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Autumn Report from the University of Oxford FOP Research Team

Research News

Cancer and FOP researchers join forces

This year has witnessed a new and highly unexpected twist for the Oxford research that has spawned a new collaboration with Dr Chris Jones at the Institute of Cancer Research, London (Figure 1). Specifically, we learnt from Chris and his team that an entirely separate group of children are also affected by the rogue FOP gene. He identified a faulty copy of the *ACVR1* gene in one in four children diagnosed with the incurable brain stem tumour diffuse intrinsic pontine glioma (DIPG for short). It is important to stress that there are other major genetic differences between this cancer and FOP, and that there are no known cancer risks in the FOP community. Conversely, DIPG patients are not prone to develop the symptoms of FOP. Their rogue copy of the *ACVR1* gene is found only in the tumour, rather than throughout the body. It is also accompanied by a number of other genetic mutations that are not found in FOP patients. Thus, FOP and DIPG share a common enemy in *ACVR1*, but are otherwise largely distinct and unrelated. Importantly, a drug targeting *ACVR1* may have potential therapeutic benefit in both conditions. All this means that there is now even more interest in these diseases which will encourage even more research on the FOP gene.

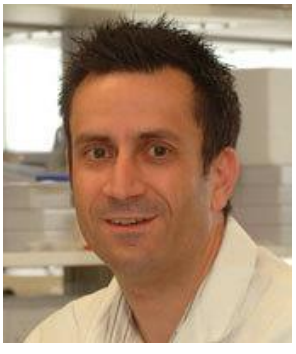

Figure 1. Dr Chris Jones heads a team whose research aims to find the genes which drive the development of childhood brain tumours. His laboratory is based at the Institute of Cancer Research based in Sutton, London.

The DNA encoding our genes can be described by the four letters A, C, G and T (see Figure 2 below). The ACVR1 gene has 1530 “coding” letters that are arranged in a precise sequence order. In FOP, the most common fault is one DNA letter change from G to A at position 617 out of 1530. This error is converted later into a faulty ACVR1 protein (also known as ALK2). Three DNA letters encode each piece of a protein so that the protein error is renumbered as R206H. This means that H, which in the protein chemistry field stands for the amino-acid histidine, replaces a R, which stands for the amino-acid arginine, at a position 206 in the normal ACVR1 protein chain. This simple change causes all the problems seen in FOP. Remarkably, the same R206H change is found in DIPG as well as some of the same variant mutations associated with FOP. However, for reasons we still don’t understand the variants are found more commonly in DIPG than in FOP.

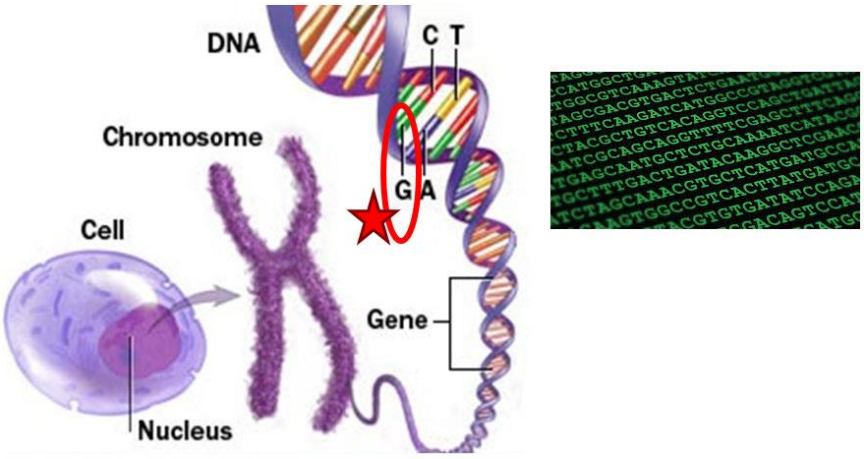

Figure 2. Schematic representation of a cell containing the 23 pairs of human chromosomes, each containing highly twisted DNA. In FOP, one letter in the DNA code is faulty.

As mentioned above, the discovery of this linkage of the *ACVR1* gene to two distinct, but highly important diseases has important implications for research and drug development. Both patient groups are eager for new breakthroughs and share a common endeavour in their fundraising. Thus, FOP and cancer researchers are coming together to share their knowledge and expertise to fight these conditions together. Moreover, there is added incentive for the drug industry to consider this area of research.

We have written two publications with Dr Chris Jones to report these exciting findings. Details are provided below.

- ACVR1 mutations in DIPG: lessons learned from FOP. (2014) Taylor KR, Vinci M, Bullock AN, Jones C. Cancer Research volume 74, pages 4565-70. http://cancerres.aacrjournals.org/content/74/17/4565.abstract

- Recurrent activating ACVR1 mutations in diffuse intrinsic pontine glioma. (2014) Taylor KR, Mackay A, Truffaux N, Butterfield YS, Morozova O, Philippe C, Castel D, Grasso CS, Vinci M, Carvalho D, Carcaboso AM, de Torres C, Cruz O, Mora J, Entz-Werle N, Ingram WJ, Monje M, Hargrave D, Bullock AN, Puget S, Yip S, Jones C, Grill J. Nature Genetics volume 46, pages 457-61. http://www.nature.com/ng/journal/v46/n5/full/ng.2925.html

A report was also featured on the Cancer Research UK website here: http://scienceblog.cancerresearchuk.org/2014/04/07/when-muscle-turns-to-bone-clues-for-treating-deadly-childhood-brain-tumours/

Further, a video of Chris explaining this discovery is also available to watch: http://www.icr.ac.uk/news-archive/video-dr-chris-jones-on-the-discovery-of-the-genetic-flaw-that-may-hold-the-key-to-devastating-childhood-cancer

Continued progress towards a drug for FOP

We previously reported a new promising starting point for drug development for FOP. This molecule, called K02288, had several superior properties to dorsomorphin, including its potency and its greater selectivity for the FOP protein ACVR1 (ALK2). However, optimising such a starting point into a viable drug candidate remains an enormous challenge. Much of this process involves systematically modifying individual parts of the drug molecule and recording if a particular desirable property is improved, unchanged or worsened. Of course many different critical properties have to be optimised in this way for a drug to be successful (for example solubility, metabolic stability, potency, toxicity, mass, specific activity to disease target and so on). Unfortunately, it is frequently observed that molecules achieve three or four of these good properties, but fail on the remainder. Most frustratingly, introducing fixes to the bad points changes the overall make up of the molecule which can cause upsets to the previously good points! The key is therefore to calculate the best compromise of all the required characteristics. This is not straightforward as success can ultimately only be determined by testing the drug in patients i.e. in carefully controlled clinical trials.

Our initial work exploring different chemical changes to K02288 identified a suspected liability in the molecule that meant that its activity in human cells was less than that observed in the laboratory test tube. This flaw was minimized by substituting in a different chemical group. However, the resulting increase in potency was also accompanied by a gain in drug binding to other unwanted proteins (so called "off-target" effects that may harm the safety profile). Pleasingly, chemical changes made elsewhere in the molecule helped to correct this. As a result, some of these second generation derivatives have become the new "preferred" molecules. A summary is shown in Figure 3.

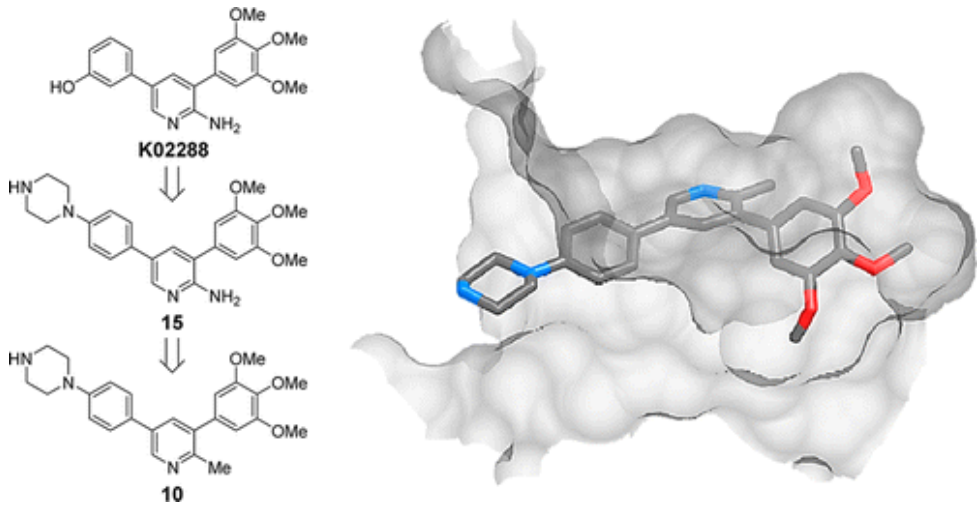

Figure 3. Summary of chemical changes to K02288 that improve cellular activity (left). The fit of the "preferred molecule" to the drug binding pocket in ACVR1/ALK2 is shown (right). Grey, red and blue correspond to carbon, oxygen and nitrogen atoms, respectively.

This work was published recently. Details are provided below.

- Structure-Activity Relationship of 3,5-Diaryl-2-aminopyridine ALK2 Inhibitors Reveals Unaltered Binding Affinity for Fibrodysplasia Ossificans Progressiva Causing Mutants. (2014) Mohedas AH, Wang Y, Sanvitale CE, Canning P, Choi S, Xing X, Bullock AN, Cuny GD, Yu PB. Journal of Medicinal Chemistry volume 57, pages 7900-15. http://pubs.acs.org/doi/abs/10.1021/jm501177w

Expansion of the University of Oxford FOP Research Team

The optimisation of medicinal chemistry requires special expertise and training that was lacking in the University of Oxford FOP Research Team. We are delighted to announce the recruitment of a new staff member to our team, Dr Aicha Boudhar (Figure 4). Aicha obtained her PhD in organic chemistry from the University of Strasbourg, France. She comes to us from a subsequent post held at the National University of Singapore where she was developing chemical tools to understand drug resistance in malaria treatment. Her work in Oxford is funded by a grant from FOP Friends with the support of the Amateur's Trust and Roemex Ltd. We are extremely grateful to all at FOP Friends as well as Richard Simcox for securing this welcome support.

Aicha's work in medicinal chemistry will help to advance the development of the K02288 scaffold further. Her efforts will be supervised on a day to day basis by Dr Paul Brennan who leads chemistry research at the Target Discovery Institute, part of the new Li Ka Shing Centre at the University of Oxford (Figure 4). Paul is also a key member of the SGC (Structural Genomics Consortium) and is therefore a close colleague of Alex Bullock. He trained at the University of California, Berkeley as well as at the University of Cambridge, UK. Critically, he has a decade of experience of drug development in the pharmaceutical industry and joined us directly from Pfizer. He is also collaborating closely with our friends at the Institute of Cancer Research, London bringing further synergies to the project. Most excitingly, we have also gained access to further drug libraries for screening. This has led us to several important new leads that provide fresh ideas for AIcha's work and the perfect molecule. We hope to provide more information on these in the next report.

Figure 4. Drs Paul Brennan (left) and Aicha Boudhar in the new chemistry facility at the Target Discovery Institute (right).

Further characterisation of the FOP protein

We continue to apply the techniques of protein crystallisation and X-ray diffraction to understand the 3D structures of the normal and faulty versions of the FOP protein ACVR1 as well as their binding to small molecule inhibitors. The most recent completed structure included a derivative of our latest drug molecule described in Figure 3. These data help us to better understand how the FOP protein functions as well as how to shape our drug molecules to achieve good binding. Our current work continues to look at the faulty version of the FOP protein to characterise how it becomes over-activated to make the unwanted bone that is progressively observed in FOP patients. Again, we hope to provide a more detailed update on the learnings from this work in our next report.

Scientific conferences

During the past months we have had frequent scientific meetings with our colleagues from across the world to discuss our findings and to review scientific understanding in FOP, general bone biology and drug discovery. In April, Alex Bullock and Jim Triffitt attended the "8th International Meeting on Fibrodysplasia Ossificans Progressiva" held in Genoa, Italy. This annual event organised by FOP Italia has continued to grow in its outreach with separate days of talks targeted at disease experts and FOP families (see Figure 5 below).

Figure 5. FOP Italia meeting hosted by Professor Roberto Ravazzolo of Genoa (left). Chris Bedford-Gay (FOP Friends), Alma Triffitt (wife of Jim) and Antoine Lagoutte (FOP France) were also attending (right).

Of course this summer we were also delighted to attend and present our work at the 2014 UK FOP Conference and Family Gathering held in Manchester, UK. This event was made possible with the support of Genetic Disorders UK and was another major success. Congratulations again to all at FOP Friends for being such welcoming hosts and for organising such an insightful and entertaining event. We were so pleased to meet again with our national and international friends and colleagues and to have a great opportunity to make many new ones. Such events can only inspire us to return to the laboratory and work even harder to find a new medicine.

The team have also presented their work at other scientific meetings in Oxford as well as in Berlin.

Final acknowledgements

Of course this summer we were also delighted to attend and present our work at the 2014 UK FOP Conference and Family Gathering held in Manchester, UK. We are currently working with other European FOP Consortium members to seek funding from the EU, which has special funds to support drug discovery for rare diseases. We are tremendously excited about the potential of some of the new molecules we have identified in screening. We look forward to reviewing our hopefully rapid progress in this area in the next report. The whole Oxford FOP Research Team join us in wishing everyone a safe and very happy Christmas, with all good wishes for 2015.

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).