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Autumn Report from the University of Oxford FOP Research Team

Research News

This year has seen some exciting developments in the search for new treatments for FOP. We have been fortunate to benefit from the talents of our new chemist Dr Aicha Boudhar as well as our long serving team members Dr Ellie Williams, Dr George Kerr, Dr Alex Bullock and Professor Jim Triffitt. Aicha joined the team in the summer of 2014 following a generous donation from Roemex Ltd and a new grant from the Amateurs Trust to FOP Friends. Aicha has provided us with additional expertise in medicinal chemistry that nicely complements our core skills in molecular and cellular biology.

We have known for a number of years now that the FOP gene *ACVR1* encodes for the making of a protein (sometimes called “ALK2”) which functions as a growth factor receptor. Growth factors include hormones, such as human growth hormone and insulin, as well as inflammatory cytokines, which function in immunity. ACVR1 is a receptor for growth factors called BMPs, which control the growth of bone and muscle as well as body shape and stem cell renewal. Most receptors sit in the fatty membranes that form the walls around our cells. These membranes are needed to keep DNA, essential chemicals and water in our cells. Hormones and other growth factors allow cells to communicate with each other. They are secreted by one cell and received as a “message” by another cell, which may be distant or nearby. Receptors such as ACVR1 are the receivers. They bind the growth factor on the outside of the cell (BMPs cannot freely diffuse across the fatty membrane) and then pass on the message by changing their shape or activity on the inside of the cell (i.e. the other side of the membrane). Imagine a dog wagging its tail after a pat on the head! In the case of FOP, a genetic mutation causes the ACVR1 receptor protein to send more message than it should – the dog’s tail wags too much (Figure 1 below). This is known as a “gain of function” mutation.

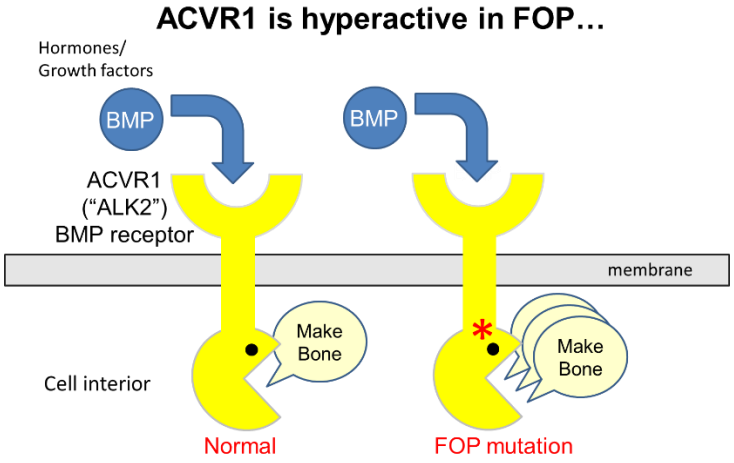

Figure 1. The FOP gene product is a growth factor receptor called ACVR1 (shown yellow above). This receptor sits in the cell membrane (grey bar) waiting for BMP growth factors (blue) to bind on its exterior. When this happens the inner part of the receptor is activated to relay the message to make bone (see left side). However, the ACVR1 receptor is subtly altered in FOP causing it to send more message than normal (see right side). This extra signalling drives the formation of unwanted bone.

There are multiple ways that we could try to correct this faulty activity in FOP and stop the unwanted bone formation (Figure 2 below). Fortunately, we can take lessons from decades of cancer research. Just like the bone in FOP, many tumours grow because of the excessive signals from the growth factor receptors on their cells. The first strategy, which we have been researching, is to develop a drug molecule that will bind on the inside of the cell to the messaging 'mouth' of the receptor and prevent it from sending any more signal that will make bone. The dose of the drug can then be modulated to allow just the normal level of signal to be transmitted. A second related strategy is to use a therapeutic antibody that will block the BMP growth factor from binding to the receptor protein ACVR1. This is the principle behind the breast cancer drug Herceptin. A third distinct approach is being tested in the clinic by Clementia and builds on the work of Professor Maurizio Pacifici and others in Philadelphia. They are using an experimental retinoid drug called "palovarotene" that helps to bolster "stop" signals along the path of building cartilage and bone. Finally, it is possible to stop the FOP gene from being used to make the receptor protein ACVR1. If less receptor is made, the signals it can send to make bone are reduced. This approach can work using a number of different technologies, but the most common is called "RNA silencing". Unfortunately, it is difficult to target drugs of this class to the sites of bone formation in FOP.

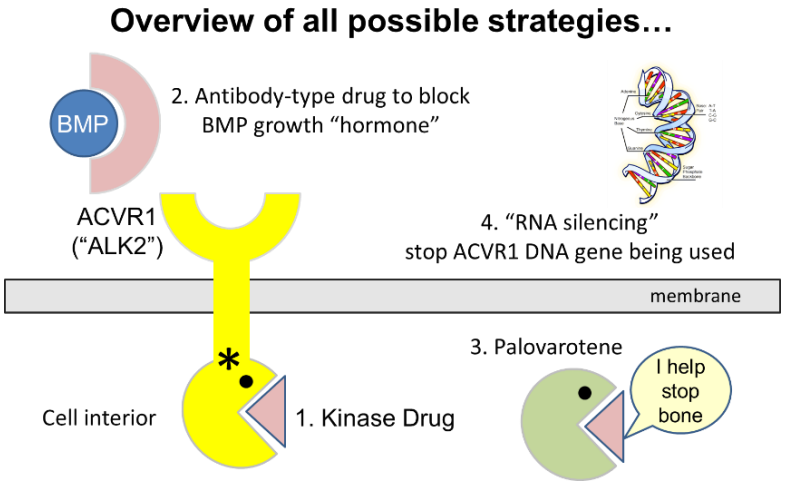

Figure 2. Cartoon showing four possible approaches to developing treatments for FOP. (1) A drug binding inside the cell to the "mouth" of the ACVR1 kinase domain can stop it from sending more signals to make bone (compare to Figure 1). (2) An antibody can block BMP ligands from binding to the exterior surface of ACVR1. (3) Palovarotene helps a retinoic acid receptor protein make signals that stop cartilage and bone formation. (4) Finally, antisense DNA-like molecules can be used to interfere with the synthesis of the ACVR1 receptor protein.

Progress towards strategy (1)

The interior side of the receptor protein ACVR1 contains a messaging region called a protein kinase domain (corresponding to the mouth above). It works much like adding a postage stamp to an envelope, except that it uses a phosphate molecule as the stamp. Only when the letter is stamped can the message be passed on. Drugs that block this phosphate transfer site are called "protein kinase inhibitors". Over twenty protein kinase inhibitors have now been approved as cancer drugs. Over the past few years we have been working with Drs Paul Yu and Greg Cuny in Boston to progress some early-stage inhibitors of the ACVR1 kinase domain, including dorsomorphin, LDN-193189 and K02288. More recently, our chemist Aicha has been making new variants of these molecules with the hope that they will have improved drug-like properties. These are currently being tested by Ellie and George. Such optimisation is a key step in the development of all new drugs. Figure 3 below shows some of the main properties that have to be considered. For example, any new drug must be sufficiently potent that it binds to its target efficiently and has a lasting biological effect. It must also be selective as ACVR1 is just one of 500 kinase proteins that each perform an important biological function. Drug molecules must also be stable to metabolism and break down and of course non-toxic.

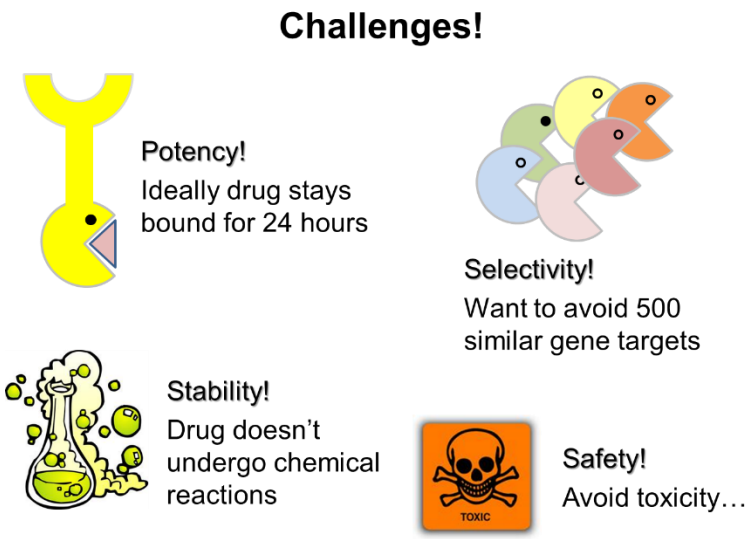

Figure 3. The challenges faced in developing a successful drug molecule.

Exciting breakthrough!

Through our work with the SGC at the neighbouring Target Discovery Institute in Oxford we have had access to a large number of new kinase inhibitors and drug libraries, including many clinically-tested molecules from the pharmaceutical industry. These libraries include approved drugs as well as clinically-tested compounds that are deemed safe, but remain under evaluation in their respective diseases. Most excitingly, we have found that one of these clinical molecules, designated compound "K04284" in our library, was an extremely potent inhibitor of the FOP protein ACVR1 (see Figure 4 below). This compound was developed by a large pharmaceutical company and has advanced to late stage clinical trials for various human cancers. Encouragingly, it meets all of the criteria we have been striving to achieve i.e. it is potent, selective, stable and safe to use.

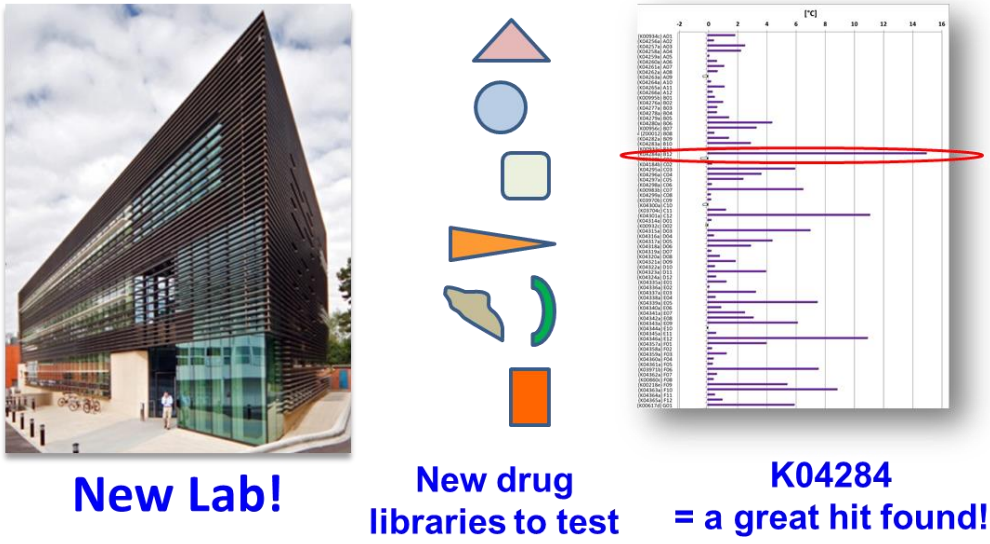

Figure 4. Aicha, Ellie and George have been working in the Target Discovery Institute (left) to access to new drug libraries (centre) resulting in the discovery of a potent inhibitor of ACVR1 that will be safe to use in clinical trials.

Following this discovery we have performed a number of additional experiments to validate the suitability of K04284 for clinical trials in FOP. Ellie confirmed its potency, selectivity and mechanism of binding to ACVR1, while George demonstrated its ability to block the exaggerated BMP signalling in cells. We also asked our colleague Dr Paul Yu in Boston to test K04282 in FOP mice. After a nervous wait, we were thrilled to learn that K04282 had successfully prevented these mice from forming the bone lesions typical of FOP. Aicha has now completed the synthesis of a few variants of K04282, which Ellie and George will test shortly to see if any further improvements can be made.

This is an incredibly exciting development for FOP research as it provides us with a new drug candidate that is safe to take into human clinical trials. The pharmaceutical company that originally developed K04282 has generously agreed to make more of this drug so that it can be available for our FOP study. We are now preparing grant applications to get both the government approval and financial backing that is needed to support such a clinical trial. This scrutiny will take some time and involves important discussions with clinical trial experts as well as with yourselves. In particular, a clinical trial must be carefully designed to ensure that any beneficial effects of a drug are properly recorded. We hope to get this approval within the next 12 months. We are also preparing a scientific paper to share our findings with the wider research community. Our other recent research publications are listed below.

Mutations in Known Monogenic High Bone Mass Loci Only Explain a Small Proportion of High Bone Mass cases. (2015) J Bone Miner Res. [online ahead of print] Gregson CL, Wheeler L, Hardcastle SA, Appleton LH, Addison KA, Brugmans M, Clark GR, Ward K, Paggiosi M, Stone M, Thomas J, Agarwal R, Poole K, McCloskey E, Fraser WD, Williams E, Bullock AN, Smith GD, Brown MA, Tobias JH, Duncan EL.

Clinical Utility Gene Card for: Fibrodysplasia ossificans progressiva. (2015) Eur J Hum Genet. Volume 23, page 1431. Bravenboer N, Micha D, Triffit JT, Bullock AN, Ravazollo R, Bocciardi R, di Rocco M, Netelenbos JC, Ten Dijke P, Sánchez-Duffhues G, Kaplan FS, Shore EM, Pignolo RJ, Seemann P, Ventura F, Beaujat G, Eekhoff EM, Pals G.

A small molecule targeting ALK1 prevents Notch cooperativity and inhibits functional angiogenesis. (2015) Angiogenesis. Volume 18, pages 209-17. Kerr G, Sheldon H, Chaikuad A, Alfano I, von Delft F, Bullock AN, Harris AL.

Small molecules dorsomorphin and LDN-193189 inhibit myostatin/GDF8 signaling and promote functional myoblast differentiation. (2015) J Biol Chem. Volume 290, pages 3390-404. Horbelt D, Boergermann JH, Chaikuad A, Alfano I, Williams E, Lukonin I, Timmel T, Bullock AN, Knaus P.

Staff news

During the past months we have been in close contact with other FOP researchers and have had the chance to present our work at a number of scientific conferences. Alex, Jim and Ellie were delighted to attend the 2015 FOP Italia patient meeting, which was held in Rome in March. This annual event has grown year on year and now attracts an impressive selection of experts in FOP from clinicians to academics and industry members.

Alex and Ellie also presented their work at the Biochemical Society's 78th Harden Conference held in Winchester, which was a meeting appropriately entitled "Protein Kinases in Health and Disease". Ellie presented a detailed poster on her discoveries in FOP research and was awarded with a certificate for the best poster presentation at the meeting (see the photograph below in Figure 5). Many congratulations to Ellie for this well deserved honour.

Figure 5. Dr Ellie Williams is presented with a certificate to celebrate her winning of the best poster prize.

As part of her career progression, Ellie has also taken on an additional role in public engagement that will be funded by the Wellcome Trust. As part of this role, she will spend 30% of her time visiting schools and other public events to educate others about medical research and to enthuse the next generation of scientists.

Following her maternity leave, George has also been awarded a 3 month grant from the University of Oxford's returning carer's fund for training in the latest stem cell and gene editing (CRISPR-Cas) technologies. These skills will be a valuable addition to the team.

Sadly, following the end of her 12 month placement with us, Aicha has decided to return to her home country of Germany to be with her family. She has had a highly successful and productive time with us, producing a variety of drug-like molecules that we will be testing in due course. She has helped us immensely in driving our research forward. We wish her all the best for her future career.

Working with Aicha for the last few months has been Mattias Basel, an Erasmus exchange student from France (see the photograph in Figure 6 below). Mattias has been making chemical compounds that will help us to investigate how the kinase domain of ACVR1 interacts with other proteins to pass on the phosphate signals that it uses to induce bone formation. Following the successful completion of his master's degree he is now moving on to start a PhD course in October and we also send him our best wishes for these future studies in Nottingham.

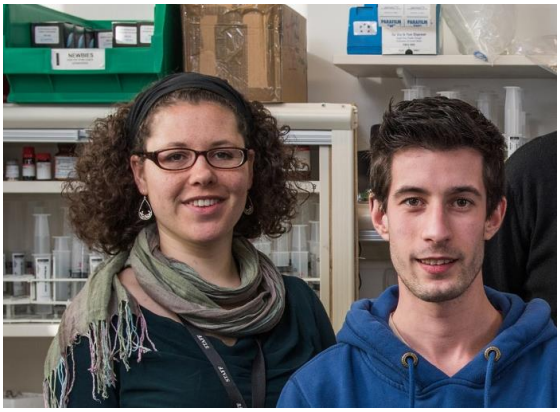

Figure 6. Our chemists Dr Aicha Boudhar (left) and Mr Mattias Basel (right). Photograph provided by John Cairns.

Fortunately, the ranks will be filled again by Dr Caroline Sanvitale, who will join us for a 3 month placement to help Ellie and George advance our current projects and discoveries. Some of you may remember that Caroline worked with us previously as a PhD student and made many important contributions that have led to our current position.

Final words

This year we have been delighted to welcome a number of you to our laboratory in Oxford. For example, back in April we met with Rachel Winnard and her husband Paul. In June we also welcomed Richard and Gail Simcox together with their American guests Maureen and Frank Pullano, who have a daughter with FOP (see the photograph in Figure 7 below).

Figure 7. Gail Simcox, Richard Simcox, George Kerr, Ellie Williams, Alex Bullock, Jim Triffitt, Frank Pullano and Maureen Pullano on a visit to the Oxford FOP laboratory.

We greatly look forward to our future meetings and in particular the next UK FOP Family Gathering, which will be held again at the Radisson Blu Airport Hotel in Manchester in May 2016. Once again, from all of us here in Oxford we would like to thank you all for your generous ongoing support, which is enabling our research to continue. We look forward to some exciting times ahead.

Sincerely

Dr Alex Bullock
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Professor Jim Triffitt
Nuffield Department of Orthopaedics,
Rheumatology and Musculoskeletal Sciences

University of Oxford FOP Research Team

(From left to right): Dr Alex Bullock (SGC), Dr Caroline Sanvitale (SGC), Dr Ellie Williams (Roemex postdoctoral fellow), Dr George Kerr (Roemex postdoctoral fellow), Professor Jim Triffitt (Botnar Research Centre).