Programme and abstract booklet

Pharmacology Futures 2018
Exploring future drug development

17 May 2018
National Museum of Scotland, Edinburgh, UK

BRITISH
PHARMACOLOGICAL
SOCIETY

smr
The Society for Medicines Research

Current trends in drug discovery

Young scientists and tomorrow's medicines

Thursday 7 June 2018, King's College London, UK

This joint meeting is aimed at all young researchers and life scientists with an interest in drug discovery. It features presentations from three internationally recognised speakers alongside poster and oral presentations from young scientists.

Plenary speakers

Professor Chas Bountra, University of Oxford, UK
How do we de-risk new or 'undruggable' targets, quickly?

Professor Steven Charlton, The University of Nottingham, UK
The kinetics of drug action; signalling in a new era

Professor Maria Belvisi, AstraZeneca
Discovering new therapeutic options for chronic respiratory disease

Final registration deadline: **31 May**

Members: **£120**
Student members: **£75**
Non-members: **£135**
Non-member students: **£85**

Members of the Society for Medicines Research should email **meetings@bps.ac.uk** to obtain a promotional code to be able to register at the British Pharmacology Society member rate.

For more information visit **www.bps.ac.uk/drug-discovery-meeting**

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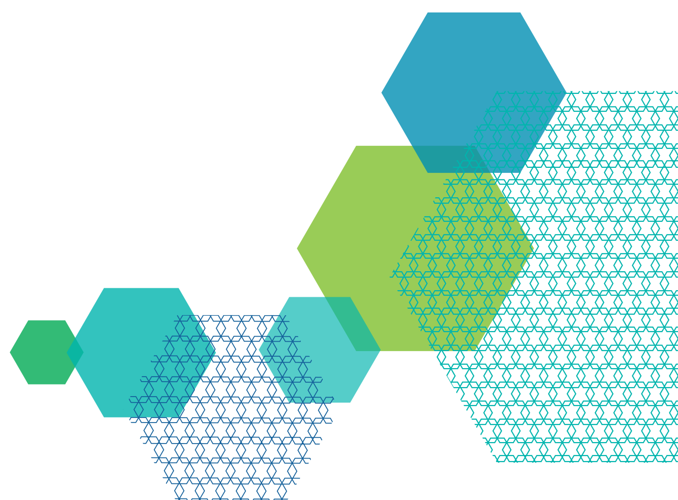

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For more information on attending or presenting at other Society meetings find us at the registration desk, email **meetings@bps.ac.uk** or visit **www.bps.ac.uk**

Information for participants

Key timings

Thursday 17 May 2018

08.30–09.00	Registration and refreshments
09.00–17.35	Scientific programme
19.00–19.45	Drinks reception (ticketed event)
19.45–22.00	Conference dinner (ticketed event)

Poster presenters

Poster presenters are asked to put up their posters during registration and to accompany their posters during the review session at lunchtime. This is the opportunity for attendees to ask questions and for reviewers to provide feedback.

Oral communications

Oral presenters will have seven minutes to present their work followed by their ideas on the future of pharmacology and of drug development.

Certificates of attendance/CPD certificates

Attendees will receive certificates of attendance and CPD certificates by email, after the event.

Internet access

Delegates may use the complimentary NMS public wi-fi network throughout the day. No registration or password is required.

Refreshments

Scheduled tea and coffee breaks, and lunch will take place in the Event Space and South Hall.

Social programme

Welcome drinks:	19.00–19.45
Dinner:	19.45–22.00
Location:	Grand Gallery, National Museum of Scotland

The social programme is a ticketed event, not included within your registration. If you are unsure if you have purchased a ticket, or would like to purchase one, please visit the registration desk.

Cloakroom

A cloakroom will be available and can be used free of charge and at your own risk.

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Please note that photographs taken at this meeting may be used on our website, social networking sites, or in other publications. If you do not wish to have your image used for this purpose, please speak to a Society staff member or email meetings@bps.ac.uk.

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Meeting programme

08.30–09.00 Registration and refreshments

09.00–09.10 Welcome and introduction
Professor David Webb, Past President, British Pharmacological Society and Professor of Clinical Pharmacology, The University of Edinburgh, UK

SESSION 1
Chair: Professor Sue Fleetwood-Walker, University of Edinburgh, UK

09.10–09.40 How do we make the UK a world leader in drug discovery?
Professor Chas Bountra, University of Oxford, UK

09.40–10.10 Cardiovascular diseases and drugs: Where are we with hiPSC models?
Professor Christine Mummery, Leiden University Medical Centre, The Netherlands

10.10–10.50 Minding the gap: Linking preclinical studies to the development of novel therapeutics at the National Institute of Mental Health
Professor Susan Amara, National Institutes of Health, USA

10.50–11.20 Refreshment break

SESSION 2
Chair: Professor Mark Evans, University of Edinburgh, UK

11.20–11.50 Drugging transcription: Progress and potential for treating human immune dysfunction
Dr Rab Prinjha, GlaxoSmithKline, UK

ORAL COMMUNICATIONS SESSION
Future of pharmacology competition, early career researchers*

11.50–12.00 OC001: ALDH1 bio-activates nifuroxazide to eradicate ALDHhigh melanoma-initiating cells
Sana Sarvi, University of Edinburgh, UK

12.00–12.10 OC002: Investigating SARMs as novel therapeutics in women
Douglas Gibson, University of Edinburgh, UK

12.10–12.20 OC003: Non-invasive in human *in vivo* target engagement biomarkers to strengthen GO/NO-GO decisions
Linde Buntinx, Center for Clinical Pharmacology, UZ Leuven, Belgium

12.20–13.40 Lunch and poster presentation

* Early career researchers: oral communications – for those with up to seven years' full-time equivalent post-doctoral experience as of May 2018

SESSION 3
Chair: Dr Sally Argyle, University of Edinburgh, UK

13.40–14.10 The pharmacology of turning thoughts into local blood flow in the brain
Professor Mark Nelson, University of Vermont, USA

ORAL COMMUNICATIONS SESSION
Future of pharmacology competition, PhD student oral communications

14.10–14.20 OC004: Unravelling the effect of metamizole, paracetamol and ibuprofen on TRPA1 using the cinnamaldehyde target engagement biomarker model
Dorien Bamps, Center for Clinical Pharmacology, KU Leuven, Belgium

14.20–14.30 OC005: Aldehyde dehydrogenase: 5-nitrofuran *in vivo* gene-drug interactions
Andrea Coates, University of Edinburgh, UK

14.30–14.40 OC006: Selective Interleukin-6 trans-signalling antagonism with sgp130Fc reduces infarct size in a rat model of myocardial infarction whereas pan-blockade with an anti-IL-6 antibody does not
Marc George, University College London, UK

14.40–15.20 The role of biomarkers in translational pharmacology
Dr James Dear, University of Edinburgh, UK

15.20–15.50 Refreshment break

SESSION 4
Chair: Professor David Wyllie, University of Edinburgh, UK

15.50–16.20 GPCR allostery: From theory to medicine
Professor Arthur Christopoulos, Monash University, Australia

16.20–16.25 Prize giving: Future of pharmacology competition

Cameron Prize
Chair: Professor Sir John Savill, Regius Professor of Medical Sciences, University of Edinburgh, UK

16.30–17.30 2017 Cameron Prize for Therapeutics lecture: Building and maintaining a global response to AMR
Professor Dame Sally Davies, Department of Health, UK

17.30–17.35 Closing remarks

19.00–19.45 Drinks reception (ticketed event)

19.45–22.00 Delegate dinner (ticketed event)

Invited speaker abstracts

How do we make the UK a world leader in drug discovery?

Professor Chas Bountra, University of Oxford, UK

The discovery of new medicines is too risky, too slow and too costly. There is massive duplication and wastage in biomedical research, there are questions being raised about the reproducibility of published work, and for many increasingly important therapeutic areas we are simply not producing what society and patients want. In my lecture I will discuss how we are trying to address these challenges.

The validation of pioneer targets for drug discovery remains a major challenge. We are therefore:

- working with a large number of pharmaceutical companies to develop high quality small molecule inhibitors, using structure based drug design
- focussing only on novel targets, or those deemed to be 'difficult' or intractable
- and giving these inhibitors to a large and growing international network of academic collaborators, to crowd source new biology, disease understanding and 'target discovery'

We have already facilitated proprietary efforts in pharma, catalysed the creation of new biotechs, and accelerated numerous clinical studies.

We are now

- generating 'Target Enabling Packages' (comprising purified proteins, biophysical or biochemical assays to assess function, three dimensional X ray structures and chemical starting points) for novel, high priority, disease linked genes
- building platforms of primary human cells, for screening novel inhibitors, in order to identify new 'better' targets for drug discovery
- building major collaborations with patient groups and hospitals, in order to catalyse these studies and enhance dissemination into the best disease labs across the world

Together, we are creating a new ecosystem for drug discovery. One which we believe will accelerate the generation of more novel medicines, more quickly. We hope these medicines will also be more affordable.

Cardiovascular diseases and drugs: Where are we with hiPSC models?

Professor Christine Mummery, Leiden University Medical Centre, The Netherlands

Derivation of cardiovascular cell types from human pluripotent stem cells is an area of growing interest as a platform for drug discovery and toxicity. Most particularly, the recent availability of methods to introduce specific disease mutations into human pluripotent stem cells and/or to derive these cells as hiPS cells by reprogramming from any patient of choice, are creating unprecedented opportunities to create disease models "in a dish" and study ways to treat it or slow down its rate of development. Most recently our lab has been investigating organs on chip solutions in which multiple cardiac and vascular cell types into microtissue formats. Crucial has been methods to promote cardiomyocyte maturation and to quantify the outcomes of drug and disease mutation responses in situ. The use of isogenic pairs has proven very important since variability between "healthy control" hiPSC lines is often greater than the difference between a diseased cells and its isogenic control. We have shown that iPSC derived cardiomyocytes with mutations in ion channel genes can accurately predict changes in cardiac electrical properties and reveal drug sensitivities also observed in patients.

Minding the gap: Linking preclinical studies to the development of novel therapeutics at the National Institute of Mental Health

Professor Susan Amara, National Institutes of Health, USA

The National Institute of Mental Health (NIMH) at the National Institutes of Health in Bethesda is committed to reinvigorating the development of new therapies for the treatment of psychiatric disorders. The current reduction in psychiatric drug discovery and development in the pharmaceutical industry provides an opportunity for the Intramural program at the NIMH to contribute to the de-risking of novel therapeutics and engage industry in an effort to get more effective treatments to patients. This talk will provide an overview of drug discovery initiatives at the NIMH.

Drugging transcription: Progress and potential for treating human immune dysfunction

Dr Rab Prinjha, GlaxoSmithKline, UK

Dramatic progress in the understanding and insights into the potential for drug-discovery against epigenetic targets has enabled the rapid transition of many agents from preclinical evaluation to studies in patients. I will discuss the scientific framework that's informing our excitement about epigenetics, in particular BET bromodomain containing proteins and highlight advances in understanding of the broader bromodomain containing family of proteins. As these epigenetic agents start to transition for the first time from oncology studies into other diseases it's increasingly apparent that epigenetics is crucially important in defining immune cell lineage and function. Innovative use of epigenetic inhibitors in studying myeloid cell activation is helping us understand the chromatin associated mechanisms controlling this and I will discuss data pertaining to this.

The pharmacology of turning thoughts into local blood flow in the brain

Professor Mark Nelson, University of Vermont, USA

Blood delivery to active neurons (functional hyperemia) is rapidly and precisely controlled through a process. Brain capillaries act as a neural activity-sensing network with capillary endothelial cells (cECs) initiating an electrical (hyperpolarizing) signal in response to neural activity that rapidly propagates upstream to cause dilation of feeding arterioles and increase blood flow locally (Nature Neuroscience, 2017). We have established the mechanistic basis for this electrical signal, showing that extracellular K+—a byproduct of every neuronal action potential—is the critical mediator and identifying the cEC strong inward rectifier K+ channel, Kir2.1, as the key molecular player.

Cerebral small vessel diseases (SVDs) correspond to various pathological processes that affect the microvasculature—arterioles, capillaries and venules—in brain. SVDs are major contributors to stroke, disability, and cognitive decline that develop with aging and hypertension. The disease processes and key biological mechanisms underlying these disorders remain largely unknown. Functional and structural alterations in the cerebral microvasculature have early and deleterious consequences on the brain prior to or in association with the occurrence of focal ischemic or hemorrhagic lesions. Currently, there is no effective treatment.

CADASIL is the most common monogenic inherited form of SVD, caused by mutations in NOTCH3. Transgenic mouse models of CADASIL recapitulate many clinical and histopathological hallmarks of the disease. Our recent studies using transgenic mice expressing a human CADASIL mutation (TgNotch3R169C) implicate ADAM17/HB-EGF/EGFR signaling axis in TIMP3-sensitive cerebrovascular dysregulation via perivascular accumulation of mutant Notch3 in vascular smooth muscle and pericytes. Importantly, mutant mice exhibit signs of SVD including impaired functional hyperemia. Our data support the concept that downregulation of Kir2.1 channels in the cECs cripples sensing of neural activity and is the major contributor to compromised functional hyperemia in CADASIL. Furthermore, we found that hypertension, the major driver of the much more common sporadic SVD, also leads to age-dependent deterioration of this major mechanism for functional hyperemia. We found that loss of phosphatidylinositol 4,5-bisphosphate is responsible for the degradation of Kir2.1 channel functionality. The 'Holy Grail' of this effort is to restore perfusion in an SVD setting and following ischemic stroke. Important in this context, we are able to rapidly reverse functional hyperemia deficits in CADASIL by re-introduction of elements of the comprised extracellular matrix pathway (e.g., HB-EGF) or through genetic correction, an accomplishment directly relevant to ischemic stroke.

The role of biomarkers in translational pharmacology

Dr James Dear, University of Edinburgh, UK

In 1998, the National Institutes of Health Biomarkers Definitions Working Group defined a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention." The number of molecules described as biomarkers has increased enormously as a result of developments in genomics, proteomics and metabolomics. However, only a relatively small number have utility in pre-clinical or clinical drug development. Recently, formal pathways for biomarker rolling qualification have been described which adopt the same level of scrutiny as formal drug development. Using drug-induced liver injury as an exemplar, this talk will describe the potential uses of new biomarkers and the hurdles to be overcome if they are to have an impact on patient health and commercial drug development.

GPCR allostery: From theory to medicine

Professor Arthur Christopoulos, Monash University, Australia

G protein-coupled receptors (GPCRs) are the largest class of receptors encoded by the human genome and have long been exemplar models for the development of classic pharmacological principles, as well as highly fruitful drug targets for orthosteric agonist and antagonist medicines. Since the term of the millennium, however, the rate of drug attrition in GPCR discovery programmes has dramatically declined and this may, in part, reflect a failure to appreciate and capture novel paradigms of GPCR drug action. Specifically, it is now well established that all GPCRs possess spatially distinct allosteric sites that can be found throughout the entire region of the receptor, depending on the location of the cognate agonist orthosteric site. Allosteric sites are conformationaly linked to the orthosteric site, and have the potential to lead to novel modes of GPCR subtype selectivity, signal-pathway-selective (biased) modulation and, importantly, a "saturability" to the allosteric effect that can be exploited to fine-tune drug responsiveness in a positive or negative direction, akin to the actions of a "dimmer switch" as opposed to the "on/off" effect of classic agonists and antagonists. However, many of these theoretical advantages of allosteric drugs are only now being explored in the context of pathophysiology, and this represents a significant next step (and opportunity) for the field. For instance, allosteric modulators can have different effects on orthosteric ligand affinity relative to signaling efficacy, as well as directly activating the receptor themselves, and it is likely that such differences in mode of action will affect the successful targeting of different disease states with allosteric drugs. In addition, the sensitivity of allosteric ligands to cellular "context" is increasingly being recognized as a major consideration in the appropriate preclinical translation of such molecules (either alone or as potential "add-on" therapies), and data are emerging to suggest that endogenous allosteric substances may have a major, hitherto unappreciated, impact on GPCR functionality in health and disease. Excitingly, structural and computational biology studies are starting to identify the molecular mechanisms that underlie the pharmacological effects of allosteric modulators, and chemical biology approaches are generating novel tools for manipulating GPCR functionality, including "bitopic" ligands that concomitantly bridge orthosteric and allosteric sites.

2017 Cameron Prize for Therapeutics lecture: Building and maintaining a Global response to AMR

Professor Dame Sally Davies, Department of Health, UK

Antimicrobial Resistance (AMR) is currently one of the leading global health priorities in the world today, as demonstrated in September 2016 when 193 United Nations Member States committed to take action to address this complex health and economic issue. This escalating global threat puts millions of lives in jeopardy through limiting our ability to treat and prevent infections as well as putting global food security and agricultural livelihoods at risk. Tackling this issue demands sustained action both nationally and internationally and across all sectors. A key part of the solution is ensuring we feed the pipeline with new antibiotics, alternatives to antibiotics, diagnostics and preventative measures, but equally that we preserve the effectiveness and ensure access and implementation of existing options. To do this we must take a one health approach, and work together across disciplines and countries in humans, animals, agriculture, and the environment.

This talk will summarise the complexities of the problem, the actions taken to date drawing from examples from the UK and the global community, and the need for sustained global and national efforts to tackle AMR for the foreseeable future.

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Oral communication listing

OC001 ALDH1 bio-activates nifuroxazide to eradicate ALDH^{high} melanoma-initiating cells
S. Sarvi[1], R. Crispin[1], T. D. Hurley[2], A. von Kriegsheim[1], C. Chen[3], M. Ranzani[4], M. E. Mathers[1], X. Xu[5], D. J. Adams[4], N. O. Carragher[6], M. Fujita[7], A. Unciti-Broceta[1], V. G. Brunton[1], E. Patton[8].
[1]University of Edinburgh, United Kingdom, [2]Indiana University, USA, [3]University of Stanford, USA, [4]Sangar Institute, United Kingdom, [5]University of Pennsylvania, USA, [6]Cancer Research UK, United Kingdom, [7]University of Colorado Hospital, USA, [8]MRC University Unit for Human Genetics, United Kingdom

OC002 Investigating SARMs as novel therapeutics in women
D. A. Gibson, I. Simitsidellis, O. Kelepouri, A. Esnal Zufiaurre, F. Collins, P. T. Saunders.
University of Edinburgh, United Kingdom

OC003 Non-invasive in human *in vivo* target engagement biomarkers to strengthen GO/NO-GO decisions
L. Buntinx[1], L. Chang[1], A. Amin[1], M. Depré[1], E. Ampe[1], A. Vanhecken[1], B. Morlion[2], J. de Hoon[1].
[1]KU Leuven, Belgium, [2]The Leuven Centre for Algology & Pain Management, Belgium

OC004 Unravelling the effect of metamizole, paracetamol and ibuprofen on TRPA1 using the cinnamaldehyde target engagement biomarker model
D. Bamps, L. Macours, L. Buntinx, J. de Hoon.
KU Leuven, Belgium

OC005 Aldehyde dehydrogenase: 5-nitrofuran *in vivo* gene-drug interactions
A. L. Coates[1], Y. Zhou[2], L. Boulter[1], M. Arends[2], E. E. Patton[1],[2].
[1]MRC Human Genetics Unit, United Kingdom, [2]Cancer Research UK Edinburgh Centre, United Kingdom

OC006 Selective interleukin-6 trans-signalling antagonism with sgp130Fc reduces infarct size in a rat model of myocardial infarction whereas pan-blockade with an anti-IL-6 antibody does not
M. J. George, D. Stuckey, N. H. Jasmin, V. Taylor, A. D. Hingorani, D. Gilroy.
University College London, United Kingdom

Abstracts: Oral communications

OC001 ALDH1 bio-activates nifuroxazide to eradicate ALDH^{high} melanoma-initiating cells

S. Sarvi[1], R. Crispin[1], T. D. Hurley[2], A. von Kriegsheim[1], C. Chen[3], M. Ranzani[4], M. E. Mathers[1], X. Xu[5], D. J. Adams[4], N. O. Carragher[6], M. Fujita[7], A. Unciti-Broceta[1], V. G. Brunton[1], E. Patton[8]. [1]*University of Edinburgh, United Kingdom,* [2]*Indiana University, USA,* [3]*University of Stanford, USA,* [4]*Sangar Institute, United Kingdom,* [5]*University of Pennsylvania, USA,* [6]*Cancer Research UK United Kingdom,* [7]*University of Colorado Hospital, USA,* [8]*MRC University Unit for Human Genetics, United Kingdom*

Introduction
Majority of melanoma patients despite initial response to treatments develop drug resistance with melanoma recurrence. ALDH^{High} enzyme activity marks subpopulation of cells that are drug resistant with tumour-initiating potential and are shown to be the cause of treatment relapse[1].

5-Nitrofurans, commonly used as antibacterial pro-drugs, are recently shown to have anti-cancer activity in neuroblastoma[2], however their mechanism of action is still unknown. We have previously shown 5-Nitrofurans are bio-activated by ALDH2. However, we hypothesize the anticancer effects of 5-Nitrofurans is due its bio-activation by ALDH1 enzyme which is highly expressed in the ALDH^{High} melanoma-initiating subpopulations.

Material and Methods
Mass spectrometry was used to study the chemical interaction of Nifuroxazide with ALDH1 and oxidation of ALDH1 enzyme. Aldefluor assay was used to quantify ALDH1 activity in cells.

The role of ALDH1A3 in Nifuroxazide cytotoxicity was assessed in ALDH1A3 Crispr/Cas9 knockout clones and ALDH1A3 over-expressing cells. Sensitivity to Nifuroxazide was measured in 3D colony assays in which cells were grown in semi-solid media in the presence or absence of the compounds.

The anti-cancer effect of Nifuroxazide was evaluated by injecting 10,000 melanoma cells subcutaneously into NOD/SCID mice (n=12). Mice were treated with 150mg/kg Nifuroxazide via oral gavage for 10 days. The tumour initiating potential of Nifuroxazide treated tumours was assessed by injecting 10,000 FACS sorted, RFP labeled melanoma cells from control or drug treated tumours into second-generation mice (n=6). The clinical importance of targeting ALDH^{High} cells was shown by quantifying the expression of ALDH3 in patient samples before and after BRAF-inhibitor treatment.

Results
Nifuroxazide activity in melanoma cells is via a two-hit mechanism: bio-activation by ALDH1 leads to toxicity in cells, coupled with oxidation and inhibition of ALDH1. Nifuroxazide by selectivity targeting the ALDH^{High} subpopulation significantly reduces tumours growth and abolishes tumour initiation in serial transplant studies. We show that ALDH^{High} subpopulations are enriched in patients with recurrent melanoma following BRAF inhibitor treatment and that the combination of Nifuroxazide with Vemurafenib is synthetic lethal.

Conclusion
Currently there are no treatments for melanoma patients who relapse following treatment. Targeting subpopulations in melanoma based on the distinctive phenotypic properties of tumor-initiation, rather than targeting the molecular activity of cancer mutations, is an orthogonal therapeutic approach to current targeted and immune therapies, and opens up new avenues in melanoma therapy.

References
1) Luo Y *et al*. (2012) *Stem Cells* **30**: 2100-2113.
2) Sholler S *et al*. (2011). *J Pediatr Hematol Oncol* **33**: 25-30.

OC002 Investigating SARMs as novel therapeutics in women

D. A. Gibson, I. Simitsidellis, O. Kelepouri, A. Esnal Zufiaurre, F. Collins, P. T. Saunders. *University of Edinburgh, United Kingdom*

Introduction

Androgens modulate key processes within the endometrium which are targets in the treatment of infertility, endometriosis and heavy menstrual bleeding (1). However the ubiquitous expression of androgen receptor (AR) has thus far limited its utility as a drug target in women due to side-effects. Selective Androgen Receptor Modulators (SARMs) are novel non-steroidal agents that bind in a tissue-selective manner without masculinizing effects in females. The aim of this study was to investigate the impact of SARMs on endometrial function.

Methods

Using a mouse model, we have both defined the impact of the potent AR agonist DHT on uterine physiology (Simitsidellis et al 2016) and identified an androgenic 'signature' as a platform for assessment and comparison of SARM candidates. Eight to ten week-old mice (C57BL/6J) were ovariectomised to deplete endogenous steroids then administered with vehicle solution [VC; 0.4% methycellulose/5% ethanol] or the SARMs GTx-007 (Andarine;0.5mg) or GTx-024 (Ostarine; 0.5mg) subcutaneously, daily, for 7 days [N=7/treatment group].

Uteri were collected and analysed by qPCR and by immunohistochemistry with automated image analysis.

Results

Treatment with GTx-024 but not GTx-007, significantly increased uterine weight as well as endometrial, stromal and glandular area compared to the control group (VC; n=7, $p<0.05$). GTx-024 significantly increased uterine AR expression in myometrial (n=7, $p<0.01$), stromal (n=7, $p<0.01$) and epithelial (n=7, $p<0.01$) cells compared to VC. GTx-024 treatment increased the absolute number of endometrial glands compared to VC or GTx-007 treatment (n=7, $p<0.05$). Gene expression analysis revealed GTx-024 altered expression of genes associated with stromal-epithelial interactions and proliferation/cell-cycle regulation including *Igf1* ($p<0.001$), ***Mki67*** ($p<0.01$) and ***Rb1*** ($p<0.01$). AR activation by GTx-024 was confirmed using ARE-luciferase mice (2) and IVIS imaging system (figure 1).

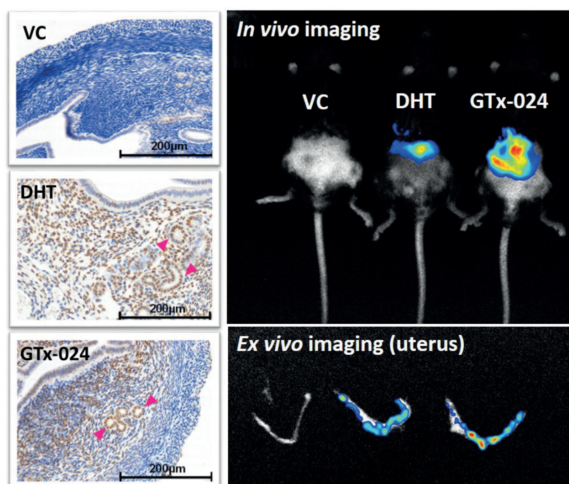


Figure 1. AR activation by GTx-024. Uterine AR expression (brown stain) and activation of ARE-luciferase were increased by DHT or GTx-024.

Conclusion

The impact of GTx-024 on the uterus mirrored that of DHT (3). SARMs offer a beneficial therapeutic strategy in women by targeting AR-dependent processes without undesirable masculinizing side-effects. Our data reveal distinct impacts of different SARMs (GTx-024, GTx-007) on the uterus. This study is the first to identify a role for SARMs in modulating endometrial function.

References

1) Simitsidellis I, Saunders PTK, Gibson DA. Mol Cell Endocrinol. 2017 Sep 15
2) Dart DA et al (2013) PLoS ONE 8(8): e71694
3) Simitisdellis I, Gibson DA et al (2016) Endocrinology.157(5):2116-28.

OC003 Non-invasive in human *in vivo* target engagement biomarkers to strengthen GO/NO-GO decisions

L. Buntinx[1], L. Chang[1], A. Amin[1], M. Depré[1], E. Ampe[1], A. Vanhecken[1], B. Morlion[2], J. de Hoon[1]. [1]*KU Leuven, Belgium*, [2]*The Leuven Centre for Algology & Pain Management, Belgium*

Introduction

Only about 1 in 10 compounds entering phase I reaches market authorization and eventually the patient. The highest attrition rate is caused in the proof-of-concept phase due to lack of efficacy and/or safety in the target population[1]. Development of the capsaicin model proved to be useful for evaluating target engagement and dose selection of calcitonin gene-related peptide (CGRP)-(receptor) antagonists in early clinical drug development[2]. Likewise, we developed 2 new non-invasive, safe and reproducible target-engagement biomarkers for potential future analgesics: the transient receptor potential Ankyrin-1 (TRPA1) channel (activated by cinnamaldehyde) and the pituitary adenylate cyclase-activating polypeptide type I receptor (activated by maxadilan). These are referred to as the cinnamaldehyde (CA) and maxadilan (MAX) model, respectively.

Methods

For both models, dose finding and reproducibility assessments were performed during single-centre, open-label, placebo-controlled studies in healthy subjects. For CA (n=11): 3%, 10%, and 30% cinnamaldehyde (CA) and placebo (=vehicle) were topically applied on the forearm. For MAX (n=10): 0.9, 3, 10ng maxadilan and placebo (=vehicle) were injected intradermally in the forearm. For both models, reproducibility between arms and between periods was assessed. CA and MAX induced dermal blood flow (DBF) was assessed by laser Doppler imaging (LDI) at baseline and every 10 minute post-challenge. To assess reproducibility, the concordance correlation coefficient (CCC) and Bradley- Blackwood test (BB-test) were used. In addition, sample size calculations (SSC) were performed to evaluate the use of the model in future clinical studies comparing active treatment with TRPA1 or PAC1 antagonists with placebo treatment.

Results

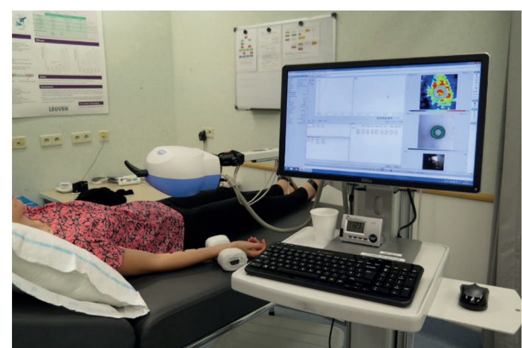
For both CA and MAX, all 3 doses increased DBF compared to vehicle at all time-points. 10% CA and 0.9ng MAX were chosen as effective and safe doses to evaluate the reproducibility. DBF responses to 10% CA and 0.9MAX were found to be reproducible between arms (CCC>0.7) and visits (CCC>0.7). Based on SSC, these models allow to detect a change in DBF of 30-50% between 2 independent groups of maximum 10-15 (CA) and <10 (MAX) subjects with 80% power.

Conclusions

In order to tackle the high attrition rate in clinical drug development, "the future of pharmacology" should consider implementing more target-engagement biomarkers, such as the CA and MAX model, early in the development of new drugs, to guide go/no-go decisions.

DBF response	Test-Retest	Mean Difference (95% CI)	CCC	SSC 10% shift	SSC 30% shift	SSC 50% shift	BB-test*
CA model AUC_{0-50} (%*min)	Inter-arm (n=10)	-1121 (-7490, 5249)	0.91	79	10	4	0.30
	Inter-period (n=23)	-848 (-3881, 2185)	0.83	117	14	6	0.18
MAX model AUC_{0-180} (PU*min)	Inter-arm (n=10)	1363 (-26341, 29068)	0.88	48	7	4	0.41
	Inter-period (n=10)	-7300 (-46455, 31854)	0.77	102	13	6	0.9246

Test-retest inter-period and inter-arm reproducibility data for DBF response expressed as percentage change in baseline at 30min (t_{30}) and as AUC of percent change from baseline from 0 min to 50 min (AUC0-50).
CCC: concordance correlation coefficient
CCC>0.9, almost perfect; CCC 0.8-0.9, substantial; CCC 0.65-0.8, moderate; CCC<0.65, poor
SSC: sample size calculation to detect an X% shift between two independent groups with an 80% power, a 5% significance level, and an active/placebo ratio of 1.
*Shapiro-Wilk test: p>0.05 indicates normal distribution of data
**Bradley-Blackwood Test: p<0.05 indicates evidence of unequal means or unequal variances between visits or arms



OC004 Unravelling the effect of metamizole, paracetamol and ibuprofen on TRPA1 using the cinnamaldehyde target engagement biomarker model

D. Bamps, L. Macours, L. Buntinx, J. de Hoon. *KU Leuven, Belgium*

Introduction
Transient receptor potential Ankyrin 1 (TRPA1) is an emerging target for pain therapy, hypothesized to play a role in different pain conditions including migraine and neuropathic pain. Recently, an *in vivo*, in human target engagement biomarker assay was developed for TRPA1 using cinnamaldehyde (CA) (1). Similar to the capsaicin model earlier developed for TRP Vanilloid 1 (TRPV1), changes in dermal blood flow (DBF) following topical application of a selective agonist, are used to assess target engagement (2). In this study, the CA model was employed to investigate the effect of marketed analgesics paracetamol, metamizole and ibuprofen on the TRPA1 response in healthy volunteers.

Method
A randomized, three-way cross-over study was conducted using metamizole (500mg), paracetamol (1g) and ibuprofen (600mg). Study visits, in which drugs were administered orally, were separated by a wash-out period of at least seven days. At Tmax of the administered drugs, CA (10% solution) was applied topically on the volar surface of subjects' right forearm. Capsaicin (1000µg/20µl) was applied on the left forearm to activate TRPV1 as a positive control. Changes in DBF were measured using laser Doppler imaging (LDI) at baseline and at 10, 20, 30, 40 and 60 minutes post CA and capsaicin application. DBF responses were expressed as arbitrary perfusion units (PUs). The area under the curve over a 60 minutes period ($AUC_{0\text{-}60min}$) was analyzed using one-way ANOVA with post-hoc Bonferroni correction.

Results
As shown in figure 1, metamizole and paracetamol had no significant effect on the CA-induced DBF response compared to no drug treatment, while after ibuprofen administration this response was partially reduced. The capsaicin-induced DBF response was not altered after treatment with ibuprofen, metamizole or paracetamol compared to no drug treatment.

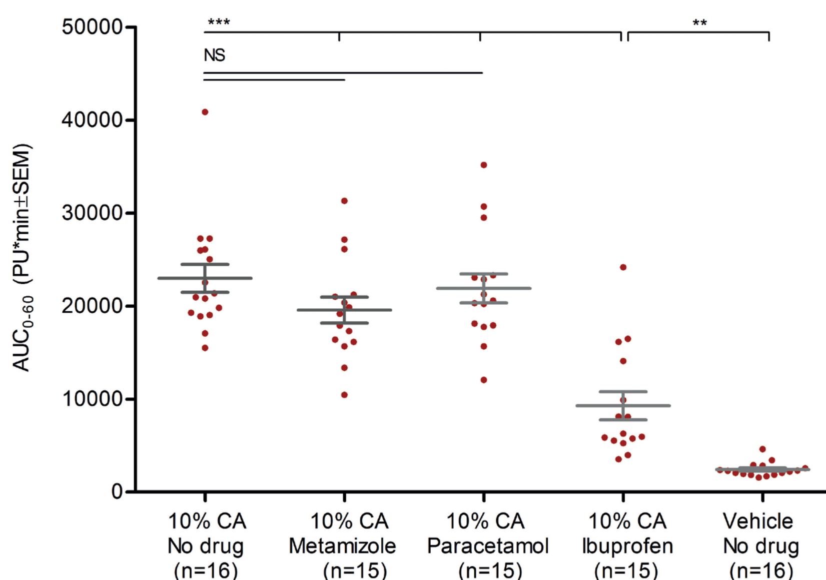


*Figure 1: Influence of metamizole, paracetamol and ibuprofen on the CA-induced DBF response. One-way ANOVA with post-hoc Bonferroni NS: Non-significance ($p > 0.05$), ** : $p < 0.01$, *** : $p < 0.001$*

Conclusion
While metamizole and paracetamol have no effect on the CA-induced TRPA1 response in humans, non-selective COX-inhibitor ibuprofen partially reduces the DBF increase induced by CA. This is in line with earlier unpublished research suggesting a role for vasodilating prostaglandins after TRPA1 activation. As expected, these secondary mediators do not seem to play a role in the capsaicin-induced DBF response.

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BRITISH PHARMACOLOGICAL SOCIETY

OC005 Aldehyde dehydrogenase: 5-nitrofuran *in vivo* gene-drug interactions

A. L. Coates[1], Y. Zhou[2], L. Boulter[1], M. Arends[2], E. E. Patton[1,2]. [1]*MRC Human Genetics Unit, United Kingdom,* [2]*Cancer Research UK Edinburgh Centre, United Kingdom*

Introduction

Aldehyde dehydrogenases (ALDHs) are key aldehyde-detoxifying enzymes. ALDH2 bio-activates 5-nitrofuran (5-NFN) prodrugs in yeast and zebrafish (1). The relevance of this interaction in mammals and influence of genetic mutation on this interaction is unknown. Despite recommendation as a WHO essential medicine for Chagas disease and inclusion in Phase 2 clinical trial for neuroblastoma (2), nifurtimox (NFX) produces side effects in humans that prevent treatment completion (3). Side effects are similar to those during exposure to the ALDH inhibitor, disulfiram and are exacerbated with alcohol consumption, suggesting that alcohol and NFX share a molecular target. We hypothesise that 5-NFNs are competitive substrates for mammalian ALDH enzymes *in vivo*, ALDH:5-NFN interactions drive side effects of 5-NFNs and these might be alleviated with ALDH inhibition, chemically with disulfiram or genetically with possession of the ALDH2*2 polymorphism, which is present in 8% of the world population.

Method

Liver homogenates, organoids and precision cut liver slices (PCLS) were prepared from CD57BL/6 mice before treatment with 5-NFNs (NFX; nifuroxazide, NAZ; nitrofuran 1, NFN1) in DMSO. *Aldh1b1*-null heterozygous and homozygous mice were treated with 20% ethanol in drinking water alongside 150 mg/kg NAZ in sunflower oil, delivered daily by oral gavage for 4 days (n = 2-6 / group). Liver ALDH activity and circulating acetaldehyde levels in blood plasma were quantified.

Results

5-NFNs are competitive substrates for ALDH and differentially inhibit ALDH activity in mammalian liver. ALDH activity in PCLS treated with NFN1 (120, 400 μM) is reduced in a dose-dependent manner 83.3% ($p = 0.0143$) and 90.6% ($p = 0.0426$) compared to control. Liver cancer cells are sensitive to 5-NFNs and ALDH substrates alter sensitivity to 5-NFNs. Exploration of the ALDH metabolic pathway in *ALDH1b1*-null mice reveals that NAZ and ethanol co-treatment increases circulating levels of the ALDH substrate, acetaldehyde more than two-fold ($p = 0.0174$).

Conclusion

5-NFNs are competitive substrates for mammalian ALDH enzymes *in vivo*, ALDH1B1 is a key enzyme for acetaldehyde detoxification in the liver and elevated levels of DNA-damaging aldehydes suggests a mechanism underpinning 5-NFN side effects *in vivo*.

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OC006 Selective interleukin-6 trans-signalling antagonism with sgp130Fc reduces infarct size in a rat model of myocardial infarction whereas pan-blockade with an anti-IL-6 antibody does not

M. J. George, D. Stuckey, N. H. Jasmin, V. Taylor, A. D. Hingorani, D. Gilroy. *University College London, United Kingdom*

Introduction

Interleukin-6 (IL-6) is elevated during Acute Myocardial Infarction (AMI) particularly after reperfusion and is associated with the development of heart failure and mortality (1). Therefore IL-6 is a potential therapeutic target in AMI. IL-6 has both pro and anti-inflammatory effects; the anti-inflammatory effects are mediated by classic signalling (2), whereas the pro-inflammatory effects are mediated by trans-signalling (3) (Figure 1). We hypothesised that selective blockade of IL-6 trans-signalling with the novel sgp130Fc protein during AMI would result in reduced infarct size (IS) whereas pan-IL-6 blockade with an anti-IL-6-Ab would not (Figure 2).

Methods

AMI was induced in male Sprague-Dawley rats by occluding the left-anterior descending artery for 50 minutes prior to reperfusion. The model was characterised by measuring IL-6 and sIL-6R within heart tissue and plasma by ELISA at 6 time-points post reperfusion (2h-7 days, n=3-4/group). In addition, cardiac leukocyte infiltration (flow-cytometry of cells obtained from heart digests) was measured. In therapeutic experiments, rats received either 0.5μg/g of sgp130Fc, 0.1μg/g anti-IL-6-Ab or vehicle alone given intravenously immediately prior to reperfusion. IS (as a percentage of area-at-risk (AAR)) was measured histologically at 24 hours (n=7-8/group). LVEF was measured by cardiac magnetic resonance imaging at 28 days in a group receiving sgp130Fc and compared to age-matched naïve rats and vehicle-controls (n=6-8/group).

Results

Characterisation (Figure 3): IL-6 levels in the heart were biphasic; with peaks at 4 and 72 hours. Only the early peak was associated with elevated circulating IL-6. The early peak was temporally associated with neutrophil influx and the second with mononuclear phagocytes (MPs). Plasma sIL-6R peaked at 24 hours. **Therapeutic studies (Figure 4):** IS/AAR was significantly reduced by the administration of sgp130Fc but not by the anti-IL-6-Ab (vehicle: 46.1%, anti-IL6-Ab: 45.6%, sgp130Fc: 26.3%, one-way ANOVA with multiple comparisons: sgp130Fc v vehicle $p<0.001$). At 28 days sgp130Fc ameliorated the reduction in LVEF associated with AMI (naïve 72.35%, vehicle: 62.58%, sgp130Fc 69.51%, one-way ANOVA with multiple comparisons: naïve vs vehicle $p<0.01$, naïve vs sgp130Fc ns).

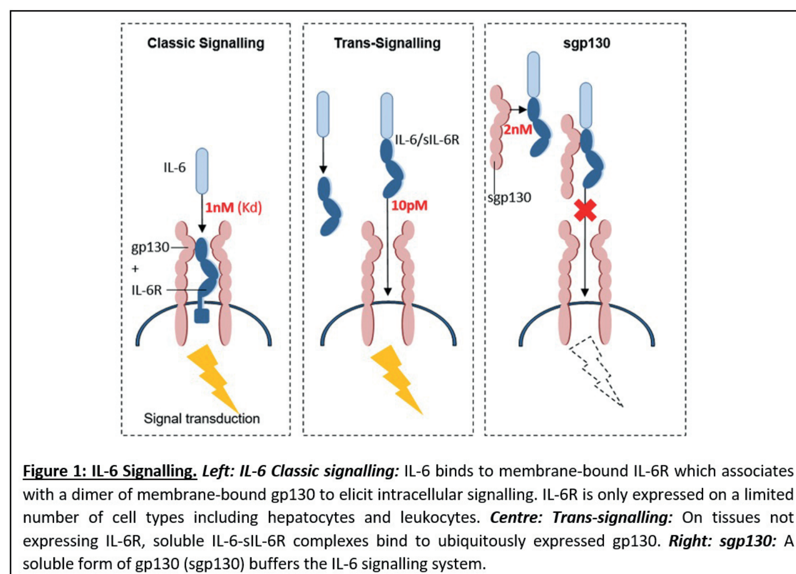

Figure 1: IL-6 Signalling. ***Left: IL-6 Classic signalling:*** IL-6 binds to membrane-bound IL-6R which associates with a dimer of membrane-bound gp130 to elicit intracellular signalling. IL-6R is only expressed on a limited number of cell types including hepatocytes and leukocytes. ***Centre: Trans-signalling:*** On tissues not expressing IL-6R, soluble IL-6-sIL-6R complexes bind to ubiquitously expressed gp130. ***Right: sgp130:*** A soluble form of gp130 (sgp130) buffers the IL-6 signalling system.

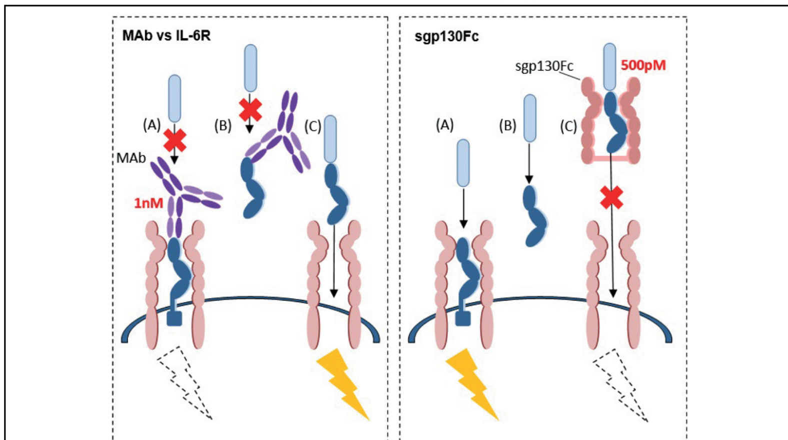

Figure 2: ***Left: MAb vs IL-6R (or IL-6)***: Blocks classic signalling (A) and prevents new IL-6/sIL-6R complexes from forming, thereby attenuating trans-signalling (B). However, pre-formed IL-6/sIL-6R complexes are not blocked (C) and therefore some trans-signalling may still occur. ***Right: sgp130Fc:*** A recombinant dimer of sgp130 does not block classic signalling (A) or the formation of new IL-6/sIL-6R complexes (B) but specifically binds to pre-formed IL-6/sIL-6R complexes with high affinity blocking trans-signalling (C).

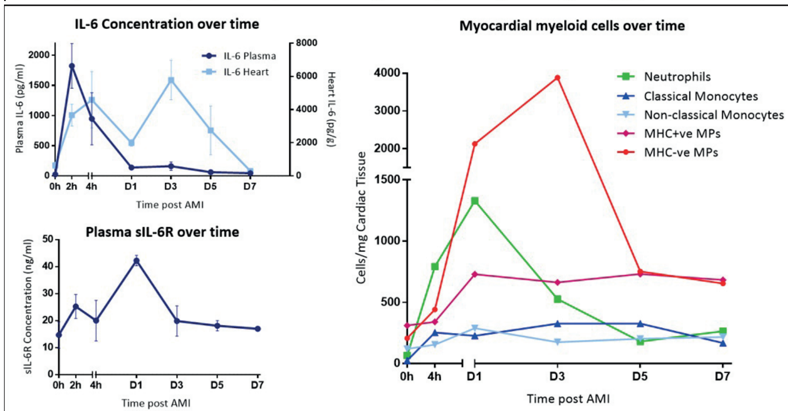

Figure 3: AMI was induced in rats by surgically occluding the LAD for 50 minutes prior to reperfusion. IL-6, sIL-6R (ELISA) and myeloid cells (flow-cytometry) were measured in circulation and/or the myocardium at 6 time-points post reperfusion (2h – 7 days, n=3-4/time-point).

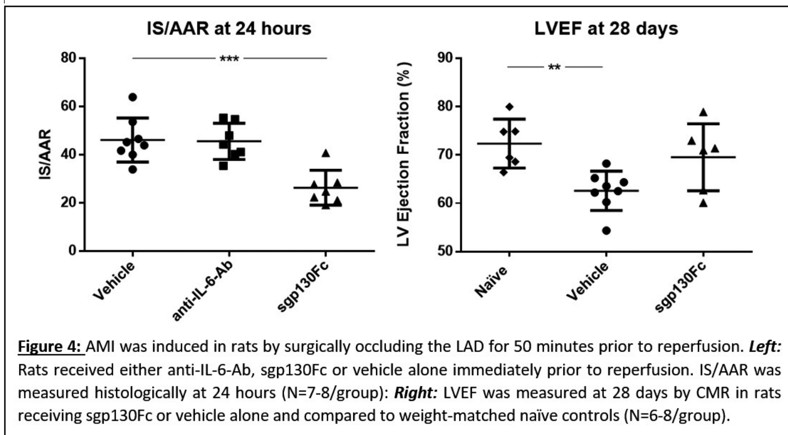

Figure 4: AMI was induced in rats by surgically occluding the LAD for 50 minutes prior to reperfusion. ***Left:*** Rats received either anti-IL-6-Ab, sgp130Fc or vehicle alone immediately prior to reperfusion. IS/AAR was measured histologically at 24 hours (N=7-8/group): ***Right:*** LVEF was measured at 28 days by CMR in rats receiving sgp130Fc or vehicle alone and compared to weight-matched naïve controls (N=6-8/group).

Conclusions

Our results suggest that specific targeting of IL-6 trans-signalling with the novel sgp130Fc protein reduces IS in an animal model of AMI with reperfusion, whereas pan-blockade with an anti-IL-6-Ab does not. Furthermore, administration of sgp130Fc preserves LVEF at 28 days.

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3) Tilg H *et al.* (1994). *Blood* **83**:113-8.

Poster session listing

Thursday 17 May 2018, 12.20–13.40

PB001 Effect of *Justicia adhatoda*leaf extract on drug induced coagulopathy in mice and *in vitro* platelet aggregation of human blood
S. Zaffar. *CMH Medical College, Pakistan*

PB002 The IUPHAR/BPS Guide to PHARMACOLOGY database (GtoPdb) in 2018: New features and updates
A. J. Pawson[1], J. L. Sharman[1], J. Armstrong[1], E. Faccenda[1], S. D. Harding[1], C. Southan[1], S. P. Alexander[2], A. P. Davenport[3], M. Spedding[4], J. A. Davies[1]. *[1]University of Edinburgh, United Kingdom, [2]University of Nottingham, United Kingdom, [3]University of Cambridge, United Kingdom, [4]Spedding Research Solutions SAS, France*

PB003 Antioxidant potency of the trans-resveratrol some its splinters and combinations
N. Bizunok. *Belorussian State Medical University, Belarus*

PB004 Sumoylation of the β_2ARinfluences receptor internalisation, desensitisation and downstream signaling
J. Ling[1], L. Wills[2], G. Baillie[1]. *[1]Institute of Cardiovascular and Medical Science, United Kingdom, [2]Cardiovascular Research Center Mount Sinai, USA*

PB005 Use of Au-functionalized devices for the specific activation of a bioorthogonal Belinostat prodrug
B. Rubio-Ruiz, A. Pérez-López, A. Unciti-Broceta. *University of Edinburgh, United Kingdom*

PB006 The IUPHAR Guide to IMMUNOPHARMACOLOGY portal
S. D. Harding[1], E. Faccenda[1], C. Southan[1], J. L. Sharman[1], A. J. Pawson[1], A. J. Gray[2], S. Ireland[1], S. P. Alexander[3], S. Anderton[1], C. Bryant[5], A. P. Davenport[5], C. Doerig[7], D. Fabbro[8], F. Levi-Schaffer[6], M. Spedding[4], J. A. Davies[1]. *[1]University of Edinburgh, United Kingdom, [2]Heriot-Watt University, United Kingdom, [3]University of Nottingham, United Kingdom,[4]Spedding Research Solutions SAS, France [5]University of Cambridge, United Kingdom, [6]Hebrew University of Jerusalem, Israel, [7]Monash University, Australia, [8]PIQUR Therapeutics, Switzerland*

PB007 Gold-mediated anticancer drug release
A. Perez-Lopez[1], B. Rubio-Ruiz[1], V. Sebastian[2], L. Hamilton[1], C. Adam[1], T. Bray[1], S. Irusta[2], P. Brennan[1], G. Lloyd-Jones[1], D. Sieger[1], J. Santamaria[2], A. Unciti-Broceta[1]. *[1]University of Edinburgh, United Kingdom,[2]University of Zaragoza, Spain*

PB008 Using genetic data to identify gene targets for drug repositioning to aid in the treatment of depression
D. Howard, J. Hafferty, A. McIntosh. *University of Edinburgh, United Kingdom*

PB009 The anti-cancer and anti-inflammatory effects of curcumin in multiple human cancer cells
M. A. Baghdadi[1], H. Almosa[2,3], Z. Almalki[2,3], M. Alqriqri[4], I. Denetiu[4], M. H. Ucisik[5,6], S. Damiati[2]. *[1]King Faisal Specialist Hospital & Research Centre, Saudi Arabia, [2]King Abdulaziz University (KAU), Saudi Arabia, [3]Jamjoom Pharmaceutical, Saudi Arabia, [4]King Fahed Medical Research Center, Saudi Arabia, [5]Istanbul Medipol University, Turkey, [6]Medipol Regenerative and Restorative Medicine Research Center (REMER), Turkey*

PB010 Modulation of angiotensin II signalling by cannabinoid receptor 1 antagonism in human coronary artery smooth muscle cells
W. Al Abdullah. *University of Leicester, United Kingdom*

PB011 Bioorthogonal activation of cancer prodrugs with catalytic palladium implants for treatment of glioma
C. Adam, L. Hamilton, J. Weiss, B. Rubio-Ruiz, A. Perez-Lopez, P. M. Brennan, D. Sieger, A. Unciti-Broceta. *University of Edinburgh, United Kingdom*

PB012 Surrogate outcomes supporting conditional marketing authorisations and accelerated approvals by the european medicines agency, 2011-2018

C. S. Schuster Bruce[1], P. McGettigan[1], P. Brhlikova[2]. [1]*William Harvey Research Institute, United Kingdom*, [2]*Newcastle University, United Kingdom*

PB013 Systematic review and meta-analysis using SyRF- a tool to accelerate drug development

G. L. Currie, J. Liao, E. S. Sena, C. T. Sena, A. Bannach-Brown, K. Hair, S. K. McCann, Q. Wang, M. R. Macleod. *University of Edinburgh, United Kingdom*

PB014 Novel synthetic superenhancers for drug screening in cancer stem cells

U. Koeber, S. M. Pollard. *University of Edinburgh, United Kingdom*

PB015 Investigation of the novel selective SRC kinase inhibitor eCF506 in preclinical models of breast cancer

C. Temps, C. Fraser, X. Li, J. C. Dawson, H. Beetham, V. G. Brunton, B. Qian, N. O. Carragher, A. Unciti-Broceta. *University of Edinburgh, United Kingdom*

PB016 Atorvastatin activates skeletal ryanodine receptors: Design of next-generation statins

C. Lindsay, A. D. Wilson, E. Venturi, A. J. Russell, R. Sitsapesan. *University of Oxford, United Kingdom*

PB017 Expansion of the druggable genome in the IUPHAR/BPS Guide to PHARMACOLOGY and other drug target resources: a key substrate for future medicines

C. Southan, J. L. Sharman, E. Faccenda, S. D. Harding, A. J. Pawson, J. A. Davies. *Edinburgh University, United Kingdom*

PB018 Evidence that human $P2Y_1$ and $P2Y_{12}$ receptors form heterodimers

M. Safar. *SIPBS, University of Strathclyde, United Kingdom*

PB019 Effect of pirfenidone on endothelium-dependent vasodilatation in type-2 diabetic (db/db) mice

E. Pinilla[1,2], L. Beck[1], D. Arcanjo[1,3], R. Hernanz[1,4], A. Petersen[1], S. Comerma-Steffensen[2], M. Sheykhzade[5], L. Rivera[2], U. Simonsen[1]. [1]*Aarhus University, Denmark*, [2]*Complutense University of Madrid, Spain*, [3]*Universidade Federal do Piauí, Brazil*, [4]*Universidad Rey Juan Carlos, Spain*, [5]*University of Copenhaguen, Denmark*

Abstracts: Poster presentations

PB001 Effect of *Justicia adhatoda*leaf extract on drug induced coagulopathy in mice and *in vitro* platelet aggregation of human blood

S. Zaffar. *CMH Medical College, Pakistan*

Introduction

Oral antiplatelet and anticoagulant drugs are commonly prescribed to patients with cardiovascular diseases. Despite being life-saving, these drugs have one major adverse effect that they can cause spontaneous hemorrhage, which can be fatal. Development of a hemostatic agent can help in quick and effective management of drug-induced hemorrhages.

Objective

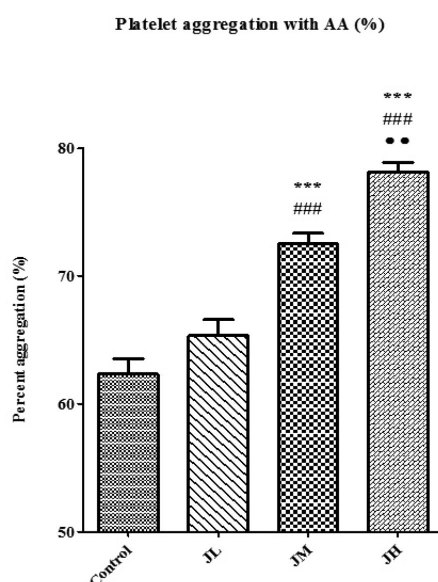
This study was devised to observe the effect of leaf extract of *Justicia adhatoda* on coagulation profile in mice and to evaluate its effect on in-vitro platelet aggregation.

Methods

The study was divided into three parts. First part was designed to evaluate the effect of different doses of leaf extract of *J. adhatoda* on bleeding time in mice. Dose that produced maximum decrease in bleeding time was chosen for further in-vivo coagulation studies. Second part of the study was to observe the effect of *J. adhatoda* leaf extract on coagulation parameters. Three drugs were used to induce coagulopathy viz., warfarin, aspirin and dabigatran. Bleeding time, platelet count, PT, INR and APTT were estimated. Third part of this study was devised to observe the effect of *J. adhatoda* leaf extract on *in vitro* platelet aggregation. Percent aggregation was recorded by light transmission aggregometer for three minutes.

Results

Leaf extract of *J. adhatoda* decreased bleeding time in mice. There was no effect on the coagulation parameters. Platelet count increased significantly only in the aspirin treated group that received the extract. Platelet aggregation increased in a dose dependent manner.

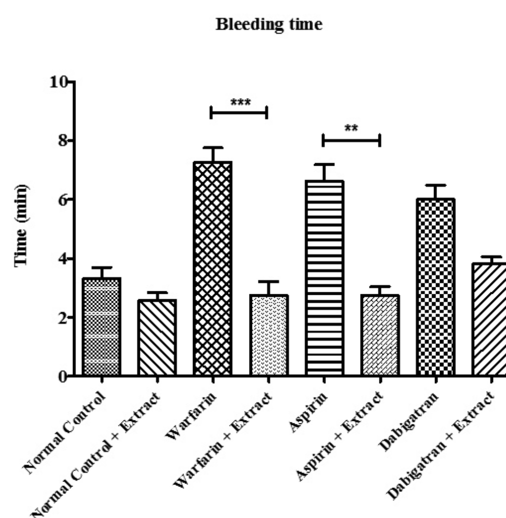


Effect of different concentrations of *Justicia adhatoda* on in-vitro platelet aggregation (mean ± SE) in human subjects (n=6)

*** = p < 0.001 (vs. control),

= p < 0.001 (vs. JL),

•• = p < 0.01 (vs. JM)



Effect of *J. adhatoda* leaf extract on bleeding time (mean ± SE) in mice (n=8)

** = p < 0.01, *** = p < 0.001

Conclusion

J. adhatoda leaf extract is effective in controlling excessive bleeding due to aspirin induced platelet dysfunction, but has no role in reversing the anticoagulant effect of warfarin and dabigatran.

PB002 The IUPHAR/BPS Guide to PHARMACOLOGY database (GtoPdb) in 2018: New features and updates

A. J. Pawson[1], J. L. Sharman[1], J. Armstrong[1], E. Faccenda[1], S. D. Harding[1], C. Southan[1], S. P. Alexander[2], A. P. Davenport[3], M. Spedding[4], J. A. Davies[1]. [1]*University of Edinburgh, United Kingdom,* [2]*University of Nottingham, United Kingdom,* [3]*University of Cambridge, United Kingdom,* [4]*Spedding Research Solutions SAS, France*

Introduction

The IUPHAR/BPS Guide to PHARMACOLOGY (GtoPdb) (1), is an open-access, expert-curated, online database of human drug targets and their ligands (2). It provides succinct overviews, key references and recommended experimental ligands for 2,800 targets and related proteins organised into families. The database includes 9,100 ligand molecules, including approved drugs, investigational small molecules, endogenous or synthetic peptides, and antibodies.

Method

The development of GtoPdb is overseen by NC-IUPHAR (3) with data selected by its subcommittees and expert curators covering established drug targets as well as those of emerging interest for drug discovery. This update will provide details of recent developments to expand the data content and add new features to GtoPdb.

Results

We present a new visualisation tool to compare ligand affinity across species and explore additional targets that have been tested in the ChEMBL (4) medicinal chemistry dataset. Enhanced search facilities have been added, including a BLAST tool for sequence similarity searching, batch search allowing users to upload a set of target or ligand IDs, downloading of result sets in CSV format, and a pharmacology search tool to allow retrieval of data on ligands known to modulate sets of target protein/gene IDs. A disease list and disease summary pages are now provided, which combine data on curated targets and ligands associated to a particular disease. A major recent effort has seen expansion in the area of immunopharmacology. Relevant targets and ligands have been added and linked to immunological cell types, processes and diseases. All this information has been gathered into a new portal aimed at immunologists wishing to search pharmacological data (5). We now provide RDF files which users can download and view in a linked data browser, thereby allowing queries across the GtoPdb data in conjunction with other open linked data sources.

Conclusions

GtoPdb is a useful resource for scientists looking for expert-curated information on drug targets and recommended ligands, and we hope these new features will further enhance its utility, as well as ensure the data are as widely accessible as possible.

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5) IUPHAR Guide to IMMUNOPHARMACOLOGY, http://www.guidetoimmunopharmacology.org/

PB003 Antioxidant potency of the trans-resveratrol some its splinters and combinations

N. Bizunok. *Belorussian State Medical University, Belarus*

Introduction

The respiratory burst of phagocytes is one of the most important mechanisms of innate immunity but it plays a crucial role in some cases of the redox-dependent cell death. The goal of this investigation was to determine on the model of respiratory burst of phagocytes the antioxidant potency some derivatives of cinnamic and benzoic acids (14 substances) and trans-resveratrol (t-R). Besides, was investigate the type and potency of pharmacodynamic interactions of the t-R with ascorbic acid (AsA) and melatonin (Mt).

Method

Reactive oxygen species (ROS) generated by rat peritoneal macrophages in presents of investigated substances (*in vitro*) were detected by luminol-dependent chemiluminescence (CL) method on the model of FcγR-induced phagocytosis of the opsonized particles. Antioxidant activity was assessed by the degree of the area under CL curve suppression (AUC CL), calculating the effective inhibitory concentrations (IC_{16} - IC_{84}) by the linear regression analysis. The interaction of component combinations calculated by the Chou-Talalay combination index (CI) method [1].

Results

The highest antioxidant activity of t-R, 3,4-dihydroxycinnamic acid and 3,4-dihydroxybenzaldehyde has been confirmed, EC_{50} (*-Log, M*) were 5,74; 5,05 and 5,15, respectively. Further investigations of the t-R, Mt and AsA interactions showed their dependence on composition ingredients, molar ratios and concentrations and exchangeable from synergistic to antagonistic pattern. The most potent synergistic action demonstrated the t-R and Mt combinations in molar ratios from t-R1/ 1 to t-R1/ 100. The combination of the t-R with Mt and AsA is synergistic in range of high concentrations permitting AsA and Mt prepotency over the t-R (molar ratio t-R1/Mt100/AsA1000).

Combination	Molar ratio	CI [IC_{16}- IC_{84}]					M $[CI]_{30-70}$	Result
		IC_{16}	IC_{30}	IC_{50}	IC_{70}	IC_{84}		
t-R	1	1,54	0,79	0,39	0,19	0,10	0,56	Synergism
Mt	1							
t-R	1	0,07	0,07	0,08	0,09	0,10	0,08	Very strong synergism
Mt	10							
t-R	1	0,08	0,05	0,04	0,03	0,02	0,05	Very strong synergism
Mt	100							
AsA	1000	3,12	1,15	0,42	0,16	0,06	0,74	Moderate synergism
Mt	100							
t-R	1							

Table 1 CI values for some combinations of t-R, Mt and AsA on the model of ROS generation in macrophages $M[CI]_{30-70}=[3CI_{30}+2CI_{50}+CI_{70}]/6$.

Conclusion

These results can be interesting for the explanation of pharmacological activity of the antioxidants at their separate or combined action and for creation a new drugs and therapeutic strategies.

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PB004 Sumoylation of the β_2ARinfluences receptor internalisation, desensitisation and downstream signaling

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Introduction

Beta 2 adrenergic receptor (β_2AR) signalling can be modulated by a variety of post-translational modifications (PTMs) which include phosphorylation, ubiquitination, palmitoylation and glycosylation [1]. Following sequence analysis of the β_2AR, we discovered a putative site for SUMOylation, a previously unknown type of modification for this receptor. Our aim was to verify whether the β_2AR can be SUMOylated and how this modification affects receptor signaling and desensitization.

Method

Using peptide array we have delineated a putative SUMO site on β_2AR. Using both wild type and SUMO-null β_2AR mutants we have investigated downstream signaling of the β_2AR using western blotting. We have "forced" SUMOylation of the receptor via overexpression of the SUMO E3 ligase PIASy.

Results

We have identified a novel site of SUMOylation on the β_2AR and shown that this modification robustly alters downstream signaling in a model cell-line. Specifically, we have demonstrated that SUMOylation reduces β_2AR phosphorylation by PKA, altering the receptor driven phospho-ERK response, inhibits β_2AR ubiquitination and degradation, and delays β_2AR internalisation.

Conclusion

We report for the first time that the β_2AR can be SUMOylated and that this retards desensitization and receptor degradation. We speculate that this mechanism may be relevant to heart disease and have developed a β_2AR SUMO-site specific antibody to investigate this possibility.

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PB005 Use of Au-functionalized devices for the specific activation of a bioorthogonal Belinostat prodrug

B. Rubio-Ruiz, A. Pérez-López, A. Unciti-Broceta. *University of Edinburgh, United Kingdom*

Introduction

In the last decade, abiotic transition metals have been successfully used for different applications in, on and outside cells, e.g. for the synthesis of small molecules, the functionalization and uncaging of enzymes and *in situ* prodrug activation.(1) Regarding the latter, our group has explored new chemistries and deactivation strategies to develop novel caged chemotherapeutic agents that are specifically released *via* heterogeneous metal catalysis in order to minimize adverse effects associated to chemotherapy.(2) In this communication, we report the development of a bioorthogonal prodrug of the histone deacetylase inhibitor Belinostat and its specific activation *via* biocompatible Au-functionalized resins in cancer cell culture.

Method

O-alkylated hydroxamate of Belinostat (**2a**) was synthesised by treatment of Belinostat with the corresponding alkyl bromide in the presence of DBU at room temperature for 24 h. Solid supported gold catalysts were prepared by *in situ* generation of Au-NP within a PEG-grafted low-crosslinked polystyrene matrix as previously described.(3) The bioorthogonal [Au]-triggered release of Belinostat was investigated in culture with human lung cancer A549 cells. A549 cells were seeded in a 96 well plate (1,500 cells/well) and incubated for 48 h. Each well was then replaced with fresh media containing: [Au]-resins (1 mg/mL); **2a** (30 μM); Belinostat (30 μM) or a combination of [Au]-resins + **2a** (30 μM). After 5 d of treatment, cell viability was determined by PrestoBlue™.

Results

Derivative **2a** mediated patently lower cytotoxicity than the parent drug, with a projected EC_{50} (**2a**) / EC_{50} (Belinostat) value far beyond two orders of magnitude. Derivative **2a** elicited a potent cytotoxic effect in cancer cell culture only in the presence of the Au-catalyst while complete innocuity in its absence. The observed antiproliferative effect was equivalent to that mediated by unmodified drug, unequivocal evidence that the active drug is released *in situ* by heterogenous gold chemistry.

Conclusion

We have developed a completely inactive precursor of chemotherapeutic drug Belinostat and demostrated its Au-catalysed activation in cell culture.

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BRITISH PHARMACOLOGICAL SOCIETY

PB006 The IUPHAR Guide to IMMUNOPHARMACOLOGY portal

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Introduction

A 2016 global pharmaceutical R&D review lists the top-three general mechanisms of action as immuno-stimulant, anticancer immuno-therapy, immune-suppressant, covering 1706, 399, 221 therapeutic agents, respectively (1). The increasing dominance of these categories is reflected in growing academic/commercial research in the pharmacology of immunity, inflammation, infection (I-I-I). Data exchange between these three research communities is therefore critical to development of new drugs. Our Wellcome Trust-funded project to produce the IUPHAR Guide to IMMUNOPHARMACOLOGY (GtoImmuPdb) addresses this need by providing a new portal that is both 'immunologist-friendly' for pharmacological information and 'pharmacologist-friendly' for accessing immunological agents/targets.

Method

The project infrastructure and curation model is based on the IUPHAR/BPS Guide to PHARMACOLOGY database (GtoPdb)(2,3). The resource is a joint initiative between the International Union of Basic and Clinical Pharmacology (IUPHAR), the British Pharmacological Society (BPS) and the University of Edinburgh, with funding from The Wellcome Trust. GtoImmuPdb is being built as a major extension of the GtoPdb. New curation input tools have been designed in Java and Postgres database tables expanded to encompass GtoImmuPdb specific data.

Results

Over 500 protein targets and over 900 small-molecule ligands have been curated into GtoImmuPdb from the existing GtoPdb. The database has been extended to include biological processes and their associations to existing targets, largely through data-mining of immuno-relevant process terms from the Gene Ontology (4). The resource also includes cell type associations to targets. In this instance, the controlled vocabulary of the Cell Ontology (5) has been used to formalise connections between targets and cells. Extension have been introduced to incorporate disease associations to both targets and ligands, *via* resources such as OrphaNet (6), Disease Ontology (7) and OMIM (8).

Conclusions

Development of GtoImmuPdb has achieved the following: Immunological-relevant target information, collected from primary literature, verified and annotated by expert curators and peer-reviewed by NC-IUPHAR subcommittees; Integration with GtoPdb for reciprocal navigation; An expanding set of compounds, peptides and antibodies active in I-I-I systems; Reciprocal links with immunology-relevant databases; Immunologist-friendly interfaces and search tools; Fully downloadable data. The third public beta release was made in March 2018, with current funding supporting development and expansion to autumn 2018.

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PB007 Gold-mediated anticancer drug release

A. Perez-Lopez[1], B. Rubio-Ruiz[1], V. Sebastian[2], L. Hamilton[1], C. Adam[1], T. Bray[1], S. Irusta[2], P. Brennan[1], G. Lloyd-Jones[1], D. Sieger[1], J. Santamaria[2], A. Unciti-Broceta[1]. [1]*University of Edinburgh, United Kingdom,* [2]*University of Zaragoza, Spain*

Introduction

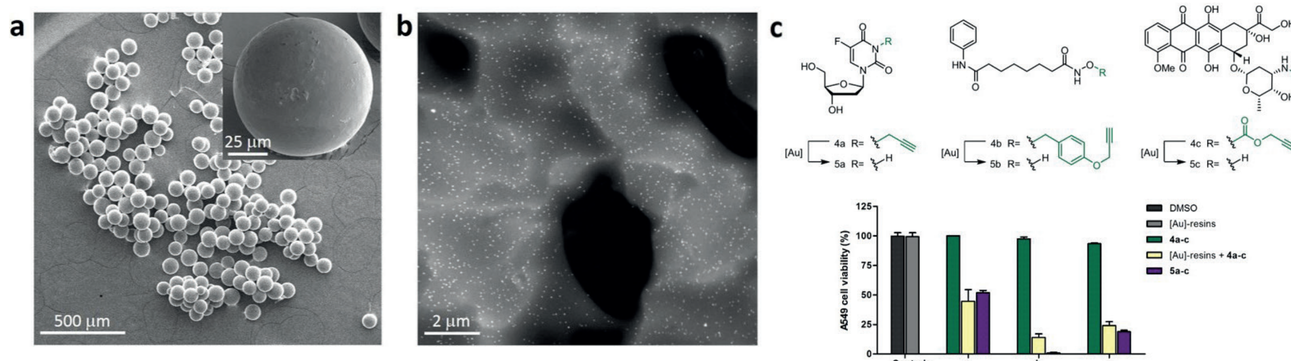
The use of foreign transition metal catalysts to mediate chemoselective transformations in cells,[1] so-called bioorthogonal organometallic catalysis,[2] has witnessed a wealth of creativity in recent years towards a variety of applications. Metallic gold is widely regarded as the most biocompatible metal. We have now shown that the catalytic properties of gold can be accessed *in vivo* if suitably protected from the environment, thus opening new possibilities to "manufacture" therapeutics in a safe manner both *in vitro* and *in vivo*.

Methods

Au^0-functionalized resins were prepared from NovaSyn TG amino resin HL as previously described.[2-3] [Au]-resins were incubated with non-fluorescent rhodamine 110 precursor. Reactions were performed in PBS at 37°C and monitored at 24h by fluorescence emission using a PerkinElmer plate-reader. A549 cells were incubated with [Au]-resins (1mg/mL), **4a-c**, **5a-c** or combination of [Au]-resins + **4a-c** (10μM, 100μM and 1μM). After 4 days treatment, cell viability was determined by PrestoBlue™. Fluorescence emission was detected using a PerkinElmer plate-reader. Implanted zebrafish larvae were treated with embryo media containing non-fluorescent rhodamine 110 precursor (20μM) for a period of 24h and imaged using a Zeiss LSM 710 confocal microscope.

Results

The bioorthogonal [Au]-triggered release of a chemically-diverse selection of prodrugs was investigated in cell culture with human lung cancer A549 cells. Remarkably, while prodrugs **4a-c** did not elicit any effect on their own, potent anticancer activity was displayed in combination with [Au]-resins (**Fig. 1c**), unequivocal evidence that the active drugs are released by heterogenous gold chemistry under physiological conditions. For the first time, this solid supported catalyst enabled the locally-controlled release of a fluorescent dye in the brain of a zebrafish.



Conclusions

We have developed a novel heterogeneous gold catalytic system that facilitates the activation of a chemically-diverse range of therapeutics in cancer cell culture and the first intracranial activation of a bioorthogonal probe in zebrafish.

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3) Pérez-López, A. M.; Rubio-Ruiz, B.; Sebastián, V.; Hamilton, L.; Adam, C.; Bray, T. L.; Irusta, S.; Brennan, P. M.; Lloyd-Jones, G.; Sieger, D.; Santamaría, J.; Unciti-Broceta, A (2017). *Angew. Chem., Int. Ed.* **56**: 12548-12552. **Figure 1. a-b)** [Au]-resins SEM. **c)** Gold-triggered activation of prodrugs.

PB008 Using genetic data to identify gene targets for drug repositioning to aid in the treatment of depression

D. Howard, J. Hafferty, A. McIntosh. *University of Edinburgh, United Kingdom*

Introduction

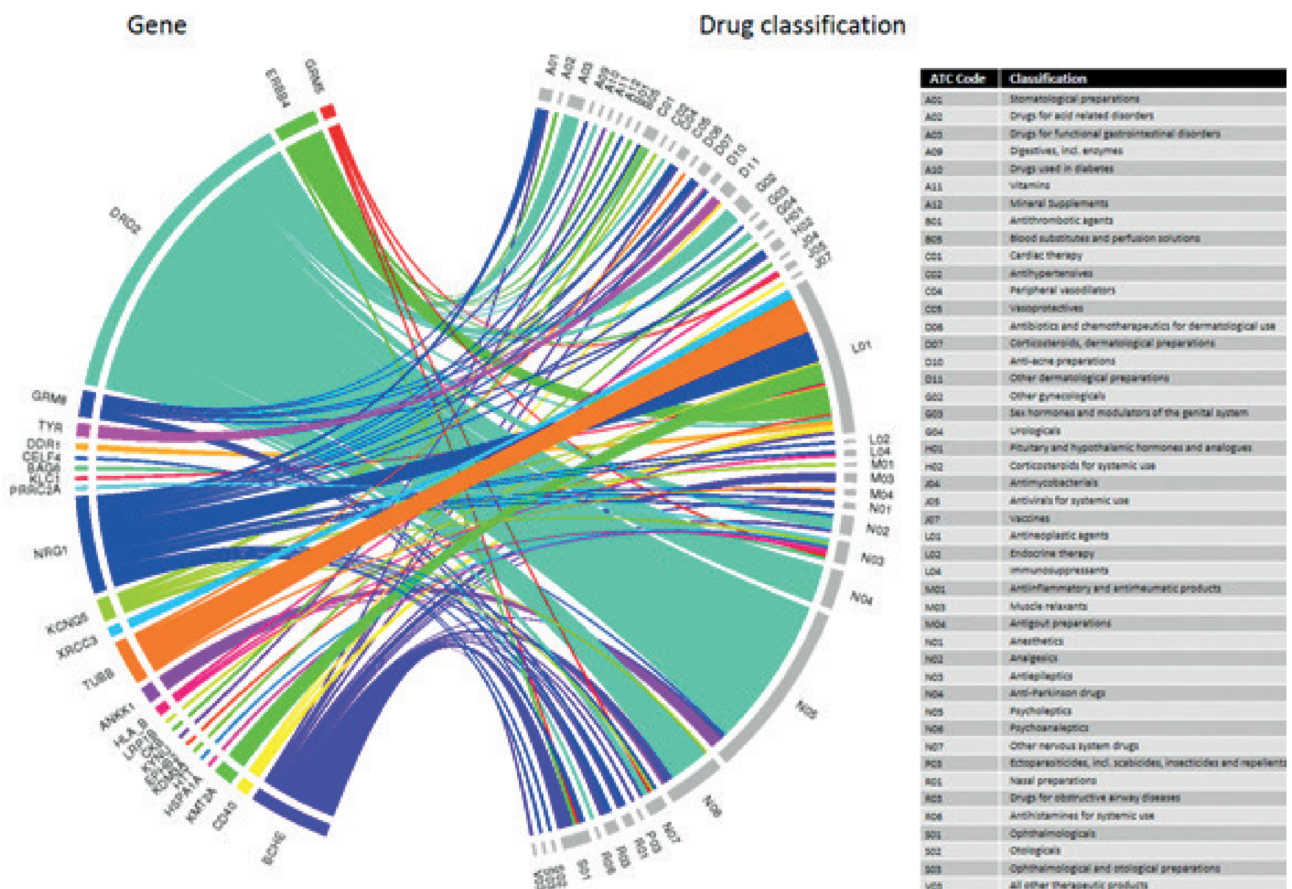
Major depression is characterised by low mood and anhedonia and is associated with substantial ill health and worldwide disability. Current medications are not always effective[1], often requiring switching of pharmacological treatments until the disorder is in remission. Genome-wide data collected on large cohorts will potentially reveal novel genes that could signpost new drug targets. These studies may also identify drug targets for which there is an existing ligand that can be repurposed.

Method

We conducted a large genome-wide meta-analysis (n = 807,553) of three studies of depression[2-4]. We used MAGMA[5] to identify the genes associated with the disease. We mined the Drug Gene Interaction Database[6] to extract the known gene x drug interactions for the genes associated with depression. The Anatomical Therapeutic Chemical (ATC) classification system[7] was used to determine the second level classification of each drug identified, with missing classifications obtained from DrugBank v5.0[8]. The number of interactions between each drug and each class are reported.

Results

A total of 177 genes were associated ($P < 2.76 \times 10^{-6}$) with depression. Of these associated genes, 26 had known interactions with 236 different drugs recorded in the Drug Gene Interaction Database. These 236 drugs belonged to 46 different drug classes (Figure 1). Dopamine Receptor D2 (*DRD2*) was shown to have the greatest number of interactions with the N05 (Psycholetpics) and N06 (Psychoanaleptics) classifications, which contain the anti-depressants class of medications.



ATC Code	Classification
A01	Stomatological preparations
A02	Drugs for acid related disorders
A03	Drugs for functional gastrointestinal disorders
A09	Digestives, incl. enzymes
A10	Drugs used in diabetes
A11	Vitamins
A12	Mineral Supplements
B01	Antithrombotic agents
B05	Blood substitutes and perfusion solutions
C01	Cardiac therapy
C02	Antihypertensives
C04	Peripheral vasodilators
C05	Vasoprotectives
D06	Antibiotics and chemotherapeutics for dermatological use
D07	Corticosteroids, dermatological preparations
D10	Anti-acne preparations
D11	Other dermatological preparations
G02	Other gynecologicals
G03	Sex hormones and modulators of the genital system
G04	Urologicals
H01	Pituitary and hypothalamic hormones and analogues
H02	Corticosteroids for systemic use
J04	Antimycobacterials
J05	Antivirals for systemic use
J07	Vaccines
L01	Antineoplastic agents
L02	Endocrine therapy
L04	Immunosuppressants
M01	Antiinflammatory and antirheumatic products
M03	Muscle relaxants
M04	Antigout preparations
N01	Anesthetics
N02	Analgesics
N03	Antiepileptics
N04	Anti-Parkinson drugs
N05	Psycholeptics
N06	Psychoanaleptics
N07	Other nervous system drugs
P03	Ectoparasiticides, incl. scabicides, insecticides and repellents
R01	Nasal preparations
R03	Drugs for obstructive airway diseases
R06	Antihistamines for systemic use
S01	Ophthalmologicals
S02	Otologicals
S03	Ophthalmological and otological preparations
V03	All other therapeutic products

Figure 1. Chord diagram of genes associated with depression and their interactions with second level Anatomical Therapeutic Chemical classifications

Conclusion

This study demonstrates plausible interactions between depression-associated genes and existing efficacious drugs. The method used also offers the potential of detecting novel drug treatments and of repositioning opportunities for depression and other diseases.

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PB009 The anti-cancer and anti-inflammatory effects of curcumin in multiple human cancer cells

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Introduction
Curcumin (Diferuloylmethane) has received widespread interest for its therapeutic effects, including antioxidant, antiseptic, anti-bacterial, anti-inflammatory, anti-cancer, and analgesic. It is an active polyphenol isolated from the *Curcuma longa* plant that has been used for centuries as a spice and as a dietary supplement, and considered pharmacologically safe [1-3]. Since curcumin showed a valuable potential against several cancers, this study aimed to analyze the cytotoxic effects of curcumin in multiple cancer cells (lung, breast, colon, head and neck, and ovarian) and its effect on the production of pro- and anti-inflammatory cytokines (for example, TNF-α and IL-10).

Method
Cell-free system for antioxidant assessment (DPPH), cell cytotoxicity (WST-1), apoptosis (Annexin) and cytokine release (ELISA) assays were carried out to investigate the effects of curcumin on cancer cells and compared to the same untreated cell lines.

Results
Free radicals scavenging activity of the curcumin was determined as a function of increasing curcumin concentration. The low IC_{50} value correlated directly to the Curcumin antioxidant properties. Determination of the *in vitro* cell viability/cytotoxicity to curcumin at 24h on the selected cancer cell lines showed different responses with varying IC_{50} concentrations. Cytokines released by different cell lines were measured by ELISA after 72h. The obtained results showed that not all cell culture medium contain measurable TNF-α (e.g. head and neck cancer cell lines), whereas other cancer cells reproducibly released different detectable levels of TNF-α and IL-10. However, the correlations between apoptosis and TNF-α were also different among the cancer cell lines.

Conclusion
The potential anti-cancer and anti-inflammatory effects of curcumin were studied in multiple human cancer cell lines. The obtained results showed that the various cancer cell lines do not respond in a similar fashion to curcumin treatment, which may be attributed to the drug-resistant cell lines used. However, these data could be exploited to assess how different tumors are likely respond to new drugs. Furthermore, they can be useful to study combination therapy, with the ability to improve the success rate for developing new effective personalized cancer therapy.

Funding
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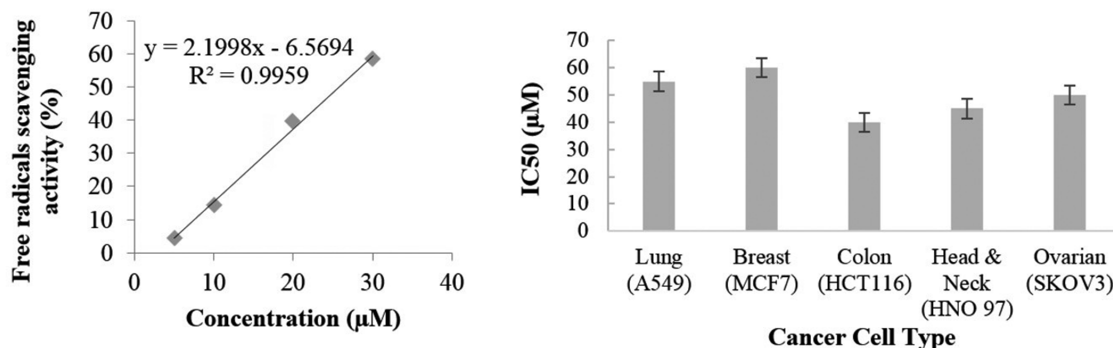

Figure 1: Left: Dose response relationship for antioxidant characteristic for Curcumin. **Right:** Average IC_{50} values of curcumin on the five cancer cell lines.

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PB010 Modulation of angiotensin II signalling by cannabinoid receptor 1 antagonism in human coronary artery smooth muscle cells

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The endocannabinoid system consists of at least two G-protein coupled receptors (GPCR), CB1 and CB2, and endogenous ligands such as anandamide (AEA) and 2-arachidonolyglycerol (2-AG). CB1 receptors are expressed in the brain but also in peripheral tissues. Several studies have demonstrated the possible importance of the peripheral endocannabinoid system, mainly in the pathogenesis of cardiovascular diseases. Angiotensin type 1 receptor (AT1R) is a GPCR that transduces the main physiological actions of the renin-angiotensin system in target cells. The major signaling events following agonist binding to this receptor are activation of phospholipase C via a $G\alpha q$ protein, mobilization of calcium from intracellular stores, and activation of other signaling pathways such as the MAP kinase pathway that participates in the hypertrophic actions of Ang II. Following characterization of CB1 and AT1R in human coronary artery smooth muscle cells (hCASMC), this project investigated evidence for functional cross talk between AT1R and CB1. CB1 and AT1R expression was examined in hCASMC using RT-qPCR and Western blotting. Phosphorylation of MAP kinase was determined using Western blotting. CB1 and AT1R were expressed in a range of hCASMC cell sources, and functional MAP kinase activity was observed following application of agonists. Peak pERK1/2 occurred at 5 minutes following 100nM Ang II and at 10 to 15 minutes after 1μM AEA. Furthermore, a concentration-dependent increase in phosphorylation of ERK1/2 in response to Ang II or AEA was observed with pEC_{50} values of 8.64±0.41 and 8.17±0.32 respectively. We examined if the presence of CB1 contributed to the response of AT1R to Ang II stimulation by directly altering pERK activation. Pre-incubation with the CB1 antagonist, SR141716A (10μM), significantly reduced Ang II-induced pERK activity. In conclusion, CB1 and AT1R receptors are expressed and are functionally active in hCASMC and it is suggested that there is cross talk between the two GPCR pathways. Future work will include study of other MAPK pathways such as p38 and Junk pathway as well as effects on Ca^{2+} mobilization.

PB011 Bioorthogonal activation of cancer prodrugs with catalytic palladium implants for treatment of glioma

C. Adam, L. Hamilton, J. Weiss, B. Rubio-Ruiz, A. Perez-Lopez, P. M. Brennan, D. Sieger, A. Unciti-Broceta. *University of Edinburgh, United Kingdom*

Introduction

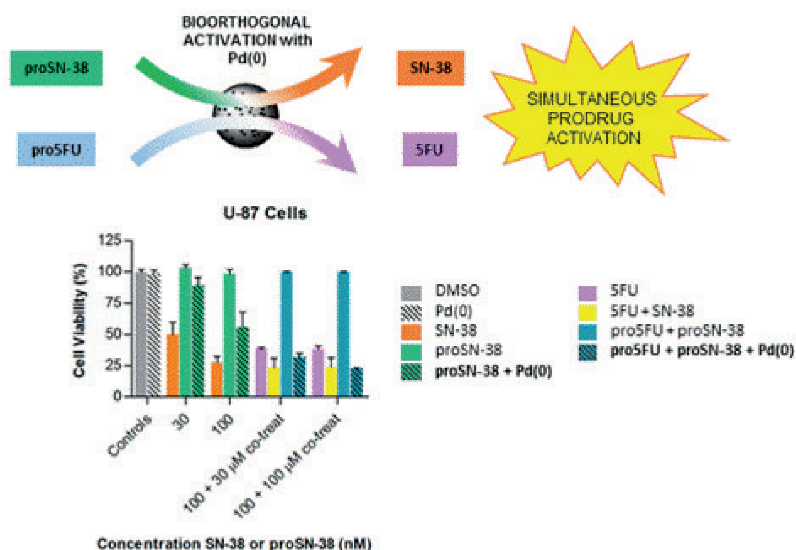
New treatments for patients with malignant glioma are needed as the current approaches - surgery, followed by radiotherapy with adjuvant chemotherapy - have significant shortcomings. Irinotecan is a cancer prodrug metabolised to the active metabolite SN-38, which is responsible for many serious side-effects.(1) We aimed to make prodrugs of SN-38 that could be activated bioorthogonally (by a chemical reaction, independent of biochemical processes) with palladium, rather than enzymatically. Further to this, we wanted to test the capacity of palladium to activate combinations of prodrugs.

Method

ProSN-38 was synthesised and tested for toxicity against U-87 and U-251 glioma cell lines, and HTC 116 (colorectal carcinoma) in comparison to SN-38 (n=3). Cells were incubated for 5 days with proSN-38 plus palladium (Pd(0)) to test the palladium-catalysed release of SN-38 in cell culture (see figure). The addition of pro5FU (2) was also tested. Experiments: 0.1% (v/v) DMSO (control); 1 mg/mL of Pd(0)-resins (-ve control); SN-38 (30, 100 nM, +ve control); proSN-38 (30, 100 nM); 1 mg/mL of Pd(0)-resins + proSN-38 (30, 100 nM); 5FU alone (30, 100 μM), 5FU (30, 100 μM) + SN-38 (100 nM co-treat +ve control); pro5FU (30, 100 μM) + proSN-38 (100 nM co-treat -ve control) and 1 mg/mL of Pd(0)-resins + pro5FU (30, 100 μM) + proSN-38 (100 nM co-treat activation assay). Cell viability was measured at day 5 using PrestoBlue reagent. Error bars: ± SEM, n=3.

Results

The cytotoxicity of pro-SN38 was lower than SN-38 against all cell lines tested, with EC_{50} values 16-, 35- and 43-fold higher for U-87, U-251 and HTC 116 respectively. When combined with bioorthogonal palladium in cell culture, cytotoxicity was restored. Co-treatment with palladium-activated pro5FU to release both 5FU and SN-38 simultaneously increased the potency of the treatment, with no increase in toxicity of the combined prodrugs alone.



Conclusions

We have demonstrated for the first time the simultaneous bioorthogonal metal-activation of two independent prodrugs in cell culture, showcasing the broad applicability of this local delivery approach. We envisage this technology could be used to control release of cytotoxic agents directly at the site of cancer in the brain, to alleviate the toxic side-effects of cancer chemotherapy. This approach using palladium has potential to simultaneously improve tolerability of existing drugs, maximise dose delivery to the tumour, and reduce side effects.

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PB012 Surrogate outcomes supporting conditional marketing authorisations and accelerated approvals by the european medicines agency, 2011-2018

C. S. Schuster Bruce[1], P. McGettigan[1], P. Brhlikova[2]. [1]*William Harvey Research Institute, United Kingdom,* [2]*Newcastle University, United Kingdom*

Introduction

In situations of unmet medical need, Conditional Marketing Authorisation (CMA) and Accelerated Assessment (AA) pathways are fast-track routes enabling early European Medicines Agency (EMA) marketing approval. CMA is conditional on completing regulator-imposed post-marketing studies. AAs require complete safety and efficacy data whilst shortening regulatory assessment time but have no imposed conditions. Surrogate outcomes (or endpoints), intended to predict desired clinical outcomes, reduce drug development time[1]. Pivotal studies supporting both CMAs and AAs commonly report surrogate outcomes, especially biomarkers. Non-validated surrogates may not reflect intended clinical outcomes. Subsequently, drugs may not provide intended benefits. We determined the numbers of CMAs and AAs granted according to clinical and surrogate outcomes, and assessed whether surrogates were validated. Fleming and Powers propose an outcomes hierarchy: Level 1 is a clinical outcome; Level 2, a validated surrogate; Level 3, a non-validated surrogate; Level 4, an un-established correlate[2]. Ciani et al. also propose a hierarchy: Level 1 requires controlled trial evidence that surrogate and clinical effects correspond; Level 2 requires 'consistent association' between surrogate and clinical outcomes across observational studies; Level 3 requires biological plausibility[1].

Methods

European Public Assessment Reports (EPARS) were used to assess CMAs and AAs issued between January 2011 and January 2018. Ethics approval was not required. PubMed searches were used to identify validated surrogate outcomes. Search terms were: ['endpoint'] and [validat* surrogate outcome OR validat* surrogate endpoint OR validat* surrogate end-point] and ['indication']. Fleming and Ciani hierarchies were used to determine surrogate validity. Fleming 'Level 2' and Ciani 'Level 1' were judged validated surrogates. Other levels were judged non-validated.

Results

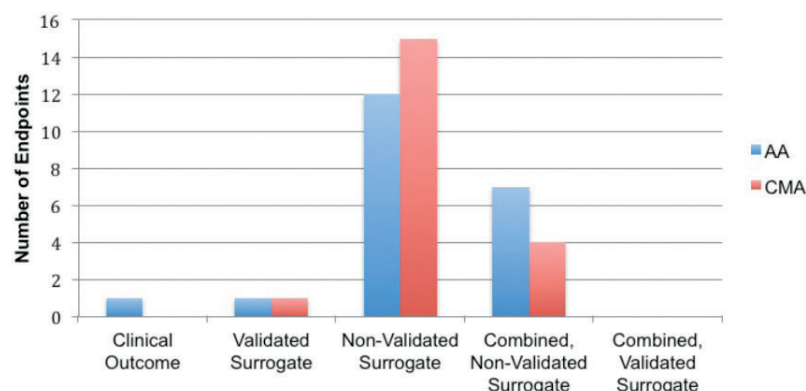
21 and 20 medicines were authorised through CMA and AA respectively (Table, Figure). 15/21 CMAs and 7/20 AAs were granted for malignant conditions. 6/21 CMAs and 13/20 AAs were granted for non-malignant conditions; 7/13 non-malignant condition AAs were for hepatitis C (HCV) medicines, all based on a non-validated biomarker, 'sustained virological response'. Most surrogate outcomes were biomarkers (Table). 13/40 (31%) EPARs did not discuss or justify the pivotal trial outcome.

Table: Summary of CMA and AA Authorisations According to Outcome Type

	CMA	AA
Number of medicines	21	20
Clinical outcome (%)	0 (0%)	1 (5%)
Surrogate outcome (%)	17 (81%)	12 (60%)
Biomarkers (% of surrogates)	15/17 (88%)	12/12 (100%)
Combined outcome * (%) Combined outcome with non-validated surrogate endpoint	4 (19%) 4 (100%)	7 (35%) 7 (100%)

*Combined Outcome e.g. Progression Free Survival whereby a single endpoint can be death (clinical) or progression of disease

Figure: Validity of CMA and AA Pivotal Trial Endpoints



Conclusions

Non-validated surrogate outcomes, most of which are biomarkers, commonly support authorisation decisions. There is inherent uncertainty with non-validated surrogate outcome use. Post authorisation obligations imposed on CMAs address this to some extent. For AAs, medicines approvals granted on the basis of non-validated biomarker outcomes should be re-evaluated as clinical efficacy evidence emerges.

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PB013 Systematic review and meta-analysis using SyRF- a tool to accelerate drug development

G. L. Currie, J. Liao, E. S. Sena, C. T. Sena, A. Bannach-Brown, K. Hair, S. K. McCann, Q. Wang, M. R. Macleod. *University of Edinburgh, United Kingdom*

Introduction
The median delay between drug discovery and licencing is 24 years[1]. A major component of this is the time it takes for translation from preclinical to clinical research. Systematic review and meta-analysis are powerful, unbiased approaches to summarising research, but at present they take months or years to complete, and this limits their utility.

SyRF (the Systematic Review Facility, app.syrf.org.uk) is an online platform that supports all stages of a systematic review and meta-analyses. SyRF levers artificial intelligence tools to enable a continually updated summary of what is known. Here we report the utility of this approach to drug development in chemotherapy-induced peripheral neuropathy (CIPN) and in stroke.

Method
As part of an ongoing systematic review of drugs tested in animal models of CIPN we assessed the performance of machine learning in citation screening; and we assessed the utility of text mining in risk of bias annotation in CIPN and in reports from animal models of stroke. We report the sensitivity and specificity, comparing the performance of the artificial intelligence tools against 2 trained human researchers.

Results
Citation screening: using 33,184 unique publications as a training set we applied a machine learning algorithm to 11,880 additional publications identified in an updated search. Compared with the human “gold standard”, the algorithm performed with a sensitivity of 97% and specificity of 67%.
Risk of bias annotation: Text mining tools developed in a psychosis dataset achieved high sensitivity and reasonable specificity in CIPN and stroke validation datasets (Table).
Future developments: We have previously used systematic review and meta-analysis to inform drug selection for the MS-SMART trial [2]. By automating key steps in the process (Figure) we will provide utterly contemporary information to inform drug selection for clinical trials.

		Sensitivity (%)	Specificity (%)
Chemotherapy-induced(n=341)	Randomisation	98	55
	Blinding	96	91
	Sample Size Calculation	75	98
Stroke(n=940)	Randomisation	100	67
	Blinding	99	77
	Sample Size Calculation	26	99

Table: Performance of risk of bias assessment using Regex. n= number of publications.

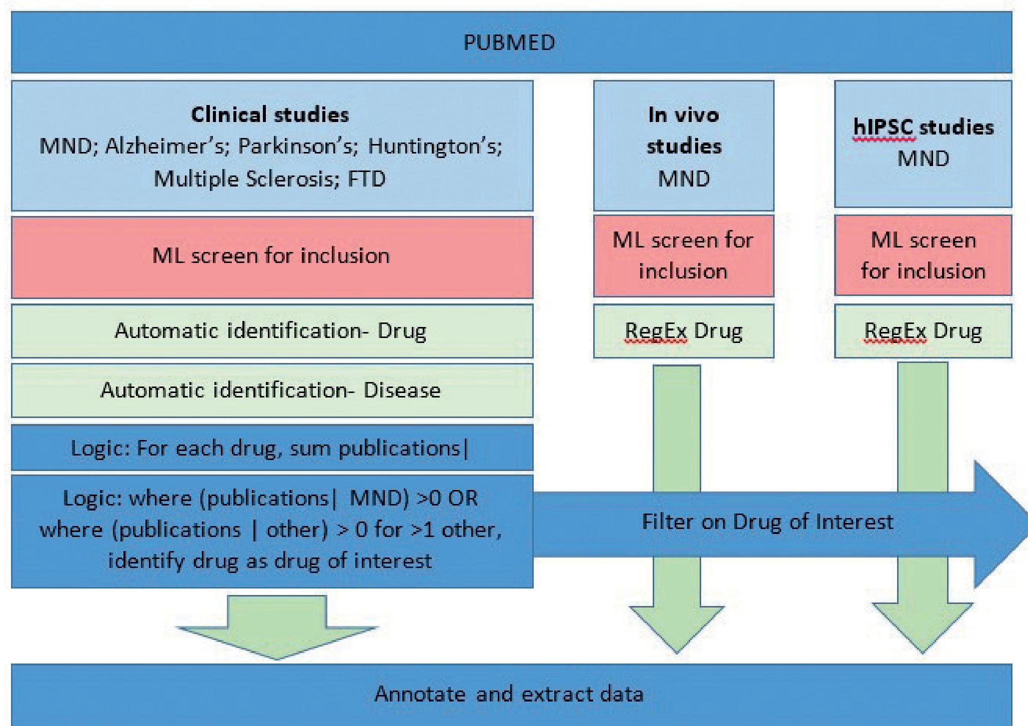

Figure: Key steps to be automated in the drug selection process. MND= motor neuron disease; FTD= frontotemporal dementia; hIPSC= human induced pluripotent stem cells; ML= machine learning.

Conclusions

SyRF - which currently hosts 220 projects, 390 users, 630,126 uploaded publications and 299,601 screening decisions - can dramatically reduce the time taken in systematic review and meta-analysis. Tools under development will facilitate living systematic reviews, provide curated current content for research areas and will help identify and rank new drugs for further investigation.

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PB014 Novel synthetic superenhancers for drug screening in cancer stem cells

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Introduction

Glioblastoma (GBM) is the most fatal malignant brain tumour. It is typically treated with a combination of surgery, radiotherapy and chemotherapy but is invariably fatal. A major driver of tumour growth is SOX2, a lineage specific transcription factor that is expressed in the GBM stem cells[1]. To date SOX2 is not amenable to structure-based drug design, as it is small transcription factor. We are able to use freshly isolated patient-derived GBM stem cell culture in cell based phenotypic screening to find new lead compounds that can disrupt GBM stem cell self-renewal.

Our goal is to develop novel neural-specific transcriptional reporters with improved sensitivity and cell type specificity that can be used to monitor SOX2 activity. These would be highly desirable and could be engineered directly into GBM stem cells with CRISPR/Cas9 knockin.

Method

SOX2 binding peaks within enhancer regions and unique for tumour-initiating cells have been identified from published ChIP-Seq datasets[2]. We have deployed the latest tools of DNA assembly and synthetic biology to create a new platform for combinatorial screening of novel clusters of SOX2 enhancer tethered to a downstream Luciferase-mNeogreen cassette. A minimal promoter was chosen based on low background expression and high inducibility. Enhancers were cloned and tested for their ability to drive reporter gene expression of NanoLuc and mNeogreen in GBM cell lines as well as in HEK, a SOX2 negative cell line.

Results and conclusions

Individual enhancers have been identified that are able to drive reporter gene expression in a cell type specific manner ($n \geq 3$). Clusters of between 4 and 8 of these enhancers ('synthetic superenhancers') are now being screened in combinatorial manner for optimal performance in GBM stem cells. These cell-type specific reporters of SOX2 activity will be valuable in future drug screening and development projects. Our overall approach provides a generic strategy that can be re-used for other transcription factors.

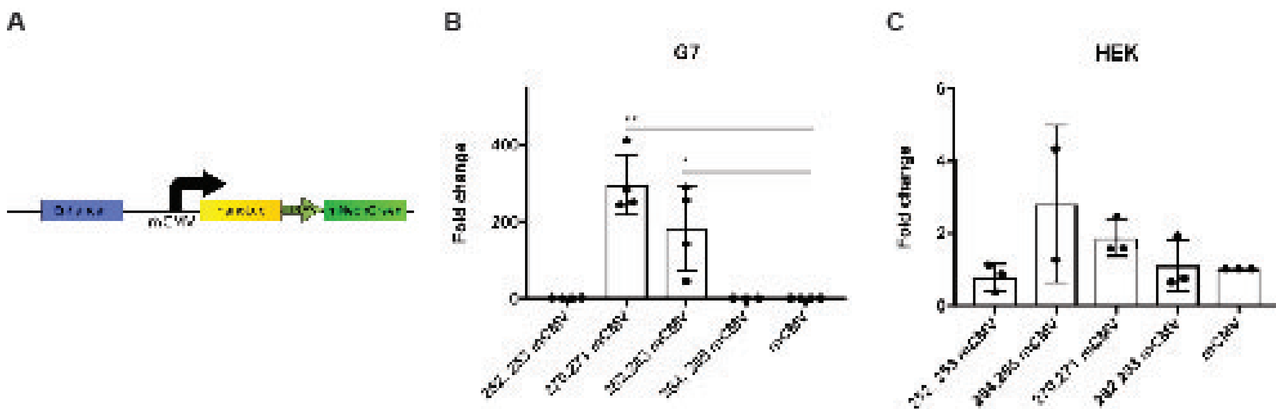

Figure 1: Enhancers are able to drive reporter gene expression in a cell type specific manner. (A) Schematic representation of a enhancer reporter activity construct. (B) Enhancers 272, 273 and 282, 283 show an up to 300-fold change in a GBM cell line. (C) Small fold change was observed in the SOX2 negative non-neural cell line. $p \leq 0.05$.

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PB015 Investigation of the novel selective SRC kinase inhibitor eCF506 in preclinical models of breast cancer

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Introduction

Activity of the proto-oncogene SRC kinase has been linked to several hallmarks of cancer (cell proliferation, metastasis, angiogenesis) and treatment resistance in solid tumours. Currently all approved SRC inhibitors also strongly inhibit ABL kinase due to their use in BCR-ABL-driven chronic myeloid leukaemia. Repurposing these drugs for their SRC activity has so far failed. Several studies suggest ABL kinase may act as a tumour suppressor in some breast cancers and its inhibition is associated with cardiotoxic events. We recently discovered the first selective and potent small molecule SRC inhibitor eCF506 (tert-Butyl N-[4-[4-amino-1-[2-[4-(dimethylamino)-1-piperidyl]-ethyl]pyrazolo[3,4-d]pyrimidin-3-yl]-2-methoxy-phenyl]carbamate). It has nearly thousand-fold selectivity over ABL kinase and superior drug like-properties compared to the current gold-standard SRC inhibitor dasatinib (2). We are currently investigating eCF506 in preclinical breast cancer models.

Method

Thermal shift assays were performed in MDA-MB-231 cells following an adapted protocol of (1), using 1 hour treatment, cell lysis with Triton-based buffer and analysis with Western blot. Cell proliferation was measured using PrestoBlue® cell viability reagent after 5 days of treatment and the GI_{50} was calculated from three biological repeats. eCF506 (50 mg/kg oral gavage, once daily for 21 days, n=6) was tested in an *in vivo* bone metastases model (intracardiac injection of MDA-MB-231 cells in nude mice) by Bin-Zhi Qian's group at the QMRI (Edinburgh).

Results

Thermal shift assays show that eCF506 and dasatinib have opposing effects on the heat stability of SRC, with eCF506 stabilising but dasatinib destabilising the protein. This is related to a differential phosphorylation status of the kinase at the auto-inhibition site Y527, as the compounds are stabilising it in an active (dasatinib) or inactive (eCF506) conformation.

Cell viability assays and Western blot analysis show that eCF506 is just as good as or superior to dasatinib at inhibiting SRC Y416 and proliferation in sensitive cell lines (Figures 1-2). Preliminary results of *in vivo* testing show it inhibits growth of bone metastases in breast cancer and further studies are planned to assess combination treatments.

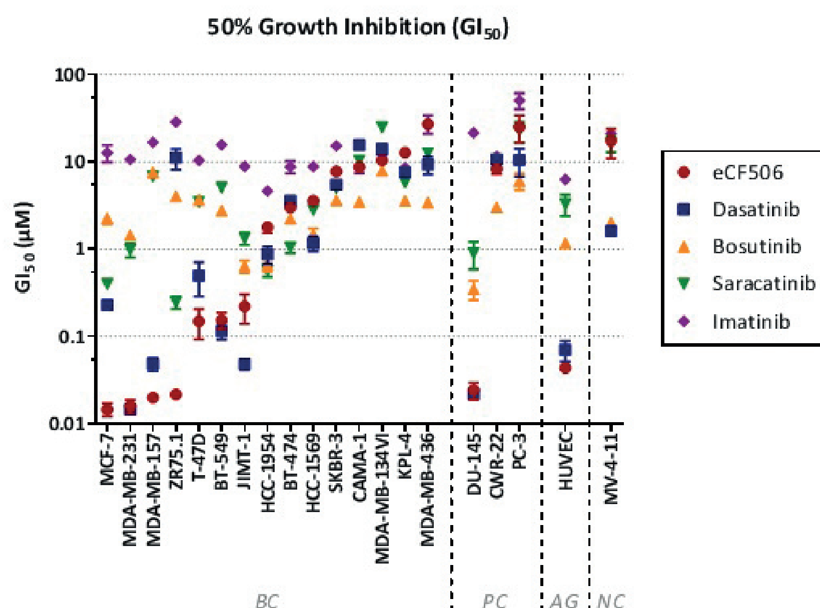

Figure 1. Anti-proliferative effects of five kinase inhibitors on a panel of breast cancer (BC), prostate cancer (PC), endothelial angiogenesis model (AG) and one negative control (NC) cell line. The average GI_{50} of three biological replicates with standard error was ranked by eCF506 sensitivity.

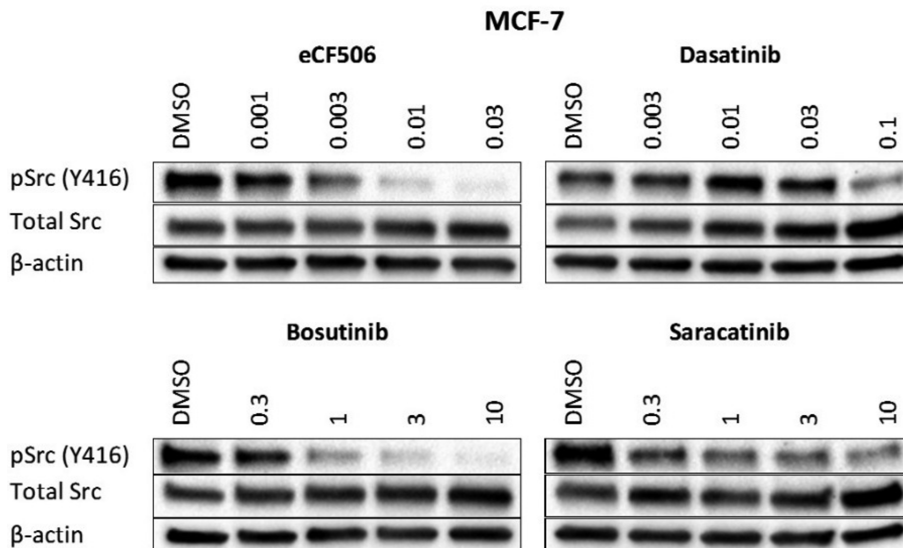

Figure 2. Western Blot analysis of eCF506, dasatinib, bosutinib and saracatinib on pSRC (Y416) in MCF-7 cells after 24 hours of treatment. eCF506 was the most potent SRC inhibitor in across both cell lines and time points.

Conclusion

Data obtained with the selective SRC inhibitor eCF506 suggests it has strong anti-proliferative and anti-metastatic properties in a subset of breast cancers. In comparison to dual SRC/ABL inhibitors, eCF506 is superior at inhibiting SRC kinase activity. Thermal shift assays revealed that, unlike dasatinib, eCF506 stabilises SRC in a closed, inactive conformation.

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PB016 Atorvastatin activates skeletal ryanodine receptors: Design of next-generation statins

C. Lindsay, A. D. Wilson, E. Venturi, A. J. Russell, R. Sitsapesan. *University of Oxford, United Kingdom*

Introduction

We have recently reported that simvastatin can activate single skeletal muscle ryanodine receptor (RyR1) channels, stimulating sarcoplasmic reticulum (SR) Ca^{2+} release from isolated skeletal muscle cells.[1] We suggested that the interaction of statins with RyR1 may contribute to their muscle-related side effects, including fatal rhabdomyolysis. The aim of this study was to investigate whether this effect was also seen with atorvastatin, a more recently developed and, in contrast to simvastatin, fully synthetic drug.

Methods

Sheep skeletal heavy SR membrane vesicles containing RyR1 channels were incorporated into planar phospholipid bilayers under voltage-clamp conditions as previously described.[2] Atorvastatin sodium salt was added to the cytosolic or luminal channel side at the concentrations indicated. Binding of [^{3}H]ryanodine to RyR1 was also used as an indirect measure of the open probability (Po) of populations of channels in their native membranes. Heavy SR vesicles were incubated for 90 min at 37°C with 200 μg of protein/ml. Nonspecific [^{3}H]ryanodine binding was determined in the presence of 1000-fold excess unlabelled ryanodine.

Results

Low concentrations of cytosolic atorvastatin, at 10 μM free Ca^{2+}, significantly and reversibly increased Po from 0.029±0.0098 (mean±SEM, n=22) in control conditions to 0.077±0.023 (mean±SEM, n=16, $p<0.05$) with 100 nM atorvastatin and 0.083±0.027 (SEM, n=13, $P<0.05$) with 1 μM atorvastatin. The addition of atorvastatin to the luminal (trans) side of RyR1 did not activate the channels even at high concentrations (≤100 μM). Atorvastatin also significantly increased [^{3}H]ryanodine binding to sheep skeletal SR membranes.

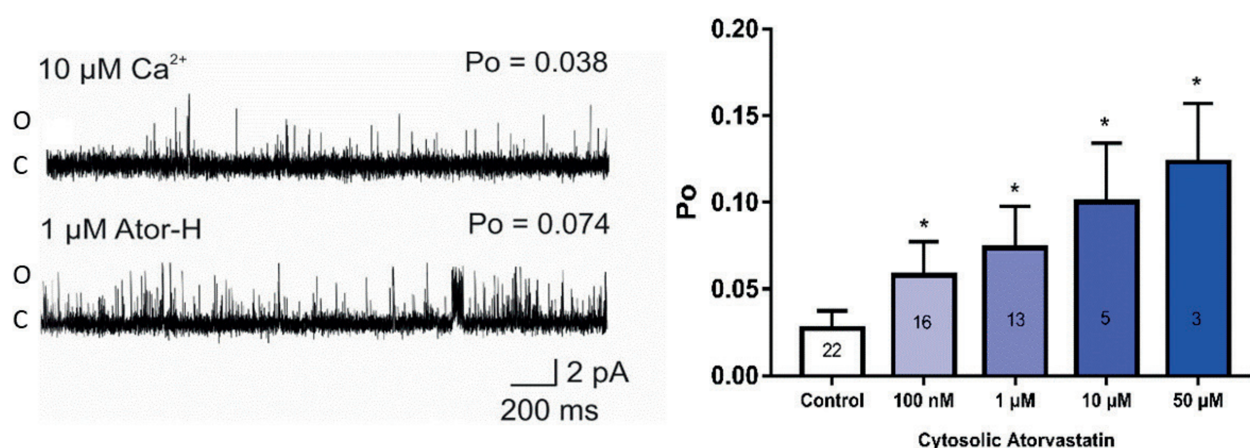

Figure 1 Activation of single RyR1 channels by atorvastatin

Conclusions

Cytosolic application of atorvastatin reversibly activates single skeletal RyR1 channels in a concentration-dependent manner and does so at concentrations low enough to be clinically relevant. Addition of luminal atorvastatin had no effect, indicating that atorvastatin interacts with RyR1 via cytosolic binding sites. We therefore suggest that RyR1 channel activation is a common property of clinically relevant statins, and that removal of this interaction will lead to the development of a statin drug with reduced muscular side-effects. We now aim to utilise a medicinal chemistry approach to design a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor that can effectively lower blood cholesterol levels yet does not activate RyR1.

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PB017 **Expansion of the druggable genome in the IUPHAR/BPS Guide to PHARMACOLOGY and other drug target resources: A key substrate for future medicines**

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Introduction

The landmark concept of the druggable genome (DG) defines the landscape of successful and potential new targets for human diseases (1). This is becoming increasingly data-supported by publications that expand the range of proteins with activity-modulating starting points. As an expert-curated online database of approved, clinical or research level pharmacological targets and their molecularly defined ligands, the IUPHAR/BPS Guide to PHARMACOLOGY (GtoPdb) provides its own internal statistics on DG coverage (2). As a DG-relevant cross-reference in UniProtKB/Swiss-Prot, we are joined by the additional target-to-chemistry resources of DrugBank, ChEMBL and BindingDB. This work compares overlaps and differences, functional coverage as well as cumulative target expansion of the data-supported DG between these four key databases.

Methods

Comparative analysis of these four resources was initiated via the UniProt query interface. Intersects and differentials were generated using the online Venny tool. Gene Ontology (GO) categories for selected protein identifier lists were displayed using the PANTHER resource. Further details, such as coverage by 3D structures, were analysed via the UniProt interface.

Results

While exact numbers will be updated for the presentation, in order of their mention above, the human Swiss-Prot cross reference counts were 1496, 2336, 3303 and 2462, respectively. This produced an outer limit total of 4223 (representing 21% of the canonical human proteome of 21,136) but the four-way consensus drops to 738. This core set thus has a well-corroborated likelihood of tractability for small molecule drug discovery or possibly biologics. These metrics underline the differential coverage of these sources but encouragingly, the consensus had increased from 568 two years ago. Analysis by GO categories indicates the different data capture selectivity and curatorial stringencies between the sources. Results will also be presented on coverage of structures, pathways and diseases for consensus sets as determined by additional UniProt queries.

Conclusions

The four resources compared here provide accessible, complementary and cumulative coverage of the DG. As we know, this does not obviate the major challenges of target validation. However, increasing delineation of the DG expands the number of potential starting points for the development of new medicines.

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PB018 Evidence that human P2Y$_1$ and P2Y$_{12}$ receptors form heterodimers

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Introduction
P2Y$_1$ and P2Y$_{12}$ receptors belong to the class A family of transmembrane GPCRs that are activated by endogenous nucleotides[1]. There is growing evidence that many GPCRs, including P2Y receptors, can exist as dimers or higher-order oligomers[2]. For example, P2Y$_{12}$ and PAR4 receptors were recently reported to dimerise[3]. Our previous studies indicated that hP2Y$_1$ and hP2Y$_{12}$ receptors may form a functional heterodimer with novel pharmacological and signalling properties[4]. The aim of this project was, therefore, to characterise the physical interaction between hP2Y$_1$ and hP2Y$_{12}$ receptors.

Method
tSA201 cells were transfected or co-transfected with hP2Y$_1$ and hP2Y$_{12}$ receptors, tagged with HA or a fluorescent protein. Cellular localisation and co-localisation of the receptors were determined using confocal microscopy. Transfected cells were cultured in the absence or presence of the N-glycosylation inhibitor tunicamycin (2.0 µg/ml) for 16 hours to determine the role of N-glycosylation in receptors expression. Receptor cell surface expression was quantified using ELISA. To investigate physical interaction between the two P2Y subtypes, co-immunoprecipitation was performed using anti-HA-agarose beads followed by immunoblotting with anti-GFP, anti-HA then alpha-Tubulin antibodies.

Result
Following transfection on their own or together, both receptors were localised mainly at the cell membrane, and this was unaffected by tunicamycin. Co-immunoprecipitation confirmed that P2Y$_1$ and P2Y$_{12}$ receptors associate physically. Each subtype enhanced the other›s surface expressions. In particular, expression of the P2Y$_{12}$ receptor more than doubled that of P2Y$_1$ receptors at the cell surface.

Conclusion
These results show that P2Y$_1$ and P2Y$_{12}$ receptors are physically associated at the cell membrane and that they enhance each other›s cell surface expressions. These results are consistent with our previous data indicating that P2Y$_1$ and P2Y$_{12}$ receptors form a functional heteromer.

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PB019 Effect of pirfenidone on endothelium-dependent vasodilatation in type-2 diabetic (db/db) mice

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Introduction

Both endothelial cell dysfunction and vessel stiffness are associated with worsening of the prognosis in patients with cardiovascular disease[1]. In this study, we investigated the effect of the antifibrotic drug, pirfenidone, on vascular tone. Moreover, we examined whether it restores endothelial function in arteries from diabetic animals.

Methods

18-20-Week old wild type mice and normoglycemic (db/+), and type 2 diabetic (db/db) male mice were euthanized by cervical dislocation. Aorta, coronary and mesenteric small arteries were isolated from diabetic db/db and db/+ control mice. The vascular segments were mounted on wires in microvascular myographs and normalized as previously described[2] for functional studies.

Results

In coronary arteries from wild type mice contracted with U46619, pirfenidone induced concentration-dependent relaxations which at 10^{-4} M pirfenidone were 71±8% (n=10). Relaxations induced by 10^{-4} M pirfenidone were reduced to 18±6% ($P<0.05$, n=7) in the presence of an inhibitor of nitric oxide (NO) synthase, N^G-nitro-L-arginine (L-NOARG, 1 mM) and to 28±12 % ($P<0.05$, n=6) in the presence of high extracellular potassium (30 mM). Relaxations induced by 10^{-4} M pirfenidone were reduced to respectively, 14±7% ($P<0.05$, n=6), 37±9% ($P<0.05$, n=6), and 9±6% ($P<0.05$, n=6) by a blocker of voltage-gated Kv7 channel XE991 (10 μM), and by blockers of large-conductance calcium-activated K channels, tetraethylammonium (1 mM) and iberiotoxin (10 nM). Weight (50±2g, $P<0.05$, n=24) and blood glucose levels (27±2 mM, $P<0.05$, n=24) were higher in db/db mice compared to db/+ control mice that weighed 30±1g (n=24) and had blood glucose levels of 8±1 mM (n=24). In aorta, coronary and mesenteric small arteries from diabetic db/db mice and db/+ control animals, relaxations induced by the endothelium-dependent vasodilator, acetylcholine, were markedly reduced. In aorta segments pirfenidone and an opener of K_V7 channels, flurpirtine (10^{-5} M) leftward shifted concentration-response curves for acetylcholine. A blocker of K_V7 channels, XE991 reduced the effect of both pirfenidone and flurpirtine and further reduced acetylcholine relaxations in aorta. In contrast, in mesenteric small arteries the potentiating effect of pirfenidone was absent despite impaired endothelium-dependent vasodilation to acetylcholine in arteries from db/db mice.

Conclusions

These findings suggest that pirfenidone is a direct vasodilator and that it improves endothelium-dependent vasodilatation by a mechanism involving K^+ channels in large arteries from diabetic animals. Thus, at relevant therapeutic concentrations the antifibrotic drug pirfenidone may restore endothelial function.

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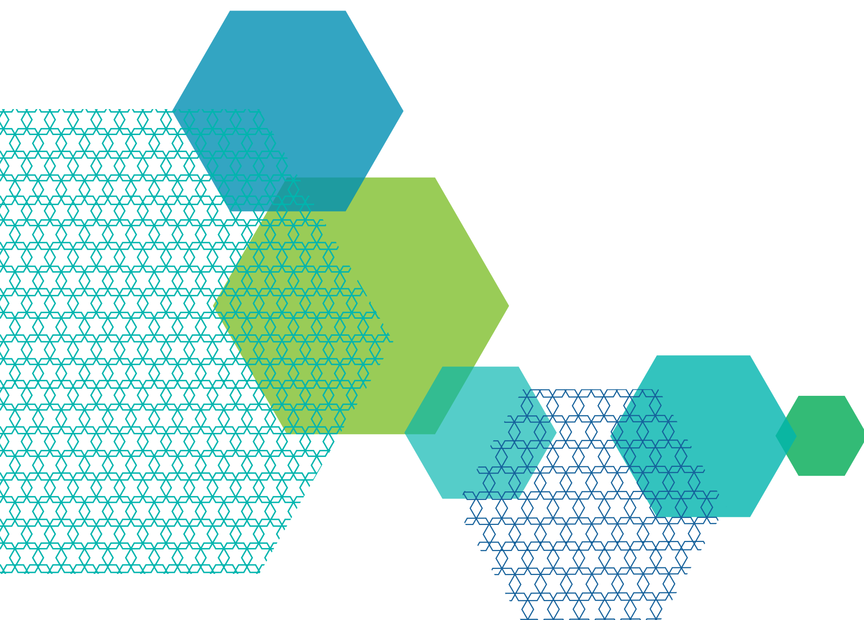

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