Monitor

***Monitor* provides an insight into the latest developments in the pharmaceutical and biotechnology industries. *Chemistry* examines and summarises recent presentations and publications in medicinal chemistry in the form of expert overviews of their biological and chemical significance, while *Profiles* provides commentaries on promising lines of research, new molecular targets and technologies. *Biology* reports on new significant breakthroughs in the field of biology and their relevance to drug discovery. *Business* reports on the latest patents and collaborations, and *People* provides information on the most recent personnel changes within the drug discovery industry.**

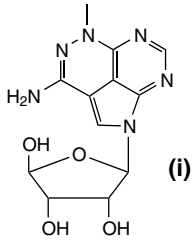
Chemistry

Antitumour molecules

Selective small-molecule inhibitor of Akt-signalling

Data associating the Akt pathway with cell survival, malignant transformation, tumour invasiveness and chemoresistance have established Akt (also known as protein kinase B) as an attractive therapeutic target for anticancer drug development [1]. The Akt family (Akt1, Akt2 and Akt3) is a subfamily of serine and threonine kinases that is activated by phosphatidylinositol 3′-kinase (PI3k) and is negatively regulated by the phosphatase and tensin homologue (PTEN) tumour suppressor (mutated in a variety of tumours). Inhibition of Akt activity induces apoptosis and hinders tumour growth in a range of mammalian cells [2].

The Diversity Set established by the National Cancer Institute (NCI; http://www.nci.nih.gov) is a chemical library comprising approximately 2000 compounds; the library is designed to be representative of the much larger NCI chemical database (~140,000 compounds). Yang and co-workers [3] screened the Diversity Set against Akt2-transformed NIH3T3 cells *in vitro* (using LXSN vector-transformed NIH3T3 cells as a negative control). From an initial set of 32 compounds that inhibited growth selectively in Akt2-transformed cells, the most potent compound, API-2 (**i**), suppressed cell growth at a concentration of 50 nM. API-2 did not inhibit known upstream activators of Akt, such as PI3k or phosphoinositide-dependent kinase-1 (PDK-1), and was highly selective for Akt over a range of related kinases. In addition, API-2 selectively inhibited the growth of tumour xenografts that overexpress Akt in nude mice (1 mg kg^{-1} day^{-1}), which reinforces the potential clinical benefit of selectively targeting the Akt pathway.



(i)

In Phase I and II clinical trials, it was observed that API-2, which has been previously identified as tricyclic nucleoside, triggered some side effects, including hepatotoxicity, hypertriglyceridaemia, thrombocytopenia and hyperglycaemia, which are effects that are closely related to dose size [4]. In this study, a low dose of API-2 was found to selectively induce apoptosis and inhibit growth of tumour cells that had elevated levels of Akt. Additionally, in the xenograft studies, no visible side effects were observed at doses (1 mg kg^{-1} day^{-1}) that resulted in significant inhibition of tumour growth. The re-establishment of a previously tested agent as an Akt pathway inhibitor not only lends credibility to the target but also provides a rationale for dosing and patient selection in future clinical trials. The discovery also raises the possibility of a future generation of API-2 analogues that retain potent Akt inhibition but simultaneously suppress dose-limiting side effects in the clinic.

1 Datta, S.R. *et al.* (1999) Cellular survival: a play in three Akts. *Genes Dev.* 13, 2905–2927
2 Jetzt, A. *et al.* (2003) Adenoviral-mediated expression of a kinase-dead mutant of Akt induces apoptosis selectively in tumor cells and suppresses tumor growth in mice. *Cancer Res.* 63, 6697–6706
3 Yang, L. *et al.* (2004) Akt/protein kinase B signaling inhibitor-2, a selective small molecule inhibitor of Akt signaling with antitumor activity in cancer cells overexpressing Akt. *Cancer Res.* 64, 4394–4399
4 Feun, L.G. *et al.* (1993) A Phase II trial of tricyclic nucleoside phosphate in patients with advanced squamous cell carcinoma of the cervix. A Gynecologic Oncology Group study. *Am. J. Clin. Oncol.* 16, 506–508

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Medicinal chemistry

Antisense PNA averts normal splicing

Despite all the hurdles that are inherent to the development of antisense techniques, this 30-year-old technology still attracts a great deal of attention in medicinal chemistry because it enables the rational

design of gene-targeted drugs [5–7]. The promise of this technology rests on the expectation that synthetic 'gene-blocking' oligomers, which have a sequence that is complementary to corresponding mRNA or pre-mRNA targets, will become a generic therapy of the future for various diseases. After common oligonucleotides and their modified analogues, a third generation of antisense oligomers – the artificial DNA and RNA mimics – is emerging, with the peptide nucleic acid (PNA) oligomers showing significant promise [8].

Recent studies performed at ISIS Pharmaceuticals (http://www.isip.com) with the murine cellular receptor CD40, which has a key role in immune response, have demonstrated that specific downregulation of protein expression can be efficiently achieved with the PNA antisense inhibitor ISIS208529; this molecule targets the exon 6 splice junction within the primary CD40 transcript [9]. When delivered to murine cells (primary macrophages and lymphoma B-cells) by electroporation, binding of ISIS208529 interferes with pre-mRNA splicing and results in the accumulation of a defective protein that lacks the transmembrane domain. Conjugation of ISIS208529 with oligolysine yields an even more potent antisense drug, ISIS278647, which is effective in murine cells via 'free uptake' (i.e. without transfection or electroporation). Importantly, cells that have been treated with PNA inhibitors exhibit a decrease in the CD40-mediated production of the cytokine interleukin-12 (IL-12) as a result of inhibition of the CD40-signalling pathway.

These results show that antisense PNA oligomers can be employed as new immunomodulatory agents. Given that just a few antisense drugs have entered clinical trials [10], and with only one such drug currently approved for clinical use (antiviral vitravene or fomivirsen; ISIS2922), these novel drug candidates are valuable additions to the small antisense family.

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7 Crooke, S.T. (1999) Molecular mechanisms of action of antisense drugs. *Biochim. Biophys. Acta* 1489, 31–44
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9 Siwkowski, A.M. *et al.* (2004) Identification and functional validation of PNAs that inhibit murine CD40 expression by redirection of splicing. *Nucleic Acids Res.* 32, 2695–2706
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Biology

Microbiology

Novel *Streptococcus pyogenes* exotoxin disrupts cytoskeleton

Exotoxins with ADP-ribosyltransferase (ADPRT) activity are produced by many bacterial pathogens. ADPRTs transfer ADP-ribose from β-NAD^+ onto host target proteins such as transcription factors, signaling molecules, and cytoskeletal proteins and thereby interfere with their function. In the important human pathogen *Streptococcus pyogenes* there are two known ADPRTs (GAPDH and SPN), but no cellular targets have been identified.

Coye *et al.* identified a novel putative ADPRT, SpyA, which is present in several genomes of various serotypes [1]. Recombinantly expressed SpyA, but not SpyA mutated in a putatively catalytic glutamic acid residue, has NAD-glycohydrolase activity and ribosylates poly-L-arginine. Besides auto-ribosylation, SpyA ribosylates several proteins when incubated with cellular extracts. Two-dimensional electrophoresis and mass spectroscopy identified the cytoskeletal proteins actin, vimentin and tropomyosin as SpyA targets. Expression of SpyA in HeLa cells and fluorescence microscopy demonstrated that actin microfilaments were disrupted. The authors hypothesize that this modification could interfere with phagocytosis of the bacteria by professional phagocytes. It is still unclear how SpyA enters host cells, but the authors speculate that cytolysin mediated translocation, which is crucial for delivery of SPN, could be involved. Another possibility not mentioned in this paper is that SpyA could be expressed by intracellular bacteria within the phagocytes and contribute to survival by cytoskeletal rearrangements.

This study describes a novel ADP-ribosyltransferase and for first time identifies cellular targets for an ADPRT from *S. pyogenes*. Even though there are still several questions to be answered regarding *in vivo* expression and cellular translocation/intracellular expression, this report indicates that SpyA and other ADPRTs could be important for the molecular pathogenesis of *S. pyogenes.*

1 Coye, L.H. and Collins, C.M. (2004) Identification of SpyA, a novel ADP-ribosyltransferase of *Streptococcus pyogenes*, *Mol. Microbiol.* doi:10.1111/j.1365-2958.2004.04262.x. (EPub. ahead of print; http://blackwell-synergy.com)

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A bit of Lov for HIV-1 patients

HIV-1 entry into and exit from target cells requires adequate cholesterol levels in host and viral membranes. Protein co-aggregation is also needed at the host cell surface: CD4 and chemokine receptors for entry, Gag and gp160 for budding. The HIV-1 infection process induces receptors clustering with lipid rafts, which necessitates actin cytoskeleton rearrangements. Rho is suspected to play a key role during this reorganization process.

Del Real *et al.* now provide evidence that statins prevent HIV-1 infection in cultured primary cells, in animal models and in chronically infected individuals [2]. Statins, currently used to treat hypercholesterolemia, inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. HMG-CoA reductase produces mevalonic acid, a precursor for cholesterol.

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