coupling reaction, PS-maleimide resin **ii** was added. Diels-Alder reaction of **i** and **ii**, followed by filtration, removed the catalyst from the reaction mixture. An anthracene-tagged boronic acid was used in a similar fashion to remove excess aryl bromide. Polymer-bound carbonate was used as a base, a strategy that replaced the usual aqueous workup; the polymer-bound carbonate also effectively scavenged excess boronic acid and other borane-containing byproducts. Workup after scavenging consisted of filtration and evaporation of solvents. This anthracene-tagging strategy could conceivably be applied to many other organic transformations.

Solution-phase split/mix synthesis facilitated by fluorous tagging

For the combinatorial chemist, the split/mix technique is one of the strengths of solid-phase synthesis: a 10x10 split/mix sequence can yield 100 compounds from only 20 synthetic manipulations. Using various techniques, active compounds can be deconvoluted relatively easily. In recent years, the emphasis has shifted to solution-phase parallel synthesis, and the power of the split/mix technique has been lost. Recently, Curran and coworkers demonstrated that the use of fluorine-tagged protecting groups can facilitate solution-phase split/mix synthesis [2] and reported an elegant example of this technique to synthesize 16 stereoisomers of the natural product (+)-murisolin.

All three known murisolins possess the same stereochemistry about a hydroxy butenolide. The split/mix strategy was utilized to obtain all 16 possible stereoisomers of the dihydroxy tetrahydrofuran fragment **iii** (represented here in its protected form). The synthetic transformations employed were straightforward and included hydroboration/oxidation, Negishi coupling, Shi epoxidation and Mitsunobu inversion. The advantage of the present strategy is the use of the split/mix technique in solution phase. In pre-mix stage, all four isomers of the protected diol **iv** were synthesized. Each isomer was tagged with a different fluorous protecting group, with the fluorine content of the tag serving as a code for the configuration at the hydroxyl positions. The four isomers of **iv** were mixed together and carried through several synthetic steps to intermediate **v**, which is still a single mixture of four isomers at this point. Mixture **v** was divided in two, and Shi epoxidation with enantiomeric catalysts was performed. These two epoxidation reaction mixtures were each split in two, and one was subjected to Mitsunobu inversion. Ultimately, four mixtures of four isomers of fragment **iii** were obtained.

The mixtures of fragment **iii** were carried on to the final natural product murisolin in parallel fashion, so four mixtures of four isomers of murisolin were obtained. Each mixture was deconvoluted by chromatography on FluoroFlash silica gel. At this stage, the individual isomers separate based on the fluorine content of their tags. The fluorous tag is then removed. The identity of each stereoisomer is provided by its synthetic series coupled with its elution order on demixing. Using this strategy, all 16 isomers of murisolin were synthesized in a total of 39 synthetic steps, followed by four fluorous phase columns to demix. If performed in traditional solution-phase parallel fashion, this would have required 156 synthetic steps and 16 purification steps. If proven to be generally applicable, this technique could potentially bring the power of the split/mix technique to solution-phase chemistry, facilitating the synthesis and deconvolution of mixtures of compounds without resorting to synthesis "on-the-bead".

1 Lan, P. *et al.* (2003) Polymer-assisted solution-phase (PASP) Suzuki couplings employing an anthracene-tagged palladium catalyst. *J. Org. Chem.* 68, 9678–9686

2 Zhang, Q. *et al.* (2004) Fluorous mixture synthesis of stereoisomer libraries: total syntheses of (+)-murisolin and 15 diastereoisomers. *J. Am. Chem. Soc.* 126, 36–37

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Biology

Cancer Biology

A new cancer drug in the pipeline?

Antibodies directed against growth factor receptor molecules possess strong therapeutic potential in the treatment of cancer. The anti-Her2 antibody Trastuzumab (Herzeptin) is currently used for the treatment of metastatic breast cancer. Evidence suggests that resistance to Herzeptin in some forms of breast cancer could be due to activation of insulin-like growth factor I receptor (IGF-IR) signalling. The IGF-IR activates mitogenic and antiapoptotic pathways and has been shown to play a pivotal role in the development and progression of cancer. Apart from breast cancer, overexpression of the IGF-IR can be observed in several tumour types and is correlated with bad prognosis. Burtrum *et al.* now report the engineering of a new fully human monoclonal antibody directed against IGF-IR [1].

A candidate, 2F8, for optimal binding to IGF-IR and competitive ligand locking was identified by screening a naïve human Fab phage display library. The light-chain shuffling technique was used to improve binding properties. The resulting Fab (A12) was chosen for further investigation and converted into IgG.

Western blot analysis and cell culture experiments revealed inhibition of IGF-IR induced mitogenesis and cell proliferation. Mechanistically, A12 was shown to prevent binding of receptor ligands IGF-I and IGF-II and to induce receptor internalization and degradation, thus blocking downstream signalling cascades. The first *in vivo* data from xenograft tumour models in mice show >70% tumour growth inhibition and no detectable toxicity.

The published data suggest that the new IGF-IR antibody A12 might be a good candidate for a broad-spectrum anticancer drug. Clinical investigation will show if it lives up to the promise.

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1 Burtrum, D. *et al.* (2003) A fully human monoclonal antibody to the insulin-like growth factor I receptor blocks ligand-dependent signaling and inhibits human tumor growth *in vivo*. *Cancer Research* 63, 8912–8921

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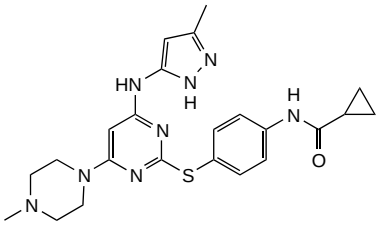
First light on small molecule inhibition of Aurora kinases

The mammalian Aurora kinases have a functional role in mitosis, particularly in spindle formation, cytokinesis and cell-cycle progression. However, they are also thought to be oncogenic, having been found to be overexpressed in tumour cells. Harrington *et al.* have now made significant steps in validating Aurora kinases as potential targets for cancer therapy using VX680 [2]. This compound is reported to inhibit all three Aurora kinases (Ki 0.6–18nM) with >100-fold selectivity over several other kinases, including the closely related Src and cyclin-dependent kinase-2 (CDK-2).



(i) VX-680

VX680 **(i)** is also shown to inhibit proliferation in a range of cancer cell lines, including leukaemic, colorectal and breast, with arrest in the G2 phase of the cell cycle, which is consistent with peaks in expression and activity of the Aurora kinase enzymes in this mitotic phase. Further experiments have focused on the subsequent fate of cells following incubation with the compound. Cell viability and apoptosis assays in cancer cell lines or primary leukaemic cells confirmed that VX680 is not cytostatic but cytotoxic, resulting in cell death via apoptosis. This effect was specific to actively proliferating cells and no toxicity was observed in non-cycling cells. VX680 was also well tolerated and demonstrated dose-dependent suppression of tumour growth in several *in vivo* xenograft rodent models at doses of 50mg kg^{-1} twice-a-day i.p. or following a 1mg kg^{-1} $hour^{-1}$ infusion over two weeks.

These studies suggest that targeting the Aurora kinases could be a new strategy in the treatment of cancer by inducing tumour cell death.

2 Harrington, E. A. *et al.* (2004) VX-680, a potent and selective small-molecule inhibitor of the Aurora kinases, suppresses tumor growth *in vivo*. *Nat. Med.* 10, 262–267

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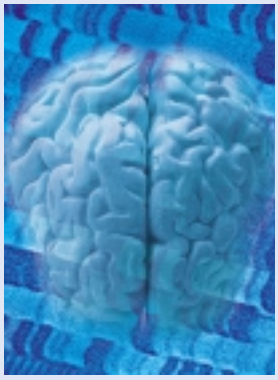
Neuroscience



CHOP kills brain cells

The specific biochemical events that lead to neuronal degeneration after brain ischaemia are still not fully understood; identification of these events, which initiate the changes leading to ischaemia-induced pathophysiology, remains an important objective. In brain ischaemia, insufficient glucose and oxygen for protein modifications in the endoplasmic reticulum (ER) can lead to ER stress in neuronal cells. C/EBP homologous protein (CHOP or GADD153) is an ER stress-associated proapoptotic protein that might function as a transcriptional factor, regulating genes involved in either survival or death. However, a role for CHOP in ER stress-mediated neuronal apoptosis following brain ischaemia has yet to be determined.

Tajiri *et al.* [3] investigated the role of CHOP by producing transient cerebral iscahemia by the bilateral common carotid arteries occlusion (BCCAO) procedure. At 48 h recovery following 15 min of occlusion, apoptotic neuronal cell death was detected in the striatum and in the hippocampus by morphological analysis, TUNEL and propidium iodine stainings. RT-PCR analysis demonstrated the induction of BiP and XBP1 mRNAs, both markers of ER stress. Immunohistochemical analysis indicated that brain ischaemia also induced the expression of the CHOP protein.

Although the ER stress response pathway is activated in CHOP-deficient mice following 15 min of BCCAO, as indicated by the expression of BiP mRNA, neuronal cell death was attenuated in both the striatum and the CA1 subfield of the hippocampus. In addition, primary hippocampal neurons from CHOP-knockout mice were more resistant to hypoxia-reoxygenation-induced apoptosis than those from wild-type animals.

Together, these results indicated that ischaemia-induced cell death is mediated by an ER stress pathway involving CHOP. It should be noted that although the disruption of the CHOP gene delayed ischaemia-induced neuronal apoptosis, this protection was only partial. Therefore, at least within the context of CHOP-deficient mice, ischaemia-induced neuronal apoptosis might be attributed to alternative pathways that are JNK-dependent or caspase-mediated. Moreover, it remains to be determined whether or not the rescued neuronal cell populations are functionally normal. Nevertheless, CHOP and other components of ER stress-mediated apoptosis are emerging as therapeutic targets for the prevention of the pathogenesis of brain ischaemia.

3 Tajiri, S. *et al.* (2004) Ischemia-induced neuronal cell death is mediated by the endoplasmic reticulum stress pathway involving CHOP. *Cell Death Diff.* DOI:10.1038/sj.cdd.4401365 (E-pub ahead of print; http://www.nature.com/cdd)

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Targets and Mechanisms

Structure of MutY adenine DNA glycosylase reveals how it recognises mismatches between adenine and 8-oxoguanine

Reactive oxygen species that are generated during aerobic respiration can lead to the oxidation of guanine bases in DNA to form 8-oxoguanine (oxoG). DNA polymerases that replicate opposite this lesion insert adenine, so both bases must be removed to prevent mutagenesis. The adenine is removed from these mismatches by MutY adenine DNA glycosylase (hMYH in humans). However, it is not known how this enzyme discriminates the adenines within A-oxoG mismatches from the normal A-T base pairs, which are present at a much higher concentration.

Fromme *et al.* [4] have solved the crystal structure of a MutY–DNA complex, showing how this enzyme distinguishes the mismatched adenines from those that are correctly base paired. The structure of full-length MutY from *Bacillus stearothermophilus* was solved bound to DNA containing an A-oxoG mismatch by using disulphide crosslinking to bond the DNA and protein together. To prevent the enzyme turning over within the crystals, the authors used MutY with a single point mutation, making it catalytically inactive.

MutY forms a two-domain structure; the catalytic domain is homologous to other proteins of the same family and the C-terminal domain is unique. The DNA backbone of the strand containing the adenine binds along a cleft on the catalytic domain, whereas the C-terminal domain contacts the other strand. The DNA helix is bent ~55° at the site of the lesion and the adenine base to be excised is flipped out of the DNA helix into a pocket within the enzyme. Interestingly, the glycosidic bond of the oxoG is in the anti orientation (it is in the syn position to pair with adenine), indicating that it has rotated 180° about the bond. This rotation could drive the flipping out of the adenine, because with oxoG in the anti orientation the two bases would sterically clash.

The DNA distortions are caused by several residues penetrating into the helix. These include tyrosine 88 intercalated 5′ to the oxoG, and glutamine 48 inserted into the space for the adenine. The oxoG base itself is contacted by residues from the C-terminal and catalytic domains, which form hydrogen bonds, such that every available face of the base is specifically recognised. It is these contacts that ensure the selection of MutY for adenine bases within the context of an A-oxoG mismatch, as they are unsuitable to bind a thymine. Thymine is presumably also not selected because the A-T base pair is more stable, preventing the adenine from flipping out of the DNA helix in the first place.

4 Fromme, J.C. *et al.* (2004) Structural basis for removal of adenine mispaired with 8-oxoguanine by MutY adenine DNA glycosylase. *Nature* 427, 652–656

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Business

Collaborations

Collaboration between Y's Therapeutics and Abmaxis

Y's Therapeutics (http://ysthera.com) and Abmaxis (http://www.abmaxis.com) have announced an agreement to collaborate on the development of a monoclonal antibody anticancer therapeutic targeting antigens that are overexpressed in tumours. It is also expected that this approach might be efficacious in autoimmune disease.

As a result of the details of the agreement, Abmaxis will be responsible for the discovery of high affinity, human antibodies directed against and specific for the antigen. Y's will be involved in the downstream development of the potential product. The agreement gives Abmaxis manufacturing, sales and marketing rights in China while Y's Therapeutics retains rights in the USA, Europe, Japan and ROW.

Masanori Murayama, scientific co-founder, President and CEO of Y's Therapeutics commented: 'We are very favourably impressed by the Abmaxis AISIM™ technology platform, which has already generated a number of humanized antibody candidates.' He continued: 'We look forward to working with Abmaxis in our quest to deliver therapeutic antibodies to patients as quickly as possible.'

Shirley Liu Clayton, CEO of Abmaxis, was equally optimistic about the collaboration, adding; 'We have a high degree of confidence in the potential drug development capability of Y's Therapeutics. We are pleased that Y's Therapeutics has selected Abmaxis to develop therapeutic antibodies to their antigen and we are eager to work with Y's Therapeutics and Dr. Morimoto, a world-renowned expert in molecularly targeted drugs.' She added that they had the ability to custom design an antibody for the specific therapeutic requirements of the project, using their proprietary library of sequence/structure information. She believes that the Abmaxis approach will rapidly identify appropriate optimal therapeutic antibodies.

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