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Timely lessons for target-based discovery of anti-inflammatory drugs

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Inflammation is currently a hot topic, generating interest among both the scientific community and the general public, a fact that was highlighted by the recent cover article in *Time Magazine* describing how this process underlies many common chronic diseases [1]. To the attendees of the 12th *International Conference of the Inflammation Research Association* (IRA), which was held on 3–7 October 2004 at Lake George (NY, USA), it was certainly no surprise that uncontrolled inflammation is associated with diseases including rheumatoid arthritis, cancer, heart disease, stroke and neurodegenerative disorders. These therapy areas and more were represented at the IRA meeting.

The rise and fall of rofecoxib

In addition to the usual talks focused on the development of SAR for specific targets and synthetic routes for small-molecule drugs, a significant number of presentations addressed the discovery and clinical development of protein drugs. With the announcement of the safety-based withdrawal of Vioxx® [rofecoxib (Merck)] from the global market just three days before the conference commenced [2], the timing of this juxtaposition of small-molecule medicinal chemistry and large-molecule medicinal biology was highly appropriate. The keynote address, 'Lessons and Opportunities from Drug Development in the Arachidonic Acid Cascade', by Garret Fitzgerald (University of Pennsylvania) was thus particularly relevant. Given the decision made by Merck, Fitzgerald emphasized the 'Lessons' aspect of the title of his presentation more than the 'Opportunities', saying that the cardiovascular risks associated with inhibiting cyclooxygenase 2 (COX2) have been recognized for many years. In his review of COX2 target rationale and clinical data from the original VIGOR (Vioxx® gastrointestinal outcomes research) study and more recent APPROVe (adenomatous polyp prevention on Vioxx®) trials of rofecoxib, Fitzgerald emphasized that 'the system worked' and that Merck made the 'ethical, appropriate and very painful decision' to withdraw the drug based on long-term safety results from the APPROVe study.

The crucial question now is whether the increased risk of cardiovascular events associated with the use of rofecoxib is likely to apply to the two remaining coxibs on the market [celecoxib and valdecoxib (both Pfizer drugs)]. Ironically, given that the development of COX2 inhibitors is frequently used as the exemplar for successful target-based drug discovery, Joe Feczko (President of Worldwide Development at Pfizer) stated in a press release issued the day after the withdrawal of rofecoxib that 'each COX2 inhibitor has a distinct chemical structure and we would not expect them to have the same side effect profile'. By contrast, Fitzgerald concluded his plenary lecture with a few lessons regarding all the COX2 inhibitors, the most important being that 'absence of evidence is not evidence of absence'. Fitzgerald also suggested that drug companies should 'show humility and introspection' regarding these events.

The withdrawal of an effective and highly profitable drug is not unique; rofecoxib is the fourteenth small-molecule drug pulled from the US market because of safety considerations during the past seven years [3]. This list includes major drugs such as cerivastatin (Bayer), alosetron (GlaxoWellcome), troglitazone (Parke-Davis, a division of Warner-Lambert) and mibefradil (Roche), which should make those who embrace the target-based approach to drug discovery contemplate its validity. This approach has been effective at developing potent small-molecule inhibitors for many targets; however, given the extreme complexity of animal systems (including humans), the withdrawal of rofecoxib demonstrates that the assessment of target selectivity *in vitro*, or even by safety-testing in animal models, is simply not feasible to predict completely the safety of a small molecule for use in humans. As Mark Murcko (Vertex) stated, chemistry is relatively straightforward, but 'biology is the final frontier' in drug discovery.

Kinases and more kinases

Although the rofecoxib saga highlights that target-based drug discovery does not guarantee delivery of a safe and effective

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drug, there is currently no real alternative to a rational approach guided by a mechanistic understanding of human disease. Therefore, numerous presentations featured a logical progression from validated targets to early leads to clinical compounds, relying heavily on medicinal chemistry to develop SAR to increase compound potency, selectivity and safety. For example, kinases continue to be a major target class for anti-inflammatory drugs, and selective kinase inhibitors were a popular topic at the conference. In an impressive example, Kevin Williams (Amphora Discovery) described high-throughput screens of ~120,000 drug-like compounds against ~100 interesting kinase drug targets in the 'human kinome'. With such comprehensive screening data in hand, Williams emphasized that medicinal chemistry can advance rapidly once the desired target selectivity is chosen. However, no screening approach can identify all potential drug–target interactions; there are thought to be >1000 kinases in the human genome and designing molecules with the required selectivity profile in humans could be impossible. For this reason, Bill Metz (Sanofi-Aventis) remarked that assignment to a kinase program has been considered the 'kiss of death' by medicinal chemists. Despite this perception, chemists have made great progress in the kinase field. For example, David Goldstein (Roche) presented encouraging preclinical and clinical data on the development of the selective p38 kinase inhibitors Ro1487 and RoXXX7. Goldstein reiterated the value of understanding and engineering kinase selectivity, and credited the rapid kinase selectivity screens of Ambit Biosciences with the capability of Roche to generate useful SAR in real-time. Similarly, Katerina Leftheris (Bristol-Myers Squibb; BMS) described progress on the selective p38α program at BMS, but indicated that there is still uncertainty concerning whether it will be possible to develop truly selective kinase inhibitors when a similar purine pocket is present in thousands of other enzymes.

Matrix metalloproteinase setback

Occasionally, a rational target-based discovery effort can be thwarted simply by having an improved understanding of target biology. For example, in his talk describing the development of matrix metalloproteinase (MMP) inhibitors for osteoarthritis, Lawrence Reiter (Pfizer) commented that Pfizer, together with many others, had been passengers on an 'MMP train' since the early 1990s. Although MMPs have long been of interest to medicinal chemists, no MMP inhibitors have reached the market; by contrast, multiple clinical trials have failed because these compounds produced musculoskeletal side effects. Subsequent research suggested that compounds targeting MMP13 and sparing MMP1 would have a better safety profile than the original nonselective antagonists; therefore, chemists worked to produce the next generation of MMP13-selective inhibitors. Reiter described CP544,439 (Pfizer) as a chemistry success in that it has the intended MMP13 and MMP1 selectivity; unfortunately, this compound induced the same musculoskeletal side effects in clinical trials as first-generation nonselective compounds. In hindsight, and with a better understanding of MMP biology, it is now clear that CP544,439 potently inhibits a number of other MMPs that were not characterized at the time the compound was developed. Reiter suggested that the recent advances in biology create the opportunity to develop the third generation of even more selective inhibitors, but concluded that challenging biology and chemistry have caused the majority of companies to disembark from the MMP train.

Designing drugs with good physicochemical properties

A theme relevant to most presentations was the need to create lead candidates with good drug properties; for example, the talks and posters addressed various guidelines medicinal chemists use to improve the chances of clinical success (e.g. the well-known Lipinski Rule of Five [4]). Taking this concept further, Murcko espoused the concept of chemogenomics, commenting that pattern-recognition tools can aid in the interpretation of small-molecule screening data, which will enable an improved understanding of the cellular target and ultimately lead to the production of better drugs. For example, by extracting high-content data generated from the screening of large compound libraries, Vertex correlates biochemical and biophysical properties with biological properties, thus building early confidence in small-molecule target selectivity. Intriguingly, Murcko stated that, despite increased industry awareness of the importance of the physicochemical properties of a drug, the small-molecule compounds described in the *Journal of Medicinal Chemistry* from 1959 to 2002 are actually deteriorating with respect to accepted drug-like properties (e.g. calculated logP, polar surface area, molecular weight and rotational bond number) [5].

Contrasting approaches to discovering drugs and their targets

One challenge for target-based drug discovery occurs when the target selectivity goal is achieved but the biology of the new compound does not meet expectations. To illustrate, Ron Magolda (Wyeth) focused on the development of selective estrogen receptor (ER)-β agonists that do not target the ER-α subtype. Through an impressive medicinal chemistry effort, Magolda's group at Wyeth Women's Health identified several compounds (including ERB041 and WAY202196) exhibiting good ER-β selectivity. When tests of these selective ER-β agonists in standard *in vitro* and *in vivo* estrogen-sensitive models failed to generate any pharmacological effects, these compounds proved to be interesting puzzles. Extensive proteomics approaches were also unable to suggest a role for ER-β activation. Fortunately, WAY202196 showed activity in several animal models of inflammation (including an inflammatory bowel disease model) and is currently in Phase I trials, although its anti-inflammatory mechanism of action remains unclear.

At the opposite end of the discovery spectrum, 'me-too' drugs are always a popular focus of medicinal chemistry efforts because they greatly minimize clinical risk [6]. In this vein, Robert Townsend (BMS) discussed the development of BMS566419, an inhibitor of the enzyme inosine monophosphate dehydrogenase (IMPDH), which is intended to compete with similar marketed drugs currently used in transplant rejection (e.g. mycophenolate mofetil; Roche). Because IMPDH is crucial for *de novo* DNA synthesis, it

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is possible that inhibitors of this target could cause similar systemic side effects, regardless of structure. Improved safety is an admirable goal for me-too drugs, but, as Townsend indicated, it can be difficult to separate drug-related safety problems (which can probably be addressed in a next-generation molecule) from target-related safety (which presumably cannot be altered).

In a fascinating alternative to the me-too approach, James Mobley (Pfizer) described a historical epidemiological approach to uncover targets involved in inflammation [7]. Mobley postulated that the high incidence of rheumatoid arthritis observed in the USA and Europe is a consequence of intense genetic pressure to increase population resistance to tuberculosis (TB), in the same way that sickle-cell anemia in Africa is associated with increased population resistance to malaria. Mobley presented the audience with evidence that, in human history, rheumatoid arthritis rates are highest where TB has been endemic and lowest where TB is absent. Most intriguingly, Mobley used these correlative epidemiological and genetic data to identify useful anti-inflammatory targets. Tumor necrosis factor (TNF) is perhaps the most compelling example mentioned because a polymorphism in its promoter has been linked to TB susceptibility. Furthermore, all the anti-TNF biologics indicated for rheumatoid arthritis also increase the risk of developing TB.

Proteins are drugs too

Not all modern drug discovery involves medicinal chemistry. Indeed, given the challenges of developing selective, safe and effective small molecules, the potential of proteins to be highly selective and safe drugs is increasingly recognized [8]. Michael Vitek (Cognosci) reported on the development of COG1410, a peptide that inhibits inflammation in severe brain trauma. Based on the hypothesis that apolipoprotein E3 (ApoE3) is involved in neurological inflammation, Vitek and his team engineered and screened novel peptides derived from the receptor-binding interface of ApoE3, and ultimately showed that COG1410 reduced loss of cognitive function in sophisticated animal models of brain trauma.

Moving on to larger proteins, the TNF inhibitors are perhaps the most successful disease-modifying anti-inflammatory agents for rheumatoid arthritis, Crohn's disease and psoriasis [e.g. etanercept (Amgen), infliximab (Centocor) and adalimumab (Abbott)]. Although TNF is without doubt a well-validated drug target at the top of an important inflammation cascade, the cytokine itself is essentially unavailable as a target for small-molecule antagonists, which are considered incapable of blocking the large protein–protein interactions between the TNF ligand and its receptors. David Szymkowski (Xencor) described a new class of 'dominant-negative' (DN) anti-TNF biologics with potential safety, efficacy and cost advantages over antibody and decoy receptor strategies. Using medicinal biology-based SAR, libraries of DN-TNFs and other protein drugs can be designed, screened and optimized by a strategy similar to that used for small molecules. An excellent example of this process was provided by Scott Batty (BMS), who discussed the clinical development of abatacept (BMS188667), a cytotoxic T-lymphocyte-associated (CTLA)-4–IgG 1 fusion protein under evaluation for several inflammatory indications. Batty emphasized that, despite the introduction of new immunosuppressive agents (e.g. mycophenolate mofetil), long-term graft survival in solid organ transplantation has not improved in the past 16 years. Through a large medicinal biology effort to create and screen 2300 CTLA4 mutant proteins, the BMS group identified LEA29Y (BMS224818) as a second-generation drug optimized for high-affinity binding to CD80/86 on antigen-presenting cells (APC), thus improving blockade of APC-mediated T-cell activation via CTLA4 and CD28. Abatacept and LEA29Y are currently in advanced clinical trials – abatacept is in a Phase III trial in rheumatoid arthritis and LEA29Y is in a Phase II trial in kidney transplant rejection. Batty's description of the development of abatacept and LEA29Y is a clear example of how structure-based medicinal biology enables the development of SAR and the optimization of the drug properties of novel biopharmaceuticals.

Finally, as a fitting close to a meeting that opened with a small-molecule withdrawal, Tony Arulanandam (Biogen Idec) described late-stage clinical data on natalizumab, a selective $\alpha 4\beta 7$ integrin inhibitor. Natalizumab could become the first new drug approved for multiple sclerosis (MS) in seven years; the FDA has accepted its Biologics License Application for priority review and accelerated approval. Arulanandam showed that natalizumab was efficacious in the guinea pig experimental allergic encephalomyelitis (EAE) model and ultimately in Phase III human trials. Animal models of MS can be challenging and even misleading (e.g. lenercept, a TNF inhibitor developed by Roche, worked well in EAE models but failed in the clinic [9]); however, there is an urgent need for better MS therapies, and considering the safety and efficacy profile of natalizumab, this monoclonal antibody could be approved rapidly.

Current and future prospects for inflammation research

In conclusion, in the 24 years since the first biennial meeting, the IRA has grown from a small informal gathering of medicinal chemists representing large pharmaceutical companies to an international meeting with biotechnology and pharmaceutical scientists describing small- and large-molecule drug discovery. The photographs taken during many talks, as well as of the posters presented, suggest that SAR series and synthetic routes described by rival researchers are sure to be analyzed in project meetings, which is a good indication of the competitive nature of the pharmaceutical and biotechnology industries. Despite this competition, the meeting format encouraged open discussion and debate. A major theme of the meeting was that medicinal chemistry is a powerful drug discovery tool, but it must be applied to the right targets. In the wake of the withdrawal of rofecoxib, the debate for the coming year should focus on strategies to choose the optimum targets, refine compound selectivity and increase the probability of clinical success. Also noteworthy was the small, but increasing, emphasis on highly selective protein drugs. With the possible approval of natalizumab and abatacept in 2005, it will be interesting to observe if and how pharmaceutical companies adapt their portfolios to balance small-molecule and protein development programs in the two years leading up to the next meeting.

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feature

High-throughput drug discovery: what can we expect from HTS?

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Fuelled by the successes and optimism of the 1980s, the concepts of HTS and combinatorial chemistry, which heralded a new age of drug discovery, were embraced by the pharmaceutical industry. However, attrition rates in later stages of drug discovery soon led to questions being raised about the viability of the high-throughput paradigm. Here, examples from the experience at Pfizer (Sandwich, UK) are used to illustrate how the quality of the compound file, the target and the screening process act in concert to define the output from HTS. This is discussed within the context of the available literature, taking into account opinions from across the pharmaceutical industry.

HTS fuels the drug discovery pipelines of the majority of pharmaceutical companies and is one of the central paradigms of modern drug discovery. The need for HTS is a consequence of our limited knowledge – it is impossible to make accurate predictions about the interactions of biological macromolecules with small compounds for the assessment of the activity of new chemical entities. Biological screening is the practical answer to this conundrum, and the concept of scale has been introduced by the proposal that screening a greater number of compounds should provide more leads of improved quality. It was expected that this strategy would deliver multiple new starting points for drug discovery projects and would sustain the double-digit growth of major pharmaceutical companies. Despite the continuous introduction of more sophisticated screening technologies and ever increasing compound collections (predominantly driven by combinatorial chemistry), HTS has, in the minds of many scientists and analysts, fallen short of this target [1–3]. Although there is the widespread belief that, in the absence of alternative solutions, HTS will remain an important tool for the foreseeable future, it is now often perceived as a costly necessity rather than a method of choice. More importantly, attrition in later stages of drug development, as a result of poor physicochemical properties, has been attributed to the nature of HTS itself [4].

Here, HTS is dissected to give a better understanding of how the compound file, target and screening process act in concert to define the output from HTS and how changes in our knowledge can have a direct impact on this output. Understanding trends and drivers within biological screening should help to set the expectations about what HTS can and cannot deliver to the business.

Physicochemical properties of compounds and HTS

Poor solubility, permeability and metabolic instability of compounds are a major source of attrition in early development [5,6], and it has been suggested that the introduction of HTS and combinatorial chemistry contributes to this through the selection of compounds with properties outside ADMET space [4,7]. To assess this impact, changes in physical properties indicative of *in vivo* properties, such as absorption and permeability, were monitored over time. This process involved the analysis of the molecular weight, calculated logP (clogP) and predicted solubility of compounds registered at Pfizer in a time-dependent manner (grouped by registration date) from 1960 to 1997, when, after Lipinski

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