Translational Medicine: **fundamental research, drug discovery and more!**

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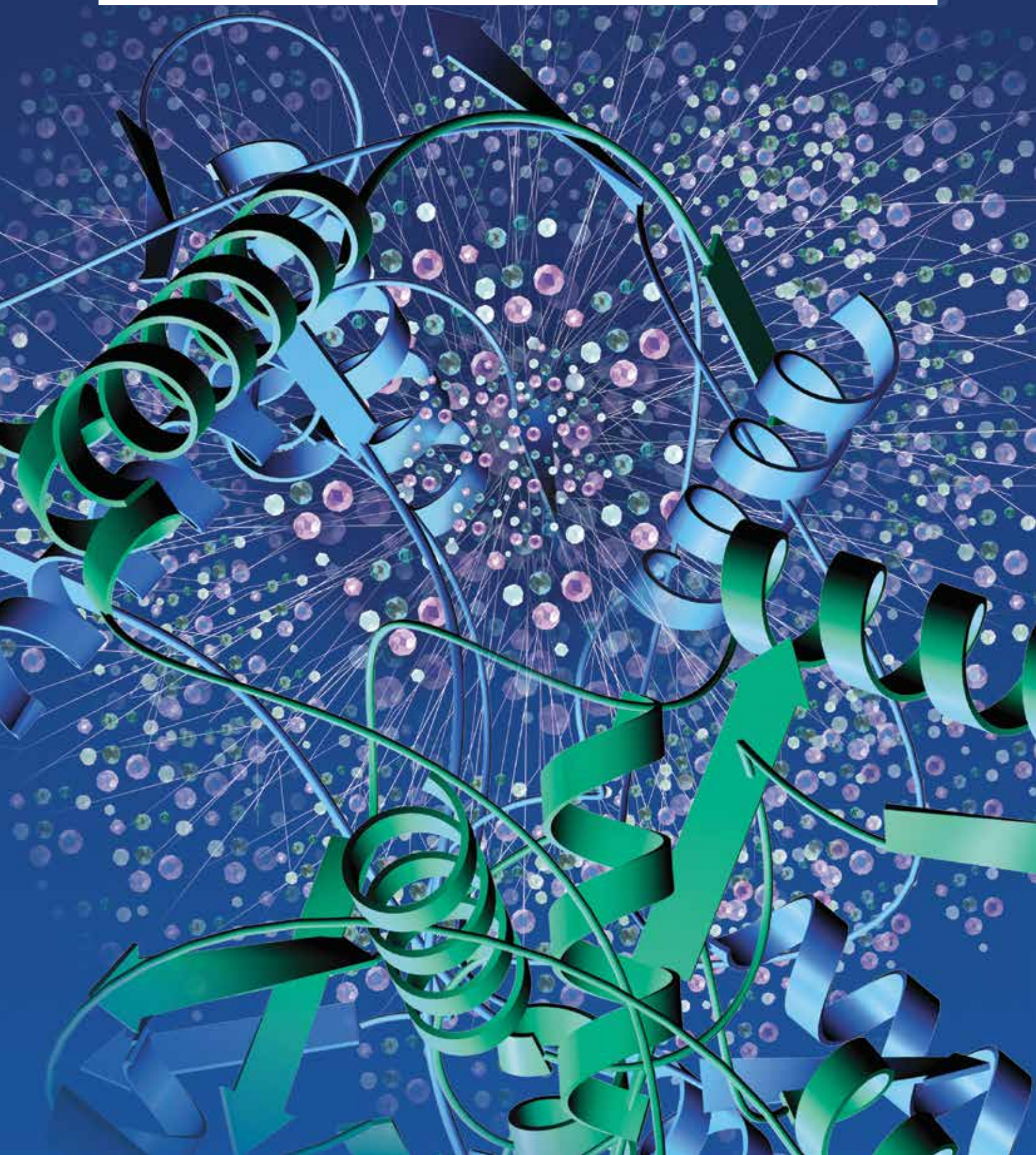


TRANSLATIONAL MEDICINE: FUNDAMENTAL RESEARCH, DRUG DISCOVERY AND MORE!

Professor Seamas Donnelly and Dr Ciaran O'Reilly are currently working together in a cross-disciplinary team, uncovering the role of macrophage migration inhibitory factor in inflammation and cancer, and investigating promising small molecule therapeutic approaches.

Your team seems to represent the archetype of Translational Medicine. How is it like for two researchers from such distinct backgrounds to collaborate?

When doing translational research, I think it's important to surround oneself with scientists from many different backgrounds. Each person brings with them a unique set of skills and tackles problems from a slightly different perspective. Having this wealth of experience together also allows us to develop novel ideas and exploit any potential opportunities that arise over the course of our projects. We are fortunate in our group to have clinicians, immunologists, microbiologists, cell biologists and medicinal chemists working together resulting in a productive synergy within the team. Translational Medicine is a very exciting space to be in. The opportunity to make a positive impact on the lives of patients is the key driving force behind everyone in the group.

CADD generates enormous amounts of candidate compounds with good predicted therapeutic characteristics. How do you select which ones to develop further? What are the main reasons for CADD hit compounds to be excluded? Does your CADD software also include predictions pertaining to drug metabolism/safety?

We tend to conduct virtual screening campaigns using commercially available libraries to look for hits against our target of choice, in this case, the pro-inflammatory protein, MIF. Within each program there are scoring functions that will rank compounds based on the likelihood it will bind to your target. We validate the accuracy of these scoring functions using training sets and docking of the virtual library into the binding site on the protein of interest follows this. Once the compounds have been docked, we cluster the compounds based on their similarity and then choose based on their rank within the clusters in order to get as many different chemotypes as we can for screening. We do filter out a lot of potentially reactive compounds and also any compounds which have moieties which may interfere with an assay (these compounds are called PAINS-pan assay interference compounds). We also explore the IP space around the structure to make sure it's not too crowded. Some of the programs do have a toxicity prediction facility integrated within them but our group rarely uses them, we tend to test the compounds in house once they've arrived from the vendors.

Is there typically a great difference between the *in-vitro* and *in-silico* properties of the compounds you chose to pursue further?

In the past, we have found a strong correlation between our *in-silico* leads and in vitro validation experiments which suggests our models are quite accurate at predicting which molecules will bind to our target of interest.

Given that MIF has both intracellular and extracellular activity, have there been efforts towards modulating the inhibitor's diffusion/transport across the cellular membrane?

Drug discovery is a big part of our work. It complements one of out strategic objectives, namely, enhancing our understanding of the mechanisms behind the development of chronic inflammation and our drug discovery programs have evolved from that space. Our group seeks to examine antibody drug conjugates and nanoparticle delivery systems to target organs and specific cells.

Are the small molecule MIF inhibitors expected to be delivered as IV formulations or is there hope for oral formulations? Are their safety profiles compatible with out-patient regimens?

We are a respiratory science group and have demonstrated a role for MIF in many respiratory diseases including ARDS, cystic fibrosis, asthma and lung cancer so a particular interest to us is exploring aerosolised systems for delivery of anti-inflammatory/anti-cancer compounds directly to the lungs.

How does the synthesis of novel small molecules compare with the development of monoclonal antibodies? Are new compounds that eventually make it to oncologist's arsenals expected to break the trend of escalating prices?

From our perspective, low molecular weight small molecules can usually be synthesised through well-defined routes and purified to give rise to a single product that is always identical. Small molecules are also more stable than their monoclonal antibody counterparts. In terms of cost, development of antibody based therapies is more expensive compared to their small molecule counterparts – so that has certain advantages in cost-containment strategies.

TARGETING MACROPHAGE MIGRATION INHIBITORY FACTOR, IN CANCER

New therapeutic approaches must focus on increasingly less conspicuous targets, seeking weak links in the chain of oncogenesis. The research conducted by Professor Seamas Donnelly and Dr Ciaran O'Reilly at Trinity College Dublin is expanding our understanding of cancer, while developing new, potentially lifesaving drugs.

Targeting MIF in Cancer Therapeutics

The Macrophage Migration Inhibitory Factor (MIF) is a protein that plays a key role in the body's natural inflammatory response. This strange molecule has been known to be an integral part of the inflammatory process since the 1950s, but its behaviour and properties are so unlike any other cellular messenger molecules that it has become a class of its own.

Contrary to other immunomodulatory molecules, MIF is present in cells even before any inflammatory processes have begun. It lies dormant, waiting to play its role in the inflammatory cascade, whenever the need may arise. Furthermore, this molecule acts not only as a messenger, but also as an enzyme in its own right.

Chronic inflammation and cancer, two sides of the same coin

From an evolutionary perspective, defending the body from invading microorganisms is the immune system's top priority, with protection from cancer cells playing a somewhat secondary role. The inflammatory cascade seeks to deploy a massive counter-response to external stresses, recruiting immune cells to infection sites, promoting the development of additional blood vessels, and preventing cells from dying before their defensive or support capabilities have been exhausted. The fact that MIF drives a number of pro-inflammatory pathways, explains why it has such a key role within inflammation. It is well recognised that many chronic inflammatory diseases predispose one to the development of cancer. Exaggerated MIF activity has been shown to be is a key driver in the development of chronic inflammation, causing persistent cell damage and stress long after the initial triggering stimulus has been removed. In addition, many of the unique biological activities of MIF are harnessed by cancer for its survival advantage. Consequently, MIF has become a prime pharmacological target in our fight against cancer.

The small molecule paradigm

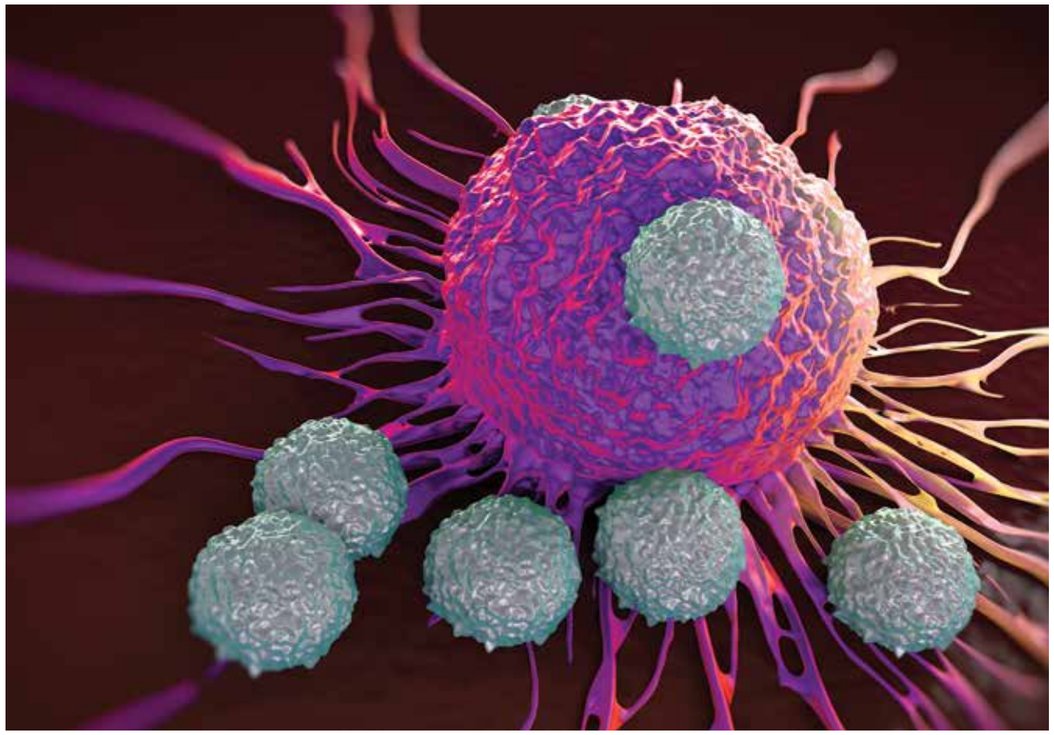
While monoclonal antibodies are renowned for their selectivity and effectiveness, there is also something to be said about the new generation of small molecule drugs. These relatively simple compounds of low molecular weight, are able to interact with biologically active sites. These compounds are small and often stable enough to be delivered orally. Furthermore, small molecules build upon a foundation of decades of chemical synthesis, making them relatively inexpensive to produce and purify, and of high value from a pharmacoeconomic perspective.

When compared to monoclonal antibodies, small molecules also provide a more rational approach to drug design. In the case of antibodies, candidate molecules are the product of random variation, that may eventually produce affinity to biologically relevant sites. For small molecules, the process of drug design is carefully directed, and aimed towards producing a specific biological effect, better pharmacokinetics or greater shelf life.

Lastly, it is also possible to benefit from the best of both worlds. By binding small molecule drugs to monoclonal antibodies or encapsulating them within nanoparticles, it is also possible to achieve systemic distribution, with very precise delivery to target cells.

Targeting MIF with small molecules

One of the classical methods for studying biological function is to structurally disrupt your protein of interest, and trace the consequences in cellular systems. Professor Seamas Donnelly and Dr Ciaran O'Reilly and their team at Trinity College Dublin are using tailored molecules to temporarily perturb the function of MIF. In this way, the researchers have been able to better ascertain its role in inflammation and in the development and progression of cancer. By disrupting the



catalytic function of MIF and/or its binding to both extracellular and intracellular receptors, these candidate drugs in-vivo have been able to significantly reduce tumour lethality.
Alternative strategies include utilising covalent inhibitors that bind irreversibly with MIF's active site, destroying its functionality permanently. 4-Iodo-6-phenylpyrimidine is an example of one of these chemicals which, although normally shunned by researchers for fear of unwanted reactions, actually has the same mechanism of action as naturally occurring dietary isothiocyanates, present in vegetables such as Brussels sprouts and watercress. Having an irreversible mechanism of action allows for much lower effective dosages, placing significantly less metabolic burden on the patient's system.

Finally, it is also possible to target other proteins responsible for stabilising the MIF trimer, like Heat Shock Protein 90, as is the case of 17-(alkylamino)-17-(demethoxygeldanamycin). This approach also benefits from the fact that other pro-inflammatory factors are stabilised by this protein, magnifying a drug's potential effect.

The virtual lab

The simplicity of small molecules makes them prime candidates for Computer Aided Drug design (CADD), highlighting once again the power of working in a cross-disciplinary research environment. Using this technique, the cancer fighting properties of never before seen compounds, can be assessed long before they are even synthesised, allowing researchers to bypass potentially expensive and time consuming projects.

In a fashion that is both faster and cheaper than the drug discovery of old, thousands of compounds are screened for their potential usefulness and are sieved by algorithm design to find patterns that equate to clinical effectiveness. Once the virtual world has yielded a great diversity of potentially effective new drugs, they can then be synthesised and assessed in the real world.

'The opportunity to make a positive impact on the lives of patients is the key driving force behind our research group'

The physical lab

In a world of perfect computational models, the entire process of drug discovery could be conducted in the black and white realm of computer programs. Unfortunately, although both commercial and open source CADD software are improving their ability to mimic and predict the real world, there is still no substitute for lab work.

Cell culture studies and animal models are an invaluable part or the team's efforts. These experiments provide a picture of the sometimes unforeseeable behaviour that compounds exhibit in actual living systems. Cell culture work allows researchers to probe the intricacies of a drug's interaction with the multitude of intracellular metabolic and signalling pathways, confirming software predictions more often than not. Animal models yield insights into how drugs behave in complex biological systems, as well as into how normal bodily function arises. Using genetically engineered animals has allowed researchers to shed light on the actual mechanism of MIF's action – a non-trivial feat, given the protein's inherently complex nature.

Meet the researchers

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Professor Seamas Donnelly is the Head of Medicine at Trinity College Dublin and leads the Translational Medicine Group. He holds an Honorary Professorship from the University of Edinburgh and has obtained significant external funding from the Wellcome Trust, Science Foundation Ireland (SFI), the European Union and other national and international funding bodies. He was one of the first clinicians to be awarded a Science Foundation Ireland's (SFI) Principal Investigator Programme award. A leader in medical research, he specialises in small molecule anti-inflammatory agents, with a recent focus on their role as possible cancer treatments.

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He holds a PhD from the National University of Ireland, Galway, for his work on carbohydrate and organic chemistry. Experienced in molecular design and medicinal chemistry, he current major focus is on respiratory disease.

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REFERENCES

O'Reilly C, Doroudian M, Mawhinney L, Donnelly SC, Targeting MIF in Cancer: Therapeutic Strategies, Current Developments, and Future Opportunities, Medicinal Research Reviews, 2016.

Adamali H, Armstrong ME, McLaughlin AM, Cooke G, McKone E, Costello CM, Gallagher CG, Leng L, Baugh JA, Fingerle-Rowson G, Bucala RJ, McLoughlin P, Donnelly SC, Macrophage migration inhibitory factor enzymatic activity, lung inflammation, and cystic fibrosis, Am. J. Respir. Crit. Care Med., 2012, 186, 162–9.

Mawhinney L, Armstrong ME, O'Reilly C, Bucala R, Leng L, Fingerle-Rowson G, Fayne D, Keane MP, Tynan A, Maher L, Cooke G, Lloyd D, Conroy H, Donnelly SC, Macrophage migration inhibitory factor (MIF) enzymatic activity and lung cancer, Mol. Med., 2015, 20, 729–35.

Donnelly SC, Haslett C, Reid PT, Grant IS, Wallace WA, Metz CN, Bruce LJ and Bucala R, Regulatory role for macrophage migration inhibitory factor in acute respiratory distress syndrome, Nat Medicine, 1997, 3, 320–323.

Bucala R, Donnelly SC, Macrophage migration inhibitory factor: A probable link between inflammation and cancer, Immunity, 2007, 26, 281–285.