Overcoming drug resistance in multi-drug resistant cancers and microorganisms

A conceptual framework

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Resistance development against multiple drugs is a common feature among many pathogens—including bacteria such as Pseudomonas aeruginosa, viruses and parasites—and also among cancers. The reasons are 2-fold. Most commonly-used rationally-designed small molecule drugs or monoclonal antibodies, as well as antibiotics, strongly inhibit a key single step in the growth and proliferation of the pathogen or cancer cells. The disease agents quickly change or switch off this single target, or activate the efflux mechanisms to pump out the drug, thereby becoming resistant to the drug. A second problem is the way drugs are designed. The pharmaceutical industry chooses to use, by high-throughput screening, compounds that are maximally inhibitory to the key single step in the growth of the pathogen or cancer, thereby promoting selective pressure. An ideal drug would be one that inhibits multiple steps in the disease progression pathways with less stringency in these steps. Low levels of inhibition at multiple steps provide cumulative strong inhibitory effect, but little incentives or ability on the part of the pathogen/cancer to develop resistance. Such intelligent drug design involving multiple less stringent inhibitory steps is beyond the scope of the drug industry and requires evolutionary wisdom commonly possessed by bacteria. This review surveys assessments of the current clinical situation with regard to drug resistance in P. aeruginosa, and examines tools currently employed to limit this trend. We then provide a conceptual framework in which we explore the similarities between multi-drug resistance in pathogens and in cancers. We summarize promising work on anti-cancer drugs derived from the evolutionary wisdom of bacteria such as P. aeruginosa, and how such strategies can be the basis for how to look for candidate protein/peptide antibiotic drugs from bioengineered bugs. Such multi-domain proteins, unlike diffusible antibiotics, are not diffusible because of their large size and are often released only on contact with the perceived competitor. Thus, multi-domain proteins are missed during traditional methods of looking for growth zone inhibition of susceptible bacteria as demonstrated by antibiotics, but may represent the weapons of the future in the fights against both drug resistant cancers and pathogens such as P. aeruginosa.

Introduction

Drug resistance in microorganisms,1 viruses2 and parasites3 is rampant and has created major problems in health care delivery in the United States and indeed worldwide. Such resistance development is not only confined to drugs but also to neutralizing antibodies, making it hard for effective vaccine development against bacteria such as Mycobacterium tuberculosis, viruses such as HIV-1, or parasites such as the malarial parasite Plasmodium falciparum. This concern applies to bacteria and other pathogens, but also analogously to the neoplastic cell populations which cause cancers.4 The situation is particularly difficult with bacteria such as multi-drug-resistant (MDR) or extensively-drug-resistant M. tuberculosis, methicillin, vancomycin-resistant Staphylococci, and multi-drug-resistant Pseudomonas aeruginosa. The potential crisis has captured attention on the political stage, as exemplified by the GAIN (Generating Antibiotic Incentives Now) bill introduced to the United States Senate. Much of this resistance development has been ascribed to the indiscriminate overuse of the antibiotics in areas other than medicine such as in the meat industries, and multi-drug-resistant P. aeruginosa. The potential crisis has captured attention on the political stage, as exemplified by the GAIN (Generating Antibiotic Incentives Now) bill introduced to the United States Senate. Much of this resistance development has been ascribed to the indiscriminate overuse of the antibiotics in areas other than medicine such as in the meat industry by incorporating antibiotics in the feed stocks to prevent infections in domestic or feedlot animals. Certainly, the high rates of mutations in genes conferring resistance such as the genes encoding the ABC transporter efflux pumps, and their association with transmissible plasmids for their rapid dissemination, have had an enormous impact on the increasing lack of efficacy of antibiotics in treating various life-threatening infections. In this review, we summarize the current clinical picture of increasing antibiotic resistance in MDR P. aeruginosa, and the limitations of antibiotic therapy. We include a short discussion of measures currently being undertaken to maximize the value of currently available therapeutic options. It is noteworthy that since antibiotics or small molecule anti-cancer drugs often target a single step in the disease progression pathway, any disease agent may quickly become drug-resistant with the correct mutation. We therefore subsequently introduce the concept that bacterial protein/peptide drugs with multiple domains, rather than small molecule compounds similar to antibiotics, may represent a superior strategy to inhibit key steps in the disease progression pathways of cancers and bacterial infections alike. Indeed, the pseudomonal redox protein azurin appears to act as a weapon against multiple
invaders—infected and malignant—via multiple mechanisms of action. Even as \textit{P. aeruginosa} itself produces factors which have great potential as anti-neoplastic agents, similar therapeutic development strategies are indicated to address the current situation with multi-drug-resistant strains of \textit{P. aeruginosa}.

\textbf{Pseudomonas aeruginosa: The Current Clinical Picture}

\textit{Pseudomonas aeruginosa} is an aerobic Gram-negative bacillus, growing primarily in soil but also able to survive in the human body as an extracellular producer of biofilms. It is known primarily as a nosocomial pathogen, capable of infection at all sites of the body. \textit{P. aeruginosa} is particularly prevalent in intensive care units (ICUs).\(^8\) The bacterium appears to be highly adapted to the respiratory tract, and is infamous as the most common respiratory pathogen in patients with cystic fibrosis (CF).\(^6\) Numerous other clinical infections attributable to this bacterium include bacteremia, bone and joint infection, meningitis, eye infection, and urinary tract infections.\(^6,7\) Estimates from samples collected by the Centers for Disease Control and Prevention in the 1980s and 1990s indicate that \textit{P. aeruginosa} is the second most common pathogen responsible for nosocomial pneumonia.\(^8\) The prevalence of this organism in a nosocomial setting is matched by its deadliness, with \textit{P. aeruginosa} pneumonia carrying a mortality rate of 30–40%.\(^8\)

Available anti-pseudomonal drugs include \(\beta\)-lactam drugs and antibiotics from other classes. Although most traditional penicillins are ineffective against \textit{Pseudomonas} species, certain new-generation penicillins combined with \(\beta\)-lactamase inhibitors do have anti-pseudomonal activity. Piperacillin-tazobactam (PITAZ) in particular remains a drug combination in very widespread use as a broad-spectrum agent with relatively high effectiveness. By many analyses, PIP-TAZ is the most effective non-polymyxin anti-pseudomonal drug in current use, although questions exist about whether the current thresholds for “susceptibility” are appropriate.\(^8\) Other \(\beta\)-lactams useful against pseudomonal infections include certain new-generation cephalosporins, most notably ceftazidime and cefepime. The carbapenems (i.e., imipenem and meropenem) are broad-spectrum \(\beta\)-lactams with \(\beta\)-lactamase resistance which have long been viewed as the drugs of choice for Gram-negative organisms resistant to other drugs; with the exception of etapienem, drugs in this class have strong anti-pseudomonal activity. In association with more frequent use, carbapenem resistance is now increasing among Gram-negative organisms.\(^9\)

Anti-pseudomonal drugs from other antibiotic classes include fluoroquinolones such as ciprofloxacin and levofloxacin, broad-spectrum antibiotics commonly employed not only in the ICU but also throughout both the inpatient and the community settings. These drugs can also be orally dosed and have generally favorable safety profiles, further contributing to their popularity. As discussed in greater detail below, fluoroquinolone-resistant \textit{P. aeruginosa} is now extremely common, leading some to conclude that “fluoroquinolones are no longer adequate for empiric therapy of infections caused by \textit{P. aeruginosa}.”\(^10\) The aminoglycosides (amikacin, gentamicin, tobramycin) are highly bactericidal antibiotics whose utility is limited by their nephro- and oto-toxicity. In the past decade, polymyxins such as colistin (polymyxin E) and polymyxin B have re-entered extensive clinical use due to the emergence of MDR \textit{P. aeruginosa}. The polymyxins also carry significant concern about toxicity, particularly nephro- and neuro-toxicity.

\textbf{Antibiotic Resistance in \textit{P. aeruginosa}}

\textit{P. aeruginosa} is intrinsically resistant to many anti-microbial drugs and rapidly acquires immunity to others. Additionally, “sequential resistance,” in which an isolate already resistant to one drug is then treated with another, is a likely means for the creation of MDR \textit{P. aeruginosa}.\(^11\) As a result, MDR Pseudomonas are isolated worldwide. Recent statistics show that the resistance of \textit{P. aeruginosa} isolates to different classes of antibiotics varies greatly depending on the country, ranging from 45% to less than 1% (Fig. 1), particularly when in-dwelling medical devices are used triggering biofilm formation (Fig. 2). Furthermore, multiple studies have demonstrated a correlation between past antibiotic exposure and infection with bacteria resistant to that antibiotic.\(^9,11,12\) Riou et al.\(^1\) and others further demonstrated that in an acute setting, the resistance of nosocomial \textit{P. aeruginosa}
pneumonia to multiple anti-pseudomonal antibiotics was higher at the end of an ICU course when compared with the initial culture.

Emerging resistance to fluoroquinolones is particularly common (Table 1). Worldwide, even the most potent member of the class, ciprofloxacin, was effective against only 60–75% of clinical isolates by 1999. Neuhauser et al. surveyed isolates from ICUs throughout the United States and found a decline in ciprofloxacin susceptibility from 89% to 63% of P. aeruginosa isolates between 1993 and 2000. As these authors point out, this period of time is also notable for a marked increase in the use of ciprofloxacin and other fluoroquinolones. Other estimates of fluoroquinolone resistance in the ICU suggest rates at above 30% by 2002, doubled compare with a decade earlier. Similarly, P. aeruginosa isolates from “clinically significant” infections as part of the Global SENTRY Antimicrobial Surveillance Program collected between 1997 and 1999 demonstrated a general decline in the proportion of isolates that were antibiotic-susceptible. These authors reported a statistically significant increase in resistance to nearly all anti-pseudomonal antibiotics in Europe (i.e., PIP-TAZ susceptibility declined from 90% to 74%). In the United States, PIP-TAZ also was effective in a significantly lower proportion of the 1999 isolates compared with the 1997–8; fluoroquinolones as a group also demonstrated significantly reduced effectiveness. The authors documented that no anti-pseudomonal was effective against more than 90% of the isolates obtained. The wide geographic variation found in this study may be attributable to sampling variability, differences in native resistance patterns, or differences in antibiotic treatment practices in different regions. These early studies document clearly the potential for resistant strains to spread worldwide. It is particularly noteworthy that no antibiotic exists to which resistant strains have not also emerged.

Contrary to previous belief that polymyxins, as a drug class, did not promote stable antibiotic resistance, examples of polymyxin resistance are now reported. The exact rate at which this potential epidemic is progressing is not entirely clear, particularly in light of findings from the mid-2000s suggesting that resistance to fluoroquinolones and other drugs might have reached a plateau despite a continued increase in PIP-TAZ resistant and MDR P. aeruginosa. The previously mentioned report by Riou et al. utilizing samples from multiple Belgian hospitals paints a particularly grim picture. The
On the other hand, Master et al. analyzed mixed colonization sites of ICU patients diagnosed with hospital-acquired pneumonia. Although the study was not designed as an epidemiological perspective analysis in which patients whose cultures demonstrated the presence of levofloxacin susceptible as compared with prior reports, as well as a smaller a widespread movement among clinicians to phase out ciprofloxacin for treatment in favor of other alternatives. It should be noted that few trials exist directly comparing susceptibility rates to most other antibiotics, and a small but statistically significant decrease in the percentage of isolates that were resistant to four or more drugs in 2009 compared with 2000. The authors suggest that perhaps infection-control measures and antimicrobial stewardship programs as discussed below are effective. We summarize published assessments of prevalence and change of fluoroquinolone and other antibiotic resistance by *P. aeruginosa* in Table 1. It is notable that the antimicrobial class that consistently continues to engender high and growing resistance is the fluoroquinolones, the only class of anti-pseudomonal agents that can be widely used in the outpatient community setting, raising the question of whether community antibiotic prescribing practices may be as important or more so than hospital practices. Karlowsky et al. also note that their data regarding apparent stabilization of ciprofloxacin resistance rates were collected at a time (2001–2003) shortly after a widespread movement among clinicians to phase out ciprofloxacin for *P. aeruginosa* treatment in favor of other drugs such as levofloxacin.

The existence of MDR *P. aeruginosa* leads to the intuitive conclusion that increasing resistance will lead to an increase in morbidity and mortality, as suggested by several case series and retrospective studies. An attempt to quantify the clinical impact of multi-drug resistance via literature review concluded that most studies report an increase in mortality attributable to MDR *P. aeruginosa*, but that differences in definitions and methodological limitations make the data difficult to compare or analyze. It should be noted that few trials exist directly comparing susceptible and resistant organisms. However, conducting a retrospective analysis in which patients whose cultures demonstrated the presence of levofloxacin susceptible *P. aeruginosa* as compared with resistance to any drug (2000–2009): 59.2 to 55.4% Other

### Table 1. Studies assessing percentage of *P. aeruginosa* with antibiotic resistance

| Study | Total n | Sample | Year(s) studied | Initial ciprofloxacin resistance | Final ciprofloxacin resistance | Other Δ |
|-------|---------|--------|----------------|----------------------------------|-------------------------------|---------|
| Gasiuk et al. 2006 | 4976 | *P. aeruginosa* isolates from US hospital | 1991–2000 | 15% | 41% (levofloxacin) |
| Neuhouser et al. 2003 | 8244 | Consecutive isolates from ICUs in 43 states | 1994–2000 | 11% | 32% |
| Gales et al. 2001 | 6631 | Clinical infections from international network of sentinel hospitals | 1997–1999 | 20.2% USA | 24.6% USA |
| Master et al. 2007 | 924,740 | Clinical infections from 25 hospitals | 1997–2009 | 20% | 25% |
| Livermore CD | 2,794 | Clinical infections from 25 hospitals | 1997–2000 | 29.5% | MDR isolates: 12.8 to 20.8% |
| Kafalowsky et al. 2005 | > 2,300 | Consecutive Pulmonary specimens from 27 US hospitals | 2001–2003 | 33.5% | 31.2% |
| Bennett et al. 2002 | 106 SICU, 68 MICU | Clinical infections from single hospital, MICU and SICU recorded separately | 2002–2004 | SICU: 29% | MICU: 50% |
| Rios et al. 2010 | 110 | Clinical isolates from pts with nosocomial pneumonia, 5 Belgian hospitals | 2006–2009 | PIP-TAZO, resistance: > 25% cephalosporin resistance: > 25% | 20% | |

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with those with levofloxacin-resistant \( \text{P. aeruginosa} \), Gasink et al. found higher hospital mortality and economic costs caused by resistant isolates; in multi-variate analysis, carbapenem susceptibility rather than fluoroquinolone susceptibility was significantly associated with mortality, possibly due to a delay in initiating appropriate therapy.

The Antibiotic Shortage and Strategies for the Near Future

Only one new anti-pseudomonal drug had been approved in recent years (doripenem), and as of 2009, no new anti-pseudomonal drugs were undergoing phase 3 clinical trials—a situation which limits treatment options in the foreseeable future given the length of time required for clinical drug development. The Infectious Diseases Society of America (IDSA) has lamented the decline in rate of introduction of new anti-bacterials and drug companies “decreased investment in this therapeutic area.” The results of a literature and database search also led the IDSA to conclude that drugs currently in the developmental pipeline will not address the emergence of carbapenem-resistant Gram-negative bacteria, and to express concern over the paucity of compounds with novel mechanisms of action.

As the authors of the above report point out, multiple factors limit pharmaceutical companies’ investment in anti-microbials, including the acute nature of the conditions treated and perceived regulatory hurdles (large necessary trial size, unfeasibility of placebo-controlled trials for ethical reasons). The IDSA advocates for federal action to provide incentives to encourage the development of new antibiotics. Indeed, at the time of this writing, the United States Senate is considering a bill, introduced by Senator Richard Blumenthal, D-Conn and Senator Bob Corker, R-Tenn, called GAIN (Generating Antibiotic Incentives Now), that would provide drug-makers 10 y of exclusive marketing rights and would allow the US Food and Drug Administration (FDA) to speedily approve such drugs without compromising the safety and efficacy issues, thereby minimizing regulatory complications.

Due to the concern that antibiotic resistance, particularly in the ICU, will outstrip the development of appropriate anti-microbial therapy, clinical utilization strategies of existing anti-microbials is an area of active investigation. Potential options include formal guidelines, optimized pharmacokinetics, and shorter courses of antibiotic treatment. We will now briefly discuss three additional strategies: combination therapy, antibiotic “cycling” (in which drugs used for empiric treatment are rotated on a scheduled basis), and improvement in infection-control measures.

Combination therapy. Controversy currently exists about whether antibiotic resistance rates can be affected, favorably or unfavorably, by the practice of combination therapy—the use of multiple antibiotics rather than mono-therapy to treat known or suspected pseudomonal infections. Possible but unproven benefits include synergistic therapeutic effects as well as possible prevention of resistance developing to one drug or the other. One combination in common clinical use is that of a \( \beta \)-lactam drug with an aminoglycoside, despite the fact that its therapeutic value is unclear. Although susceptibility testing generally suggests that combination therapy is superior to a \( \beta \)-lactam alone, a meta-analysis of 64 randomized trials concluded that adding an aminoglycoside provided no clinical benefit in all-cause mortality in patients with severe infection compared with \( \beta \)-lactam mono-therapy. These findings support the general notion that appropriate mono-therapy based on susceptibility data are clinically equivalent to combination therapy. In vitro studies of levofloxacin/imipenem co-administration on different strains of \( \text{P. aeruginosa} \) over a 30-h period indicated that this combination was generally superior to either drug alone, even in strains that were partially or fully resistant to one of the two agents. To our knowledge, this effect has not been further investigated in a patient setting. Other investigators propose novel antibiotic combinations incorporating drugs not traditionally thought to have anti-pseudomonal activity, such as rifampins, whose potential mechanism is unknown. It should also be noted that regardless of the ultimate treatment once cultures are grown and susceptibility data obtained, the practice of combination therapy will remain widely used by clinicians who feel that, during the empiric stage of treating a clinical infection (prior to culture growth), combination therapy may limit the mortality associated with inappropriate initial drug selection. For instance, the guidelines for empiric therapy for health-care-associated pneumonia by the American Thoracic Society and the Infectious Diseases Society of America (Guidelines 2005) do recommend using multiple anti-pseudomonal agents, i.e., a \( \beta \)-lactam plus a fluoroquinolone or aminoglycoside. As of the time of this writing, the most recent version of these guidelines was published in 2005, with an update scheduled for late 2012. A full evaluation of combination therapy is beyond the scope of this review, but any consensus appears to be far away on the question of whether and under what circumstances it is appropriate for the treatment of \( \text{P. aeruginosa} \) infection.

Antimicrobial cycling. Regular cycling of empiric therapy for presumed Gram-negative infections, such that each individual drug class spends frequent time in limited use, provides the potential to transiently reduce selective pressure and prolong a drug’s effectiveness. Some early studies employing antibiotic cycling strategies to limit ventilator-associated pneumonia (VAP) caused by Gram-negative bacteria yielded promising results, although methodological limitations have been cited—most notably lack of true control groups. Nijssen et al., focusing on ICU-based studies of antimicrobial cycling, identified numerous issues with most old and new research on the topic. The most common study design is a “before-after” model in which the “control” and “intervention” arms of the study are temporally separated. Obviously, this introduces tremendous potential for confounding variables. Specifically: (1) Resistance patterns in ICUs normally exhibit baseline variation over time; (2) Resistance patterns in the surrounding community may change over the course of a study; (3) Hospital infection control measures may change over the course of a study. Other concerns identified by Nijssen et al. include the difficulty of comparing studies of colonization (incorporating surveillance cultures) with those that limit their focus to clinical
Infection, the fact that the usual definition of nosocomial infection (>48 h after hospital admission) has not been thoroughly validated, and the fact that most studies do not directly measure levels of patient to patient transmission (this may distort data due to the fact that many standard statistical tests assume that each patient represents an independent data point).

One group of authors reports a comparison between antimicrobial cycling and intermittent use of carbapenem, a drug which may potentially limit the use of the anti-pseudomonal carbapenems, the hospital also implemented increased education promoting hand hygiene and supply of alcohol-based hand rub. Hand rub consumption was correlated with the net trend of reduction in the resistant Pseudomonas (the data were statistically significant for the overall trend, not in direct comparisons of any two time periods), whereas the association with the stewardship intervention was not significant. The literature reports a wide range of adherence to hand hygiene protocols, often very low.26,28,29 For this reason does not appear to be sufficient to prevent nosocomial infection, and in one case report,27 an ICU outbreak of MDR P. aeruginosa was only contained after the center instituting pasteurization of water taps. If infection control is to be successfully used to limit the emergence of antibiotic resistance, the combination of resources and education toward hand hygiene, personal protective equipment, environmental sterilization, and patient cohorting are likely to all be essential.

A Long-Term Approach: Beyond Small Molecule Anti-Microbials

For the more distant future, the new drugs under development include both traditional antibiotics and antibody fragments to reduce pathogenicity.27 However, it is clear that the ability of P. aeruginosa to evolve resistance to anti-microbials will continue to pose the potential to outstrip drug development. The situation exposes the fact that standard approaches to antibiotic therapy are inherently limited. Bacterial "evolutionary wisdom" and the example of azurin. Antibiotics are generally small molecules produced by slow-growing soil microorganisms such as Actinomycetes in order to compete with faster-growing neighbors.30 When a soil-dwelling organism such as P. aeruginosa also lives in a setting such as the human body, it may also be drawn into competition with viruses, parasites, cancers, and other bacterial pathogens, any of which may threaten to out-compete the bacteria or simply kill the host. It stands to reason that over three billion years of evolution, successful pathogenic bacteria will have developed the "evolutionary wisdom" to produce tools to compete with "enemies" within a eukaryotic host. Furthermore, weapons designed by evolutionary wisdom might be expected to include molecules with multiple domains to target the multiple invasive apparatuses. Due to the limited genome sizes of bacteria, an ideal weapon would also be able to attack multiple different enemies. In sum, just as evolutionary wisdom in soil-dwelling fungi produced the antibiotics currently in use, evolutionary wisdom in pathogenic bacteria may have also designed multi-domain proteins very different from commercial antibiotics, which generally have a single or limited number of target sites. In this respect, standard pharmaceutical approaches for antibiotic detection and in-hospital susceptibility testing are inadequate, relying on diffusion of small molecules in agar plates to produce zones of inhibition of susceptible bacteria or fungi. This detection of growth inhibition zones, which can only be exhibited by small molecules diffusing out of the producer.
microorganism, prevents the detection of larger molecules such as proteins. Certain bacteria employ proteins to inhibit the growth of competitors in the human body (prokaryotes, viruses, parasites or even cancer) when such a bacterium is resident in the human body in the state of extremely slow-growing biofilms. A second drawback of the current antibiotic isolation method is that it fails to detect compounds released by bacteria only when in physical contact with an enemy.

One example of evolutionary wisdom as a potential source for novel defenses against threats to the human body comes in the form of the bacterial redox protein azurin, produced by bacteria such as P. aeruginosa. Azurin demonstrates the ability to inhibit cancer growth. A chemically synthesized 28 amino-acid peptide derived from the protein, termed p28, is currently undergoing clinical trials, including a recently completed phase I study conducted in individuals with end-stage (stage IV) metastatic cancer refractory to all conventional treatment. Of the 15 patients treated with p28, stabilization of disease occurred in 6 patients, partial regression of tumor in 2 patients, and complete regression of the tumors in 2 other patients, without demonstrating any toxicity or side effects even at the highest dose (Table 2).

Besides its anti-neoplastic abilities, the azurin protein also inhibits the growth of other infectious agents, including the malarial parasite Plasmodium falciparum, the toxoplasmosis-causing parasite Toxoplasma gondii and the human AIDS virus HIV-1. Our research has also uncovered other bacterial proteins and peptides with potential activity against pathogens and cancers. This is the case with MPT63, a 16 kDa protein from Mycobacterium (M. tuberculosis, M. bovis) which, like azurin, exhibits anti-cancer and anti-HIV activity. Interestingly, it has previously been noted that multi-drug resistance actually emerges in bacteria by way of drugs. The rise of MDR P. aeruginosa represents a looming threat to patient health in the near future, conjuring fears of a return to pre-antibiotic morbidity and mortality levels for the many infections caused by P. aeruginosa. Evidence demonstrates the increasing ineffectiveness of both β-lactams and other non-β-lactams—particularly widely used antibiotics such as fluoroquinolones. Resistance of P. aeruginosa to all existing anti-pseudomonal drugs, including “new” agents such as polymyxins, is reported. This rate of decline in drug efficacy threatens to outstrip the discovery and approval of new anti-bacterial drugs, which have also been in decline over the past several decades. Strategies to maximize the lifetime of currently available drugs already in place in some clinical settings include combination therapy and antimicrobial cycling. However, in many cases, a paucity of clinical studies of these interventions and methodological limitations of those studies prevent us from drawing firm conclusions as to their effectiveness; more research is urgently needed. Improved disease-control measures are likely to prove essential in slowing or stopping the development of resistance to existing antibiotics, but the exact value of their contribution is also unknown.

Table 2. Results of human phase I clinical trials of p28 in stage IV cancer patients with metastatic, refractory solid tumors

| Patients | Type of cancer | Treatment modality | Dosage (mg p28/kg) | Adverse events | Objective response |
|----------|---------------|-------------------|-------------------|----------------|-------------------|
| 11 male  | Melanoma (7)  | ix. bolus of p28  | 0.63              | None attributable to p28 | Stable disease (6/15) |
| 15       | Colon (4)     | 3 times per week for 4 weeks | 1.66 | (grade 1) | Partial tumor regression (2/15, 1 prostate, 1 melanoma) |
| 4 female | Sarcoma (2)   | Observation 2 weeks before the next higher dose | 2.5 | | |
| 50–80    | Pancreatic (1) | in escalating doses | 3.33 | No immune response or adverse effect even at maximum dosage of p28 | Complete regression (2/15, 1 sarcoma, 1 melanoma) |
| Median   | Prostate (1)  |                   | 4.16 | | |

Data taken from a presentation made at the ASCO meeting in Chicago on June 6, 2011.

**Conclusion**

The rise of MDR P. aeruginosa represents a looming threat to patient health in the near future, conjuring fears of a return to pre-antibiotic morbidity and mortality levels for the many infections caused by P. aeruginosa. Evidence demonstrates the increasing ineffectiveness of both β-lactams and other non-β-lactams—particularly widely used antibiotics such as fluoroquinolones. Resistance of P. aeruginosa to all existing anti-pseudomonal drugs, including “new” agents such as polymyxins, is reported. This rate of decline in drug efficacy threatens to outstrip the discovery and approval of new anti-bacterial drugs, which have also been in decline over the past several decades. Strategies to maximize the lifetime of currently available drugs already in place in some clinical settings include combination therapy and antimicrobial cycling. However, in many cases, a paucity of clinical studies of these interventions and methodological limitations of those studies prevent us from drawing firm conclusions as to their effectiveness; more research is urgently needed. Improved disease-control measures are likely to prove essential in slowing or stopping the development of resistance to existing antibiotics, but the exact value of their contribution is also unknown.
In the years to come, genuinely novel approaches, as outlined briefly in this review, will be crucially necessary in the fight against development of all multiply drug-resistant pathogens. Approaches based on small molecules will always be especially against development of all multiply drug-resistant pathogens. It should be noted, however, that there are other parameters that might be independent of the nature or the size of the drug.

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