Combined pre-implantation genetic screening (PGS) for aneuploidy and haplotyping of the ANXA5 gene

Screening for chromosomal aneuploidy and miscarriage predisposition in IVF embryos in real time means no freeze/thawing and reduces the waiting time for embryo transplantation

Contact: **info@nanoporetech.com** More information at: **www.nanoporetech.com** and **publications.nanoporetech.com**

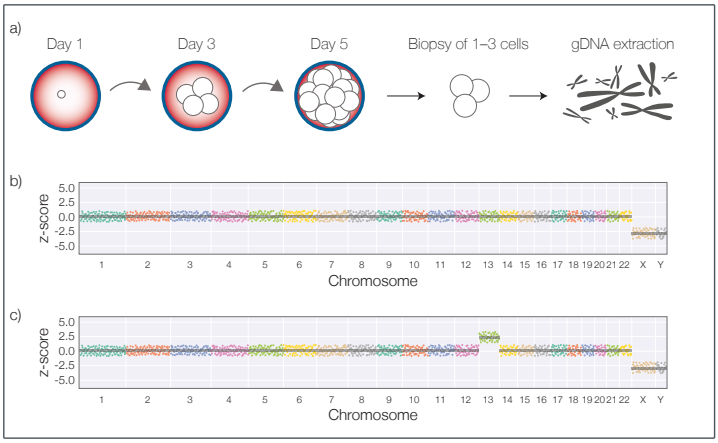

Fig. 1 PGS a) sample prep, idealised NGS results for b) a euploid male c) a male with trisomy 13

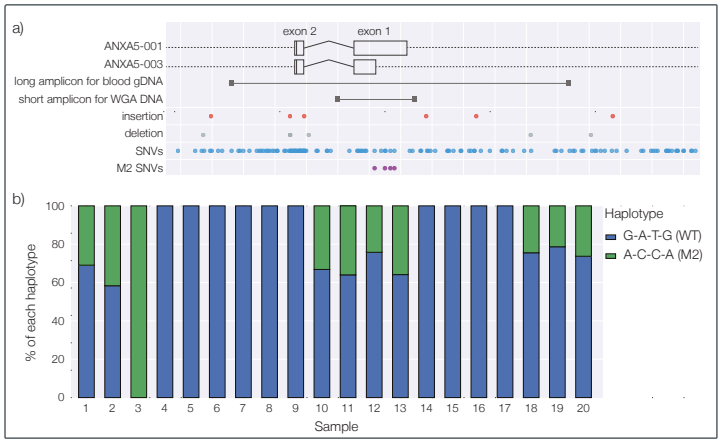

Fig. 2 ANXA5 a) locus and variants b) haplotyping results. Blue = wild-type, green = M2

PGS improves the success rate of in vitro fertilisation (IVF) by screening for aneuploidy

PGS is the process of testing an IVF embryo for an abnormal number of chromosomes (aneuploidy). It is estimated that around 70% of miscarriages in early pregnancy are due to chromosomal abnormality and that ~50% of IVF embryos are affected by aneuploidy. During PGS, a small number of cells (1–3) is taken from the embryo at the blastocyst stage on day 5/6 (Fig. 1a). At this stage, multiple cells can be removed from the trophectoderm (precursor to the placenta) without harming the inner mass cells (precursor to the foetus). gDNA is extracted from these cells, and can be tested for aneuploidy using a variety of methods (Figs. 1b and 1c).

ANXA5 M2 haplotype is associated with increased risk of miscarriage

The human ANXA5 gene is situated on chromosome 4, and the protein encoded by the gene is a member of the annexin family of calcium-dependent phospholipid binding proteins, and is a placental anticoagulant. A variant haplotype of ANXA5 contains 4 nucleotide substitutions which lie within the space of 57 nucleotides in the promoter (Fig. 2a). These substitutions reduce the activity of the promoter substantially, and the M2 haplotype, inherited from either parent, has been shown to be associated with recurrent miscarriage. By amplifying across the affected region by PCR and sequencing, we are able to identify ANXA5 haplotypes (Fig. 2b).

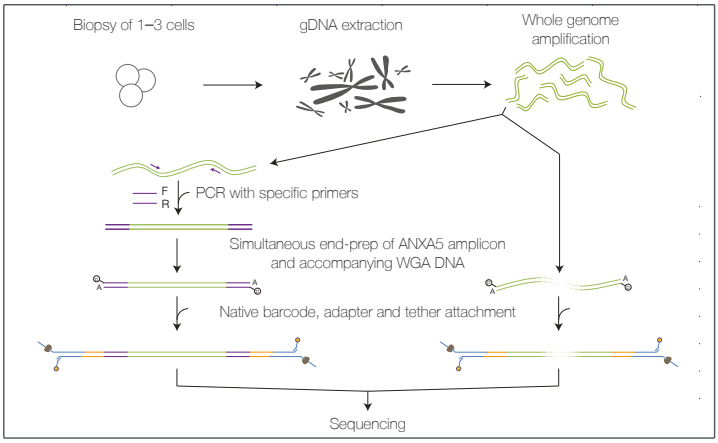

Fig. 3 Laboratory workflow for combined PGS and ANXA5 haplotyping

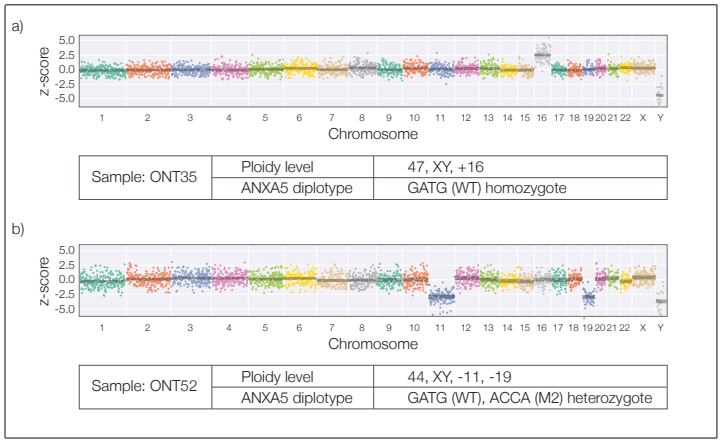

Fig. 4 PGS and ANXA5 results from two IVF embryos using the combined workflow

Workflow for combined PGS and ANXA5 haplotyping

We extracted genomic DNA from 1–3 cells taken from 5-day-old IVF blastocysts and performed whole genome amplification (WGA) of the DNA. The WGA product was amplified for a limited number of cycles using primers specific for the ANXA5 locus. Following amplification, all the DNA, including the accompanying WGA DNA were prepared for sequencing using Oxford Nanopore's Barcoded Rapid Amplicon kit. The products of each combined library prep were quantified and the WGA and ANXA5 products from 6 samples were pooled in the ratio 5 PGS : 1 ANXA5, and were sequenced on a single flowcell for 24 hours.

Single assay giving both PGS and ANXA5 status for IVF embryos

We performed WGA followed by the combined PGS and ANXA5 assay on 30 IVF blastocysts for which the ploidy level had previously been determined by array-CGH. Our results were in full concordance with the CGH results and showed that each sample was aneuploid. In addition, we determined the ANXA5 status of each sample, and confirmed the results by capillary sequencing. Combined results for two of these samples are shown in Fig. 4: panel a) shows a female sample which was found to be homozygous for the wild-type ANXA5 haplotype and triploid for Chr16; panel b) shows an M2 heterozygote that is haploid for Chr11 and Chr19.

Targeted Next Generation Sequencing

Assessing only relevant genes provides a cost-effective, useful tool for oncologists.

Cancer is a genetic disease. Understanding the precise nature of the genetic damage in each patient's own cancer can provide extremely useful information for research and even informing decisions about treatment choices – so-called "precision medicine".

Developments in precision medicine in oncology have led to a situation where often several different kinds of genetic tests have to be performed on a given biopsy specimen to check for the variety of genetic aberrations found in apparently similar tumours. This arises because similar tumours under the light microscope may contain a number of different mutations across a number of different genes, making these supplementary test(s) necessary to fully understand the tumour make up beyond its simple morphology. Searching for the full range of pathogenic mutations can be very important not only in research, but also in terms of actual diagnosis, understanding prognosis and for informing treatment choices (Table 1). This process, which today requires a number of different techniques (eg PCR, FISH, IHC), is expensive, resource and time consuming, and may not even be possible if only small pieces of tumour tissue are available.

Tumour Type	Detection Technology	Mutations	Reason
Lung Cancer	Sequencing	EGFR, KRAS, BRAF, MET (exon 14 skipping), ERBB2. (ALK, ROS1, RET)	Treatment Choices. (Kinase inhibitors, mutation specific).
	FISH or sequencing	ALK, ROS1 and RET rearrangements	Treatment Choices. (Kinase inhibitors, mutation specific).
Colorectal Cancer	Sequencing	KRAS, NRAS	Treatment Choices. (Utility of antiEGFR MAbs).
		BRAF (with loss of MLH1)	Prognosis. (Also to identify sporadic "mismatch repair".)
	IHC or other methods	MLH1, MSH2, MSH6, PMS2	Diagnosis of Lynch Syndrome/ dMMR.

Next Generation Sequencing (NGS) can identify many possible important aberrations in one experiment. The technology involves identifying the tumour sequence (base by base) and comparing it to known sequences in the normal human (reference) genome. Targeted sequencing refers to experiments in which pre-specified parts of the genome (eg panels of a few key genes or parts of genes) are "enriched" from the total genome focussing on specific regions of interest and sequenced in great detail. NGS for oncology samples must be robust to the processes of sample collection and handling including preservation in FFPE and ideally must be capable of detecting all the various forms of genetic aberration: point mutations, small insertions and deletions, copy number variations and structural alterations (translocations, inversions etc.).

Oxford Gene Technology has developed and commercialised customisable next generation sequencing panels (and associated software) that deliver reproducible and robust genetic information from oncology samples detecting mutations with a high degree of accuracy.

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SEND

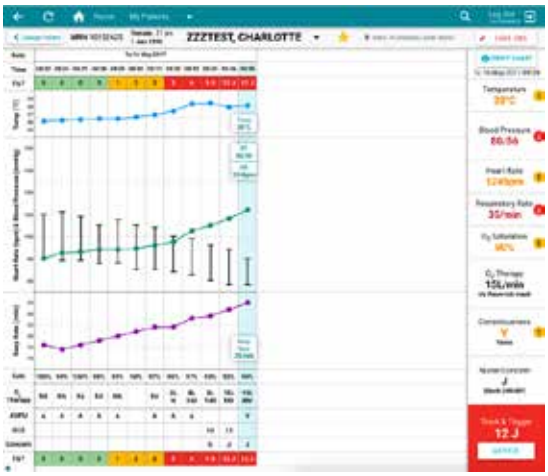
A digital patient safety system.

SEND is a system for digitally recording and reviewing vital signs. It automatically calculates an Early Warning Score every time new vital signs are entered to help staff easily identify patients who need urgent intervention.

In the UK alone, there are around 10,000 avoidable in-hospital deaths each year. The majority of cardiac arrests in hospital are preceded by a period of clinical deterioration, defined by abnormalities in patients' vital signs (heart rate, respiratory rate, blood pressure, temperature, arterial oxygen saturations and level of consciousness). Traditionally these have been recorded on paper charts, which can be inaccurate or illegible and are only accessible at the patient bedside.

The SEND system replaces the paper charts with a highly intuitive digital system that brings all the advantages of digital data storage whilst retaining the speed and usability of paper charting. Developed by a multidisciplinary team of clinicians, engineers and human factors scientists, the solution comprises a software application containing an Early Warning Score algorithm developed using machine learning techniques, and a customised hardware platform for fast, ergonomic data entry.

The ward overview screen, showing patients listed in order of priority for review.



A patient chart showing clinical deterioration. When the vital signs flag red the patient's care should be escalated

SEND improves safety at multiple levels in the healthcare system. At the bedside it helps clinical staff identify abnormal vital signs and act appropriately. At a ward level it provides senior clinicians with a real-time overview of patient sickness. At an organisational level it provides real-time auditing of clinical practice and data for clinical governance.

The research origins of SEND have strongly informed its development and future potential. Not only have we undertaken clinical trials to study the effectiveness of SEND but it also provides hospitals with a valuable data resource for research and innovation.

SEND is currently operational across all adult wards in all 4 hospitals of the Oxford University Hospitals Foundation Trust (OUHFT). It is used to record 150,000 observation sets every month. In a clinical trial SEND was found to be 30% faster than charting using pen and paper. Since the start of roll-out in 2014 over 16 million vital signs have been stored in the SEND database. These are being actively used in Oxford to develop next-generation Early Warning Scores and improve patient safety.

The SEND data entry hardware, comprising of a tablet, barcode scanner and bespoke roll stand capable of mounting most common vital signs monitors.

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Unobtrusive and automated sleep monitoring

Using the lightweight cEEGrid platform for accurate sleep staging.

Changing sleep patterns is considered a reliable marker for a large range of illnesses, from arthritis to schizophrenia. However, sleep monitoring is cumbersome and expensive, creating the need for a reliable but convenient alternative.

Sleep quality directly impacts general health. Changes in how we sleep can cause, and be caused by, a long list of illnesses.

However, the golden standard, the polysomnogram (PSG), involves electrodes spread over the whole upper body, requiring time consuming setup and expensive equipment, as well as inconvenience to the patient. This makes sleep measurements unfeasible in many cases where it could help diagnosis or treatment, and when it is used, it is often far less than what would be optimal.

A lightweight platform

The cEEGrid platform is a lightweight EEG array, which is attached around the ears using simple adhesives. This makes it highly promising as a method for performing a standard EEG measurement. The attachment of a set of electrodes at once is quicker than the current standard clinical practice of placing one electrode at a time. The cEEGrid also has the potential to be attached at home, without expert assistance. This capability would enable a real revolution in the study and treatment of a long range of chronic illnesses, in which repeated, longitudinal measurements of sleep are needed.

Figure 1: The cEEGrid array. For better signal quality, an array can be placed around each ear. The cEEGrids interphase over bluetooth with a smart phone, that stores the recording.

Comparison to current standard

Based on pilot data, we have shown that the cEEGrid contains similar information about sleep as conventional scalp electrodes (used in the PSG). The next, ongoing, step is a larger scale study, investigating the feasibility of clinical sleep measurements using cEEGrid instead of PSG.

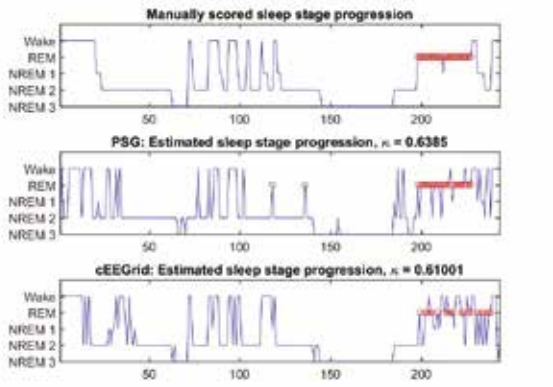

Figure 2: Comparison of estimated sleep stage progression with an automated processing pipeline. Top: expert estimates based on PSG data. Middle: Automatic estimation using PSG data. Bottom: Same algorithm used on cEEGrid data.

Kaare Mikkelsen
Post Doc at Institute for Biomedical Engineering,University of Oxford

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PepGen: Peptide drug delivery platform technology

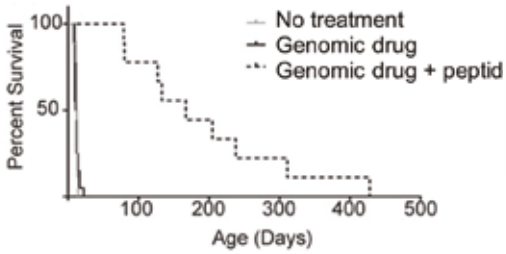
Delivery is the major barrier to the development of genomic precision medicine for human disease. We have developed a highly advanced peptide platform technology for the effective delivery of genomic medicine.

Genomic medicine (e.g. oligonucleotides, siRNAs and gene editing) is a dynamic and active field. The effects of antisense oligonucleotides (AONs) have been shown in multiple clinical trials, however due to the poor delivery of these drugs to target tissues, clinical benefit has been marginal and thus regulatory approval has proved difficult. This has left an urgent unmet need for AON therapies that are more efficacious.

Drug delivery platform technology

A highly productive collaboration stretching over ten years between Prof Matthew Wood at the University of Oxford and Dr Michael Gait at the MRC Laboratory of Molecular Biology in Cambridge has led to the development of a drug delivery platform technology that dramatically increases the efficacy of AON therapeutics.

This propriety peptide-based delivery platform has been developed with an initial focus on enhancing AON delivery for the treatment of neuromuscular diseases yet its applicability is far wider reaching with pre-clinical work in the pipeline for neurodegeneration, inflammation and oncology targets.



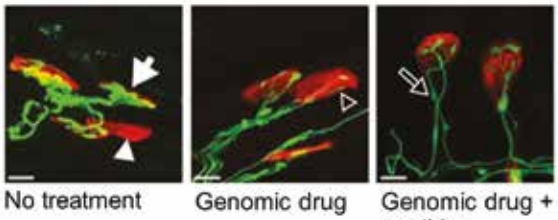
Peptide drug delivery platform significantly enhances survival in model of spinal muscular atrophy.

Neuromuscular disease

A wealth of pre-clinical work has been generated demonstrating the ability of this peptide-based platform technology to significantly enhance the delivery of AONs in two key neuromuscular diseases; Duchenne muscular dystrophy (DMD) and spinal muscular atrophy (SMA). These targets are clinically validated and commercially de-risked with data showing functional therapeutic benefits likely to translate into preventing or delaying disease progression.

Broad and robust patent position

Alongside the development of this technology we've built a unique intellectual property portfolio and working with Dr Ruth Barrett from Oxford University Innovations, are now seeking to commercialise this.



Morphological disease hallmark reduced following treatment with peptide drug delivery platform in model of spinal muscular atrophy.

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Big Data: delivering the digital medical revolution

Work within this theme aims to deliver an integrated, modular, sustainable and extensible informatics approach that facilitates delivering personalised medicine, by translating laboratory research into patient treatments. We are taking a 3 way approach to the themes mission:-

Clinical Informatics

Develop and deploy secure, interoperable methods, tools, and systems for acquisition, integration, and sharing of health data (including medical records, patient-reports, sensors, imaging, 'omics, and other biological data), and for controlled information exchange among academic and NHS organisations.

Information Governance

Develop, deliver and evaluate an ethically robust governance framework (with appropriate informatics tools and services) for capture, application and management of consents; information sharing; and transparency within and across organisations.

Big Data analytics

Develop methods for analysis of large, heterogeneous biomedical datasets, including scalable methods for evaluating phenotype and joint analysis of high-dimensional, longitudinal biological and clinical data.

Our projects include:

- Oesophageal cancer: Understanding the influence of molecular pathology and response to checkpoint blocking immunotherapy on clinical progression of early oesophageal cancer
- Chronic disease: Digital Health approaches to management of chronic disease using EHR, smartphone-enabled sensors/cameras, EHR and machine learning
- Inflammatory bowel disease: Development of clinically applicable algorithms for patient stratification in inflammatory bowel disease
- Atrial fibrillation: Assessing the impact of (often silent) atrial fibrillation on cardiovascular, stroke, and vascular dementia diseases
- Drug-resistant infections: Using combined clinical and genomic data to identify emergence of infection threats

Global Digital Exemplar

Oxford Health NHS Foundation Trust and Oxford University Hospital NHS Foundation Trust within the AHSC partnership have been designated as Global Digital Exemplars to champion the use of digital technology to drive radical improvements in the care of patients and are paving the way in the use of digital technology in the NHS, embracing digital health on a daily basis to support patient care.

Oxford Health initiatives have included offering patients remote consultations using video conferencing facilities such as Skype and FaceTime, electronic patient notes available via iPad from anywhere at any time, signposting to online wellbeing and mental health therapies and using and recommending apps such as True Colours to support patients' self-management and recovery.

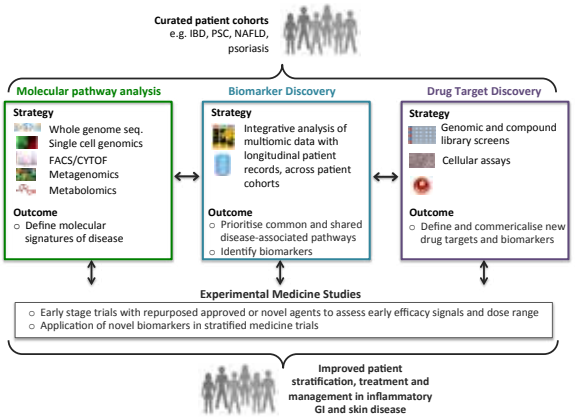
The exemplar has allowed Oxford University Hospitals to accelerate the opportunities that digital technology offers, in line with the ambition of the NHS to be 'paper-free at the point of care. The Trust has been acknowledged to be one of the most advanced NHS trusts for implementing electronic patient records (EPR), with over 1.2 million daily transactions via EPR. Administering more than 20,000 drugs every day using electronic prescribing and medicines administration, and having recently introduced a new state-of-the-art digital imaging system.

SEND, state of the art patient monitoring

Martin Landray
Professor of Medicine and Epidemiology and Deputy Director of the Big Data Institute

Modulating immune response for patient benefit

Work within this theme integrates cutting edge, multi-disciplinary basic science with first-rate clinical research. The close physical and intellectual collaboration between clinical and academic researchers provides an innovative, fast-paced, evolving approach to translational medicine that results in significant advances in patient treatment and care.



We are taking a multi-disciplinary approach to the mission.

1. Molecular pathway analysis: Using state-of-the-art technologies, including single cell genomics, CyTOF and whole genome sequencing we are identifying the underlying molecular causes of disease. These molecular signatures will improve patient stratification and optimise treatment options.

2. Biomarker discovery: Our academics and clinicians work closely with patients. Using clinical samples we can identify molecular markers of disease that can be exploited to facilitate patient stratification.

3. Drug target discovery: We are screening existing drugs and novel compounds for their ability to modify disease pathways. In vitro and cellular assays will combine with computational analysis to define new drug targets.

These three approaches will lead to Experimental Medicine Studies including early clinical trials to assess drug efficacy, the utilisation of biomarkers to identify the patients most likely to benefit from a particular therapy and the improved stratification and treatment of patient cohorts.

The stratification of patients to ensure the most appropriate and effective treatment regimens are followed is a key aim of our theme. The Gastro-Enterology and Mucosal Immunity NIHR Oxford-BRC theme is proving instrumental in this area. Using molecular pathway analysis, biomarker and drug discovery and experimental medicine, inflammatory disorders can be classified to ensure that the patients receive the right treatment at the right time.

Professor Simon Travis at the TGU is leading a programme of real-time data collection in ulcerative colitis to relate fluctuations in disease activity with the biology of the disease. TrueColours-ulcerative colitis (TCUC) is a comprehensive real-time web-based programme for patients with UC. It monitors multiple parameters via electronic questionnaires: symptoms, quality of life (QoL), outcomes (eg emergency department visits) and demographics. Medications are entered and personalised treatment guidance formulated. This information, graphically displayed on a traffic light system, is available to the patient and clinical team via the TCUC website (https://ouh.truecolours.nhs.uk/ibd/en/), and is housed on a secure National Health Service server. Proteomic and metabolomics analysis of serial biological samples collected over 6 months from patients is planned.

Paul Klenerman,
Sidney Truelove Professor
of Gastroenterology

Managing the epidemic of chronic disease

Advances in medicine over the past few decades have led to an unprecedented increase in life expectancy and reduction in major disabilities. However, these achievements have also contributed to a rise in often poorly understood chronic conditions and their co-occurrence among individuals. This trend together with growing public expectations from medicine and healthcare, has generated new challenges for patients, clinicians, researchers and health policy makers in managing the burden of chronic disease and multi-morbidity. The theme builds on Big Data and Digital Technologies to advance the science and management of chronic diseases.

Deep Medicine programme

Researchers at the George Institute UK, have collaborated with the Oxford Martin School at the University of Oxford on a major new programme looking at how machine intelligence can be used to treat chronic disease.

The recently funded programme on Deep Medicine (http://deepmedicine.medsci.ox.ac.uk/) is led by a core team of interdisciplinary researchers who work together towards the common goal of advancing "data science" in medicine and healthcare. By aligning their goals, Deep Medicine is developing a Big (Health) Data analytics platform that will bring together the silos of traditional research methods and datasets, and will employ a powerful High-Performance Computing (HPC) infrastructure for extracting insights from medical data. Deep Medicine has already accessed some of the largest and most complex biomedical datasets that have ever been collected and is applying state-of-the-art machine intelligence algorithms and statistical methods to them to generate insights into complex disease patterns and risk trajectories in a replicable and scalable way. The clusters of new correlations and previously unknown associations will lead to new insights to direct research and transform healthcare delivery.

SUPPORT-HF programme

An exemplary project is the SUPPORT-HF programme (http://supporthf.org/), which aims to support patients with chronic heart failure to manage their conditions better at home. After several years of system design and usability testing, the project is now at the trial stage. About 200 patients from across the UK have been randomised into the trial, which now tests the hypothesis that centrally supported and data driven approaches to chronic disease management can reduce the gap between evidence and practice.

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Stephen MacMahon
Head of the George Institute for Global Health and Professor of Medicine
University of Oxford

Mobile phone-based six-minute walk test

The six-minute walk test (6MWT) consists of walking as far as one can in six minutes. This simple test is a standard way for measuring exercise capacity in patients with cardiopulmonary disease. It is also used as a parameter for optimising the use of expensive treatments.

The problem:

6MWT are performed in hospitals to assess how patients are, and their response to treatment. However many factors on the day of the test can affect patients performance, including tiredness or stress. The test requires two physiologists and it can be difficult to find a quiet corridor in a hospital to perform the test

The solution:

We are developing an “app” to allow patients to easily perform the 6MWT at home (indoors or outdoors).

The app will send the results of the tests to a website accessible by the doctors under strict data protection rules. The quality and the reliability of the measurement is expected to be better than the hospital-based one.

The future:

Patients will be able to assess their physical condition more comfortably and as often as they want. Their treatment will be improved thanks to more reliable and frequent data.

Currently, we are testing this system in the clinic for pulmonary hypertension in the JR hospital.

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New methods for stratifying airway disease

New BRC-supported technology promises to distinguish between different respiratory pathologies.

A new medical device has been developed by University of Oxford researchers supported by the Oxford Biomedical Research Centre (BRC). Data from this device, used with a computational model, can quantify lung inhomogeneity.

All lungs, young or old, healthy or diseased, have some degree of inhomogeneity – the distribution of fresh gas entering the lungs with each breath does not match perfectly with the distribution of the blood perfusing the lungs. It has long been known that different diseases affect the distributions of gas and blood flow in different ways, but previous methods measuring these distributions are invasive, time-consuming, and/or insensitive to early disease.

Researchers at the University of Oxford have devised a simple, quick, and non-invasive test that makes use of a new medical device that has been under development for the last 10 years. All that is required is a 15-minute period breathing through a mouthpiece (see fig 1). This can be a standalone test, or included as part of routine lung-function tests.

Data from these tests are then used with a computational model, also developed with support from the Oxford BRC. The model generates several numerical parameters that describe the patient's lungs; this includes how inhomogeneous the lungs are with regard to air flow, blood flow, and 'deadspace' (airways that do not exchange gas with the blood).

The research team have already demonstrated that the model can distinguish between young participants, older participants, and patients with Chronic Obstructive Pulmonary Disease (COPD) (see fig 2). They are now working to sub-classify (stratify) patients with different forms of COPD. Specifically, lungs with emphysema, and lungs with small airways inflammation should show differences in deadspace and compliance distributions and the research team is hopeful of finding promising results.

This new technology, simple patient procedure, and novel computational model could lead to the application of precision medicine to diagnose early-stage lung disease and refine treatment decisions.

Fig 1) Participant breathing through Molecular Flow Sensor.

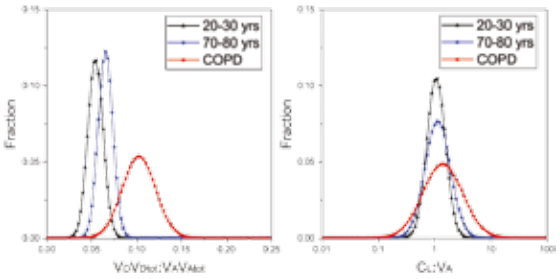

Fig 2) Distributions generated by the computational model for three participant groups (young participants, older participants, and patients with COPD). Left panel shows distribution of deadspace; right panel shows distribution of lung compliance. Lower, wider distributions correspond to less effective gas exchange within the lungs.

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Oxford Acute Referral System (OARS)

An electronic system to manage the referral of patients to specialist hospital services.

The process of complex-patient referral can be unstructured and inefficient leading to suboptimal care. OUH has developed OARS which enables transparent, real-time sharing of clinical knowledge, between teams across a care network.

Patients who require urgent or emergency care from specialist (tertiary) hospital services are often the most unwell individuals the organisation cares for. Their care is challenging:

- They are referred from many different institutions, covering a large population, over a wide geographical area.
- Multiple members of different teams are involved in their care and accurate decision-making requires the synthesis of numerous different factors in each case.
- The capacity to provide specialist care is limited, resources are expensive and outcomes are often time-critical.

Despite (or perhaps, because of) these features, the process of acute specialist referrals has typically been conducted via repeated telephone calls with limited data capture. OUH identified the opportunity to improve the quality and efficiency of specialist clinical services by digitising this acute referral pathway.

A vital aspect of this project was the involvement of the Quality, Reliability, Safety and Teamwork Unit of the Nuffield Department of Surgical Sciences, University of Oxford. Their academic Human Factors research team helped lead a user-centered design process that involving widespread stakeholder engagement.

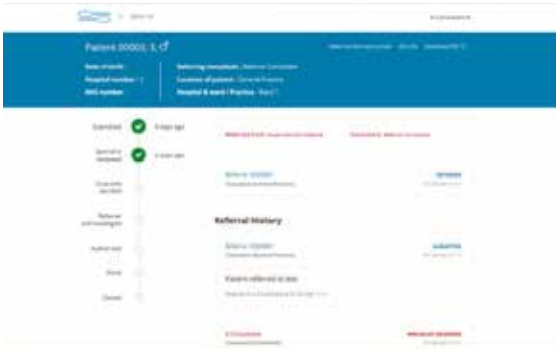
Ethnographic research and design-thinking approaches identified that other electronic referral systems that simply passed a referral from one individual to another did not fit the existing process. A new system would need recapitulate the to-and-fro of repeated voice conversations that were the basis of the existing process.

The system was developed and deployed in partnership between a local SME, OUH and the University of Oxford.

OARS went live in the Department of Neurosurgery in April 2016. Over the first year of use 3000 healthcare workers registered and over 5500 referrals have been managed by the system. During 2017 OARS will be rolled-out into further specialties in Oxford and opportunities to extend its use into other institutions will be explored.

OARS being used on a tablet

Further information can be found at http://www.ouh.nhs.uk/services/oars/default.aspx



The referral 'conversation'

Oxford University Hospitals NHS
NHS Foundation Trust

NUFFIELD DEPARTMENT OF SURGICAL SCIENCES

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Selfies to help diagnose rare diseases

Facial recognition for aiding diagnoses of rare diseases by diagnostically relevant phenotyping.

Minerva & Me is a public engagement in science effort to allow people to share photographs and selfies with researchers. From this computer vision and machine learning models can be developed to aid diagnosis of rare diseases.

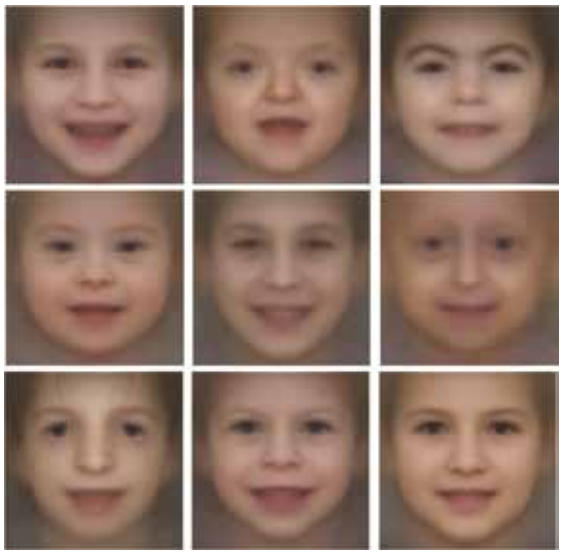
Rare diseases are collectively common, with estimates of up to 8% of the population having some type of rare disease. The clinical pathway and patient journey to get a diagnosis is frequently costly and protracted, often taking several years.

We are developing facial recognition-like approaches to produce models to aid diagnosis of rare diseases. This holds great promise as a clinical genetics tool around the world by bringing together and identifying ultra-rare diseases and conditions. However, this is a big data problem – which can only be addressed through training with broad representations of people with different diagnoses, and variation in ancestry, age and clinical settings.

To make this possible we have formed a research consortium with the purpose of allowing research collaboration and data sharing, the Minerva Consortium. The purpose is to allow a healthy competition and development of basic research into approaches.

Minerva & Me is a research web portal, designed to allow families and patients with rare diseases to participate and help drive this research forward. Through this portal it is possible to share family photographs and control how these can be used in research projects.

Minerva & Me is hosted on secure University of Oxford servers and is controlled by an Advisory Board with patient group, clinician, data security and legal representatives. At the core of the design of Minerva & Me is that participants retain control of their own data and how that data is used in research. Participants can always change the way their data can be used, or delete the data entirely if they so choose. The hope is that Minerva & Me will be a growing resource hub for patients to participate with their medical information and photographs in research projects into rare diseases.



Dr Christoffer Nellåker
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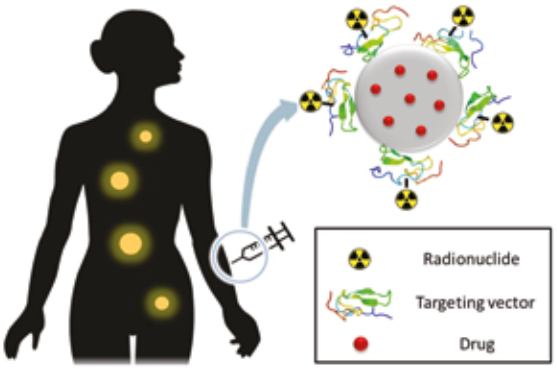
Theranostic nanoparticles

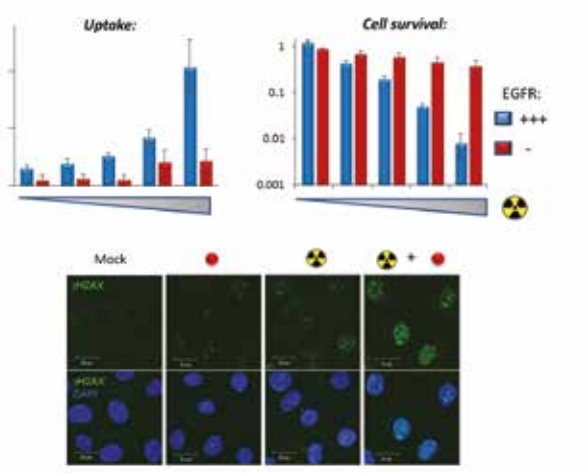
Cancer imaging and treatment.

Development of new therapeutic combinations for targeted drug delivery to sites of disease

Oncology, including the treatment of cancers of unmet need such as those of the oesophagus and pancreas, remains centred around the systemic administration of highly cytotoxic drugs such as cisplatin, usually employed in combination with radiotherapy. However, the toxicity associated with conventional chemotherapy agents severely restricts their use. Although technical advances have enabled highly precise delivery of therapeutic radiation, exposure of surrounding healthy tissue still limits the dose that can be administered to a cancer.



To address this, a multi-disciplinary research team led by Professor Katherine Vallis (Oncology) and Professor Robert Carlisle (Bioengineering) aims to develop nanoparticle-based dual therapeutic/imaging systems - “theranostics” - for cancer treatment. These drug delivery vehicles combine both a radiopharmaceutical suitable for PET/SPECT imaging and a complimentary small molecule agent as the therapeutic payload within. Cancer specific uptake is achieved by targeting receptors over-expressed by cancer – but crucially not healthy - cells. As a lead candidate we have developed nanoparticles for the synchronous targeted delivery of the radionuclide Indium-111, a SPECT imaging agent which also generates DNA double-strand breaks, and a novel ruthenium-based radiosensitizing compound, Ru1. This has been tested in oesophageal cancer cells that over-express the membrane receptor, epidermal growth factor receptor.



Working side-by-side with Theragnostics, we aim to streamline the production of radiolabelled nanoparticles employing good manufacturing practice (GMP) to develop a radiolabelled nanoparticle kit with optimised yields, minimising loss of reagents and improving preparation efficiency to a level suitable for clinical application. As all components of this system: targeting ligand, payload and radionuclide, may be varied, specific functionalities may be introduced and tailored to specific tumours. Thus our strategy enables patient-specific treatment and promotes the desirable goal of personalised medicine. Further developmental work and pre-clinical studies are ongoing.

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Oxford Biodesign

Devising innovative technology-based solution for healthcare needs.

In the UK, every 10 seconds someone experiences a potentially life-threatening asthma attack, every 8 minutes someone is hospitalised and every 8 hours someone dies due to asthma. These numbers, worst since 2003, demonstrate the increasing severity of the problem and unmet need for an effective solution. asthma attacks cause significant disruption in daily activities, panic, trauma, and even deaths. Moreover, the vast majority of these asthma attacks are preventable.

The NHS spends about £1 billion each year caring for people with asthma, while the unplanned expenditure due to emergency visits and hospitalisations alone costs the EU over €5 billion a year. The lack of reliable monitoring mechanisms leaves clinicians in the dark about their patients' condition when they are out of the clinic or hospital, making it very difficult to provide optimum treatment.

We are developing an innovative monitoring device with integrated analytics that can track objective markers of disease severity in asthma patients, empowering them to self-manage their asthma and enable timely intervention before their condition worsens considerably. The system, designed to achieve high-compliance, high-quality monitoring for long term use, would include clinicians and adaptive algorithms in the process to deliver optimal personalised asthma care.

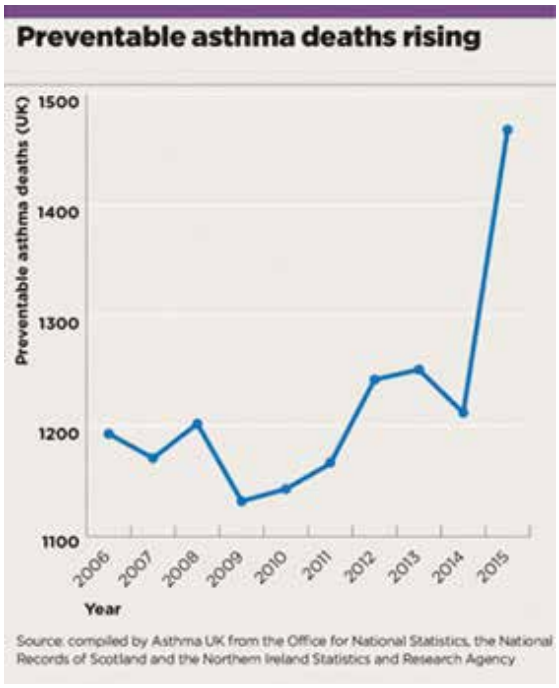
What do patients and healthcare professionals want from asthma technology?

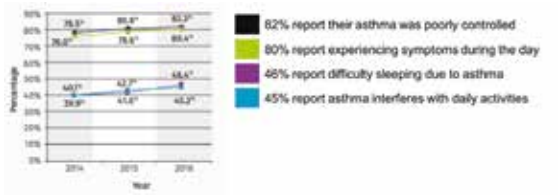
In 2015 Asthma UK asked patients and healthcare professionals for their views on what they would like from an mHealth system.[10]

Nearly three-quarters of patients wanted to see an mHealth device that would help them monitor their asthma. In addition, nearly half suggested they would value a system which could be used as part of their asthma action plan and tell them if changes to asthma medication have improved their asthma and when to seek medical attention.

Over three-quarters of healthcare professionals said they would value an mHealth system that would monitor patients' asthma symptoms over time and provide patients with an asthma action plan.

With our combined expertise in clinical practice, electrical engineering, signal processing and machine learning, we are geared up to deliver clinically effective and commercial scalable solutions for patients, clinicians and payers of healthcare.





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Mapping the personal health space

Understanding the health of a patient in the context of their environment.

As a healthcare Internet of Things company, we understand the health of a patient in the context of their environment and generate actionable information and discoveries that improve health and reduce costs.

Drayson Technologies is a healthcare IoT company addressing major global health problems. Chronic disease is the single biggest driver of ballooning healthcare costs (CDC, 2012) and air pollution is the biggest environmental health risk and a major cause of chronic disease exacerbations (WHO, Urban Air Quality Database, 2016).

We adopt a holistic patient approach to the development of digital pharmaceuticals that fuses health and environmental monitoring data across the whole care pathway from hospital to home.

Environmental monitoring

- CleanSpace™ is the world's most comprehensive air pollution monitoring platform. This IoT sensor network uses machine-learning and connected smart sensors to create hyper-local air pollution information to enable people to "see the air they breathe" and to help enterprises and municipalities improve air quality.

Health monitoring

- Drayson Technologies, Oxford University and Oxford University Hospitals (OUH) NHS Foundation Trust have signed an agreement to collaborate on the development, testing and future commercialisation of three clinically validated digital health products arising from research undertaken by engineers and doctors at Oxford University and the OUH Trust.
- SEND, GDm-health, EDGE-COPD are digital health products that use machine learning artificial intelligence software, to analyse data and provide decision support and patient safety information to both patients and healthcare professionals. The products have undergone significant clinical testing and validation involving over 100,000 patients and generated over 20 million data records to date. Results suggest that these technologies could deliver significant improvements in patient health outcomes and reduction in costs for the NHS.

Our expertise in regulated diagnostic and therapeutic product development and marketing combined with expertise in digital health sensor networking, wireless technology, Big Data and machine learning enables us to create effective new care pathways for the diagnostic and treatment of chronic disease.

CleanSpace is the world's most comprehensive air pollution monitoring platform.

EDGE (COPD management)

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Precision Medicine and Digital Health

In Silico Prediction of Drug Effects on Human Ventricular Electrophysiology

Using the Virtual Assay Software and Comparison to In Vitro Data.

- **Electrophysiological cardiotoxicity** is an important cause of drug withdrawal from the market
- **Inter-subject variability** is the key to explain the different responses to diseases and drug action

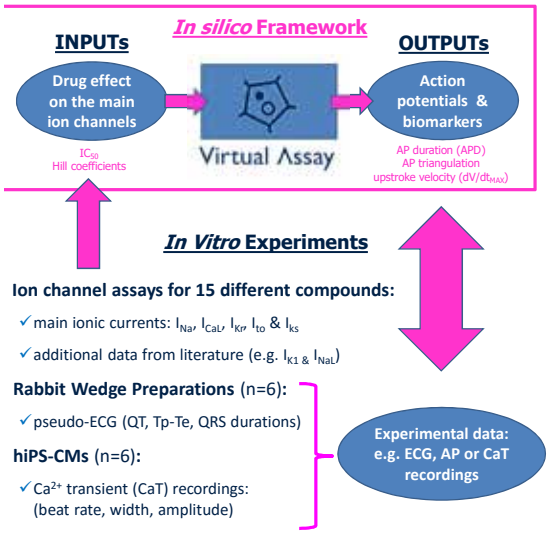
  ✓**"Who, When and Why?"** people may be at risk
- ***In Silico* models** constitute a new frontier for the assessment of drug cardiotoxicity

  ✓ Help in the **translation from animal to human**

  ✓ **Identify causes of cardiotoxicity**

  ***In Silico* models** are included in the **Comprehensive in vitro Proarrhythmia Assay (CIPA):**

  ✓ novel safety screening proposal based on in vitro and *in silico* cellular cardiac electrophysiology predictions (Sager et al. AHJ. 2014)



Conclusions

- Virtual Assay Software constitutes a user-friendly powerful *in silico* tool for safety pharmacology assessment
- *In silico* predictions with Virtual Assay are in overall agreement with rabbit wedge and hiPS invitrocalcium measurements
- *In silico* human cell populations are able to reproduce inter-cellular variability in drug responses, not captured by a single action potential model
- *In silico* human cell populations identify key factor determining inter-subject variability in cardiotoxic response

UNIVERSITY OF OXFORD

Oxford Computer Consultants

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Precision Medicine and Digital Health

Autonomous speech-based monitoring of health at scale

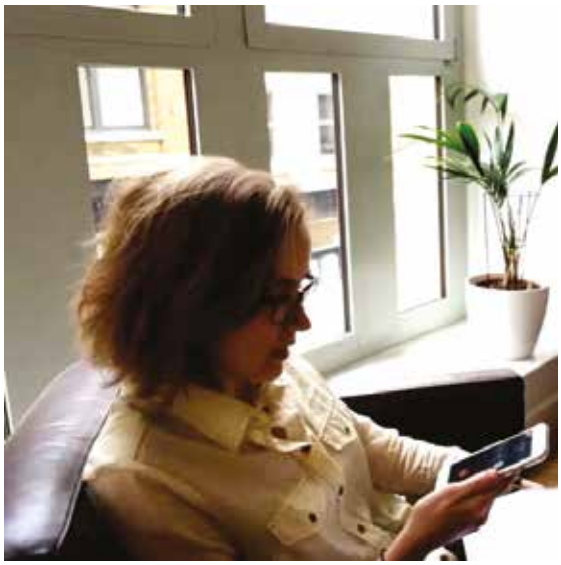
Ufonia is an Oxford University Innovation start-up company that is developing voice chat-bot systems that can monitor individuals' health and wellness across entire patient populations.

ufonia

Ufonia's initial product supports people with long-term conditions ('chronic diseases'), such as diabetes, asthma and heart failure. These patients account for 70% of health and social care expenditure. Throughout the world this sector of healthcare faces the greatest challenge from increasing demand. Forthcoming 'accountable care systems' will incentivise providers to more intensively monitor these patients. But, the additional workforce capacity to do so, does not exist. Ufonia is developing products to meet these needs. It is an automated system, delivered via a telephone, that can engage entire patient populations without the need for specific software, devices or training.

Further market opportunities exist for Ufonia to provide new and effective engagement channels for secondary care, research, pharmaceutical, medical device and insurance providers.

Ufonia has been supported by funding from the Science and Technology Facilities Councils' Harwell HealthTec Cluster and Hartree, High Performance Computing Centre, plus IBM Watson.



A user talking to the system

Hartree Centre
Science & Technology Facilities Council

Harwell

IBM Watson

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In Silico Prediction of Drug Effects on Human Ventricular Electrophysiology Using the Virtual Assay Software and Comparison to *In Vitro* Data

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INTRODUCTION

- **Electrophysiological cardiotoxicity** is an important cause of drug withdrawal from the market
- **Inter-subject variability** is the key to explain the different responses to diseases and drug action
  - ✓**"Who, When and Why?"** people may be at risk
- ***In Silico* models** constitute a new frontier for the assessment of drug cardiotoxicity
  - ✓Help in the **translation from animal to human**
  - ✓**Identify causes of cardiotoxicity**
- ***In Silico* models** are included in the **Comprehensive *in vitro* Proarrhythmia Assay (CIPA)**:
  - ✓novel safety screening proposal based on *in vitro* and *in silico* cellular cardiac electrophysiology predictions **(Sager et al. AHJ. 2014)**

AIMS

***In silico* assessment of the potential pro-arrhythmic effects of 15 compounds, considering inter-subject variability by using a human *in silico* cell population**

Comparison of simulation results against *in vitro* experimental data acquired in rabbit wedges and human iPSC-CMs

METHODS

Virtual Assay Software

- New software for ***in silico* drug testing**: user friendly
- **Population of human ventricular *in silico* cells accounting for inter-subject variability**
- **Drug assays** for different compounds and multiple concentrations
  - ✓electrophysiological changes and potential pro-arrhythmic effects

***In silico* Framework**

INPUTs: Drug effect on the main ion channels (IC_{50}, Hill coefficients)

Virtual Assay

OUTPUTs: Action potentials & biomarkers (AP duration (APD), AP triangulation, upstroke velocity (dV/dt_{MAX}))

In Vitro Experiments

- **Ion channel assays for 15 different compounds:**
  - ✓main ionic currents: I_{Na}, I_{CaL}, I_{Kr}, I_{to} & I_{Ks}
  - ✓additional data from literature (e.g. I_{K1} & I_{NaL})
- **Rabbit Wedge Preparations** (n=6):
  - ✓pseudo-ECG (QT, Tp-Te, QRS durations)
- **hiPS-CMs** (n=6):
  - ✓Ca^{2+} transient (CaT) recordings: (beat rate, width, amplitude)

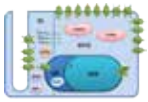
Experimental data: e.g. ECG, AP or CaT recordings

The idea behind Virtual Assay:

Population of Human *In Silico* Cell Models

- **Traditional modelling techniques:**
  - ✓**a single human AP model**, representative of the **average cell** behaviour

O'Hara et al. PLOS Comp Biol 2011

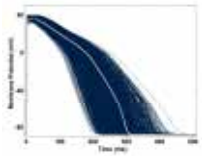


cell-to-cell variability

- **Population of models** approach: **(Britton et al. PNAS 2013)**
  - ✓**Thousands of human in silico cells**, sharing the same equations but with different parameter sets

- **1,239 human models**
- **9 randomised parameters** (maximal current conductances)
- **[50% - 150%]** range

Population of Human Ventricular Action Potential Model in Virtual Assay



Drug Studies in Virtual Assay

- **How to run a drug study in VA**
  - ✓create a population of human cell models
  - ✓insert the information about the effect of the selected compounds on the main ion channels
  - ✓run the simulation

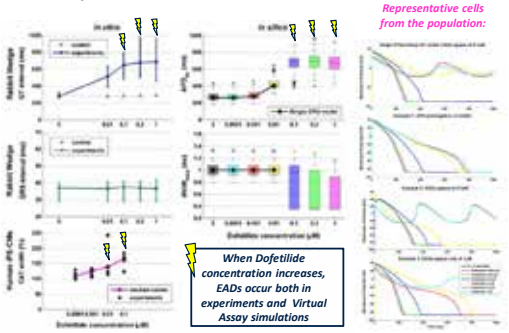
More information on VA: www.cs.ox.ac.uk/ccs/tools

RESULTS: CASE STUDIES

Case Study 1: Dofetilide

- I_{Kr} (hERG) blocker, slightly affect I_{Na} and I_{CaL}
- Dose dependent TdP risk: pro-arrhythmic for some patients
- Virtual Assay software predicts APD increase, and EADs were observed in some human in silico cells, in agreement with the experiments

Representative cells from the population:

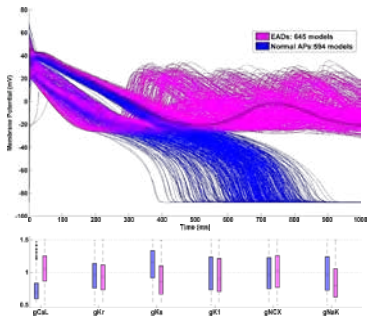


When Dofetilide concentration increases, EADs occur both in experiments and Virtual Assay simulations

- Simulation in a population of human cell models, accounting for inter-subject variability, allow to reproduce the variety of behaviours shown in the experiments, which cannot be captured by a single action potential model

What causes the difference in response to Dofetilide?

Population of human ventricular AP models with Dofetilide 0.1μM:



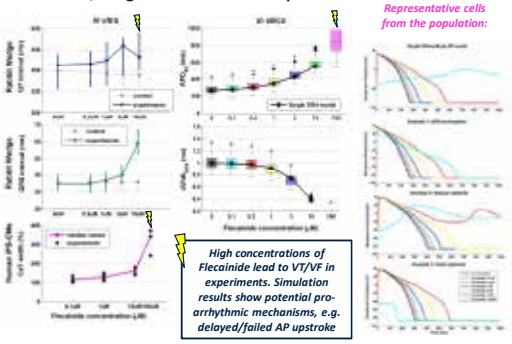
- ***In silico*, EADs occur in human cells with low repolarisation reserve:**
  - ✓**high I_{CaL}**
  - ✓**low repolarising K^+ currents**

The ionic conductances distribution in the population of models highlights the key factors underlying cell-to-cell variability in drug response

Case Study 2: Flecainide

- Fast I_{Na} blocker, also affect I_{Kr} and I_{CaL}
- It may be pro-arrhythmic in some patients
- Simulation results show APD increase and upstroke velocity decrease, in agreement with the experiments

Representative cells from the population:

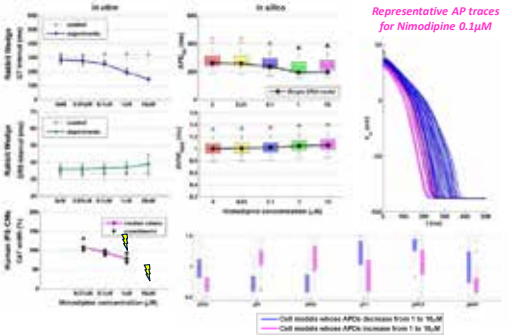


High concentrations of Flecainide lead to VT/VF in experiments. Simulation results show potential pro-arrhythmic mechanisms, e.g. delayed/failed AP upstroke

Case Study 3: Nimodipine

- Non-selective I_{CaL} blocker, also affects I_{Kr} and I_{Na}
- Up to 1μM, simulation results show APD decrease in agreement with experiments
- At 10μM, QT interval further decreases in rabbit, beating stops in hiPS-CMs (1) and *in silico* APD either increases or decreases, depending on the human cell balance between I_{Kr} and I_{CaL}

Representative AP traces for Nimodipine 0.1μM



RESULTS: SUMMARY

- 15 compounds, multiple concentrations
- *In silico* results compared with *in vitro* experiments
  - ✓simulated APD_{90} *vs* rabbit wedge QT interval & CaT width in hiPS-CMs
  - ✓simulated dV/dt_{MAX} *vs* QRS interval (opposite variations)

Tested Compounds	Main Ionic Current Block(s)	Tested Concentrations	*in vitro* Results			*in silico* Results	
			Rabbit Wedge		hiPS-CMs	Virtual Assay	
			QT	QRS	CaT width	APD_{90}	dV/dt_{MAX}
Dofetilide	hERG	0.0001-1 μM	↑	–	↑	↑	–
Sparfloxacin	hERG	0.1-100 μM	↑	–	↑	↑	–
Moxifloxacin	hERG	0.3-300 μM	↑	↑	↑	↑	↓
Procainamide	hERG/I_{Na}	0.1-100 μM	↑	↑	↑	↑	↓
Flecainide	I_{Na}/hERG	0.1-100 μM	↑	↑	↑	↑	↓
Mexiletine	I_{Na}/hERG	0.1-10 μM	–	↓	↑	↑	↓
Phenytoin	I_{Na}/hERG	0.03-30 μM	–	↑	↑	↑	↓
Lidocaine	I_{Na}/hERG	0.1-100 μM	–	↑	↑	↑	↓
Primidone	I_{Na}/hERG	0.01-10 μM	–	–	↑	↑	–
Nimodipine	I_{CaL}/hERG	0.01-1 μM	↓	–	↓	↓	–
Nisoldipine	I_{CaL}/hERG	0.01-1 μM	↓	–	–	↓	–
Bepridil	I_{CaL}/hERG	0.3-3 μM	↑	↑	↑	↑	↓
Verapamil	hERG/I_{CaL}	0.001-0.1 μM	↑	–	↓	↑	–
$BaCl_2$	I_{K1}	0.1-100 μM	↑	–	–	↑	–
Ranolazine	Late I_{Na}/hERG	0.01-100 μM	↑	↑	↑	↑	↓

- Table legend: – <5% ↑↓ 5-10% ↑↓ >10%

CONCLUSIONS

- **Virtual Assay Software constitutes a user-friendly powerful *in silico* tool for safety pharmacology assessment**
- ***In silico* predictions with Virtual Assay are in overall agreement with rabbit wedge and hiPS *in vitro* calcium measurements**
- ***In silico* human cell populations are able to reproduce inter-cellular variability in drug responses, not captured by a single action potential model**
- ***In silico* human cell populations identify key factor determining inter-subject variability in cardiotoxic response**

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