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Spring Report from the University of Oxford FOP Research Team

Research News

We are delighted to present our update on the current progress of FOP research in Oxford. Firstly, we would like to thank everyone who attended the FOP Action AGM at the Oxford Hotel on 30th January. Many of you travelled great distances, including our friends from FOP France, and we greatly appreciate this effort. It was truly rewarding for us to meet with you all and to discuss how together we can combat this relentless condition. Over the last few months there have been also several important European FOP meetings aimed at bringing together different expertise from across Europe. Brief summaries of these events are given later in this report.

This quarter we have published three collaborative research papers that address both the molecular mechanism of FOP and the continued development of FOP inhibitors:

(1) Constitutively active ALK2 receptor mutants require type II receptor cooperation.
Bagarova J, Vonner AJ, Armstrong KA, Börgermann J, Lai CS, Deng DY, Beppu H, Alfano I, Filippakopoulos P, Morrell NW, Bullock AN, Knaus P, Mishina Y, Yu PB.
Mol Cell Biol. 2013 Apr 9. [Epub ahead of print].
http://www.ncbi.nlm.nih.gov/pubmed/23572558

(2) Development of an ALK2-Biased BMP Type I Receptor Kinase Inhibitor.
Mohedas AH, Xing X, Armstrong KA, Bullock AN, Cuny GD, Yu PB.
ACS Chem Biol. 2013 Apr 30. [Epub ahead of print]
http://www.ncbi.nlm.nih.gov/pubmed/23547776

(3) A New Class of Small Molecule Inhibitor of BMP Signaling
Sanvitale, CE, Kerr, G, Chaikuad, A, Ramel, M-C, Mohedas, AH, Reichert, S, Wang, Y, Triffitt, JT, Cuny, GD, Yu, PB, Hill, CS, Bullock, AN.
PLoS ONE 8(4): e62721
http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0062721

The first paper reports a collaboration between ourselves and scientists at Cambridge, Germany, Japan, and the US (Harvard and Michigan). We know that ACVR1 (ALK2) normally functions in concert with other genes encoding type II BMP receptors, such as ACVR2 and BMPR2. For example, ACVR1 and ACVR2 can form a complex together to bind BMP molecules in the same way that two hands may work together to catch a ball (see **Figure 1** below).

Figure 1. ACVR1 normally pairs up with a type II BMP receptor like ACVR2 to bind BMP molecules. In effect, they function similarly to the two hands that work together to catch a rugby ball.

Once the BMP is bound, the receptors become activated to effect changes in bone formation, but neither ACVR1 nor ACVR2 can do this alone.

Our study, led by colleagues at Harvard, asked if this co-dependence was also necessary for constitutively active ACVR1 receptors, such as those found in FOP. These FOP ACVR1 receptors carry a genetic mutation that tricks the receptor into thinking it has already caught the BMP (or rugby ball above). Hence the study asked the question do we still need the other genes like ACVR2? Or is the FOP-activated ACVR1 now sufficient?

The answer was quite surprising and extremely interesting. Yes, the binding partners like ACVR2 and BMPR2 were still required. However, only very specific parts of them were needed. For example, the “hand” part that receives the BMP on the outside of the cell was no longer required, whereas other parts (the ‘arms’) inside the cell were. To some extent this also mirrors the requirements in ACVR1. While more work is needed to fully understand this co-dependence, we can start to draw conclusions for potential FOP therapies. Firstly, it reinforces the view that simple ACVR1 or BMP-targeted therapeutic antibodies could not succeed – these molecules cannot enter the cell to stop the aberrant signalling seen in FOP. (You may be familiar with the drug Herceptin, which acts in this manner to fight breast cancer). Similarly, it suggested that an inhibitor that worked solely on ACVR2/BMPR2 would also fail. Secondly, and most significantly, it suggested a potential new therapeutic strategy: to disrupt the coming together of ACVR1 and ACVR2 (or ACVR1 and BMPR2). Part of our work is focussed on better understanding their “handshake” to see how to this may be achieved.

The second paper, also in collaboration with Harvard, describes the continued development of their BMP inhibitor known as LDN-193189. It was found that modifying the structure by shifting the nitrogen atom (N) around the inhibitor scaffold (see **Figure 2** below) could dramatically improve the specificity of the drug for ACVR1 over other BMP/TGFβ receptors (potentially advantageous to reduce side effects).

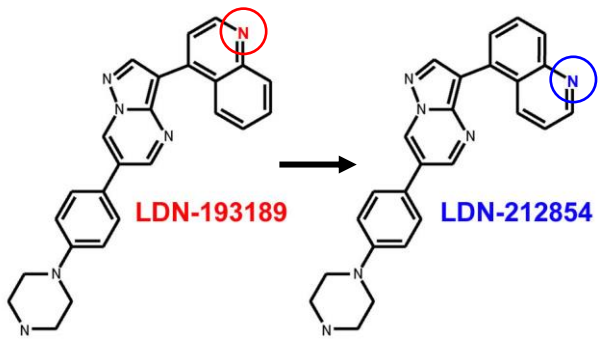

Figure 2. Further development of LDN-193189. Changing the position of the red nitrogen ("**N**") atom to the blue ("**N**") nitrogen position in LDN-212854 improved specificity for ACVR1.

Encouragingly, the Harvard team were able to show that the new improved molecule LDN-212854 could also inhibit FOP-like symptoms in mice (see **Figure 3** below):

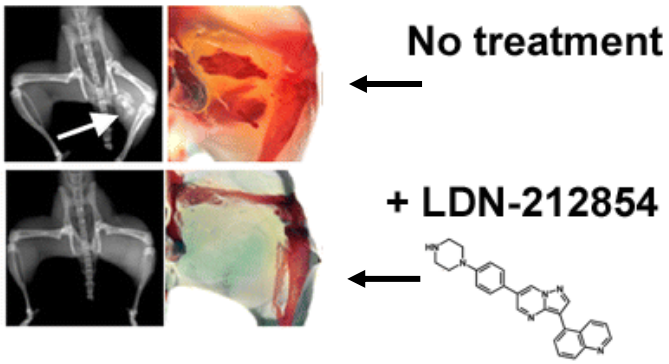

Figure 3. LDN-212854 could inhibit ectopic bone formation in mice.

We are trying to better define why this nitrogen shift is helpful for ACVR1 selectivity. It also remains likely that further upgrades to LDN-212854 would be necessary to make this an attractive drug for FOP patients. It is still a somewhat "dirty drug". Preclinical efforts in this direction are underway in the US through the NIH (National Institute of Health), as well as through the ongoing research work in Oxford, Harvard, Texas and Nashville.

The final paper reports the ACVR1 inhibitor molecule K02288 first identified in Oxford. K02288 bears little structural resemblance to LDN-193189, but still potently inhibits ACVR1. Tests against a panel of 250 gene products related to ACVR1 were conducted to assess how widely the two drugs were affecting "off-target" genes (e.g. biology unrelated to bone). This work indicated that K02288 was "cleaner" than LDN-193189 (i.e. fewer effects were seen

beyond ACVR1). It also showed that outside the ACVR1/BMP receptor group, there was little correlation between the "off-target" activities of the two drugs (see **Figure 4** below). This could mean that if one of these molecules proved to be toxic there would still be hope from the other molecule.

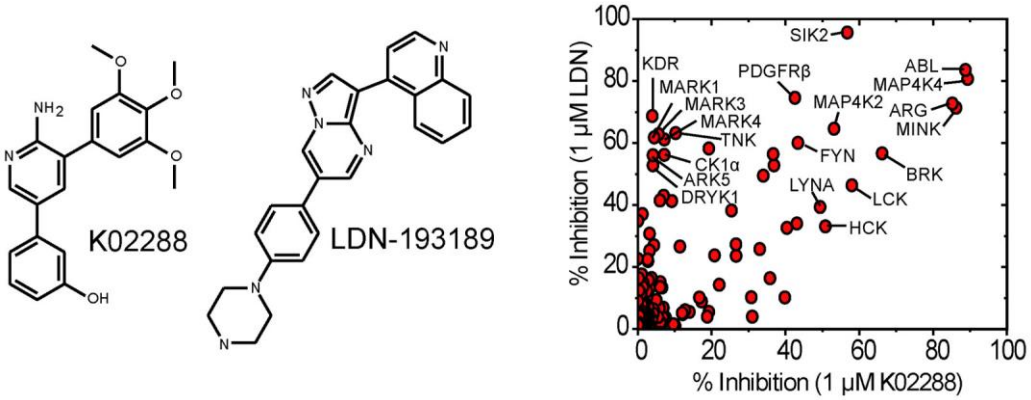

Figure 4. Comparison of K02288 and LDN-193189. The chemical structures of these two drug-like molecules (left) are quite distinct. This is also reflected in their "off-target" activities. The scatter plot (right) shows unwanted LDN-193189 off-target activities on the y-axis and unwanted K02288 activities on the x-axis. If the two molecules acted very similarly all the points would lie on the diagonal line (full correlation). However, the plot reveals substantial scatter. Overall, more unwanted "off-target" genes are affected by LDN-193189 than by K02288 (as indicated by the bias towards the y-axis).

We are continuing to work with our collaborators in Harvard and Texas to improve the properties of K02288, similar to work performed in the earlier report on LDN-212854. For example, generally drugs should be relatively stable and inert so they are not too quickly metabolised by the body or chemically modified to an inactive chemical species. Some of the oxygen-containing ("O") positions of K02288 are potentially vulnerable to these effects. K02288 was first identified by screening a library of chemicals purchased from a biotech company called BioFocus. This company has agreed to make a larger stock of K02288 available so that it may be purchased and tested also by other FOP or BMP research labs around the world.

Scientific meetings

Over the past few months several FOP research symposia have taken place to bring together the leading FOP experts from across the world. In November, the first meeting of

the European FOP Consortium took place in Amsterdam together with a meeting of the Dutch FOP foundation (**Figures 5 and 6** below).

Figure 5. First meeting of the European FOP Consortium.

Figure 6. Jim (shown) and Alex presented slides describing FOP Action UK and the FOP research in Oxford.

The European FOP Consortium currently includes scientists and patient groups from Germany, Italy, France, Holland, Spain and the UK. The consortium has the following aims:

1) To enable close collaborations to help the search for new FOP treatments
2) To support applications for European grants
3) To help members meet the growing European rules and regulations
4) To help to support patient care and epidemiology studies
5) To lay the foundation for clinical trials according to European trial regulations
6) To help share research tools and FOP cell lines according to European rules
7) To allow easy collaboration with IFOPA in research and databases
8) To organize a yearly meeting

The meeting was extremely positive with lots of new ideas to screen for potential new FOP therapies as well as the potential for closer collaborations. Indeed, following the meeting, a Masters student from the University of Amsterdam has joined the Oxford FOP Research Team for a 6 month period under the European Erasmus scheme. Further details of the Amsterdam meeting are available here:
http://www.fopstichting.nl/symposium-nov-2011.php

A second European meeting took place in Parma, Italy on March 22-23rd hosted by FOP Italia and included IFOPA representatives from as far away as Brazil and Argentina, as well as the FOP lab in Philadelphia (**Figure 7**).

Figure 7. The audience and lecture theatre at the meeting of FOP Italia.

Over two days, the Italian FOP meeting held some 25 talks, including both scientific sessions and patient group talks from Italy, Holland, France, Argentina, Brazil and the US. Jim and Alex presented our work on understanding the causes of FOP and the early drug-like molecules identified in Oxford. We had been visited briefly by Alessandra Scoglio and her friend Daniela in November of 2012 (Figure 8). Alessandra is a noteworthy ambassador for FOP Italia and the FOP team who were available (Ellie was absent as was lecturing elsewhere) were delighted to meet them and explain our research plans before we met again in Italy at the Parma meeting.

Figure 8. Alessandra and Daniela's Oxford FOP Laboratory visit.

(From the left: Alex, Caroline, Georgina, Alessandra, Daniela, Jim)

Visions for a treatment for FOP

At the Italian meeting, Dr Roberto Pignolo, a clinician at Philadelphia, presented historical data recording the average disease progression of FOP (age of onset and severity of ossification in different skeletal sites with age). IFOPA expect that these statistical data can be updated very shortly using the recent patient survey results. Any clinical trial would try to measure whether a new treatment successfully reduced unwanted bone formation versus these historical data.

Several talks, including one from Dr Fred Kaplan, addressed the future vision for small molecule FOP therapy. In the best scenario, any drug would be taken only on the first signs of a flare-up and continued until after the flare-up dissipated. This would limit the patient's long term drug exposure and therefore protect against any potential toxicity. It would also allow normal bone growth and development to occur at other times. Currently, there are two candidate drug approaches under consideration: (i) the retinoid drug class and (ii) the ACVR1 kinase inhibitor.

Retinoids prevent stem cells differentiating into cartilage, which is the first critical step en route to bone formation in a FOP flare-up. A clinical trial of the retinoid drug isotretinoin was undertaken in 21 FOP patients in the 1990s and the results published in 1998. At this time the data did not allow the determination of whether isotretinoin was effective or in fact detrimental. Over the past years, retinoid research has continued to explore ways to reduce the associated drug toxicity, which was a significant safety concern. It is now recognized that some of this toxicity is reduced by using a drug that targets only the retinoic acid receptor gamma (there are alpha, beta and gamma receptors). Palovarotene is one example drug candidate being developed by Roche Pharmaceuticals. This molecule has been evaluated in patients with emphysema in extensive past work. However, several years' of work are needed before direct application to treatment of FOP patients.

The second hope draws more directly on the discovery in 2006 of the faulty gene in FOP, *ACVR1*. As you know, this discovery was hugely significant as ACVR1 belongs to one of the most intensely studied protein families in pharmaceutical drug discovery, the kinase family. Indeed, some 15 or more drugs targeting members of this family have been clinically approved and a further 150 are currently in clinical trials. Such molecules targeting ACVR1 remain at an earlier preclinical stage, but include the Oxford molecule K02288 and the Harvard molecule LDN-193189 (as well as other derivatives such as DMH1 and LDN-2121854).

It is truly a blessing that there are multiple opportunities to fight FOP. There are many hurdles still along the way and we need all these opportunities in case one avenue for treatment fails.

Public engagement

From time to time, members of the Oxford team and its host department, the SGC, participate in public open days to engage with the public to help communicate the importance of medical research, as well as its application to rare diseases such as FOP. Last year we

reported on one such event held at the Diamond Light Source in Oxfordshire (see **Figure 9** below).

Figure 9. Last year's public open day at the Diamond Light Source. The photos show Ellie and our SGC colleagues explaining to visitors about the structure and function of human proteins, specifically how these are synthesized from our DNA and folded into all manner of shapes and sizes (e.g. plastic teaching kit, lower left). Ellie also presented a poster of some of her work on FOP (lower right).

The Diamond Light Source is a large doughnut shaped building in Oxfordshire (see **Figure 10**) that accelerates electrons to near light-speed around a 561 metre ring to generate brilliant beams of light from infra-red to X-rays which are used for academic and industry research. Our department at the SGC is one of its primary users. We use these powerful X-rays like a giant microscope to unlock the hidden 3D structure of the atoms in proteins such as ACVR1/ALK2 and to visualise how they bind to drug-like molecules. This year's open days will be held on June 1st and 2nd and will again be attended by Ellie. Further information

and booking details are available on line (see address below) should anyone be interested to visit this remarkable X-ray facility (note there are warnings that the tour involves some extensive walking and is not suitable for under 5s):
http://www.diamond.ac.uk/Home/Events/InsideDiamond.html

Figure 10. The doughnut-shaped Diamond Light Source. A 561 metre ring for accelerating X-rays to see the atomic structure of the FOP protein.

Final words

Once again we would like to thank everyone involved at FOP Action UK, as well as FOP France, for their inspirational activities and continued motivation to help us towards finding a successful treatment for FOP. We hope to be able to write to you in the summer with further progress on characterising the FOP protein and the drug molecules that might stop the rogue activity that causes the devastating bone formation in FOP.

Sincerely

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University of Oxford FOP Research Team

(From left to right): Dr Alex Bullock (SGC), Miss Caroline Sanvitale (University of Oxford FOP Research Fund DPhil student), Dr Ellie Williams (Roemex postdoctoral fellow), Dr George Kerr (Roemex postdoctoral fellow), Prof Jim Triffitt (Botnar Research Centre).