needs to grow into big science to deliver major new innovative therapies

Contributors

LC and JHR co-chaired the working party. All authors reviewed and contributed to the finalisation of the manuscript.

Declaration of interests

LC is a Director of Chemical Biology Ventures Ltd, and Abgentis Ltd and an employee of Persica Pharmaceuticals Ltd. MC consults and collaborated with AmpliPhi Biosciences Corporation. MD is a Director/ Shareholder in Novacta Biosystems Ltd and a Director of Cantab Anti-infectives Ltd. HF is a Director/Shareholder in Phico Therapeutics Ltd. VAF consults for Contrafect Inc. SF is a Director/Shareholder and Consultant for Absynth Biologics Ltd. REW has licensed projects to Elanco, holds alternative patents and is forming a new company. DH is an employee/Shareholder in AmpliPhi Biosciences Corporation. KH is a Director of TiKa Diagnostics Ltd. DK is Chairman of Absynth Biologics Ltd and Procarta Biosystems Ltd. SO is a Shareholder in Akthelia Pharmaceuticals. DP is an employee/Shareholder in GSK. SP is an employee/Shareholder MedImmune. SS is a Founder/Shareholder in Abzenza plc. JS is an employee/Shareholder in Cubist. CT is a Director of Plasgene Ltd. JHR is an employee/Shareholder in AstraZeneca, a consultant to F2G, and a consultant to Advent Life Sciences (an investor in F2G). All other authors declare no competing interests. LC and JHR received remuneration from The Wellcome Trust and the Department of Health for chairing the Working Party and managing the preparation of the report and this review.

Acknowledgments

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Tables

Table 1. Prioritised alternative approaches

Alternative Approach	Comment	Likely spectrum of activity and initial use	Recommendation over the next 5 years	Refs
Tier 1 (Primarily translational - funding to clinical evaluation at Phase 2)				
Antibodies	Antibodies that bind to and inactivate a pathogen, its virulence factors, or its toxin(s) were widely considered one of the alternative approaches most likely to have major clinical impact. Antibodies were considered a relatively low risk area with strong underpinning science, safe history of use, and a high degree of technical feasibility.	Prevent G+ and G- infection, possibly also adjunct use	Basic R&D and Translational	2, 4–7
Probiotics	Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host organism". It is considered likely that defined mixtures of bacteria or the use of non-toxigenic spores of *C. difficile* will provide therapeutic and prophylactic therapies that will improve on current clinical practice for the treatment of CDAD/AAD. Basic research to understand the mechanism of action of probiotics in different settings and how they might be used in combination with antibiotics and other A2As e.g. bacteriophages could enable their wider use in other indications.	Prevent or treat CDAD/AAD	Translational	8–13
Lysins	Phage lysins are enzymes used by bacteriophages to destroy the cell wall of a target bacterium and are potential replacements for antibiotics because of their direct antibacterial action, and also as adjuncts because they act to reduce bacterial burden and/or weaken biofilms. Emphasis on Lysins active against Gram-ve pathogens would be beneficial.	Treat G+ infection	Basic R&D and Translational	14–21
Wild-type Bacteriophages	Wild-type bacteriophage that infect and kill bacteria have the potential to replace antibiotics for some indications. Bacteriophage may be used in a comparatively small dose because they replicate when their host bacterium is present. During treatment of an infection they also evolve to infect the strains causing the disease. This replication and evolution makes them unique in pharmaceutical product development. In terms of pharmacology, the subject experiences more product than was dosed and that product can change over time so that what is sampled after dosing is not exactly what was given to the patient.	Treat G+ and G- infection	Basic R&D and Translational	22–26
Engineered Bacteriophages	The ability to genetically engineer to produce a new phage for therapeutic use is strong. Early indications are that many of the challenges associated with mixtures of wild-type phage, such as breadth of strain coverage, development of resistance, and rapid elimination after systemic administrationcould be addressed. One of the advantages of non-replicating phage might be in dose selection but there could be issues with manufacturing the larger doses required. Higher doses may also result in a greater host immune response which could limit the use of non-replicating phage to once/lifetime. One disadvantage could be that non-replicating phage cannot evolve during the course of treatment.	Treat G+ and G- infection	Basic R&D and Translational	27–30
Immune Stimulation	Successful antimicrobial therapy depends on a supportive immune response. Immune stimulation has been proposed as a potential adjunct approach in conjunction with broad-spectrum antibiotic therapy. Re-purposing of phenyl butyrate and vitamin D to enhance expression of natural antimicrobial peptides seems feasible. Oral bacterial extracts are registered and used clinically to reduce the incidence of respiratory tract infections in some at risk groups in some regions. If successful, additional clinical trials to confirm their efficacy in other populations would encourage wider use.	Prevent or provide adjunct therapy for G+ and G- infection	Basic R&D and Translational	31–37
Vaccines	With potential to substantially reduce the incidence of infection and therefore the need to use antibiotics, the long established investment in vaccines for new targets should continue. Given the ageing human population, we need better knowledge of the potential for vaccination in the elderly and how to dose to achieve protection in immune compromised individuals.	Prevention, G+ more than G- infection	Basic R&D, esp. on new adjuvants	38–52
Tier 2 (strong support for judicious funding while monitoring for breakthrough insights)				
Antimicrobial Peptides (AMPs)	The advantages of AMPs are their broad spectrum activity which includes most major Gram-negative and Gram-positive bacteria; their bactericidal and rapid action; low target-based resistance and their lack of immunogenicity. The extensive academic literature has	Treat or Adjunct for G+ and G- infection	Translational	53–61

	not led to therapeutic breakthrough for systemic treatments.			
Host/Innate Defense Peptides (HDPs/IDRs)	Host defense peptides and innate defense regulators are small natural peptides or synthetic peptides respectively, which have indirect antimicrobial effects. They act primarily by increasing expression of anti-inflammatory chemokines and cytokines, and by reducing the expression of pro-inflammatory cytokines. Additional resources are required to accelerate their preclinical evaluation and progression into clinical trials to provide clinical validation of the approach	Adjunct for G+ and G- infection	Basic R&D	62, 63
Antibiofilm Peptides	Peptides that specifically inhibit bacterial biofilm formation have been identified and are in preclinical development. Their use as adjunctive therapy could improve outcomes	Adjunct for G+ and G- infections	Basic R&D	64

G+ = Gram-positive, G- = Gram-negative, Basic R&D = provide support for fundamental research and preclinical proof of concept studies to validate approaches and extend into early translational work to characterise efficacy, pharmacology, pharmacodynamics and preliminary toxicology so that potential liabilities can be defined. Translational = focus support on bringing products into the clinic.

Table 2: Additional alternative approaches

Alternative Approach	Comment	Refs
Immune Suppression	A bacterial infection can lead to an excessive host innate immune response (ranging from the systemic inflammatory response syndrome (SIRS) to septic shock) in which the injury to the host is made much worse by the host's pro-inflammatory cytokine response. Selective manipulation of this cytokine response has the potential to be used in synergy with antibiotics to reduce pathogen induced but cytokine and neutrophil mediated tissue damage, and to accelerate the time to patient recovery. The medical need is high but past failures of Phase III clinical trials, despite promising preclinical and Phase I or Phase II data, means that this area of sepsis and septic shock has not been prioritised. New concepts are therefore needed to develop novel small as well as large molecule drugs for this high mortality area of infection whose incidence is increasing. In contrast to antibiotics, the healthcare sector would pay a large premium for a drug that was effective at reducing morbidity and mortality	65–67
Anti-resistance nucleic acids	Antibiotic resistance genes are frequently spread by highly contagious plasmids, particularly in Gram-negative pathogens. Effective removal of resistance genes could resensitise bacteria to conventional antibiotics. The approach may present substantial technical and regulatory challenges.	68–73
Antibacterial nucleic acids	The use of nucleic acids to directly kill bacteria is being explored in a variety of formats in both academia and biotech. In terms of their therapeutic potential, studies are at an early stage. At the very least, these tools will continue to be developed to support fundamental microbial genetics studies.	72–74
Toxin sequestration using liposomes	Pathogens often secrete toxins that damage mammalian cells and drive inflammation. Administration of liposomes to act as decoys for toxin binding has been shown to reduce damage to cells and reduce disease severity.	75
Antibiotic-degrading enzymes to reduce selection of resistance	When antibiotics are eliminated via the gut, exposure of the normal gut bacteria to the antibiotic may lead to development of resistance and drive CDAD/AAD. Phase 2 studies demonstrate that oral beta-lactamase can destroy the beta-lactam in the fecal stream. The clinical challenge of demonstrating these clinical effects at Phase III meant that this area was not prioritised	76-79
Metal Chelation	Bacterial pathogens require zinc, manganese and iron ions to fully express their pathogenicity/virulence, biofilm formation and multiple essential enzymatic and metallo-beta lactamase activities. Metal chelation may deny pathogens these key processes. Discussion with pharmacologists and toxicologists suggests that this approach is speculative and could present safety concerns	81-84
Alphamers	Alphamers are immune modifiers comprising an α-Gal epitope fused to a bacterial pathogen binding aptamer to redirect endogenous anti-Gal antibodies to the pathogen and hence enhance immune clearance.	85
Transcriptional regulatory compounds	The use of compounds to modify transcriptional regulation can resensitise pathogens to antibiotics e.g. the use of EthR-binding compounds to derepress expression of the ethA gene, a mono-oxygenase that activates ethionamide in *M. tuberculosis*. The compound sensitises some strains of *M. tuberculosis* to ethionamide	86
Apheresis of protective antibodies	In some patients with *P. aeruginosa* lung infection, antibodies bind to the pathogen and protect it from serum-mediated killing. Depletion of these antibodies restores the ability of serum to kill bacteria and initial clinical data suggest an improved clinical outcome.	87
Immune Stimulation by P4 Peptide	Phagocytic killing of bacteria can be enhanced by P4 Peptide, a chemically synthesised 28 amino acid peptide derived from the *S. pneumoniae* surface exposed virulence factor PsaA. P4 peptide stimulates opsonophagocytic uptake and killing in invasive disease models of *S. pneumoniae* infection in mice. The combination of P4 intranasally and i.p. IgG provided 100% survival in the mouse model and significantly reduced bacterial burden. A therapy based on P4, IgG and antibiotic is proposed. However, additional evidence may be required to support the use of iv IgG in severe pneumonia. The project recently received MRC DPFS funding to progress to Phase I studies.	88, 89

Table 3. A2A Portfolio Review as at Q1 2015

Approach	**Probability of Registration by 2025[+] and** Sponsor	**Risk Adjusted Cost of Projects** (current/subsequent phases) and Target	**Recommended pipeline investment to enable additional Phase 2 validation[#]** and Name	Phase at Q1 2015	Earliest Anticipated Registration*
Antibodies	**170%[+]**	**£54m/£106m**	-		
	Merck	*C. difficile*	MK-3415A	P3 ongoing	2017
	MedImmune	*S. aureus*	MEDI4893	P2 ongoing	2021
	Aridis	*P. aeruginosa*	AR-101	P2a complete	2021
	Aridis	*S. aureus*	AR-301	P2a ready	2022
	MedImmune	*P. aeruginosa*	MEDI3902	P1 ongoing	2023
	Aridis	*P. aeruginosa*	Aerucin	IND ready	2025
Probiotics	**124%[+]**	**£52m/£53m**	-		
	Seres	*C. difficile*	SER-109	P3 ready	2018
	Rebiotix	*C. difficile*	RBX2660	P2 ongoing	2019
	Shire (Viropharma)	*C. difficile*	VP20621	P2 ready	2022
Lysins	**26%[+]**	**£12m/£28m**	**£135m**		
	Intron Biotechnology	*S. aureus*	SAL200	P1 ongoing	2022
	Contrafect	*S. aureus*	CF-301	P1 ready	2022
Bacteriophages	**9%[+]**	**£13m/£57m**	**£135m**		
Wild-type					
	AmpliPhi	*C. difficile*	AmpliPhage-004	Pre-P1	2023
	AmpliPhi	*P. aeruginosa*	AmpliPhage-001	Pre-P1	2023
Engineered					
	Phico Therapeutics	*P. aeruginosa*	PT-3.1	Pre-P1	2024
Immune Stimulation	**43%[+]**	**£0m/£55m**	-		
	Akthelia	*C. difficile*	Phenylbutyrate/vitD	P2 ready	2021
	-	Various	Bacterial extracts	P1 ready	2022
Vaccines	**188%[+]**	**£74m/£66m**	-		
	Sanofi Pasteur	*C. difficile*	*C. difficile* toxoid vaccine	P3	2019
	Valneva	*P. aeruginosa*	IC43	P2/P3 ongoing	2019
	Valneva	*C. difficile*	IC84	P2 ongoing	2021
	Pfizer	*S. aureus*	SA4Ag	P2 ready	2021
Antimicrobial Peptides	**52%[+]**	**£16m/£104m**	**£135m**		
	Roche	*P. aeruginosa*	POL7080	P2 ongoing	2022
	Novacta Biosystems	*C. difficile*	NBV302	P1 ongoing	2022
	Adenium	*S. aureus*	AP-138	Pre-P1	2023
	Adenium	*UTI*	AP-139	Pre-P1	2023
	Adenium	*C. difficile*	AP-114	Pre-P1	2023
Other Peptides	-	-	**£575m**		
	-	Gram-ve and Gram+ve	-	Preclinical	2027

Table 4: Estimate of the project pipeline cost for Host Defence and Antibiofilm peptides

Phase	Preclinical	Phase I	Phase II	Phase III	Registration	Total
Stage probability of success	23%	45%	47%	71%	90%	
Number of projects	34	8	4	2	1	
Cost of Phase £m	12.5	6	10	45	1.3	
Portfolio cost £m	425	48	40	90	1.3	£575

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