Drug Discovery and Delivery in the 21st Century

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Key Words

Genomics • Nanotechnology • Artificial cells • Genetic code • Genomics • Chemical biology • Drug targets • Drug delivery • Pharmacokinetics • Pharmacodynamics • Pharmacogenomics • Combinatorial chemistry • Molecular evolution • Darwin in a test tube • Systems biology

Abstract

Drug discovery in the late 20th century has increasingly focused on the definition and characterization of the macromolecular substrates that serve as targets for drug design. The advent of genomics and the molecular biology revolution has permitted both the definition of new targets and the characterization of the genetic basis of disease states. The introduction of powerful new technologies should greatly accelerate the pace of new drug discovery. Although genomics, both human and nonhuman, should in principle increase the number of potential drug targets and provide a greater understanding of cellular events contributing to the pathology of disease this has yet to occur in practice, primarily because of the underlying complexity of cellular signaling processes. The emerging discipline of systems biology is attempting to bring both order and understanding to these signaling processes. Genomics has, however, impacted on drug discovery in ways that are important beyond a mere increase in potential drug target numbers. Genomics has provided the tools of contemporary drug discovery, the pharmacogenomic pathways to personalized medicine, and has greatly influenced the nature of synthetic organic chemistry, a discipline that is still the cornerstone of contemporary

drug discovery. In the future, genomics and the tools of molecular biology will have a corresponding impact on drug delivery processes and mechanisms through introduction of drug delivery machines capable of both synthesis and activation by disease-specific signals. Such machines will be based on a synthetic genome, using an expanded genetic code, and designed for specific drug synthesis and delivery and activation by a pathological signal. This essay is based upon a lecture of the same title presented at the Faculty of Medicine, Kuwait University during a visit in the spring of 2005. It is intended, as was the lecture, to be a broad, descriptive and speculative overview rather than a comprehensive and detailed review.

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'Now that the liability to and danger of disease are to a large extent circumscribed – the effects of chemotherapeutics are directed as far as possible to fill up the gaps left in this ring.'

Paul Ehrlich, 1913

Introduction

Ehrlich's comments, ironically enough written on the eve of World War I and the great influenza pandemic of 1918, are an appropriate reminder of Euripides, who wrote, 'Those whom the Gods would destroy, they first make arrogant'. Euripides was referring to politicians, but hubris, although regrettably common, is not unique to that class; it is also expressed in science and by science.

tists. Indeed, it may be argued with conviction that, Ehrlich's claims notwithstanding, the challenges to health faced today are at least as severe as those faced a century ago. However, the issues before us today are less the inadequate science and technology of Ehrlich's day than they are the continuing inequitable distribution of knowledge, wealth and opportunity, a circumstance for which the rich world remains significantly responsible [1–4].

Despite these continuing and profound inequities, it would be foolish to deny that advances in health care and research over the past half-century, including therapeutic medicines, have not been of immense, albeit unequal, benefit. And few would deny that the future should be one of at least equal promise. Children will be born with their genes profiled, 'personalized' medicines will be a routine reality, gene and stem cell therapies will be mature technologies with major implications for the degenerative diseases of an aging world. This new world will be one of artificial cells and machines, many specifically created de novo with an expanded genetic code that will perform specific tasks, including the site- and disease-specific delivery of drugs and genes. These advances will have been made possible by a remarkable series of gene-based scientific discoveries made over the past 25 years [5, 6].

In many important respects it may be argued that as the 20th century was dominated by the great discoveries of physics so will the 21st century be dominated by the paradigms of biology. This transition started many years ago and not coincidentally by the work of physicists who converted biology from a descriptive to a quantitative discipline based on a number of molecular themes – diversity, replication, evolution, targeting and self-organization. These themes are being applied increasingly to diverse disciplines from chemical engineering to synthetic chemistry, disciplines that are central to the emerging 21st century vision of the pharmaceutical sciences. These five themes are linked through the process of biological recognition – the underlying molecular processes that govern the oft exquisite sensitivity of the molecular interactions that control biological events. Such molecular specificity was, of course, central to Ehrlich's concept of the 'magic bullet' – the as yet to be completely fulfilled goal of drug discovery programs.

In reality there are three components to the design of the magic bullet – the molecular interactions that govern the specific attraction between the drug, small molecule or macromolecule, and its macromolecular target, the interactions that determine the access of the drug to its target and the interactions that represent interindividual

Fig. 1. The old and the new paradigms for drug discovery.

variability. These are typically referred to as pharmacodynamic, pharmacokinetic and pharmacogenomic components, respectively. Collectively, these components of drug design are transforming the nature of drug discovery, although their anticipated rewards are still as yet incompletely realized.

The Drug Discovery and Delivery Process

The traditional process of drug discovery has been driven largely by phenotypic observations of the effects of a natural product or a derived synthetic agent on a physiological process or pathological state. This was followed by a process of iterative synthesis and biological testing. Typically, the molecular nature of the biological target was not known, the biological testing was carried out significantly in vivo, and chemical synthesis was a hand-crafted 'one molecule at a time process'. It has been a very successful approach yielding antibiotics, antidepressants, antihypertensive and anticancer agents, to name but four principal classes of therapeutic agents.

This traditional scheme is laid out in figure 1 and is contrasted with the genomic-based approach where genome interrogation was assumed to lead to thousands of new targets, a significant increase over the approximately 500 targets in the current pharmacopoeia. This genomic methodology, coupled with an assembly line approach utilizing the tools of combinatorial chemistry and high-throughput screening, would yield not just one clinical drug candidate, but rather hundreds of candidates (fig. 2). For example, it is now estimated that there are over 500 protein kinases and a rather lesser number of phosphatases that control protein phosphorylation, a key pathway in both physiological and pathological processes in the

Fig. 2. The new model for drug discovery whereby targets are identified by genome interrogation and the technologies of combinatorial chemistry and high-throughput screening provide the compounds and the biological activities, respectively. The data from these three areas are integrated through bioinformatics [from ref. 125].

human and other genomes [7, 8]. A number of these kinases are known to be specifically associated with human cancers [9]. However, despite exponential increases in research costs the productivity of drug discovery has actually decreased and the pharmaceutical industry has focused increasingly on maintaining a 'blockbuster' business model with an emphasis on lifestyle drugs [1, 10–15].

Underlying the lack of productivity are several factors. First, the number of human genes, approximately 25,000, is far fewer than originally anticipated [16, 17]. Consequently, the complexity of human cellular organization is not based upon a simple 'one gene = one protein' model, but rather by multiple use of the same gene-splice variants, population alleles and post-translational modification, and by the combinatorial diversification of signaling pathways. From this relatively limited gene repertoire the human probably expresses in spatial and temporally heterogeneous manner some 150,000 proteins. However, a protein target may not be druggable because of its intrinsic properties or expression, but also because it may play a role in multiple signaling pathways other than the one of pathological interest. The deciphering of this signaling network is thus of critical importance to the process of target validation. Increasingly, a systems biology approach is being used whereby an integrated rather than a reductionist approach is used to understand the relationships between function of a biological system and the

effects of perturbations such as a disease or the addition of a drug or potential drug molecule. This approach suggests that there may be only a few thousand druggable targets rather than the 150,000 or so once confidently predicted. However, 3,000–4,000 targets are still a very significant increase over the number of targets currently interacting with the pharmacopoeia of 2006. Regardless of the actual number, a key issue is the extent to which validation of targets can be executed. Second, new technologies are introduced too frequently with the anticipation that each one represents *the* key to increased productivity. In practice, each of the new technologies, including combinatorial chemistry, high-throughput screening, structure-based design and in silico ADMET, serves a useful function but only when employed with the older technologies, notably in vitro and in vivo pharmacology [18–20]. Finally, many of the 'easy' diseases have already been tackled with the consequence that there are, for example, over 100 antihypertensive drugs already available, many of them in generic form. In a market-based model of drug discovery there is little incentive for improvement in this area and there is even less incentive to work in areas such as tuberculosis and tropical diseases, where payment for drugs would be problematical since these are largely diseases of the poor world.

Nonetheless, and regardless of these limitations of the genomics-based approach to drug discovery, few will doubt but that this approach has had and will continue to have a major impact on all aspects of the drug discovery and development process from synthesis to delivery. Indeed, the practice of synthetic chemistry has already been significantly affected by the paradigms of biology, the discovery and validation of potential drug targets is increasingly a genomics-based process, and the drug delivery mechanisms of the future are likely to be cell-based rather than device-based. Thus, the three principal issues where genomics and molecular biology are transforming the 20th century process of drug discovery are: finding the target, finding the molecule and delivering the molecule to the target.

Finding the Target in the Age of Biology

Genomics has provided an extremely powerful set of tools with which to generate new targets – receptors, channels, enzymes, structural proteins – that can be employed with the technologies of combinatorial chemistry and high-throughput screening to generate 'hits' of appropriately defined affinity and selectivity. In appropri-

Fig.3. The identification of targets through genomic and chemical approaches using reverse and forward approaches. The forward chemical genetic approach represents the 'classical' approach to drug discovery, whereby the target may either be not characterized during the drug discovery approach or is identified after a successful approach.

ately designed in vitro systems, functional activity can also be determined. However, these systems (such as cell lines) typically lack the signaling characteristics of 'real' cells and certainly lack the integrated functional properties of organ and organismic systems, so their predictive ability has often been found to be lacking. Ultimately, the power of genomics needs to be integrated into the classic discipline of pharmacology to generate real leads and real targets, and to observe the phenotypic manifestation of drug action as early as possible in the discovery process [18].

The critical issue for genomics is thus not the discovery of targets per se, but rather the *validation* of targets – the experimental determination of the real function and role of the proposed target in a disease state [21–27]. This can be attempted in a variety of ways.

The classic genetic approaches involve producing random mutations by, for example ethylnitrosourea treatment, and subsequent selection of the phenotype of interest (forward genetics), of mutating or eliminating a specific gene and observing the phenotypic change (reverse genetics), or of identifying mutations associated with specific human diseases [28–32]. The use of knockout and conditional knockout techniques coupled with the availability of the mouse genome has been very useful in the latter context. The potential power of such techniques is illustrated by a retrospective correlation of mouse knockout technology with the 100 currently best-selling drugs [33]. The authors conclude that the knockout phe-

notypes correlate well with known drug efficacy, a finding that the authors interpret as indicative of a positive forward path for this and related technologies. This prediction remains to be tested since knockout technologies can, given the existence of redundant and compensating signaling and regulatory pathways, deliver misleading results for the significance of a particular gene. More recently, small interfering RNA (siRNA) was proved to be of substantial value in selectively eliminating gene function and observing the phenotype [34–36]. In a veritable *tour de investigational force* some 87% of the 19,000 plus protein coding genes of *Caenorhabditis elegans* was transiently inactivated by siRNA and the roles of those inactivated genes in fat regulation (an increasingly important drug target) analyzed [37, 38].

The power of small molecules to influence biological function has long been known and was formalized by the 1800s into the discipline of pharmacology, itself a powerfully integrative method of both analyzing and predicting drug activities [18]. Although increasingly overshadowed during the past three decades by the power of genomics, the importance of molecular perturbation of biological systems is increasingly recognized in the term 'chemical genetics' [39–43]. By analogy to classical genetic approaches, chemical genetics falls into the forward and reverse modes in which the phenotypic consequences of a molecule are observed with subsequent target identification or through a library screening process a ligand is identified for a macromolecule (typically a pro-

tein) of interest and the phenotypic expression of the ligand-macromolecule interaction is subsequently determined (fig. 3). The former mode will be recognized as classical pharmacology in new clothes, whilst the latter adds pharmacological principles to genome-derived targets, for example 'orphan' receptors and proteins identified from homology screening of the genome [44].

Although genome studies have been, and will continue to be, of major significance in target identification they also illustrate Alexander Pope's dictum that, 'The proper study of mankind is man' [45]. It is now clear that very small sequence differences, sometimes a single residue only in a protein, or differences in expression level can dramatically alter ligand sensitivity. Thus, a single arginine residue determines the species specificity of the human growth hormone receptor relative to nonprimate species. Arg⁴³ in the human hormone receptor interacts with Asp¹⁷¹ of the hormone: these critical residues differ in nonprimates [46]. Serotonin 5-HT-6 receptors are widely believed to be involved in cognition processes: however, the mouse receptor differs in both regional brain expression and sequence in two critical residues (Tyr¹⁸⁸ in helix 5 and Ser²⁹⁰ in helix 6) from the rat and human equivalent, thus raising serious questions about the validity of a mouse knockout model in drug discovery for this particular receptor [47, 48].

Finally, although the contemporary paradigm of drug discovery focuses almost exclusively on highly specific molecules interacting with defined single targets, there is abundant evidence that a number of diseases are, in fact, multiple pathologies with multiple defects and that a number of therapeutically important molecules owe their effectiveness to interaction at multiple targets [49, 50]. This is true for a number of therapeutic areas, including psychiatry where drugs used in mood disorders and schizophrenia have very complex pharmacology [51]. Clozapine, for example, interacts with a number of biogenic amine receptors and transporters in the central nervous system [52]. It remains, despite a number of side effects, a very effective standard agent. However, it is likely that it would not have been discovered using the current single validated target approach. A similar situation applies to a number of therapeutic areas. Stroke pathology is mediated through a variety of processes including ionic imbalances mediated through a variety of voltage-gated and ligand-gated ion channels. Although experimental drugs may be effective in highly controlled laboratory conditions there is no effective drug treatment for stroke currently available (other than clot dissolution), almost certainly because the clinical situation demands

a drug with multiple actions or a complex cocktail of single target drugs [53].

Such promiscuous molecules offer serious challenge to the contemporary mode of drug discovery. The design of 'magic shotguns' rather than 'magic bullets' challenges both the drug designer who is faced with the problem of fitting diverse structure-activity relationships within a single molecule and the drug screener who is faced with the issue of designing a screening system that both reports multiple activities and is relevant to the clinical condition [49, 50, 54].

Finding the Molecule in the Age of Biology

The application of chemistry to drug discovery has changed dramatically over the past three decades and the rate of change continues to increase. The medicinal chemist of the mid 20th century synthesized molecules one at a time from leads typically provided by natural products and obtained biological data from *in vivo* test systems [55]. Today, the search for new structures employs combinatorial chemistry to explore more chemical space, and both the biological targets and data are likely to be derived from genomic leads and systems [56, 57]. Nonetheless, organic chemistry is still a central component of drug discovery, although the knowledge base of the medicinal chemist has expanded significantly [58–60].

Nature is, of course, the ultimate combinatorial chemist. A repertoire of 20 amino acids has generated the 100,000 or so catalytic, regulatory, immune and structural proteins that constitute the cellular catalog. In the laboratory the potential chemical space of a typical small molecule drug of molecular weight 350–500 is so large as to be unattainable. The issue then becomes one of using combinatorial chemistry to explore biologically useful space and to mimic nature, which does not synthesize nonfunctional molecules. One such approach is to focus on 'privileged' structures or pharmacophores that constitute a skeleton compatible with a component of biologically useful chemical space and which, when appropriately decorated, can generate molecules with diverse biological activities. One example is provided by the benzodiazepine nucleus which in addition to being the basis for valium and many other antianxiety and muscle relaxant agents can also be directed against other biologically distinct targets (fig. 4).

Nature has, of course, linked combinatorial chemistry with biological selection to generate the most biologically fit molecules. An interesting example of this is provid-

Fig. 4. The benzodiazepine skeleton as a 'privileged' structure generating molecules that interact at diverse and unrelated targets.

ed by the several hundred venomous mollusks of the *Conus* genus where these cone snails synthesize multiple variations of disulfide-bridged peptide toxins that exhibit, in species-dependent manner, both high affinity and selectivity for a variety of ion channels and neurotransmitter receptors to ensure maximum compatibility between venom production and prey selection [61]. This process of selection can also be mimicked in the laboratory by the process of dynamic combinatorial chemistry whereby molecular fragments self-assemble in the presence of a target or template, thus shifting a reversible equilibrium in favor of the target-assembled product [62, 63] (fig. 5).

The fundamental importance of template-guided reactions in biological systems is well known. It was the basis of the famous understatement by Watson and Crick [64] in their 1953 *Nature* paper announcing the structure of DNA: 'It has not escaped our attention that the specific pairing we have postulated immediately suggests possible copying mechanisms for the genetic material.'

The technique can be mimicked in the laboratory by using a biological molecule as a template and allowing optimum fragment assembly followed by covalent interaction to produce the final template-oriented product [65]. This technique has, for example, been used to produce an extremely potent inhibitor of acetylcholinesterase with femtomolar affinity [66] (fig. 6).

Although replicating molecules are classically described in terms of nucleotide sequences there are now an increasing number of self-replicating systems delineated for both peptides and small synthetic organic molecules [66–69]. Coupled with a selection process around a biological target these systems can be described as 'Darwin in a test tube' [70]. Biologically driven macromolecular synthesis is subject to constant evolutionary pressure: hence, the diversity of macromolecules is coupled to the changing biological environment. The translation of this fundamental biological paradigm to the *in vitro* environment of the laboratory has now been described for a number of systems [71–73]. Any evolutionary system is mini-

Fig. 5. A schematic representation of dynamic combinatorial chemistry whereby molecular fragments self-organize on a template via a reversible (dynamic) equilibrium process to generate the optimum molecule.

Fig. 6. An example of template-guided synthesis ('click' chemistry) whereby molecules that interact reversibly with adjacent sites on acetylcholinesterase are linked covalently through a 1,3-cycloaddition reaction ('click') to form an extremely potent enzyme inhibitor, significantly more potent than the starting compounds.

mally formed from three components: a source of materials of different structures and properties – the chemical population; a recognition and selection process that can differentiate between individual molecules; a mutating or randomizing process that generates the mixed population from which another round of selection can occur (fig. 7).

Thus, the 'new' chemistry applied to drug discovery may be described as depicting self-reproducing, self-organizing, self-targeting and self-evolving molecules (fig. 8). The challenge to be faced is how to translate this into the 21st century equivalent of Ehrlich's magic bullet.

Delivering the Molecule in the Age of Biology

The landscape of drug delivery has undergone considerable change in the past 20 years. Although the simple tablet, orally administered, remains dominant, even this has undergone major transformation into a variety of sustained and extended release forms and more recently with time-dependent mechanisms into chronopharmacological devices [among others, 74–78]. Simultaneously, new polymeric materials have made possible more effective release control and drug-coated stents are now in routine use in a number of obstructive vascular diseases [79]. Polymer therapeutics, including polymer-drug and polymer-protein conjugates, represent improved ways of de-

Fig. 7. A molecular evolutionary cycle (Darwin in a test tube) whereby a population of molecules is selected via target and that pool of molecules is subject to further mutation (change in structure) to create a new molecule population which is further selected and mutated until optimum fitness for the target is achieved.

Fig. 8. The 'new' chemistry.

livering both small and large molecules and these polymer conjugates can also be made 'directional' through the inclusion of specific chemical groups that recognize cellular markers [80, 81]. Additionally, these polymeric systems can be designed to reproduce physiological systems where pulsatile, rather than continuous, release is required: key examples include insulin, growth hormone and other related protein factors and nociceptive drugs in the control of pain [82]. Finally, these systems can be made response-sensitive whereby drug release occurs in response to a specific signal. A number of examples are known of stimuli-responsive hydrogels in drug delivery systems [83].

The ultimate goal of these efforts is to miniaturize drug delivery systems from the current macro level: pumps, patches and syringes – to the micro (100- to 0.1- μm) and nano (100- to 1-nm) scales, generating 'integrated systems that combine device technology with therapeutic molecules to allow the creation of implantable devices that can monitor health status and provide prophylactic or therapeutic treatment in situ' [84–88]. The limitation to such micro- and nano-devices is their limited capacity and although this can, in principle, be overcome by the addition of reservoirs (internal or external) or by the use of multiple small devices, the intrinsic value of the small-scale device is diminished significantly. This is not to argue that the technology behind such devices will not continue to be improved and that such systems lack value. However, their current limitations certainly suggest that alternatives to simply mechanical devices are worthy of pursuit.

It is likely that the next decade will see through the incorporation of more biomimetic materials, the increased creation of stimuli-sensitive systems with nano-characteristics and the use of cell-directional mechanisms steadily increasing progress to drug delivery systems that mimic very closely endogenous cellular release systems for hormones and transmitters – specific, on demand and keyed to physiological or pathological signals. Thus drug delivery systems of the future may be seen as having the following general characteristics [89–91]: (a) transition from site-nonspecific to site-specific; (b) transition from device-driven systems based on in vitro properties to pathophysiology-driven systems for in vivo performance; (c) delivery through intrinsic synthetic capacity, and (d) drug delivery systems that are an integral component of new medicine development rather than an 'add-on'.

We may thus envisage drug delivery systems of the future that will be activated by specific pathologies and cellular defects and that have built in reservoirs and/or synthetic capacity. Ultimately, these drug delivery systems will be 'custom-built cells'. Figure 9 depicts such a device capable of synthesizing and releasing growth factors in, for example, the ischemic myocardium to accelerate new blood vessel growth. In principle, several approaches are possible, and while distinct they are all based on nucleic acid-based systems – engineered viruses, bacterial or cells synthesized de novo.

In the hypothetical example in figure 9 the pathology-recognition characteristic of the delivery system is pro-

Fig. 9. A schematic representation of a drug delivery machine whereby machinery for the delivery of vascular endothelial (VEGF) and platelet-derived (PDGF) growth factors are delivered to ischemic tissue via a guidance mechanism provided by an oxygen sensor.

vided by the transcription factor – HIF – activated by hypoxic scenarios. A more general recognition process can be provided by using a disease-specific nucleotide sequence to initiate a recognition sequence that culminates in drug release or drug synthesis at the disease locus. Proof of principle is provided in figure 10, demonstrating that the association of a drug attached to a specific nucleotide sequence binds specifically to the disease sequence thus permitting the release of drug at the disease locus [92]. In principle, the same approach is applicable to nucleotide, amino acid or carbohydrate sequences.

The use of adenoviruses as potential therapeutic vehicles presents a number of advantages. They are nonintegrating DNA viruses that can readily be engineered and adenovirus infection generates a humoral immune response, although caution is necessary with respect to the latter property [93, 94]. It is this that probably underlies their current lack of clinical utility [95]. The adenovirus system has been employed in a number of oncolytic scenarios, including those where p53 activity is defective [96–99].

The p53 cellular protein serves to suppress abnormal cell proliferation leading to tumor growth and is mutated in approximately 50% of human cancers [100, 101]. In one scenario the virus was engineered as 01/PEME to express itself selectively in cells that lack functional p53 protein [99]. In the normal replication cycle of the adenovirus, a cellular transcription factor E2F is used to initiate the synthesis of viral DNA. By engineering the adenovirus to express an inhibitor of E2F under the control of a p53-responsive promoter it can be ensured that the virus will

not replicate in normal (p53-positive) cells. Thus, in normal cells that contain active p53 no vital replication occurs, whilst in p53-deficient cells replication proceeds. To ensure that the engineered virus was significantly cytopathic the virus was also engineered to overexpress a protein, E3-11.6K, involved in the lysis of cells and viral release.

The vesicular stomatitis virus has been re-engineered to take advantage of the dual receptor mode of HIV-1 entry into cells. The human immunodeficiency virus, in common with many other viruses, takes advantage of preexisting receptors to gain entry into cells [101, 102]. These ‘hijacked’ receptors present excellent opportunities for antiviral drug design because in many instances both agonist and antagonist ligands are already known. The human immunodeficiency virus enters the host’s immune cells by dual occupancy by the viral envelope protein gp120/41 of the CD4 and the chemokine CCR5/CXCR4 receptors [103, 104]. Cells infected with HIV express the specific protein gp 120/41 on their cell surface. A lytic vesicular stomatitis virus engineered with a deleted G protein, thus preventing fusion with normal cells, but expressing CD4 and CXCR4, is capable of fusing selectively with HIV-infected cells and destroying them by lytic action (fig. 11) [105]. The engineered virus can be thought of in terms of Clint Eastwood’s ‘Dirty Harry’ character who awaits the appearance of the bad guys so that he can destroy them and ‘make his day’.

Similarly, a number of attempts have been made to engineer commensal bacteria so that they secrete chemotherapeutic agents. The success of this strategy depends

Fig. 10. A schematic representation of the use of a specific nucleotide sequence to provide recognition for a specific chemical reaction – the hydrolysis of an ester ('drug') via a catalyst ('catalyst'). In an extension to a drug delivery scheme the drug could be loaded into a nanomachine or engineered cell and only when it interacted with a specific nucleotide sequence (physiological or pathological) would the delivery or synthesis of drug occur [93].

Fig. 11. A viraceutical system composed of an engineered vesicular stomatitis virus designed to interact only with an HIV-1-infected cell, and to enter the cell and destroy it [from ref. 105].

upon a number of factors including: ability to compete with indigenous bacterial strains; ability to secrete sufficient agent; be free from intrinsic pathology. Reports thus far include bacteria engineered against rat models for vaginal *Candida* infection and dental caries [106, 107], and against HIV-I infection [108–110]. The use of colonizing bacteria to secrete anti-HIV-1 compounds is particularly attractive since much HIV-1 infection occurs through the mucosal surfaces of the vagina and lower intestine. Most recently, the highly colonizing Nissle 1917 strain of *Escherichia coli* was engineered to secrete a fusion-inhibitory peptide derived from the gp41 sequence [110]. Fusion-inhibitory peptides are the latest drug in the AIDS armamentarium; these act to prevent the fusion of the viral and cell membranes achieved after CD4/chemokine receptor interaction [111]. The engineered *E. coli* were capable of colonizing mice for prolonged periods and could secrete fusion-inhibitory peptide at micromolar concentrations.

In principle, it will ultimately be possible to engineer a cell de novo through the construction of a completely synthetic genome [112, 113]. This is not yet possible although the genomes of at least two viruses have been assembled in the laboratory – bacteriophage 1 × 174 [114] and polio [115]. Progress has, however, been made in defining the minimum gene set critical to cellular function [116–119]. Thus, *Mycoplasma genitalium* possesses 480 genes, of which only some 256 may be essential for growth [116, 117]. In *Bacillus subtilis* it is estimated that, of the approximately 400 genes in this organism, 192 were indispensable and another 79 were predicted to be essential. Thus, the minimal gene set may be quite small, although nonetheless a current serious synthetic challenge. However, it is worthy of note that the assembly of a contiguous and functional 32-kb polyketide synthase gene cluster (responsible for the biosynthesis of polyketide products such as erythromycin) has been achieved [120].

The process of genome synthesis may be extrapolated one stage further with the envisioning of an artificial or expanded genetic code that is capable of incorporating novel amino acids (and ultimately amino acid homologs) other than the 20 currently generally coded for [121–123]. Considerable progress has already been made in this direction with the incorporation of several unnatural amino acids. Of particular interest is the generation of a bacterium with a 21 amino acid code that is completely autonomous and encodes *p*-aminophenylalanine through an endogenous biosynthetic pathway [124].

When this is achieved we will have the ultimate drug synthesis, homing and delivery machine sketched in figure 12.

A self-organizing, self-synthesizing, self-directing and self-evolving drug machine

Fig. 12. A self-organizing, self-synthesizing, self-directing and self-evolving drug delivery machine – the ultimate in medicine's development? [From ref. 125].

'I never make predictions, especially about the future'

Samuel Goldwyn

Conclusions for the Future

Like many other quotations attributed to the late Mr. Goldwyn this one is also probably apocryphal. It does, however, point to the difficulty in making predictions, particularly about our scientific future. It was, after all, Lord Kelvin (President of the Royal Society from 1890 to 1895), who argued that 'X-rays will prove to be a hoax', and that 'heavier than air flying machines are impossible'.

The speculations offered here may well suffer the same fate. If they do, it will probably be because of our hubris. Over the past 20 years in pharmaceutical research each new technology and advance has been hailed as 'the advance' that will generate the magic bullet. This has been true for structure-based design, combinatorial chemistry, in silico design, genomics, knockouts and gene therapy, and many other advances. In truth, all of these technologies have been useful but ultimately in an incremental manner – one more tool to be added to the scientist's toolbox. The speculations offered here will likely at best be part of incremental change and advance. That is not all bad: our aim after all is perhaps to prepare for the future, rather than to predict it.

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