Phage offer a real alternative

To the editor:

A News and Views piece by Steven Projan in your February issue offers a gratuitous, pessimistic assessment for the prospects of phage therapy *per se* (*Nat. Biotechnol.* 22, 167–168, 2004). We believe Projan's criticisms are overly broad and fail to consider the published literature and the impact that contemporary phage biology is having on the development of phage therapeutics. We would not have been moved to respond to his comments were it not for our view that the pharmaceutical industry's capacity to develop truly novel chemical antibiotics or antibacterials is being outstripped through the evolution of antimicrobial resistance by a broad array of infectious agents. Thus, in the spirit of a constructive dialogue, we—participants at a Cold Spring Harbor Banbury Conference[1]—offer the following rejoinder.

Projan argues that claims for the efficacy of phage therapy come from uncontrolled studies and individual case reports. In this regard, a close reading of the history of phage therapy is instructive. Phage therapy was used extensively in the pre-antibiotic era when phage biology was poorly understood and before properly designed randomized clinical trials had been undertaken for any medical intervention. This era nonetheless provided many proofs of concept for the safety and efficacy of phage therapy. Then, as with many other areas of infectious disease research, the advent of the antibiotic era brought clinical studies of phage therapy to an end, except in some states of the former Soviet Union where phage therapy continues to be used, although it is not systematically studied by contemporary clinical trial standards. Thus, it is wrong to infer that phage therapy has been tested and failed. Rather, it is abundantly clear that phage therapy is highly likely to cure a variety of infectious diseases; modern investigators must now conduct the same kinds of preclinical and clinical testing that serve as the metric for chemical antibiotics. There is every reason to believe such studies will identify those infectious diseases most amenable to phage therapy and generate crucial information about the administration, pharmacology and safety of phage.

Projan further argues that the size and complexity of phage may prevent them from entering sequestered sites of infection. However, nearly all antibiotic classes either fail to enter or exhibit greatly reduced activity in several important kinds of infections, most notably abscesses and bacterial biofilms on implanted prosthetic devices. The performance of phage in these or other sites is an active area of investigation, but the capacity of wild-type phage to replicate exponentially to very large numbers *in situ* (*e.g.*, at the site of infection) and their billion-year coevolution with bacteria in natural environments suggest that phage may succeed in some settings where antibiotics typically fail.

Projan states that bacteria naturally resistant to any bacteriophage pre-exist in bacterial infections consisting of $>10^6$ organisms. This puts bacteriophage on a par with the worst chemical antibiotics, rendering them unusable for treating bacterial infections involving 10^9 or more organisms. His statement is contradicted by published data. Most of the carefully conducted animal experiments using phage have used infections with 10^9 or more target bacteria, and when phage shown to be active *in vitro* against these bacteria are administered in a timely manner, they have proven effective in rescuing the infected animals. Furthermore, in a comparative study of mice given potentially lethal intramuscular and intracerebral injections of bacteria, a single intramuscular dose of phage was more effective than multiple intramuscular injections of tetracycline, ampicillin, chloramphenicol or trimethoprim plus sulphafuraxole[2]. The authors of this study, Smith and Huggins[2], note that "the therapeutic success of phage was due to its high *in vivo* activity and the failure of phage-resistant mutants to proliferate during treatment."

Projan goes on to argue that the narrow selectivity of phage for a single bacterial species or strain, and the attendant need for phage sensitivity testing, will limit the usefulness of phage for the 'empiric' therapy of an infected patient before the isolation and characterization of the responsible microbe. This concern belies the way antibiotics are actually used in clinical practice, where broad-spectrum drugs are replaced, as quickly as possible, by narrow-spectrum drugs (once the infecting microbe is identified and tested for susceptibility) to prevent the emergence of resistance. It also misrepresents the time that is currently required—ranging from 24 h to 72 h—for the identification and antibiotic susceptibility testing of an infectious agent isolated from a clinical specimen. In contrast, phage-based diagnostic tests, which simultaneously provide species identification and information about phage sensitivity, can in principle be completed in hours and in a highly cost-effective manner. Thus, except for infections harboring several different infectious agents, it is far from clear which therapeutic option—phage or antibiotics—will provide an intervention that best balances specificity and rapidity. Nevertheless, some phage are highly specific, whereas others are extremely broad in their host range. Most phage isolation techniques start by selecting phage that amplify on a single host, and so, not surprisingly, they favor the collection of phage that specialize in that single host. However, phage P1, for example, will inject its DNA into a very wide range of Gram-negative bacteria and even into *Myxococcus*. Coliphage K1-5 is a 'dual-specificity' phage that encodes two different tail proteins, allowing it to attack and replicate on both K1 and K5 strains of *Escherichia coli*. Furthermore, there is an existing scientific literature showing that phage host range can be extended or narrowed at will, or even at random by intrinsic diversity generators (*e.g.*, within *Bordetella* phage[3].

Taken together, Projan's concerns about the practical application of phage therapy perpetuate a fundamental, and we think

incorrect, assumption that, in the future, only phage alone or only chemical antibiotics alone will be available to the clinician. In contrast, we see a more complex scenario where each modality will be used where and when it is most suited and where combined therapy with both will be an option as well.

Projan also worries that by suddenly lysing large numbers of bacteria, bacteriophage might release toxic bacterial products that could cause shock or other untoward reactions in the patient or that phage might carry and/or transmit horizontally unidentified toxin genes from one bacterium to another. Yet phage have been administered to countless persons, including children, without evident toxicity. Bacteriocidal antibiotics carry at least as great a risk for the same adverse events caused by abrupt release of toxic internal bacterial contents, but except for the now rarely seen Herxheimer Reaction, the concern seems unfounded. The worry of phage carrying or transferring bacterial toxin genes might have been more credible in the premodern era of phage therapy. Today, however, it is possible to sequence phage genomes and identify or eliminate toxin genes as well as the genetic capability to transduce such genes. Moreover, contemporary phage engineering can even further reduce the probability that phage administered therapeutically will acquire or transmit deleterious genetic elements. Equally exciting for the prospect of phage therapy is the capacity to modulate phage lysis *in situ*, including the capacity to construct phage that kill, but do not lyse, their target host bacteria. Finally, eliminating all concerns about the content or effect of foreign genetic material, phage therapy research now includes a focus on the use of phage or phage components that do not replicate, can be precisely characterized as molecular entities and, in some instances, may be entirely free of phage genes. Today, modern molecular science drives phage therapeutics.

In deference to Projan's concerns, we agree that the developmental pathway for phage therapeutics may well require new therapeutic and diagnostic paradigms and new procedures and criteria for US Food and Drug Administration (Rockville, MD) approval. We do not downplay the research and development challenges that lie ahead. However, as compared to the early days of antibiotic therapy, when not even penicillin's mode of action was understood, the development of phage therapy in the modern era will have several distinct advantages: the molecular basis by which phage kill their bacterial targets is known and can be modulated by well-established genetic engineering methods; animal models of infectious disease and clinical trial design, long used to evaluate antibiotics, will prove equally useful in the evaluation of phage; and the enormous diversity of phage in nature will provide an unlimited source of novel starting materials. Finally, in contrast to the optimism that characterized the first phase of the antibiotic era, we are no longer naive about the capacity of microbes to supersede our best antibiotic discovery efforts. It seems a matter of simple prudence to pursue all promising means for the control of infectious diseases.

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COMPETING INTEREST STATEMENT
The authors declare competing financial interests.

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Gary K Schoolnik

Stanford Medical School, Beckman Center, Room 241, Stanford, California 94305, USA
e-mail: schoolni@cmgm.stanford.edu

William C Summers

Yale University School of Medicine, Biophysics and Biochemistry, 333 Cedar Street, New Haven, Connecticut 06520, USA

James D Watson

Cold Spring Harbor Laboratory, 1 Bungtown Rd., Cold Spring Harbor, New York 11724, USA

Projan responds:

I regret that Schoolnik, Summers and Watson found my News & Views pessimistic; I was attempting to outline the very real challenges in developing bacteriophage as antimicrobial therapies (challenges that, by the way, are much the same for any novel antibacterial). I did not conclude that phage therapy was a 'nonstarter', but rather that one could (and probably would) do better by investing in other approaches for the prevention and treatment of bacterial infections. It is telling that bacteriophage experts, such as those at PhageTech (St. Laurent, QC, Canada), have turned away from the concept of using bacteriophages themselves as therapy and instead are using their understanding of bacteriophage as part of a new paradigm for small-molecule drug discovery. Schoolnik *et al.* claim to offer a rejoinder to my arguments; in fact, they further underscore several of my key points.

First, we all agree that a profound understanding of each and every bacteriophage to be used in therapy is an absolute requirement, especially if one is to evade resistance mechanisms and avoid potential toxicity. I am not sure, however, what Schoolnik *et al.* are telling us about resistance: are they suggesting that there are bacteriophage, either natural or engineered, to which resistant bacteria cannot readily be selected? I think not; generating bacterial resistance to virtually any bacteriophage is a trivial undertaking.

Second, Schoolnik *et al.* argue that tissue penetration would not be an issue for bacteriophage (citing what appears to be anecdotal data) and they state that biofilms currently also represent a barrier to the penetration of small-molecule antibiotics. However, those who study bacterial biofilms no longer maintain that biofilms represent a barrier to antibiotic penetration; the fact is that most small molecules have little problem in penetrating into biofilms and in some cases antibiotics actually accumulate within biofilms (and work quite well as a result of that accumulation). On the other hand, Schoolnik *et al.* do realize that bacteriophage may well be hampered in their ability to get at the bacteria within biofilms and indicate this is an area of current study.

Third, Schoolnik *et al.* have missed my point about rapid, point-of-care diagnostics. We are all in agreement that this will be a requirement for the use of phage therapy. I would go a step further and maintain that we should already be using rapid diagnostics on a routine basis (this would be a boon for selecting the appropriate antibiotic or phage from the start). The technology already exists to obtain species and susceptibility information in minutes to hours; the fact is that such diagnostics are not used, mainly for reasons of expense. This is why relatively cheap and effective broad-spectrum, often combination, empiric therapy is used today in what can best be termed a 'shoot first and ask questions later' strategy. Given current pricing pressures on pharmaceuticals, Schoolnik *et al.* should realize that what they are proposing would be much more expensive than current antimicrobial therapy,

which is a further argument that their approach is unlikely to be adopted.

Finally, the dearth of valid clinical data supporting the use of phage therapy must be acknowledged. But the fact remains that phage therapy is still practiced in certain parts of the world, so there should be no bar to actually performing a valid clinical trial testing phage therapy versus small-molecule antibiotics. The statement that "Phage have been administered to countless persons, including children, without evident toxicity," is based—like much of the information in this field—mainly, if not totally, on anecdote. What the field needs is some validated data published in peer-reviewed journals rather than in the popular press.

Steven Projan

Wyeth Research, Protein Technologies, 200 Cambridgepark Drive, Cambridge, Massachusetts 02140, USA
e-mail: projans@wyeth.com

Drugs in crops

To the editor:

Your editorial in the February issue (*Nat. Biotechnol.* **22**, 13, 2004) expressed concerns about the use of conventional crops, mainly maize, for the production of biopharmaceuticals. With the current capacity crunch for production of biopharmaceuticals by current-day technologies, and the projected demand for therapeutic proteins (such as antibodies) in the next few years[1,2], it is imperative that the acceptance of plants for molecular farming proceed smoothly.

Throughout the debate on the use of plants as production platforms for pharmaceutical proteins, we have been puzzled by the persistent and exclusive focus on the use of existing crop varieties. This focus has, predictably, triggered and fueled concerns about this exciting new agricultural opportunity from stake-holding industry groups[3,4]. However, those participating in the debate might take note of the fact that existing commercial crop varieties are unlikely to be optimal for plant-manufactured pharmaceutical applications anyway, having been bred and selected entirely for their present-day, traditional uses. This is true of both food (*e.g.*, corn) and non-food (*e.g.*, tobacco) crops that might be used in such applications.

Fortunately, however, it may be quite unnecessary to turn to alternatives, such as undomesticated plants or rarely cultivated crops, which might require new development of appropriate gene expression technology and so on. We contend that modern plant breeding approaches can be used to develop novel and distinctive cultivars of the desired (conventional) crops, which will be optimally suited to biopharmaceutical applications in every respect. Together with appropriate crop-management protocols, the resulting crop system will completely obviate any compromise or contamination of the equivalent traditional crop.

To illustrate this approach with tobacco[5–7], which is the subject of several plant manufactured pharmaceutical platforms, we note that the US tobacco industry has voiced a zero-tolerance standard for genetically modified (GM) contamination of the conventional crop (Dean Wallace, Council for Burley Tobacco, personal communication). Transgene escape can be ameliorated through the use of male sterile varieties[8], although the proximity of fertile, non-GM tobacco nearby could result in the production of some seed and the potential for GM volunteer plants. However, we believe that through an F_1 hybrid strategy, we can take advantage of the many benefits of tobacco as a platform for producing pharmaceuticals while at the same time eliminating its drawbacks. We have found that interspecific crosses between tobacco and certain other species of *Nicotiana* produce vigorous, completely sterile hybrid plants that are morphologically distinct from conventional tobacco (thus achieving identity preservation) and can be planted and grown in a manner similar to conventional tobacco (though at a higher plant density) using existing infrastructure (float beds, machinery, fertilizers, herbicides, *etc.*), yet can be mechanically harvested three or four times during the growing season. For production of pharmaceutical or industrial proteins, the maternal (tobacco) cultivar is transformed with a gene of interest, and homozygous transformants with high-level protein expression are selected and crossed with the other species. It is the resulting F_1 hybrid offspring that are grown in the field, as with hybrid maize. Our present work involves breeding a tobacco cultivar that is optimized for plant-manufactured pharmaceutical applications, and we are in the process of evaluating the performance of our hybrids with the appropriate gene expression systems.

We feel that all parties participating in the debate over biopharmaceutical crops will benefit from consideration of developing plant varieties specifically tailored to plant manufacture of pharmaceuticals, which will permit the use of existing transformation and gene-expression technologies without imposing undue risk to conventional crops or the environment. The hybrid *Nicotiana* approach effectively addresses the main concerns about 'third-generation' GM crops[9] and could very well lead to simplified regulatory requirements in the near future.

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David Zaitlin, Orlando D Chambers, Baochun Li, Richard E Mundell & H Maelor Davies

The Kentucky Tobacco Research and Development Center, Cooper & University Drives, University of Kentucky, Lexington, Kentucky 40546, USA
e-mail: dzait2@uky.edu