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R&D technology investments: misguided and expensive or a better way to discover medicines?

Esther F. Schmid[1] and Dennis A. Smith[2]

[1] Strategic Management Group, Sandwich Laboratories, Pfizer Global Research and Development, Sandwich, Kent, CT13 9NJ, UK
[2] Drug Metabolism, Sandwich Laboratories, Pfizer Global Research and Development, Sandwich, Kent, CT13 9NJ, UK

The pharmaceutical industry is in crisis owing to spiralling costs and a lack of new product launches. It is said that expensive investments in technology have not paid off. But is this really true? In this review, we explore some of the recent medicines that were, or are being, brought to market, and we discuss how they were discovered and what difference new technologies have made during the discovery of these medicines.

After a promising recovery in 2004 with 36 new molecular entities (NMEs) approved, 2005 was a lacklustre year for NMEs. At the end of 2005, the FDA recorded the approval of only 20 NMEs (http://www.fda.gov). Against this backdrop, many voices are linking the investment in new technologies with the failure to produce new medicines. For example, an article in the *Wall Street Journal* suggested that, 'the explosion of modern technologies such as high-throughput screening and combinatorial chemistry in the mid-1990s could be responsible for the current lack of new drugs in development' [1]. The same article seeks to provide further evidence that investments in technologies have yet to pay off, by citing a recent study by David Newman of the National Cancer Institute, which suggested, 'of 350 cancer drugs now in human trials, only one drug was the product of combinatorial chemistry' [1].

However, given the cyclical nature of R&D, short-term snapshots are likely to paint an incomplete picture of overall trends. This was proved when we explored the numbers of NMEs approved by the FDA over a much longer timeframe and found no evidence of a dramatic decline in pharmaceutical industry innovation [2,3]. In fact, over six decades, we identified a steady increase in new medicines launched (Figure 1). By contrast, the cost of bringing new medicines to market exponentially increased over the same period [3]. So, although the productivity argument might still be valid, because the cost per medicine launched has increased as a result of R&D costs rising much faster than the industry's innovation rate, it appears that innovation measured by output has not been the primary problem. Nor does the overall benefit that the public receives from these innovations appear to be an issue, despite the current focus on drug safety and risk:benefit in the wake of the withdrawal of Vioxx® (rofecoxib, Merck) from the market. The benefit of new medicines can be measured at the macro level, for example using life expectancy as an outcome. Life expectancy has increased steadily in the USA, as well as in other industrialized nations (Figure 2). About 40% of this increase is thought to be directly related to NMEs (http://www.nber.org/papers/w9754).

Corresponding author: Schmid, E.F. (esther.schmid@pfizer.com)

ESTHER F. SCHMID
Dr Esther Schmid is a Research Strategist with 15 years pharmaceutical industry experience. Since 1995 she has worked for Pfizer, UK; first as Drug Discovery Biology Team Leader and now, since 1999, as a Strategist.

DENNIS A. SMITH
Dr Dennis Smith is Vice President of Pharmacokinetics, Dynamics and Metabolism at Pfizer, UK; with 27 years experience in the pharmaceutical industry. During this time he has directly contributed to the discovery and development of eight new chemical entities.

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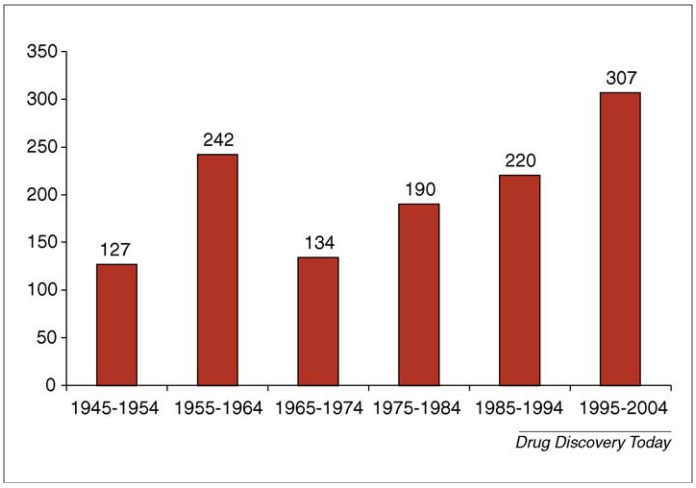

FIGURE 1

Industry performance (FDA-approved new molecular entities) over six decades.

So, clearly, the angle from which a phenomenon is observed, be it productivity- or safety-related, alters the conclusions drawn. What does this mean for new technologies used in R&D and their effect on the launch of new medicines?

What impact has technology had on the R&D process?

Over the past five decades, more and more indications and disease areas have become treatable by new medicines (Table 1). Drugs against cancer and AIDS in particular, but also those targeting cardiovascular disease, have benefited from the use of new technologies in the drug discovery and development process. Synergies at the interface between basic academic and medical research and applied drug discovery in pharmaceutical companies, in a range of areas from genetic profiling to target validation, drug delivery and imaging technologies, have further advanced the discovery of new medicines.

Several drugs that are better targeted towards specific cancer mechanisms and appear to be safer than older cytotoxic and cytostatic drugs have been brought to market over recent years [4,5]. Today, pharmaceutical companies and patients can be highly optimistic about finding new treatment options for cancer. For example, one of the earliest technologies that the pharmaceutical industry embraced in the 1990s – molecular biology – has matured. As a consequence, the so-called reductionist approach, where a molecular target is expressed, purified and screened in a multiwell plate, is now successful. Drugs like Gleevec® (imatinib, Novartis) and Sutent® (sunitinib, Pfizer), and other drugs currently at various stages in the R&D process, were discovered in this way [5]. Yet, this approach was not always successful, at least not in the early 1990s. Recollection of many early failures could be the reason why there is such a high degree of pessimism when *in vitro* HTS is discussed, despite the fact that this technology has evolved over the past two decades into a much more sophisticated, knowledge-driven operation [6].

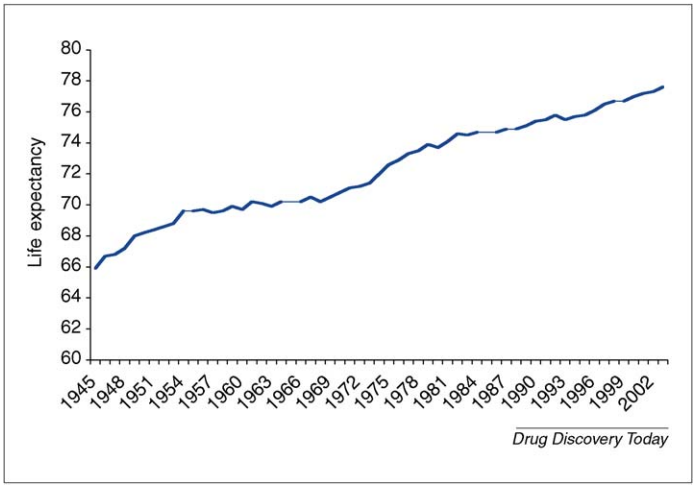

FIGURE 2

Improvements in life expectancy in the USA. The blue line represents estimated life expectancy in the USA.

An evolving knowledge-base as the key driver for successful use of technology in the R&D process

Target selection

A condition for the emerging success with recombinant drug targets was better target selection based on druggability through computational algorithms. Many of the early failures were caused by screening targets for which there were no known active molecules, often because they were outside the traditional aminergic G-protein-coupled receptor (GPCR) space. The new space explored in the early 1990s included non-aminergic GPCRs, adhesion molecules and other protein–protein or lipid–protein interactions. This space, although often intriguing to project teams because of novelty and established links to human disease, proved to be much tougher than anticipated in terms of druggability. Lipinski's Rule of Five [7], which advocates keeping drugs small with a low logP, is a testimony to the kinds of leads that emerged from these screens – large, often lipophilic and badly absorbed. In short, such molecules were difficult, if not impossible, to convert into drugs, because the targets screened could only be modulated by non-druglike compounds. In other cases, no hits emerged because current compound collections do not contain appropriate chemical matter. Recombinant technology has stepped into this gap over the past twenty years. Today, humanization reduces the potential of a host immune response against therapeutic antibodies, and represents an approach embraced by small biotechnology and large pharmaceutical companies alike, particularly when the drug target is extracellular and not amenable to small-molecule intervention. Antibodies, such as Herceptin® (trastuzumab, Genentech), demonstrate the impact such an approach can have on providing new treatment options for cancer patients.

Molecular mechanisms of disease

A second condition for success was an improved understanding of the molecular mechanisms underlying the disease, such that when HTS has produced a lead against a novel drug target the chance that it will work in human disease is maximized. A case in point regarding this is (again) cancer, where many different causes for various types of cancer have been found over the years. Genetic profiling, disease association studies and pathway mining contributed to the discovery of many basic molecular mechanisms

TABLE 1

Disease areas that have become treatable by new medicines over the past five decades

1955–1964	1965–1974	1975–1984	1985–1994	1995–2004
Tuberculosis	Tuberculosis, bacterial infections	Bacterial infections	Bacterial infections, leprosy	Bacterial infections, leprosy, tuberculosis
Schistosomiasis	Worms[a]	Schistosomiasis, worms	Parasites	Worms, parasites
Parkinson's disease	Parkinson's disease	Parkinson's disease	Parkinson's disease	Narcolepsy[a]
Anxiety	Viral infections[a]	Viral infections	Viral infections, **AIDS**[b]	Viral infections, **AIDS**[b]
Depression	Fungal infections[a]	Fungal infections	Fungal infections	Fungal infections
Psychosis	Psychosis	Psychosis	Psychosis	Obsessive-compulsive disorder[a]
Convulsions	Convulsions	Convulsions	Convulsions	Convulsions
Analgesia	Analgesia	Spasticity[a]	Anxiety	Arthritis[a]
Anaesthesia	Anesthesia	Anesthesia	Anesthesia	Acromegaly[a]
Hypertension	Hypertension (β-blockers)	Hypertension (ACE inhibitors, CCB)	Hypertension	Hereditary metabolic disorders[a]
Diuresis	Diuresis	Diuresis	Depression[a]	Diabetes
Heart failure	**Hyperlipidemia**[b]	**Hyperlipidemia**[b]	Developmental disorders[a]	**Hyperlipidemia**[b]
Obesity	Obesity	Growth (growth hormone; GH)[a]	Hormones	Obesity
Diabetes	Diabetes	Thyroid[a]	Alzheimer's disease[a]	Alzheimer's disease
Inflammation	Inflammation	Inflammation	Inflammation	Inflammation
Thyroid	Fibrinolysis[a]	Arrhythmia[a]	Arrhythmia	Thyroid
Cough, mucolysis	Asthma[a]	Asthma	Asthma	Asthma
Cancer[b]	**Cancer**[b]	**Cancer**[b]	**Cancer**[b]	**Cancer**[b]
Gynaecology	Gynaecology	Gynaecology	Irritable bowel[a]	Lower gastrointestinal tract, irritable bowel syndrome
	Contraception[a]	Contraception	Blood clots, fibrinolysis	Blood clots, fibrinolysis
	Sedation[a]	Sedation	Emesis[a]	Emesis
		Pain	Pain	Pain
		Nasal congestion[a]	Allergy[a]	Anemia[a]
		Transplantation[a]	Kidney disease[a]	Angina[a]
		Gastric acid[a]	Gastric acid	Pulmonary hypertension[a]
		Bone metabolism[a]	Bone metabolism	Bone metabolism
		Constipation[a]	Immunomodulators[a]	Immunomodulators
		Ophthalmology[a]	Ophthalmology	Ophthalmology
		Malaria[a]	Malaria	Diarrhoea[a]
			Migraine[a]	Shock[a]
				Osteoporosis[a]
				Movement disorder (ALS)[a]
				Impotence[a]

Each column represents one decade. Where new mechanisms or improvements in compound class improved treatment markedly in the subsequent decade(s), the disease area continues to be shown in following columns.

[a] New disease areas opened by new medicines are represented.

[b] Highlighted in bold text are cancer, AIDS and Hyperlipidemia as examples of indications with sustained efforts, and where new technologies have started to impact novel medicines in recent years.

involved in cancer formation or progression. These include the *bcr-abl* gene aberration causing a type of leukaemia (leading to the discovery of Gleevec®) and HER-2 overexpression in breast cancer, which gave rise to Herceptin® (Table 2).

In addition, pathway mining has led to R&D efforts against more than one target within a given pathway, which also widened treatment options. For example, estrogen-sensitive breast cancer can be treated by blocking the effects of estrogen through estrogen receptor antagonists, such as Nolvadex® (tamoxifen, AstraZeneca), whereas patients with disease progression after Nolvadex® therapy can switch to aromatase inhibitors, such as Aromasin® (exemestane, Pfizer), which inhibit the formation of estrogen upstream of the receptor. Other targeted treatments already on the market include Tarceva® (erlotinib, OSI), a

TABLE 2

Representative examples of new, targeted cancer treatments brought to market over recent years (http://www.fda.gov)

Novel target mechanism	Primary cancer indication	Small molecule or biological	Trade name and generic name	Company
HER-2 receptor	HER-2-positive breast cancer	Biological (antibody)	Herceptin® (trastuzumab)	Genentech
ErbB1 receptor tyrosine kinase	Non-small-cell lung cancer	Small molecule	Iressa® (gefitinib)	AstraZeneca
Epidermal growth factor (EGF) receptor	Colorectal carcinoma	Biological (antibody)	Erbitux® (cetuximab)	Imclone
Bcr-Abl tyrosine kinase	Chronic myeloid leukemia (CML)	Small molecule	Gleevec® (imatinib)	Novartis
Vascular endothelial growth factor (VEGF) receptor tyrosine kinase	GI stromal tumours and renal cell carcinoma	Small molecule	Sutent® (sunitinib)	Pfizer
Several kinases	Renal cell carcinoma	Small molecule	Nexavar® (sorafenib)	Bayer
Estrogen receptors	Estrogen-sensitive breast cancer	Small molecule	Nolvadex® (tamoxifen)	AstraZeneca
Aromatase	Estrogen-sensitive breast cancer	Small molecule	Aromasin® (exemestane)	Pfizer
CD20	B-cell non-Hodgkin's lymphoma (CD20 also occurs on normal B cells)	Biological (anti-CD20 antibody associated with radiochemical agent)	Zevalin® (ibritumomab tiuxetan)	IDEC
CD33	Acute myeloid leukemia (AML). (CD33 also occurs on normal hematopoietic cells)	Biological (anti-CD33 antibody conjugated with cytotoxic agent)	Mylotarg® (gemtuzumab)	Wyeth
Asparagine	Asparagine-dependent acute lymphocytic leukaemia	Biological (enzyme)	Elspar® (asparaginase)	Merck
VEGF	Colon carcinoma	Biological (antibody)	Avastin® (bevacuzimab)	Genentech
Proteasome, nuclear factor κB (NFκB)	Multiple myeloma	Small molecule	Velcade® (bortezomib)	Millennium

small-molecule inhibitor of the ErbB1 receptor tyrosine kinase, Erbitux® (cetuximab, Imclone), Bexxar® (tositumomab, Corixa), Herceptin®, and many others (Table 2).

Random and subset screening and targeted libraries

The increased knowledge-base described previously meant that HTS of drug targets, predicted to be amenable to small-molecule intervention, became more focused and successful. Yet, reductions in the costs of screening and improvements in the quality of compounds screened were still necessary. In fact, screening costs have been reduced significantly through miniaturization and automation. In parallel, most large pharmaceutical companies have tried to set up and enrich compound subsets that are more likely to modulate certain target types. These include collections of molecules that are likely to modulate aminergic GPCRs, kinases or ion channels, allowing subset screening to reduce the cost and increase the chance of finding lead material. Iressa® (gefitinib, AstraZeneca), Gleevec® and Tarceva® were discovered using subset screening and will be studied as case examples later in this review. Moreover, chemistry automation, combined with computational methodologies and improved logistics, has helped to enrich compound files with more-attractive higher-quality molecules. It is hoped that the increase in compound numbers will further the chance of finding active leads, whereas the improvement in quality should make lead optimization easier, once a potential drug has been identified. Indeed, advances in computational chemistry, X-ray crystallography and other chemistry technologies allow the synthesis of successive rounds of target-specific libraries with increasingly higher potency, selectivity and better ADME–Tox profiles, thus contributing to the success of lead optimization. However, all of this came at a price – through the 1990s the investment in these technologies and the associated learning processes undoubtedly contributed to the rising costs of R&D. However, there can be no doubt that many of today's marketed cancer drugs, and the hundreds of potential cancer therapies in clinical development, have benefited from the previously described investments. Table 3 outlines some of the novel mechanisms that the top ten pharmaceutical companies currently explore.

A PhRMA survey in 2005 found 399 cancer drugs in development (http://www.phrma.org), a clear testimony not only to the synergies between academic and industrial research but also to the evidence that the investment and learning accumulated over the past decade is starting to pay off.

Case studies that demonstrate the impact of technology on novel medicines

Herceptin®: a humanized antibody against the HER-2 receptor for the treatment of HER-2-overexpressing breast cancer

Breast cancer is the most common cancer in women in the USA, and the second leading cause of death from cancer according to the statistics of the American cancer society (http://www.cancer.org). It is also a type of cancer that is increasingly treatable with a range of new drugs, such as the humanized HER-2 blocking antibody Herceptin®. HER-2 is a member of the ErbB/HER family of receptor tyrosine kinases and occurs in normal tissue where it plays a role in cell proliferation. It appears to have no natural ligand [8], instead acting as a cell growth promoter – through dimerization with other HER family members. Overexpression of the HER-2 receptor on the cell surface occurs in 15–30% of metastatic breast cancers and is linked to poor prognosis for affected patients [9]. The discovery of HER-2, the link to breast cancer, and the discovery and the

TABLE 3

Examples[a] of novel, targeted cancer treatments currently in advanced clinical stages by the top ten pharmaceutical companies (2005 annual reports[b])

Novel target mechanism[c]	Primary cancer indication	Small molecule or biological	Name	Company
Farnesyltransferase	Acute myeloid leukemia	Small molecule (Phase III)	Zarnestra (tipifarnib)	Johnson and Johnson
Cytotoxic T-cell-associated antigen-4 (CTLA4)	Melanoma	Antibody (Phase II)	CP-675,206	Pfizer
Kinesin spindle protein (KSP) Dual ErbB2 and ErbB1 receptor kinases	Non-small-cell lung cancer Breast cancer	Small molecule (Phase II) Small molecule (Phase III)	Ispenesib Tykerb® (lapatinib)	GlaxoSmithKline
FMS-like tyrosine kinase 3 (Flt-3) Mammalian target of rapamycin (mTOR)	Acute myeloid leukemia Endometrial cancer	Small molecule (Phase II) Small molecule (Phase II)	PKC-412 RAD001 (complexed with endogenous FKBP-12)	Novartis
Cyclin-dependent kinase (CDK) Sigma receptor Neurotensin receptor Therapeutic vaccine (not a target mechanism)	Chronic lymphocytic leukaemia Prostate cancer Small-cell lung cancer Melanoma	Small molecule (Phase III) Small molecule (Phase II) Small molecule (Phase II) Vaccine (Phase II)	Alvocidip SR 31747 Meclinertant Uvidem	Sanofi-Aventis
HER-2 dimerization	Breast cancer	Antibody (Phase II)	Omnitarg® (pertuzumab)	Genentech (same portfolio for Roche)
Endothelin receptor A	Solid tumours	Small molecule (Phase II)	ZD4054	AstraZeneca
Histone deacetylase	Various cancers	Small molecule (Phase II)	Vorinostat	Merck

[a] Only first-in-class mechanisms are listed that are in Phase II or III.
[b] http://www.jnj.com; http://www.pfizer.com; http://www.gsk.com; http://www.roche.com; http://www.novartis.com; http://www.sanofi-aventis.com; http://www.amgen.com; http://www.astrazeneca.com; http://www.merck.com.
[c] Similar mechanisms pursued by Amgen: many follow-on cancer drugs in Phase II and III.

development of Herceptin® would have been impossible without the advances in molecular biology. For example, the HER-2 oncogene was observed in a rat model of chemically induced carcinogenesis, and HER-2 overexpression transformed normal cells into cancer cells. Although these initial discoveries occurred in academia [9], the same technologies were used in Genentech during the Herceptin® R&D process.

In effect, HER-2 became a drug target and a biomarker to identify the patient subpopulation most likely to respond to Herceptin® treatment [10]. Given the low incidence of HER-2-positive breast cancers, the development of Herceptin® might not even have succeeded without selecting the clinical study population on the basis of this biomarker. Even the development of the humanized antibody would have been impossible without molecular biology technologies. Today, Herceptin® provides a new standard for treatment of breast cancer by selectively targeting the extracellular domain of the HER-2 kinase receptor, preventing cell proliferation and stopping cancer progression. A recent study showed impressive disease-free survival statistics of 85% for patients treated with Herceptin®, compared with 67% in those treated with chemotherapy alone [11]. However, even Herceptin® is far from being a panacea for patients with HER-2-positive breast cancer, owing to the possibility of Herceptin® resistance developing through tumour mutations, and potential cardiovascular side effects (http://www.herceptin.com).

Iressa® and Tarceva®: small-molecule inhibitors of intracellular ErbB receptor tyrosine kinases for the treatment of lung cancer

In addition to antibodies against the extracellular domains of members of the ErbB/HER family, small-molecule ErbB receptor tyrosine kinase inhibitors have also been developed. Iressa® and Tarceva® both target non-small-cell lung cancer. The original lead structures were discovered through screening compound libraries against an isolated enzyme *in vitro* [12].

Although the efficacy of Iressa® and Tarceva® appears much less impressive than that of Herceptin®, it is conceivable that both drugs have yet to find the patient subpopulation most likely to benefit from either mono- or combination-therapy with small-molecule ErbB1 receptor tyrosine kinase inhibitors [13]. In addition, multi- and pan-ErbB receptor tyrosine kinase inhibitors are also under development and might provide efficacy gains.

Gleevec®: a small-molecule inhibitor of Bcr-Abl tyrosine kinase for the treatment of leukaemia

Gleevec® is a small molecule that inhibits the abnormal, constitutively active Bcr-Abl tyrosine kinase that leads to cell proliferation in chronic myeloid leukaemia (CML). This drug clearly prolongs life: 78.5% of patients that respond to Gleevec® are still alive eight years after diagnosis, compared with just 22.6% in the control group and 6.2% where patients were not responsive to Gleevec®. Median survival for patients in blast crisis (an advanced phase of CML) receiving standard chemotherapy is only 2–3 months [14]. The discovery of Gleevec® is a prominent example of R&D technology delivering the goods: the target was identified through genomic analysis of a chromosome (Philadelphia chromosome) that was shorter than usual in leukaemia patients. This genomic analysis showed chromosomal translocation and fusion of the Bcr and Abl genes into an abnormally active Bcr-Abl fusion kinase, which is present in 95% of patients with chronic myeloid leukaemia [15]. Subsequent transgenic validation in mice showed that Bcr-Abl expression causes malignant transformation [16]. Novartis randomly screened privileged libraries enriched with attractive leads

against Protein Kinase C (PKC), and used traditional rational medicinal chemistry and *in vitro* screens to follow up the weak, dual PKC and platelet-derived growth factor (PDGF) receptor kinase inhibitors (which happened to also have Abl kinase inhibitory activity) that were identified [17,18].

Most of the small-molecule kinase inhibitors listed in Tables 2 and 3 were discovered via similar routes or through medicinal chemistry alterations to existing lead series (e.g. those that gave rise to Gleevec®, Iressa®, Tarceva® and other kinase inhibitors) to create novel selectivity profiles.

Following Gleevec®: allosteric inhibitors for Bcr-Abl-dependent cell proliferation

Rather than screen against the recombinant kinase, which is likely to lead to ATP-competitive inhibitors, Adrian *et al.* [19] screened for differential cytotoxicity between cells transformed with Bcr-Abl and their isogenetic parental cell line. A library of 50,000 compounds representing 30 different heterocyclic scaffolds was screened. Active compounds binding to the ATP-binding site were discarded, leading to the identification of 4,6-pyrimidines exemplified by the preferred compound GNf-2 (shown in Figure 3, where it is compared with Gleevec®). This compound binds at the myristic-binding pocket rather than the ATP-binding pocket, and the compound is highly selective. Such a compound could potentially be active against Gleevec®-resistant Bcr-Abl mutants. Moreover, the compound shows synergistic and antiproliferative effects combined with Gleevec®. The ability to rationalize resistance, design screens and libraries and produce druglike compounds rapidly illustrates the maturation of the technology over the past 15 years.

VEGF: an example of pharmaceutical innovation following laborious biology

Innovation covers many fields but pharmaceutical innovation occurs when biology has matured to a point where a viable druggable target emerges. Even then, it is a long process from the identification of a potentially druggable target to the production of a successful drug. Angiogenesis, the growth of new blood vessels, has been linked to health and disease for over a century. For cancer, the recognition of blood supply and tumour growth was made in 1945, and the possibility of potential drug targets was identified in 1971. As the knowledge of pathways increased, new viable targets emerged, such as vascular endothelial growth factor (VEGF) in 1980 [20]. VEGF is a glycoprotein that exists in several isoforms capable of binding to two homologous VEGF receptors that are transmembrane tyrosine kinase receptors [FMS-like tyrosine kinase 1 (Flt-1) and kinase insert domain receptor (KDR)] [21]. VEGF induces angiogenesis via a direct effect on endothelial cells, through which it maintains an immature vasculature, rendering it more permeable and, hence, allowing the development of an extravascular matrix [21]. VEGF is crucial to tumour growth and, ultimately, metastatic potential. VEGF expression is elevated in many cancers such as colorectal, breast and lung [21].

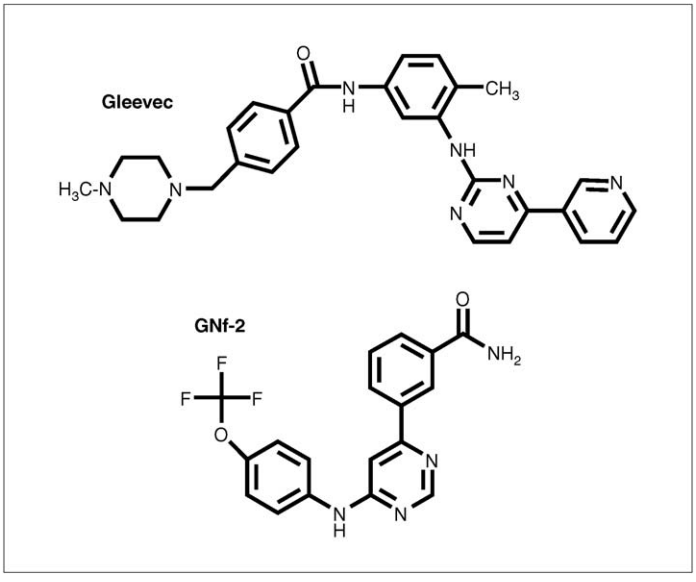

FIGURE 3

Inhibitors of Bcr-Abl kinase. Structures of Gleevec®, which binds at the ATP-binding site of Bcr-Abl kinase, and GNf-2, which binds at the myristic (allosteric) site of Bcr-Abl kinase.

Drug discovery programmes for cancer have focussed on targeting VEGF itself via antibodies and soluble receptor constructs (large molecules) and the receptors themselves via antibodies and tyrosine kinase inhibitors (small molecules). Later in this review we will indicate how radical pharmaceutical innovation can be, once the biology has matured to a sufficient position for drug discovery, by examining the case of Macugen® (pegaptanib, Eyetech and Pfizer) the first approved aptamer.

Targetting VEGF, or its receptor, has led to marked success. Avastin® (bevacizumab, Genentech), a humanized anti-VEGF-A monoclonal antibody, was approved in 2004 for the treatment of metastatic colorectal cancer, and results in a marked improvement in median survival (i.e. a 30% increase in survival). Recent results show the drug has significant efficacy in advanced breast cancer, opening up huge avenues of medical research that will hopefully provide further substantial benefits to patients.

Sutent® represents a small-molecule approach that targeted the tyrosine kinase receptors, not only of VEGF but also of PDGF and other receptor tyrosine kinases. The discovery of Sutent® utilized the strategy of targeted libraries, which led to lead molecules with a novel indolinone core (Figure 4). The small nature of these lead molecules indicates how a fragment-based screening programme can utilize a minimum binding template, leaving large scope for medicinal chemistry. Early on in the programme, using X-ray crystallography, some of the lead molecules were examined in complexes with receptor tyrosine kinases, which allowed structure-based drug design to influence the chemistry direction. These experiments showed that the oxindole occupied the site in which the adenine moiety of ATP binds [22]; and substituents at position 3 on the oxindole contacted residues in the hinge region between the two kinase lobes. These studies suggested that modifications at positions 5 and 6 could generally enhance affinity, whereas selectivity could mainly be modified by substitution at position 3. The subsequent programme (Figure 4) optimized the oxindole series for selectivity, potency and ADME–Tox properties [23]. Sutent® was approved in 2006 for its breakthrough efficacy in gastrointestinal stromal tumour (GIST) and metastatic renal cell carcinoma (MRCC). Studies in GIST showed that the tumour progression time improved, from 6.4 weeks to 27.3 weeks, and similar results were seen in MRCC.

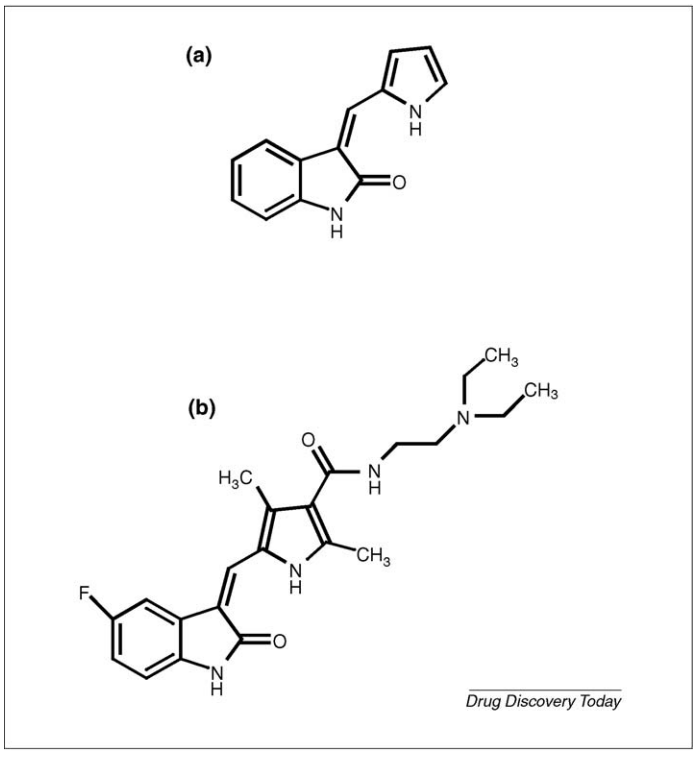

FIGURE 4

Structures of an early lead molecule and Sutent®. (a) This early lead molecule has a molecular weight (Mw) = 210 Da, solubility <1 μg/ml and potency against vascular endothelial growth factor (VEGF) = 12 μM and against platelet-derived growth factor (PDGF) = 0.39 μM. **(b)** Sutent®: Mw = 398 Da, solubility = 364 μg/ml, VEGF potency = 0.08 μM and PDGF potency = 0.002 μM.

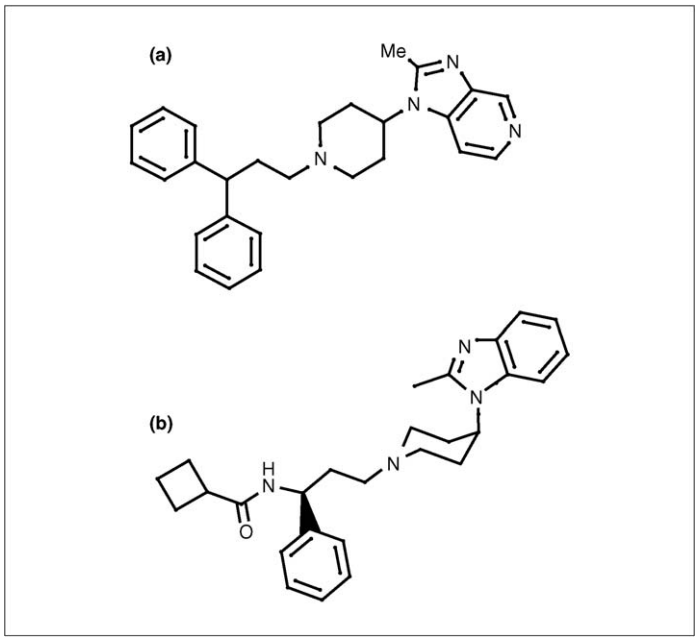

FIGURE 5

Chemical structures of an original HTS lead and UK-374,503. An original HTS lead **(a)** that binds to C–C chemokine receptor 5 (CCR5) but does not have antiviral activity [macrophage inflammatory protein (MIP)-1β IC_{50} = 0.6 μM]. Note should be taken of the lipophilicity (cLogP 4.7) and the molecular weight (410 Da), making this compound less than optimum for chemistry follow-up. UK-374,503 **(b)** is an early example of chemistry follow-up. The compound possesses sufficient potency for antiviral activity but poor pharmacokinetic properties.

Maraviroc: a small-molecule antagonist of the CCR5 receptor for the treatment of HIV and AIDS

Discovering new medicines when the drug target is located at the extreme end of druggable space is challenging, and in a resource-constrained environment such projects can easily be abandoned. As a consequence, there are probably more examples of new medicine failure than there are of success in such instances. Some early signs indicate that the boundaries of druggable space might well be expanding. For example, kinases were, for a long time, regarded as difficult targets because of concerns over selectivity and associated safety issues. The dominant school of thought assumed that ATP-binding sites were the only target site for small-molecule inhibitors of kinases and that the intrinsic conservation of this site would not allow selectivity. As outlined previously, this view was incorrect and the kinases represent a rich class of drug targets. Suddenly druggable space has extended to include an entire target class.

Similarly, the efforts made in screening more and better chemical structures can help to extend target space. One example is the C–C chemokine receptor 5 (CCR5). Its endogenous ligands are relatively large polypeptides, such as RANTES, macrophage inflammatory protein (MIP)-1α and MIP-1β. The involvement of CCR5 in HIV and AIDS was demonstrated through genomic analysis of individuals who had never showed evidence of HIV infection, despite high-risk occupations and/or behaviours [24]. A rare mutation in the CCR5 gene leads to a deletion (delta 32) and the lack of functional receptor, conveying almost complete resistance against HIV infection. The hypothesis subsequently developed was that small-molecule antagonists of CCR5 might be able to reproduce the protection conveyed by this genetic polymorphism. During HIV infection, CCR5 acts as a co-receptor with CD4 for the viral attachment glycoprotein gp120. Historically, such protein–protein interactions that involve large surface areas between the protein partners are bad news for drug discovery efforts that try to identify small-molecule antagonists. Therefore, the discovery of maraviroc (Pfizer) [25] involved extensive screening efforts with >500,000 compounds, including privileged libraries in a miniaturized high-throughput format. The hits that emerged (Figure 5) were large and lipophilic, making chemistry follow-up difficult and challenging. Lipophilicity, high metabolic lability and, therefore, dose projections of >1 g per day rendered the early leads, such as UK-374,503 (Figure 5), unsuitable for development. Benzimidazole replacements in UK-374,673 and UK-408,030 (Figure 6) were intended to increase metabolic stability and absorption. However, it took >1000 analogues and many setbacks, for example inward rectifying potassium channel (Ikr) selectivity issues, before all of the required properties were present in maraviroc (Figure 7). Despite these difficulties, and despite only average resource spend for the project, maraviroc was discovered and entered clinical development faster than many other druggable projects. An early study addressing the efficacy and safety of 10-day monotherapy with maraviroc in 63 HIV-1-positive individuals showed compelling reduction in viral load [26]; this occurred at a median of 10–15 days, with a mean reduction of $\geq 1.6 \log_{10}$ copies/ml. Today,

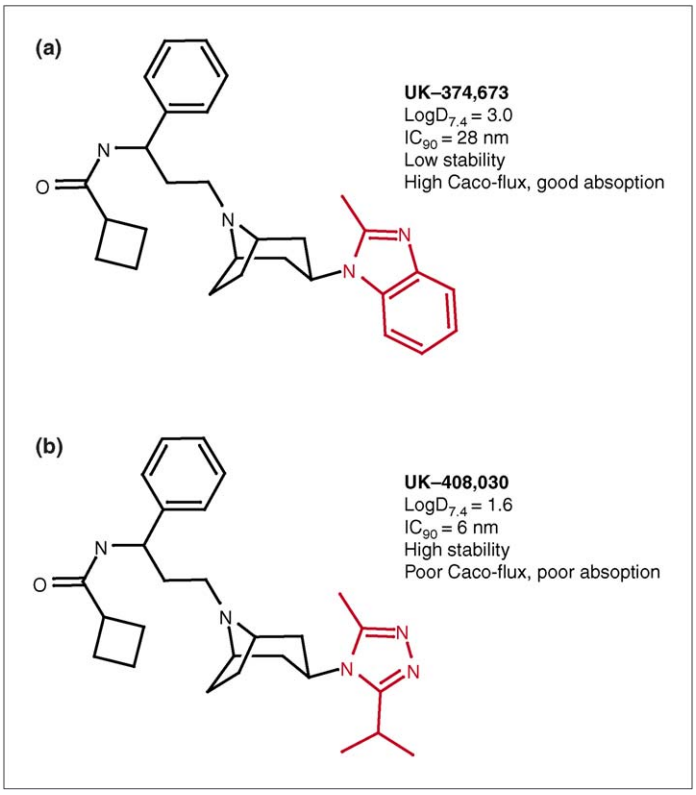

FIGURE 6

Later chemistry optimization. Structures of UK-374,673 **(a)** and UK-408,030 **(b)** are illustrated. The two compounds represent the balancing act between metabolic stability and absorption, in which lipophilicity is key ($LogD_{7.4}$).

maraviroc is in Phase III clinical trials and is showing excellent antiviral activity in patients with HIV.

Torcetrapib: a CETP inhibitor for the treatment of dyslipidaemia – and a case study for formulation technologies and imaging technologies impacting R&D

Cholesteryl ester transfer protein (CETP) was first associated with dyslipidaemia in humans in 1989, when researchers in Canada, the USA and Japan discovered that individuals with genetic deficiencies in CETP had favourable plasma lipid levels [27]. Although the full picture has yet to emerge as to what extent and how CETP deficiencies link to longevity and the absence of cardiovascular disease, there is evidence that individuals with hereditary CETP deficiencies are protected from cardiovascular disease [28]. Thus, in theory, a small-molecule inhibitor of CETP should protect against cardiovascular disease by increasing plasma high-density lipoprotein cholesterol (HDL-c). However, a thorny path lies between the concept and the production of an eventual medicine. Since the original paper in 1989, it took ten years until the first patient study started; a study that demonstrated the safety and efficacy of torcetrapib (Pfizer). One of the reasons why it took so long was the need for many innovations along the way, because the nature of the drug target – CETP – dictated the properties of torcetrapib. Cholesteryl esters, the substrate of CETP, typically have extremely high lipophilicity (clogP ~18) and relatively high molecular weight (Mw 650 Da) coupled with conformational flexibility. CETP is not an enzyme but a transfer protein that mediates a protein–protein interaction. The binding pocket is large with a solvent-accessible area of 573 $Å^2$, of which 91% can be considered hydrophobic. HTS identified a 10 μM lead with a logP of 4.84 and a Mw of 456 Da. Subsequent modifications resulted in torcetrapib, with 5 nM potency but a logP of 8.2 and a Mw of 600 Da (Figure 8). The fivefold increase in the affinity of CETP for HDL following treatment with torcetrapib probably represents a shift to a binding state that is non-permissive for lipid transfer and that blocks all of the major lipid transfer functions of plasma CETP by inducing a non-productive complex between the transfer protein and HDL [29].

Clearly, with a logP of 8.2 and a Mw of 600 Da, torcetrapib does not have conventional druglike properties. Given the lipophilic nature of the substrate for CETP, it seemed unlikely that potent,

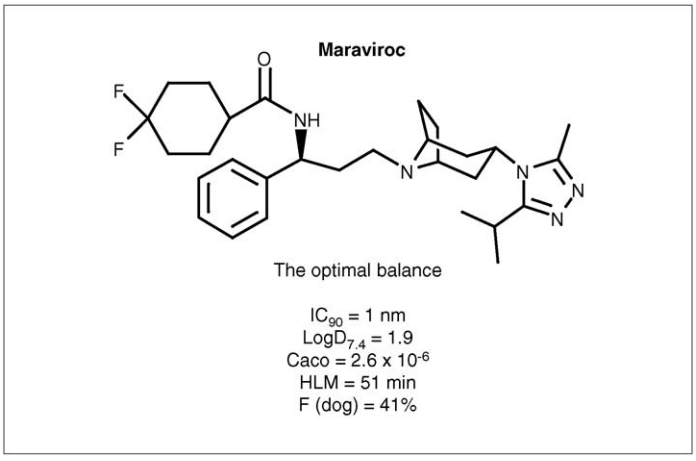

FIGURE 7

The chemical structure of maraviroc. A potent, selective, orally available C–C chemokine receptor 5 (CCR5) antagonist for the treatment of HIV and AIDS.

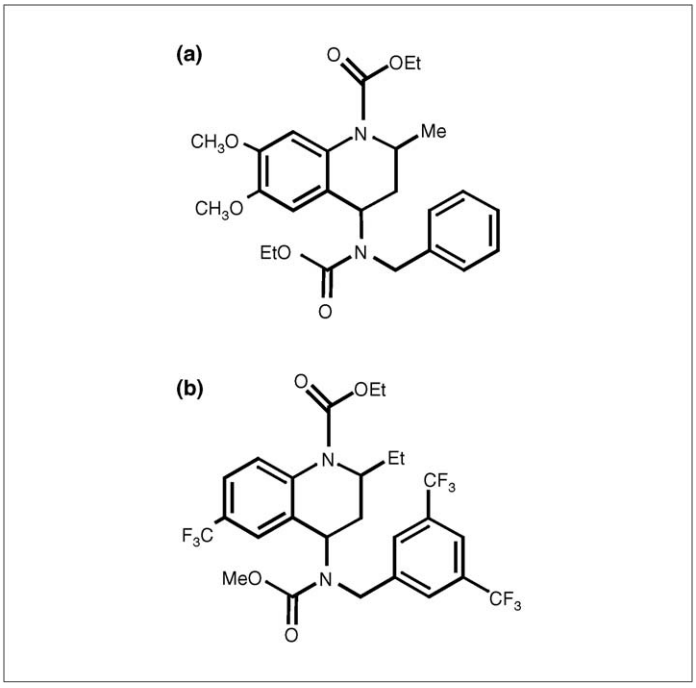

FIGURE 8

Structures of the original HTS hit and torcetrapib. The original HTS hit **(a)** and torcetrapib **(b)** are extremely lipophilic molecules with properties dictated by their target, cholesteryl ester transfer protein (CETP).

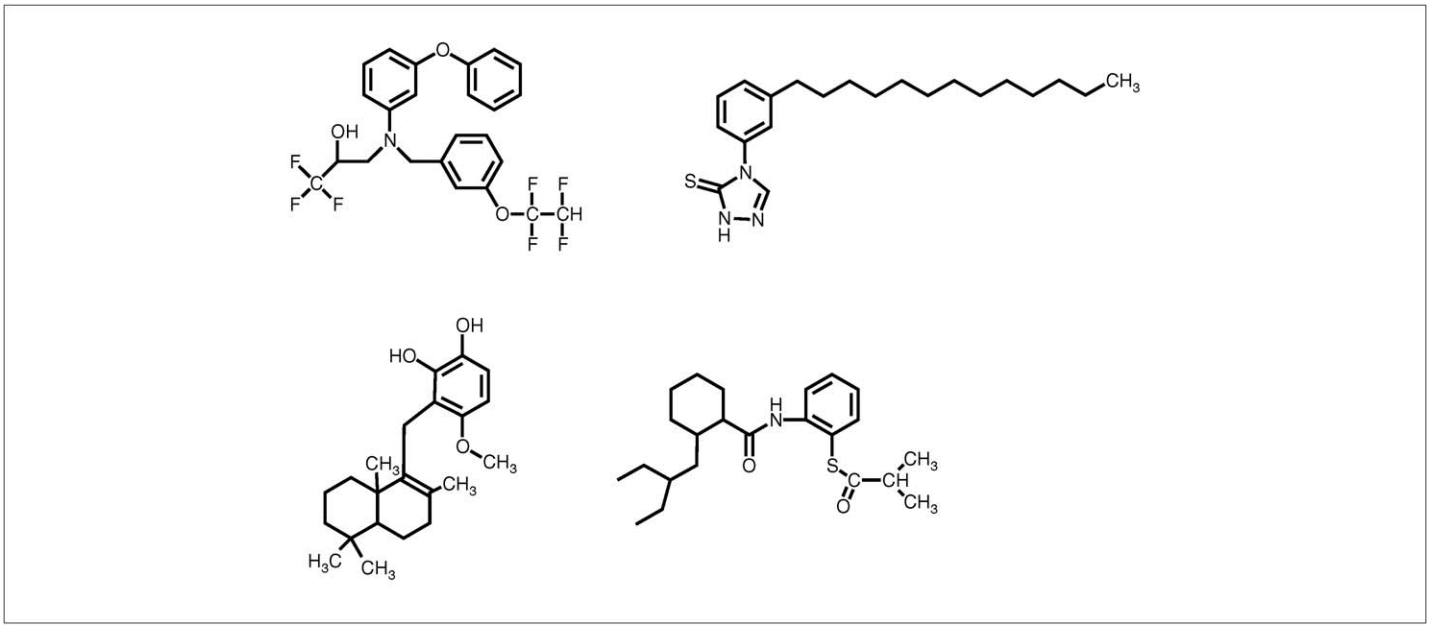

FIGURE 9

Structures of cholesteryl ester transfer protein (CETP) inhibitors from other research programmes. These structures emphasize that lipophilicity is an inherent property in a CETP inhibitor. The compounds illustrated have cLogP values of 6.8 or greater.

non-lipophilic inhibitors would be found – other research programmes at different companies yielded molecules that were equally lipophilic (Figure 9). To overcome this inherent property, efforts were focused on identifying and applying new, solid dosage form technologies to enhance torcetrapib in a way that would make it suitable for development and commercialization. In collaboration with Bend Research (BRI, Oregon, USA), spray-dried dispersion technology was successfully developed. However, the next hurdle in this project is the need to prove that raising HDL-c does result in a clinical benefit. Measuring the standard endpoint, myocardial infarction, requires the recruitment of thousands of patients over a period of five years or more. To get faster clinical results for torcetrapib, novel cardiovascular imaging technologies are currently being applied (intravascular ultrasound) to demonstrate that, by raising HDL-c via torcetrapib in combination with Lipitor® (atorvastatin, Pfizer), the size of atherosclerotic plaques in the coronary artery (coronary atheroma) can be reduced. A recent Pfizer-sponsored 500-patient study that took just 18 months demonstrates the viability of this surrogate endpoint approach. The REVERSAL study showed that aggressive treatment with high-dose Lipitor®, compared with lower-dose Pravachol® (pravastatin, Bristol-Myers Squibb), could reduce the progression of, or even reverse, atheromas and this could be reliably measured using intravascular ultrasound [30].

Beyond small-compound file screening: DNA and RNA aptamers as drugs

Oligonucleotides and proteins show highly specific interactions and this principle has been extended to new limits with aptamer drug discovery. The technology behind this is the SELEX process, which allows the systematic evolution of ligands by exponential enrichment. Random RNA and DNA oligonucleotides (20–40 nucleotides long) are produced. These oligonucleotides are flanked by binding sequences for reverse transcriptase (RT) and PCR, promoter sequences for T7 RNA polymerase, and restriction endonuclease sites for cloning. A standard library will contain 10^{15} different aptamers. Those that bind are amplified by RT–PCR and reselected, and this process is repeated until the final potency is achieved.

Macugen® was discovered with libraries of stabilized nucleotides: 2′,F-substituted pyrimidines and 2′,OMe-substituted purines [31]. These substitutions render the nucleotide more resistant to nucleases. The specific target was VEGF-165, which promotes ocular neorascularization and increased vascular permeability leading to macular degeneration. The final, selected aptamer (t44-OMe) was modified further to improve tissue residence by the addition of a 5′-linked 40 kDa polyethylene glycol. This drug is administered by intravitreous injection at a dose of 0.3 mg. Efficacy of the drug is dramatic, for instance patients receiving Macugen® showed a 45% relative benefit in mean change in vision at the end of 102 weeks compared with those receiving the usual care.

Conclusion

In an industry, where success rates from development candidate to market at the best of times are no higher than 10%, it is very easy to be pessimistic – and to be proven right. Indeed, new technology in biomedical research has taken a long time to impact R&D in a way that is measurable in output terms. However, we are now seeing the first products emerge, many of which have not only been enabled by technology but also, in fact, would not have been possible without it. Whatever we might mean when we use the term technology, and in pharmaceutical R&D that can be a very broad term indeed, there is absolutely no doubt that the millions of dollars of investments made over the past two decades by pharmaceutical companies are starting to pay off. A key requisite in this achievement was the evolution of a knowledge-base in a range of areas. These areas include better understanding of pathways and interactions

of small-molecule modulators with receptors and enzymes, from discovery to clinical evaluation of patient genotypes and novel biomarkers. These advances, in academia and in industry, were necessary to lay the foundation for the successful integration and application of technology in the R&D process. The evidence for this success, in the form of case studies of approved medicines and advanced development compounds, has been presented in this review.

References

1 Landers, P. (2004). Human element: drug industry's big push into technology falls short. *The Wall Street Journal* 24 February, http://www.wsj.com
2 Schmid, E.F. and Smith, D.A. (2005) Is declining innovation in the pharmaceutical industry a myth? *Drug Discov. Today* 10, 1031–1039
3 Schmid, E.F. and Smith, D.A. (2005) Trends in pharmaceutical innovation. *Ann. Rep. Med. Chem.* 40, 451–459
4 Roberts, T.G. *et al.* (2004) Trends in the risks and benefits to patients with cancer participating in Phase 1 clinical trials. *JAMA* 292, 2130–2140
5 Druker, B.J. and Lydon, N.B. (2000) Lessons learned from the development of an Abl tyrosine kinase inhibitor for chronic myelogenous leukaemia. *J. Clin. Invest.* 105, 3–7
6 Macarron, R. (2006) Critical review of the role of HTS in drug discovery. *Drug Discov. Today* 11, 277–279
7 Lipinski, C.A. *et al.* (1997) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* 23, 3–25
8 Lonardo, F. *et al.* (1990) The normal erbB-2 product is an atypical receptor-like tyrosine kinase with constitutive activity in the absence of ligand. *New Biol.* 2, 992–1003
9 Slamon, D.J. *et al.* (1987) Human breast cancer: correlation of relapse and survival with amplification of the Her-2/*neu* oncogene. *Science* 235, 177–182
10 McCormick, S.R. *et al.* (2002) HER2 assessment by immunohistochemical analysis and fluorescence *in situ* hybridization: comparison of HercepTest and PathVysion commercial assays. *Am. J. Clin. Pathol.* 117, 935–943
11 Romond, E. *et al.* (2005) Doxorubicin and cyclophosphamide followed by paclitaxel with or without trastuzumab as adjuvant therapy for patients with HER-2 positive operable breast cancer. Combined analysis of NSABP B13/NCCTG N931. *Proceedings of the 2005 annual meeting of the American Society of Clinical Oncology* (http://www.asco.org/ac/1,1003,_12-002511-00_18-0034-00_19-005817-00_21-001,00.asp)
12 Wakeling, A.E. *et al.* (1996) Specific inhibition of epidermal growth factor receptor tyrosine kinase by 4-anilinoquinazolines. *Breast Cancer Res. Treat.* 38, 67–73
13 Agrawal, A. *et al.* (2005) Overview of tyrosine kinase inhibitors in clinical breast cancer. *Endocr. Relat. Cancer* 12, S135–S144
14 Faderl, S. *et al.* (1999) Chronic myelogenous leukemia: biology and therapy. *Ann. Intern. Med.* 131, 207–219
15 Druker, B.J. (2002) Perspectives on the development of a molecularly targeted agent. *Cancer Cell* 1, 31–36
16 Voncken, J.W. *et al.* (1995) BCR/ABL p210 and p190 cause distinct leukaemia in transgenic mice. *Blood* 86, 4603–4611
17 Lesney, M. (2003) Focus on phosphate: high throughput screening improves the odds by using kinases as targets for drug discovery. *Modern Drug Discovery* May, 38–45
18 Zimmermann, J. (1996) Phenylamino-pyrimidine (PAP) derivatives: a new class of potent and selective inhibitors of protein kinase C (PKC). *Arch. Pharm. (Weinheim)* 329, 371–376
19 Adrian, F. *et al.* (2006) Allosteric inhibitors of Bcr-abl-dependent cell proliferation. *Nat. Chem. Biol.* 2, 95–102
20 Ferrera, N. and Kerbel, R.S. (2005) Angiogenesis as a therapeutic target. *Nature* 438, 967–974
21 Ferrera, N. (2005) VEGF as a therapeutic target in cancer. *Oncology* 69 (Suppl. 3), 11–16
22 Mohammadi, M. *et al.* (1997) Structures of the tyrosine kinase domain of the fibroblast growth factor receptor in complex with inhibitors. *Science* 276, 955–960
23 Sun, L. *et al.* (2003) Discovery of 5-[s-Fluoro-2-oxo-1, 2-dihydroindol–3Z)–ylidenemethyl] –2, 4-dimethyl– H-pyrrole-3-carboxylic acid (2-diethylamino ethyl) amide, a novel tyrosine kinase inhibitor targeting vascular endothelial and platelet-derived growth factor receptor tyrosine kinase. *J. Med. Chem.* 46, 1116–1119
24 Liu, R. *et al.* (1996) Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell* 86, 367–377
25 Dorr, P. *et al.* (2005) Maraviroc (UK-427,857), a potent, orally bioavailable, and selective small-molecule inhibitor of chemokine receptor CCR5 with broad-spectrum anti-human immunodeficiency virus type 1 activity. *Antimicrob. Agents Chemother.* 49, 4721–4732
26 Fatkenheuer, G. *et al.* (2005) Efficacy of short-term monotherapy with maraviroc, a new CCR5 antagonist, in patients infected with HIV-1. *Nat. Med.* 11, 1170–1172
27 Brown, M.L. *et al.* (1989) Molecular basis of lipid transfer protein deficiency in a family with increased high-density lipoproteins. *Nature* 342, 448–451
28 Barzilai, N. *et al.* (2003) Unique lipoprotein phenotype and genotype associated with exceptional longevity. *JAMA* 290, 2030–2040
29 Clark, R.W. *et al.* (2006) Description of the torcetrapib series of cholesteryl ester transfer protein inhibitors, including mechanism of action. *J. Lipid Res.* 47, 537–552
30 Nissen, S.E. *et al.* (2004) Effect of intensive compared with moderate lipid-lowering therapy on progression of coronary atherosclerosis. *JAMA* 291, 1071–1080
31 Ng, E.W.M. *et al.* (2006) Pegaptanib, a targeted anti-VEGF aptamer for ocular vascular disease. *Nat. Rev. Drug Discov.* 5, 123–132