University of Oxford FOP Research Team

A Q & A with Prof Alex Bullock

Spring 2022

Who makes up the current team?

Over the past few years the funding of the FOP community has supported 3 full time team members, including two senior staff members, Dr Ellie Williams and Dr Jong Fu Wong, and a PhD student, Miss Liz Brown who are managed by myself. While retired, Emeritus Professor Jim Triffitt also retains an interest in the team. We are now in a state of renewal as Ellie, Jong Fu and Liz are all leaving their current roles to progress their careers. Ellie has been promoted internally to a managerial role, Jong Fu is leaving for a position in a local biotech company, while Liz is about to complete her PhD. We are just advertising to recruit their replacements. All are very sad to be moving on, but are keen to stay in contact and to ensure a smooth handover to the new recruits.

What collaborations do you have around the world?

The FOP research community is a small and close-knit group that meets regularly enabling collaborations across the globe. Examples of our collaborations by country are listed below.

UK: Richard Keen (STOPFOP clinical trial); Chris Jones/Swen Hoelder Institute of Cancer Research (ACVR1 inhibitors for FOP/DIPG); Various clinical geneticists (FOP diagnosis); AstraZeneca (STOPFOP clinical trial); Charles Rivers Labs (ACVR1 inhibitors for FOP/DIPG)

France: the biotech company Oncodesign (ACVR1 inhibitors for FOP)

The Netherlands: Gonzalo Sánchez-Duffhues and Peter ten Dijke (ACVR1 inhibitors for FOP/DIPG); Marelise Eekhoff (STOPFOP clinical trial).

Germany: Clemens Stockklausner (STOPFOP clinical trial); Petra Knaus (ACVR1 inhibitors for FOP)

Spain: Ángel Montero Carcaboso (ACVR1 inhibitors in DIPG mouse models)

Canada: Ontario Institute for Cancer Research, M4K Pharma and Jerome Fortin (ACVR1 inhibitors for FOP/DIPG)

Japan: Takenobu Katagiri (understanding the functional consequences of ACVR1 variants in FOP)

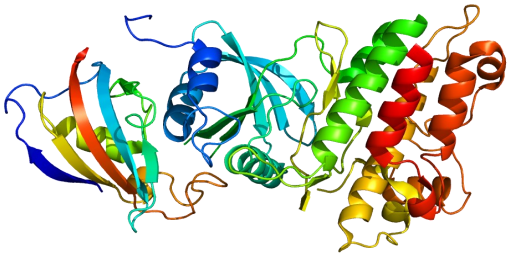
USA: Eileen Shore (testing ACVR1 inhibitors from M4K Pharma), Aris Economides/Regeneron (FOP mouse model); Paul Yu (ACVR1 inhibitors for FOP, STOPFOP clinical trial)

Research Update

What is your current research focus and what does that mean for us?

Our work can be described around the three areas below:

- Diagnosis: assisting clinical geneticists
- Treatment: the STOPFOP clinical trial
- Research: Understanding of how ACVR1 gene variants cause FOP and using this knowledge to help develop additional drug treatment strategies for FOP



Recently, our research has involved liaising with clinical geneticists to help with FOP diagnoses or to investigate genetic similarities between variants found in FOP and those identified in some cancers or other conditions. All DNA is considered to be made up of four letters (A, T, G, C) representing different chemical constituents (nucleotides, also known as bases) that form chains of information. The coding sequence for ACVR1 contains 1527 copies of these letters in a specific order. All cases of FOP are associated with a change in this code involving at least one letter position. The DNA code is translated into a protein, alternatively called ACVR1 or ALK2, that contains 509 chemical parts called amino acids. We think 95% of FOP cases involve the DNA letter at position 617 changing from “G” to “A”, which results in the ACVR1 protein changing amino acid position 206 from “R” to “H” (i.e. the R206H variant). From time to time individuals present with other DNA letter changes in the ACVR1 gene that have not been seen before. For example, in the past we analysed DNA changes resulting in an L196P variant in the ACVR1 protein. New letter changes can happen by chance and can either be benign with no damaging effect, or act similarly to the known R206H variant which results in overactive ACVR1 protein activity. Thus, to establish between these possibilities we make the variant proteins in human cells in a dish in the lab, provide them with nutrients and then measure how active the ACVR1 protein is after adding a stimulating growth factor such as the activin protein. If the new variant ACVR1 protein becomes overactive after activin is added then we have good evidence that the new variant is causative of FOP and this is relayed back to the clinicians and doctors.

A key highlight of our past research was the discovery of saracatinib as candidate drug molecule for the treatment of FOP. ACVR1 belongs to a class of protein known as a protein kinase. These proteins are molecular machines that stamp other proteins with phosphate molecules as a form of message transfer. Saracatinib is a known protein kinase inhibitor originally developed by AstraZeneca. It acts like a key in a lock. It binds directly to the ACVR1 protein and switches its activity off. This stops it from becoming the overactive protein responsible for the unwanted bone formation in FOP. Importantly, saracatinib successfully stopped the development of FOP in mice, as shown by our collaborator Paul Yu in Boston, USA, and has previously

proven to be safe in phase I and phase II clinical trials. This allowed us to win grant funding from the EU to start the STOPFOP clinical trial. We are still meeting weekly with the STOPFOP clinical team to discuss its continued roll out, as well as its longer term funding and future development.

Our ongoing research is heavily focussed on the ACVR1 protein and its role in causing FOP. To function, ACVR1 must switch between ON and OFF states. The ON switch occurs when a sister protein kinase such as ACVR2 attaches phosphate molecules (small chemical groups) onto ACVR1. The ACVR1 variants causing FOP appear to be more easily turned ON. We are studying this biochemically and structurally and can replicate it in "test tube" conditions in the lab. It seems to behave like the childhood game "Whack-a-mole" where the phosphates can be added at alternative different sites. Thus, if we remove some of them, they just appear elsewhere. Nonetheless, if we can simplify the phosphate pattern there is a better chance for us to take a snapshot picture of its 3D structure to learn how it works more precisely. For example, how do the added phosphates on ACVR1 enable it to grab hold of the SMAD protein molecules that then pass on instructions from ACVR1 for more bone formation in this body part?

Using the knowledge of ACVR1 as a switch and our current 3D structural understanding, we aim to design a wrench that can jam the ACVR1 ON/OFF switch shut. We previously identified a starting molecule to build on and now have 50 new designs to test. Each explores slightly different chemistry so we can learn iteratively to improve the chemical scaffold towards a future drug candidate. This wrench approach targets a different part of the ACVR1 protein to that of saracatinib in the STOPFOP trial. We hope this wrench strategy could make it a safe drug approach as this part of the ACVR1 ON/OFF switch is quite different to other human proteins so will hopefully reduce the risks of side effects. This will be one of the tasks to be addressed by the next team members to be hired in Oxford.

What are your significant goals / milestones in the next 1/2/3 years?

Over the next year we would like to (i) show that the wrench idea for ACVR1 inhibition is tractable for further chemistry; and to (ii) define the wider set of proteins that help to regulate the ACVR1 ON/OFF switch (i.e. they may act to further lock it, or make it easier to activate). Over the next 3 years we aim to (i) complete the STOPFOP clinical trial; and (ii) have a 3D structure for the active ON state of ACVR1 to learn more about its mechanism.

If there was one single piece of information you would like to know that would progress the potential success of their research, what would it be?

We would like more 3D structural information showing how our wrench-type drug precursors act to block the ACVR1 ON switch. Armed with this information, chemists would know how best to amend the drug precursors to improve their properties (akin to a locksmith fine tuning a key).

Fundraising update

How is the fund-raised money from FOP Friends with FOP France being spent?

All the monies generously donated to FOP research at the University of Oxford are spent on research. About 80% of the money is spent on personnel while 20% is spent on the raw materials needed to perform experiments (antibodies, chemicals, plasticware, DNA and protein purification kits, nutrients for growing cells etc). Perhaps 1% is spent on attendance at scientific conferences where research results are reported to other scientists and clinicians.

If FOP Friends found £1,000,000 what could we achieve?

Such a figure could be used to further develop the STOPFOP clinical trial. For example, to perform safety experiments (toxicology studies) to check the suitability of saracatinib for use in children. Alternatively, if the wrench idea for ACVR1 inhibition proved tractable, this money would likely allow us to develop it sufficiently for a proof of concept study in mice, which would be a necessary step to before considering any use in humans.

Are there any other roles you would like to add to the team, if money was no object?

Based on the current expertise, I would say complementary roles relating to medicinal chemistry. This would allow us to design new molecules within the team and rapidly explore their effectiveness at blocking the ACVR1 ON/OFF switch.

Is the research scalable i.e. could you increase from 3 staff to 6 based on the size of the lab? If there were 10 people, would a treatment take a 10th of the time?

Certainly, the University of Oxford/AstraZeneca vaccine against Covid shows what can be done. In agreement with stereotypes, scientific research is often associated with eureka moments, as well as common setbacks along the way. The eureka moments keep us motivated to continue and make up for all the setbacks; they typically open a path forward where extra staff resource is beneficial to exploit it. We seek to further validate our wrench for ACVR1 inhibition to give us confidence that this qualifies as a eureka moment. At this next potential eureka point, the team would require extra chemistry resource. Under this event, the time to a treatment would likely be

roughly proportional to the number of staff, although it is still a long and difficult journey. Chemistry resource would likely be needed in a separate laboratory to ours as it is a skillset we lack. However, we have a number of chemistry collaboration partners when required.

Are there grants available which we could apply for to help fund a new member of staff?

Government or other funders typically required grants to be authored by the scientists. I am not aware of grants open to families or small charities, such as FOP Friends, that would directly fund staff positions in research labs. There may be small monies available to support charity activities in the community, as exemplified by the National Lottery. We hope that the petition to parliament for FOP funding can be helpful for all future FOP grants.

STOPFOP

Do you have a timeline for the StopFOP trial?

The STOPFOP trial has been active throughout the pandemic period and is openly recruiting across Europe. There have been inevitable delays as hospitals and regulatory bodies experienced intermittent closures to all activities outside of Covid-19 work. Clinical sites are currently open in Amsterdam and Garmisch, Germany. We hope Dr Richard Keen can open the London site this summer. Overall, we expect the trial work to continue over the next two and half years.

Are you able to disclose the side effects of the StopFOP drug?

Saracatinib has been used in multiple clinical trials providing excellent knowledge of the tolerated dose. We are using a relatively low dose of the study medication “saracatinib” as a preventative treatment for FOP with the aim to minimise the side effects as much as possible. At this low dose, we expect the main side effect to be occasional mild gastrointestinal irritation (i.e. a stomach ache or loose bowel movement). Part of the study aim is to monitor these safety indicators to learn how FOP can be safely managed in the future. At high drug doses (e.g. double), the medication can reduce a person’s blood count. Thus, it is important that patients follow the prescribed dosing and this general advice applies to any condition and any medication (too little drug could be ineffective, while too much drug can be harmful).

Do you foresee (based on mode of action) potential for combination treatments in the future with other treatments in development?

It would be fantastic to see more than one drug approved as safe and effective for use in FOP. In this case two drugs could be used in combination (taking both together), or sequentially e.g. you could spend 3 months taking one drug and then switch to spend 3 months taking a different drug (and then switch back again and repeat the cycle). A combination approach would increase the risk from side effects as you would experience the different side

effects of two different drugs together. Thus, this approach, if possible in future, may not necessarily become the long term standard of care, but it could potentially be used for short time periods – for example, around dental surgery. The sequential approach has the benefit that any specific set of side effects from one drug would be temporary allowing the body to recover. As an analogy, it would be similar to taking shorter periods of a high protein or high carb diet and switching between them rather than sticking to one only.

Living with FOP

Does you have views on the Covid vaccine and the new anti-viral oral drug being given to vulnerable people (cancer patients). Can it only be used once a patient has tested positive, or does it offer any protection as an oral vaccine would?

Your FOP-specialist clinicians would likely to be best placed to advise on the status of vaccines and new delivery methods (e.g. nasal spray) that may help to make them safer for someone with FOP. Anti-viral drugs (e.g. of the protease inhibitor class) would be highly recommended for anyone in a vulnerable group, such as the FOP community, but these should only be taken after a diagnosis with Covid-19 (and as soon as possible).

Is it common for bone marrow to be affected, following a flare?

This is outside of our specific expertise. It is known that a bone marrow transplant cannot cure FOP. The immunosuppression associated with this can temporarily dampen FOP flare activity only.

Is there any indication or research into diet? Does a certain diet have a better effect on someone with FOP for example less protein?

Everyone is probably aware of occasional media stories pronouncing the benefits of anti-oxidants or other chemicals in certain foods. For the most part, these are present at low levels that would not be equivalent to any form of medication. We assume a balanced diet is best. In other words, the advice for someone with FOP is the same as for anyone else in their family.

Likewise, research by Dr Paul Yu at Harvard suggests that manageable exercise is also beneficial.

An invitation...

Will you be holding an open day for us to meet the team, see the lab etc?

Everyone is welcome to visit the lab and indeed several families have done so in the past. Due to the pandemic, it is likely to be easier to arrange visits for individual families rather than a mass open day. We can of course see how this changes with time.

Enquiries can be made to alex.bullock@cmd.ox.ac.uk

Dr Ellie Williams will also be taking her science-based escape room to the FOP Friends UK Conference and Family Gathering at the Radisson Blu Airport Hotel, Manchester on Saturday 21st May.

The escape room is designed to give the experience of our lab with a range of photo-realistic scenes.

www.fopfriends.com

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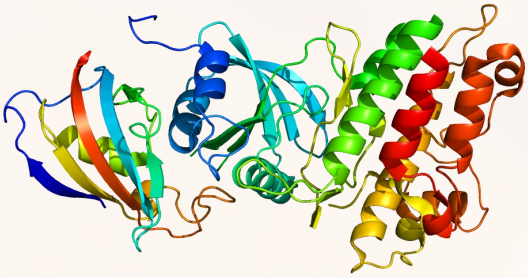
FOP Research Update from Dr Ellie Williams

Spring 2022

Introduction



FOP is caused by a small change in a single protein in your body. This protein is known as ACVR1 (or sometimes ALK2). Normally this protein is involved in making bone in the right places at the right times, and in response to the correct signals from the rest of the body. The FOP mutation causes ACVR1 to respond to the wrong signals at the wrong times, causing bone formation in the wrong places.

The way ACVR1 works is by adding what we call a phosphate group to another protein (called SMAD1) to switch it on. SMAD1 then goes on to activate other processes that ultimately lead to bone formation. This is like adding a stamp to a letter that would allow it to be posted with ACVR1 acting as the stamping machine. In FOP this stamping mechanism is switched on not only by the usual signals that are used to generate bone but also by other signals that are usually involved in cell division and not bone formation. This cross talk is a major contributor to FOP.

One major focus of our work has been on trying to find something that could be used as an 'inhibitor' of ACVR1 – something that would bind to the main binding site of ACVR1 and stop it stamping it's activating signal on SMAD1, and hopefully thus stopping the mistaken 'make bone' signal from being passed before it could get to the point of actually making bone.

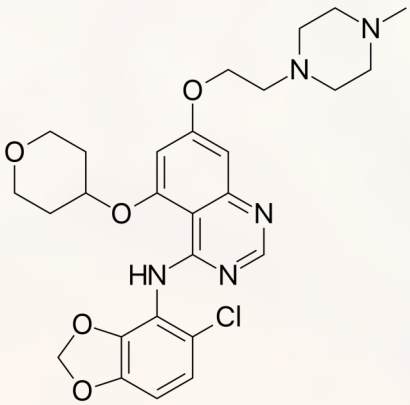
One complication with trying to find an inhibitor to bind to the main site of ACVR1, is finding something that will stop, or inhibit, ACVR1 from working but not affect any of the very similar proteins that exist in your body. These other proteins are responsible for things like muscle formation and normal cell growth so it's really important to be selective.

We have been looking at and testing hundreds of inhibitors to try and find ones that could potentially become safe medicines. Quite often the inhibitors we look at in the lab seem to be very effective at stopping ACVR1 selectively, but may have side effects when taken by a person. They need modification to make them safe enough to be taken as a medicine by a patient.

This can be a very difficult step and is sometimes even impossible, so finding several options that could be developed and made safe gives us the best chance of success for FOP patients.

Saracatinib:

There is however another approach that we've taken to searching for an inhibitor that might work against FOP. Many inhibitors are developed by companies and go through a series of stages of trials to make sure they're both safe and effective. A phase 1 trial is where a potential medicine is tested in healthy volunteers to see if it's safe before being taken to a phase 2 trial in a small group of patients to see if it actually works. Some medicines make it through phase 1 (they're safe) but fail at phase 2, potentially meaning that the therapeutic hypothesis was wrong often due to a lack of disease understanding.



Part of our work looked at screening a library of these 'clinical medicines' that were shown to be safe but didn't work for their original indication. As part of this we identified an inhibitor called 'saracatinib' that showed very good safety data but didn't help in the cancer it was originally targeted at.

Saracatinib was originally designed to inhibit two proteins called Src and Abl which are both in the same protein family as ACVR1. As well as inhibiting Src and Abl, saracatinib bound to ACVR1 just as well and warranted further investigation. We looked at exactly how well it bound and what happened when you added Saracatinib to cells that we use to model FOP in the lab. This all looked very promising in stopping the aberrant signalling seen in FOP on a single cell scale, and so we worked with a collaborator to test saracatinib on mice used to model FOP. This also gave promising results in stopping bone formation occurring under circumstances where FOP bone growth would otherwise be seen.

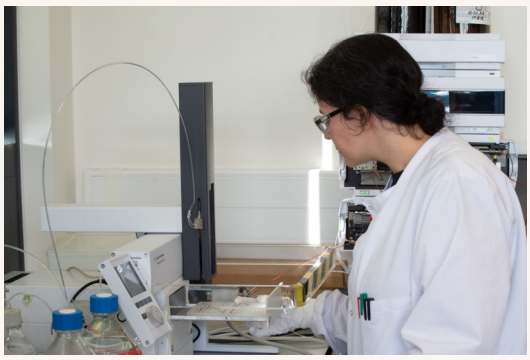
Crucially this means that this is an inhibitor which seems to stop FOP bone formation in our experiments that has already been shown to be safe when taken by people. This means that saracatinib would be able to go straight into phase 2 trials in FOP patients to see if it actually works in treating the condition.

STOPFOP:

From all this, the STOPFOP trial started in early 2020, looking to test whether saracatinib was able to treat FOP in patients.

It is expected to run for 3 years however due to the pandemic there have been delays and so results are not expected to be released from the trial for a few years more.

A second binding site:



Meanwhile, back in the lab we've been planning our next steps to see if we can make something even better. The failure rate in making a new medicine is over 90%. Therefore, we've been looking at alternatives and new approaches to treat FOP.

One option we've been exploring recently is to see if we can find a second binding site somewhere else on the protein that might let us find an inhibitor that binds better to ACVR1 than to the other proteins. The idea is to find a second site unique to ACVR1, with other proteins either lacking this second pocket completely or where the second pocket is such a radically different shape that any inhibitor we find for ACVR1 won't fit inside any other proteins. This second site forms our wrench binding site. Molecules binding here would stop the ACVR1 protein from switching ON.

One challenge is that the second pocket isn't well studied at all as it isn't the key part of the stamping mechanism, and so finding a basic starting point is one of the big issues to tackle. One way we've been looking at this is through a method called 'fragment screening'. Instead of taking large molecules and seeing which ones fit most exactly or not at all, we instead take very small molecules and see if they bind to any part of the second site. On their own they won't bind that tightly or that specifically, but these small fragments give us a starting point for building something bigger and better. This puzzle piece approach means we don't need to search through thousands of large compounds looking for something that fits in all the nooks and folds of the second pocket, but instead can look at a much smaller number of building blocks which will fit inside these spaces much more easily. We can then look at joining them up or building out from them to make a more complex and useful molecule that might work to switch off ACVR1 and not any other protein.

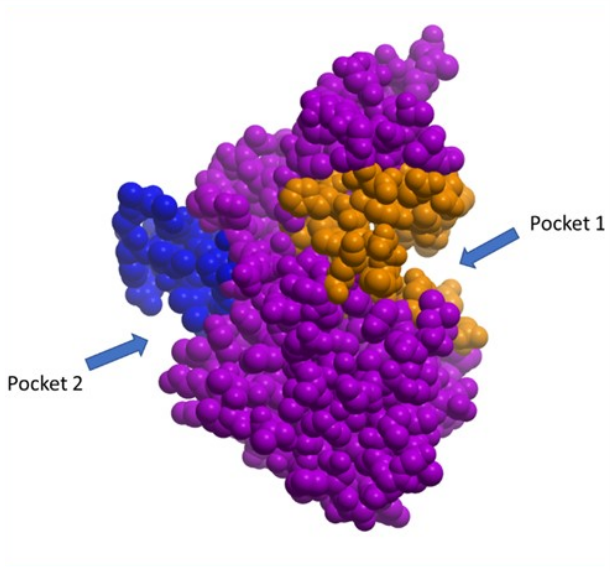


Figure shows a 3D structural model of the ACVR1 kinase domain. Each little sphere represents an atom in the protein molecule.

After testing over a hundred fragments, we found several that bind in various places across the protein and of those, one that binds in the second pocket. This gives us a starting point to build out from to try and develop this fragment into a strong inhibitor.

With two approaches to developing new inhibitors against ACVR1, we can learn more about how the mutation causes FOP and better understand how we can use that to try and develop new medicines.