Green fingers, molecular pathology and the art of drug design

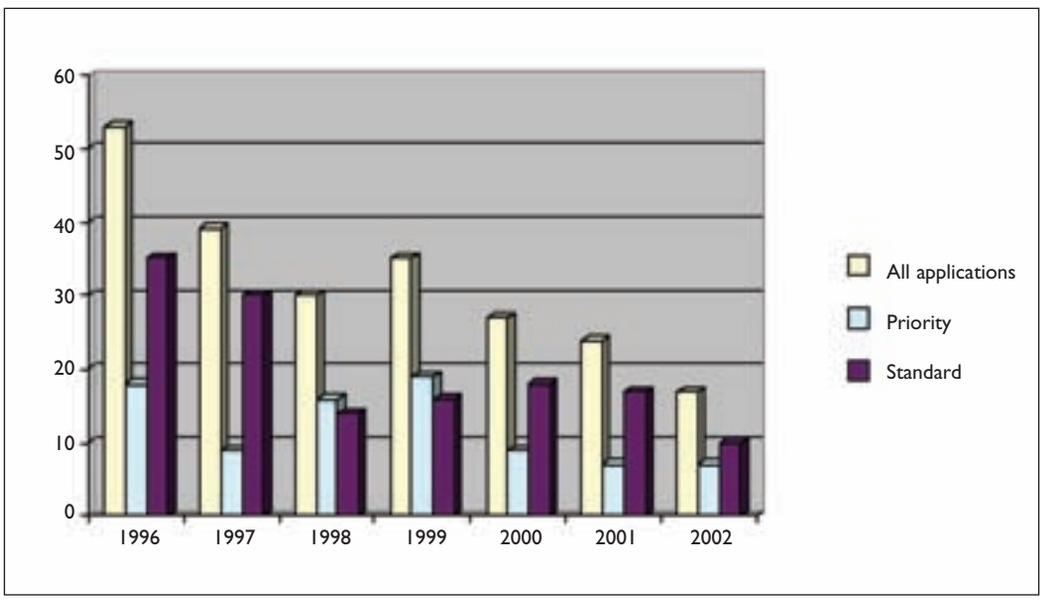
The current explosion of interest in sophisticated genomic technologies, computerised robotics and high throughput screening of diverse molecular libraries appears to have been associated with a reduction in research productivity in recent years, as judged by the rate of new drug approvals. Analysis of today's largest selling drugs shows that most have come from knowledge of key rate limiting enzymes or pathways in the causes of disease and the rational design of selective inhibitors to block them. Current genomic research techniques appear to be producing fewer breakthrough drug candidates than the 'green fingers' of more thoughtful innovative chemists and biologists of the past. If the biopharmaceutical industry is to continue to produce breakthroughs in medicine, for the benefit of patients and shareholders alike, it is important that attention be focused on the molecular causes of disease and more reliance placed on creative medicinal chemistry and biochemistry in the rational design of specific inhibitors.

By Dr Keith McCullagh

The explosive growth of bioscience information and technology of the past decade has placed powerful tools in the hands of pharmaceutical and biotech research leaders. The sequencing and genetic analysis of the genomes of man and a variety of his fellow creatures has stimulated a new lexicon for our growing knowledge: genomics (functional or otherwise), transcriptomics, proteomics, metabolomics and now the somewhat prosaically named, systems biology, to quote but a few. Such names imply that the entire universe of biological data relating to a living entity is knowable which, in concept at least, is probably true. However, at present these names remain largely aspirational – in most cases we are still at a geographic level, describing and creating atlases to bring order to our minds. The reality of our knowledge at this early point in demystifying the riddles of biology is that we have a pile of data but understand very little of it.

Why then have pharmaceutical companies around the world invested so heavily in technologies and expertise aimed primarily at cataloguing and defining bioscience rather than do something more commercially exciting and creative? That is, to think and imagine how disease might occur, how it is mediated in molecular terms and how the symptoms or causes might be slowed or corrected by pharmacological means. After all, the money which flows so readily within big pharma from revenues to R&D

Figure 1
The decline in FDA (Center for Drug Evaluation and Research) approvals of new molecular entities 1996-2002



expenditure does so because of the implicit assumption that more investment into R&D will drive innovation and enhance the flow to market of novel proprietary medicines capable of changing patient outcomes in today's poorly treated diseases. If, today in mid-2003, one walks the halls and corridors of pharma and biotech research centres worldwide, peering through security glass at gleaming laboratories with micro-array robots, high throughput assay instrumentation, bioinformatics databases on multiple LCD screens, one is left with the thought: has building new drug molecules been pushed to one side by the drive to measure, record, archive and retrieve information?

Have we lost interest in creating medicines in the rush to uncover and store ever more complex data sets? The argument is often made that the sheer volume of bio-data being discovered and the sophistication of the bio-informatics tools used to uncover relationships within it will inevitably lead over the years to the identification of a vast array of new drug targets. While it is true that the first draft sequence of the human genome was published only recently[1], the tools of micro-spotted DNA and RNA arrays, SNP analysis, expression sequence tagging, proteomic mapping, antibody diversity and many others, coupled to combinatorial chemistry and high throughput screening, started to gain prominence in pharma bioscience research laboratories during the 1990s. If such techniques enable enhanced productivity in pharmaceutical research, we should at least be seeing early signs of an increased wave of new molecular entities emerging through development pipelines.

Table 1: DATA for Figure 1. *(Source: FDA Center for Drug Evaluation and Research)*

CALENDAR YEAR	NUMBER OF NMEs APPROVED BY FDA		
	Priority	*Standard*	*All applications*
1996	18	35	53
1997	9	30	39
1998	16	14	30
1999	19	16	35
2000	9	18	27
2001	7	17	24
2002	7	10	17

Decline in new drug approvals

On the basis of evidence provided by regulatory agencies in the US and Europe, this does not appear to be happening. On the contrary, since hitting a peak in 1996, the number of new molecular entities (NMEs) approved as new medicines each year by the US Food & Drug Administration has been a steadily declining (see **Table 1** and **Figure 1**).

The decline in approvals has been manifest in both Standard applications (those considered by the FDA to be drugs with similar therapeutic qualities to those already marketed) and Priority applications (those thought to represent an advance over available therapy). The decline does not appear to be related to the speed of review. The FDA has a target of six months for review of Priority applications and by and large during the years 1997 to 2001 it has achieved this, although there was a glitch last year (2002) when the median review time shot up to 13.8 months. For Standard applications, the median review time has been pretty constant at 12 months over the period.

The numbers shown in **Figure 1** are those for NMEs handled by CDER, the FDA's Center for Drug Evaluation and Research. However, most new genetically engineered therapeutics are dealt with separately by CBER, the Center for Biologics Evaluation and Research. Perhaps then, we might anticipate that the emergence of new medicines from the genomics revolution is more likely to be seen in data from CBER. However, there is not much evidence of this. Numerical data for Biologicals approvals is shown in **Table 2**.

The rate of approvals from CBER has stayed within the range 5-9 for each year since 1996. It is tempting to single out 2002 as the start of an upturn but in fact two of the approvals were for PEG-modified versions of existing drugs (Neupogen and Interferon-alpha), one was for a version of Interferon-beta from a new manufacturer, two were for combination diphtheria, tetanus and pertussis vaccines, two were purified enzymes (human alpha-1 proteinase inhibitor and rasburicase) and only two were for truly novel treatment modalities (Zevalin – the radioactive combination antibody regimen version of IDEC's Rituxan for the treatment of non-Hodgkins B-cell lymphoma; and Humira – the Cambridge Antibody Technology antibody for treatment of Rheumatoid arthritis marketed in the US by Abbott Laboratories).

I have quoted the approval rates for the US because it is the largest pharmaceutical market in the world (see below). However, the situation in Europe is no better. The European Medicines Evaluation Agency approved only 31 NDAs in 2002, a 47% drop from the 58 approved in 2001[2].

Table 2: Therapeutic Biologicals (including vaccines) approved by the FDA between 1996 and 2002.
(Source: FDA Center for Biologics Evaluation and Research)

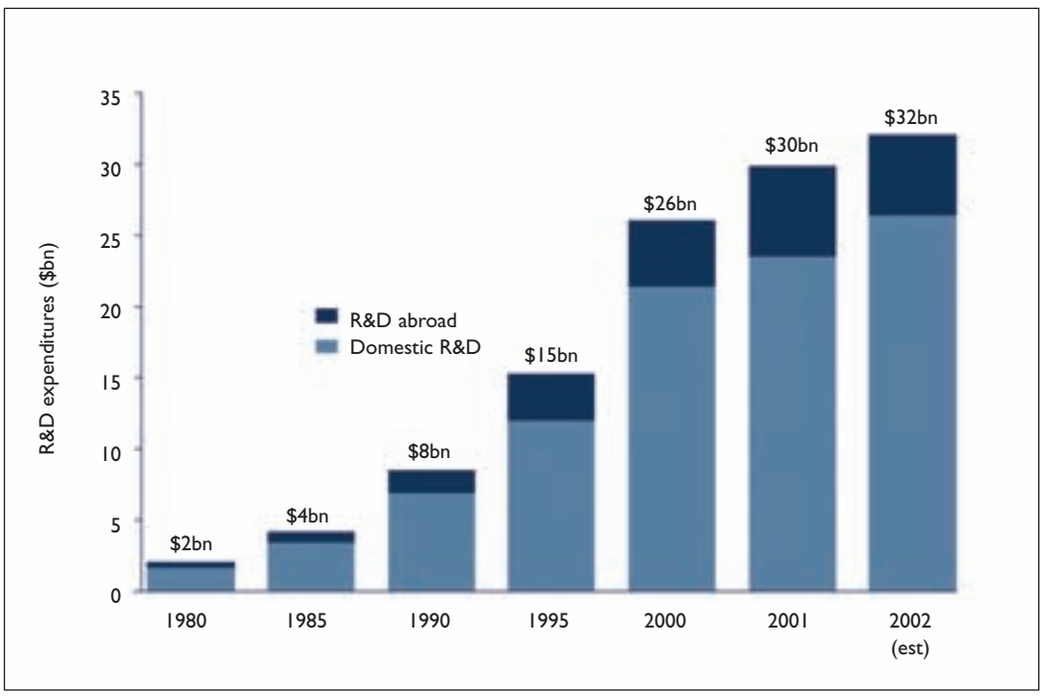
CALENDAR YEAR	NUMBER OF APPROVED BIOLOGICALS
1996	5
1997	8
1998	8
1999	5
2000	6
2001	7
2002	9

Increasing research and development expenditures

The declining approval rate for NCEs and the low approval rate for NBEs has occurred against a background of huge increases in R&D investment by the world's pharmaceutical and biotech industry. Since 1995 the R&D spend by US pharma companies has more than doubled from $15 billion in 1995 to $32 billion in 2002 (see **Figure 2**). This figure is now substantially higher and increasing more rapidly than the entire operating budget of the US National Institutes of Health ($24 billion in 2002[3]). R&D spend by public biotech companies has also been expanding rapidly, from $7.2 billion in 1998 to $12.3 billion in 2001[4]. Judged by how many innovative new medicines are getting to market (and hence benefiting patients), the cost to benefit ratio has moved strongly to the left. In other words pharmaceutical research productivity appears to have declined dramatically in recent years.

Fortunately for the industry, worldwide sales of pharmaceuticals are continuing to grow. In 2002 global sales increased by 8% to reach a total of $400.6 billion, of which just over half ($203.6 billion) were achieved in the US. The US market also showed stronger growth at 12%[5]. Not surprisingly, in order to achieve these results, industry expenditure on sales and marketing is also growing steadily (total promotional spend by US companies was $19 billion in 2001, an increase of nearly 19% on the previous year). Nevertheless, for the moment at least, the pharmaceutical industry can continue to afford the extraordinary largess of its investment in

Figure 2
The growth of research and development spending by US pharmaceutical companies 1980-2002. *(Source: PhRMA[3])*



R&D. But the message is clear – productivity needs to return to higher levels and in particular, new breakthrough drugs commanding large markets are urgently needed.

Today's 'blockbuster' drugs

Any company seriously interested in how best to achieve such breakthroughs would be well served by examining the histories of today's best selling drugs worldwide (**Table 3**). Eight of the 10 largest selling drugs are rationally designed inhibitors or antagonists of specific molecular targets. That is, the pharmacological target for drug action is a clearly defined biological enzyme or membrane receptor and the drug has been synthesised with this target in mind.

The astonishing rise of the 'statins', cholesterol lowering drugs which inhibit the conversion of HMG-CoA to mevalonate, a rate limiting step in cholesterol biosynthesis, is in part due to clinical studies showing first that their use had a profound effect on low density lipoprotein cholesterol, a major risk factor for heart disease. This led to large-scale clinical trials which proved that long-term statin use can lower mortality rates from coronary heart disease and that most drugs in the class had relatively few side-effects. With more statins and 'superstatins' entering the market, most recently Crestor (rosuvastatin) from AstraZeneca, there will soon be in excess of 14 companies with launched drugs in this class[6].

Rational drug design

The basic science research which has led to the success of the statins started with unravelling the steps in the pathway of cholesterol biosynthesis, work conducted in the 1950s and by Konrad Bloch at Harvard, John Cornforth and George Popjak in the UK and Feodor Lynen in Germany. The seminal nature of this work was recognised when Bloch and Lynen shared the Nobel Prize in Physiology or Medicine in 1964 with Cornforth being awarded the Nobel Prize in Chemistry in 1975. However, it was the work of Jesse Huff and his colleagues at Merck Sharpe and Dohme laboratories studying bacteria which do not synthesise sterols, which in 1956 identified the formation of mevalonic acid as a necessary step in cholesterol synthesis chain. Subsequently in 1959 the enzyme responsible, HMG-CoA reductase, was discovered by scientists at the Max Planck Institute. For the next two decades scientists at Merck and others around the world tried to identify or build inhibitors of the enzyme. Finally, in 1979 Carl Hoffmann, who had been a member of the team at Merck which discovered mevalonic acid 23 years earlier, isolated the first HMG-CoA reductase inhibitor from a strain of the fungal micro-organism, Aspergillus terreus. Merck filed a patent for the compound, which subsequently became the first drug of its class, Mevacor (lovastatin) in June that year.

The development of Mevacor was not uneventful. An earlier compound, compactin, discovered

by the Japanese company Endo, was rumoured to cause cancer in dogs and Merck was forced to suspend studies on Mevacor. However, Roy Vagelos, Merck's pioneering Research Director and later CEO, persuaded the FDA to allow Merck to give Mevacor to patients suffering from a severe form of hypercholesterolaemia which was unresponsive to other drugs. The results were dramatic, substantially reducing their blood cholesterol levels with few side-effects. In a courageous personal decision, Vagelos recommenced development of Mevacor and committed a substantial part of the company's R&D resources to fast track the programme which, given the Japanese data, required long-term carcinogenicity studies. The drug proved clean and was approved by the FDA in 1987.

Mevacor achieved the highest level of sales of any newly launched prescription drug, reaching approximately $260 million in the US market for the first 12 months from launch, and by 1991 it

Table 3: Worldwide sales of top 10 leading drugs in 2002. *(Source of sales data: IMS World Review 2003 [published Feb 28, 2003])*[5]

TRADE NAME AND COMPANY	GENERIC NAME (INN)	THERAPEUTIC CLASS	MOLECULAR MECHANISM OF ACTION	WORLDWIDE SALES 2002 ($BN)	% GROWTH IN 2002
Lipitor *Pfizer*	atorvastatin	Cholesterol reducer	HMG-CoA R Inhibitor	8.6	+20%
Zocor *Merck*	simvastatin	Cholesterol reducer	HMG-CoA R Inhibitor	6.2	+13%
Losec/Prilosec *AstraZeneca*	omeprazole	Anti-ulcerant	Proton-pump Inhibitor	5.2	-19%
Zyprexa *Eli Lilley*	olanzapine	Anti-psychotic (atypical)	Multiple neuroreceptor antagonist	4.0	+21%
Norvasc *Pfizer*	amiodipine	Antihypertensive /anti-angina	Calcium antagonist	4.0	+6%
Erypo/Procrit *J & J (Amgen)*	Epoetin-α	Red blood cell generator	r-human Erythropoietin	3.8	+18%
Ogastro/Prevacid *TAP Pharma*	lansoprazole	Anti-ulcerant	Proton-pump Inhibitor	3.6	+3%
Seroxat/Paxil *GlaxoSmithKline*	paroxetine	Anti-depressant	SSRI	3.3	+13%
Celebrex *Pfizer (Pharmacia)*	celecoxib	Anti-arthritic/NSAID	COX-2 Inhibitor	3.1	-1%
Zoloft *Pfizer*	sertraline	Anti-depressant	SSRI	2.9	+12%
Total 2002 WW sales for 10 leading therapeutic products:				44.7	+11%

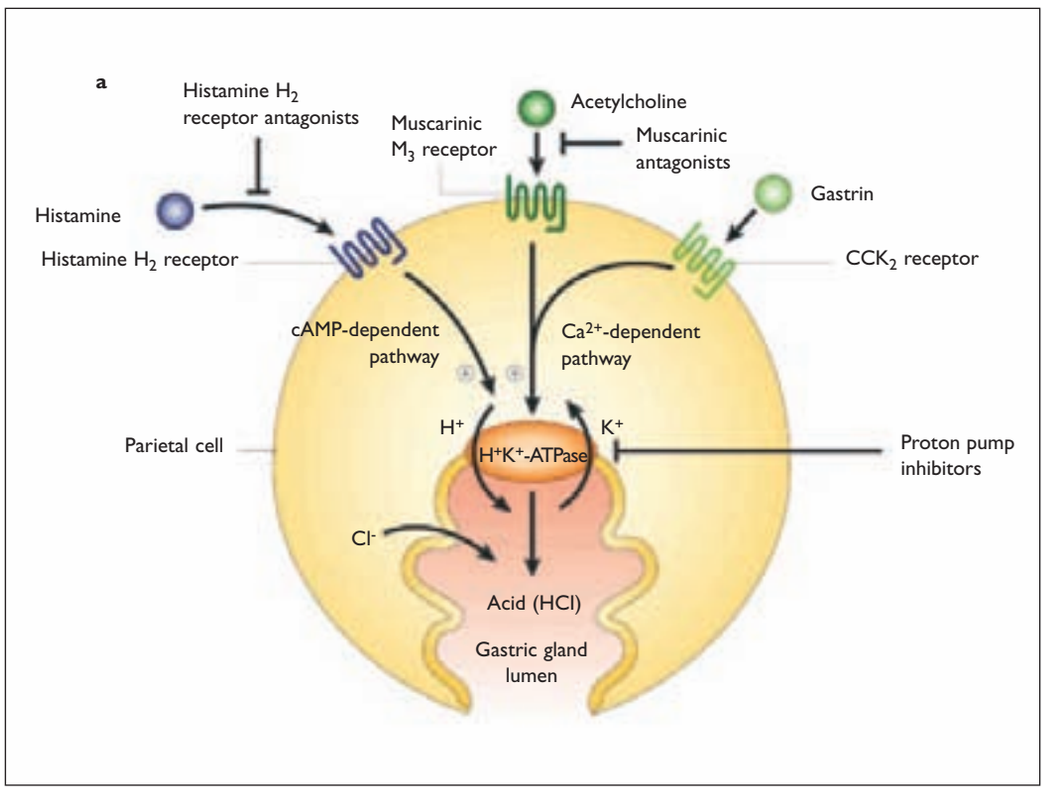


Figure 3
Mechanism of action of the histamine H_2 antagonists, Tagamet and Zantac, and the proton pump inhibitors, Losec and Nexium, on acid secretion by stomach parietal cells[8]

had topped $1 billion. Nevertheless, many competitors had targeted HMG-CoA reductase as a major opportunity in pharmaceutical research and teams of medicinal chemists around the world were soon successful in designing and patenting superior, more potent semi-synthetic inhibitors of the enzyme. Bristol Myers Squibb won FDA approval for Pravachol (pravastatin) in October 1991 and Merck itself achieved FDA approval for a second-generation product Zocor (simvastatin) three months later. Both were marginally more effective than Mevacor but there was little scientific difference between them. The resultant competition in the marketplace drove clinical studies and marketing spend over following years.

This story is interesting from two standpoints. First, although the initial drug candidates were extracted and purified from micro-organisms, the research at Merck and other companies was clearly and unambiguously focused on a specific molecular pathology target – a key enzyme which controls cholesterol synthesis and is upregulated in patients with high blood cholesterol levels. Secondly, the Merck approach was not random. Rather, it was the result of Roy Vagelos's rationalisation of the drug research process. When he was appointed as R&D Director, Vagelos insisted that rather than hedge their bets by working on multiple projects which may or may not lead somewhere, Merck scientists should prioritise their work in areas where:

(1) there were no therapies currently available,
(2) the science was advanced enough to allow the possibility of a breakthrough, and
(3) they had sufficient understanding of the disease to have an idea of how to arrest it[7].

This approach, particularly the focus on a specific target for drug action, has become widely known as *rational drug design*. It is remarkable to realise that nearly all of the drugs listed in **Table 3**. together with many more which would justify the label 'blockbuster' have resulted from such rational drug design research.

To take another disease area, it has been apparent for many years that inappropriate levels of gastric acid secretion are the basis of many widespread gastro-intestinal pathological conditions, including gastro-esophageal reflux disease (GERD), whose major symptom is heartburn, and peptic ulceration, a major and common cause of pain and suffering which until the late 1970s was also life-threatening. The elucidation of the H_2-receptor on the surface of gastric parietal cells and design of specific antagonists of histamine selective for this receptor (**Figure 3**) is now one of the classic stories in rational drug design. Under Sir James Black's

leadership, Bill Duncan's biochemistry and Robin Ganellin's intensive medicinal chemistry led to the rational design of potent selective H_2 blockers. The subsequent launch of Tagamet (cimetidine) by SmithKline & French in 1987 met a significant medical need and did much to transform SK&F into the successful international company SmithKline Beecham.

In a similar manner, Sir David Jack's subsequent selection of the H_2-receptor as a rational drug design target and his championship of medicinal chemistry at Glaxo led to the launch of Zantac (ranitidine) five years later. In an analogy with the impact of Tagamet, Zantac became the driving force for the growth of Glaxo into a truly international business and provided the financial strength for its subsequent acquisition of Wellcome.

H_2-blockers are no longer placed in the top 10 drugs worldwide although they certainly contributed to anti-ulcerants remaining the top selling therapeutic category in 2002 ($21.9 billion[5]). As shown in **Table 3**, the leading drugs in this class are now the proton pump inhibitors, Losec/Prilosec (omeprazole) and Ogastro/Prevacid (lansoprazole). The development of proton pump inhibitors is also a story of rational drug design and was described recently in detail[8].

Scientists at Astra's Hässle subsidiary commenced work on inhibition of gastric acid secretion in 1972, developing a series of novel benzimidazole inhibitors of stomach acid secretion in the dog. In 1977, evidence began emerging that the activation of a newly discovered enzyme pump (an H^+K^+-ATPase) in the membranes of the parietal cell was the final step in acid secretion. Meanwhile, Astra scientists were rationally analysing the reasons for thyroid toxicity in the early compounds and designed in mercapto-derivatives which removed such side-effects. In 1981, Astra showed that its substituted benzimidazoles did indeed selectively inhibit the gastric proton pump enzyme[9]. Since the proton pump inhibition mechanism impacts the acid secretion mechanism at a later point in the pathway than the H_2-antagonists, omeprazole and its analogues are more universal inhibitors of gastric acid secretion than cimetidine or ranitidine, blocking alternate gastrin and acetylcholine stimulated acid production as well as histamine (see **Figure 3**).

Astra was also fortunate in that the half life and duration of action of omeprazole was superior to that of the H_2-antagonists. The result was that following their market launch in 1988, the omeprazole brands Losec and Prilosec grew rapidly in market share and became the world's largest selling drug in 1996. Omeprazole is now off patent but AstraZeneca's research team continued to apply rational drug design principles to define its successor. Recognising that omeprazole was a mixture of two optical isomers at the suphoxide nucleus, in a ratio of 1:1, the company discovered that while each of the isomers had identical activity in inhibiting the proton pump, one of them, the S-isomer, had improved bioavailability and therefore enhanced potency in man. This patented new entity, esomeprazole, was launched in 2000 as AstraZeneca's new second generation product, Nexium.

There are many more examples of the power of rational drug design, allied to an understanding of the molecular pathology of the disease, to create innovative drug molecules with new and different mechanisms of action. Frequently, such new and different agents create breakthroughs in clinical medicine, improving patient outcomes so much that they change the way medicine is practised.

This applies not only to medicinal chemistry-derived drugs but also to biologics. Erythropoietin is a classic example. Not only is it a member of the top 10 list (**Table 3**) but it is undoubtedly the most successful recombinant therapeutic protein produced to date with worldwide sales from all versions (Epogen and Aranesp from Amgen and Erypo and Procrit from J&J) of $8.1 billion in 2002. Although recombinant human erythropoietin was not rationally designed as a molecule, the protein's role in stimulating red blood cell formation was recognised by Amgen scientists in the 1980s and it was rationally selected as one of a number of human proteins whose gene was targeted for recombinant cloning. It was George Rathman, Amgen's CEO at the time, who made the key decision to focus resources on patenting and manufacturing EPO, based on a rational appraisal of its potential medical benefit. Like that of Roy Vagelos at Merck, it was a courageous decision due to the financial commitments involved. No-one today can doubt it was a correct choice.

Molecular pathology

In 2003, at the beginning of the post-genomic century, I am not proposing that research should return to old techniques. Quite the reverse. The power of modern bioscience is that we now have the tools to study and reveal enormous detail about the molecular interactions and cause and effect pathways which characterise normal and abnormal cell functions. By focusing our sophisticated modern bioscience tools on a particular disease pathology and using them to unearth its

regulatory steps, we should today be able to identify rate limiting enzymes, receptors and ligands as targets for new drug action more effectively than in the past. If that is not happening at the speed we would wish, it is perhaps because we have allowed technology to seduce us into thinking that analysing genes, gene expression patterns and translated protein maps associated with a disease is a short cut to novel drug targets. It may be but it is also non-rational. Association does not prove cause and effect and the challenges of resolving useful from spurious information become more daunting the more data is processed.

If this sounds far-fetched, think for a moment about the words used to describe favoured modern techniques. Combinatorial chemistry, high-throughput screening, compound libraries, micro-array technologies, numbers of 'hits' and even the term 'drug discovery' sound as if the process is a random 'needle in a haystack'-like search. Conceived in this way, pharmaceutical research harks back to the early days of antibiotic discovery when many companies, particularly in Japan, randomly screened thousands of soil and other natural samples for anti-bacterial or anti-fungal agents. Not only is it an unpredictable and risky approach, such random screening is highly likely to lead to unspecific and therefore toxic compounds.

By contrast, rational drug design techniques lead to selectively targeted agents with fewer side effects. It is also the case that successful drugs which create new markets tend to arise from much more targeted and focused questions related to particular disease pathways – in fact from rational drug *research* rather than random drug *discovery*.

Conclusion

The formula for success is therefore to focus on a single disease, to understand its molecular pathology, to identify a key rate limiting step (the target drug 'receptor') in the causation of the disease or disease symptoms, to elucidate its structure at the molecular level and by three-dimensional modelling, iterative synthesis and testing for potency and selectivity, design a chemical or biological entity which can block, inactivate or down-regulate its action in a highly selective way. This process is time-consuming and requires shared knowledge among multi-disciplined teams of researchers all passionately focused on a single target.

The selection of the right 'target' is not a matter for bioinformatics. It comes from an understanding of disease pathogenesis at the molecular level. Such understanding involves imaginative scientific deduction and often visionary leadership. As I have illustrated in this article, pharmaceutical research teams are often led by an entrepreneurial scientist who has taken the time and the trouble to understand something of the complex etiology of a particular disease. He is like a seasoned gardener, whose 'green fingered' ability to grow strong plants from unpromising situations comes from years of integrating experience with reason. Despite the pharma industry's current fashion for investing in the genomic sciences and laboratory automation, I believe we need more scientists with 'green fingers' in molecular pathology, pharmacology and medicinal chemistry and a rational approach to drug design if the decline in the productivity of pharmaceutical research is to be reversed and the industry's reputation for creating breakthroughs in medicine is to be maintained. DDW

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