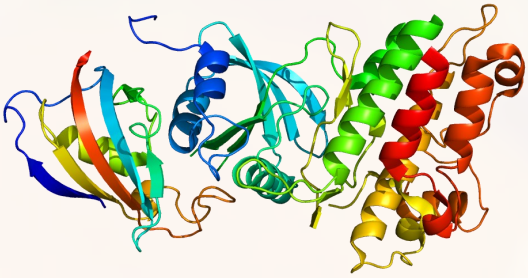
University of Oxford FOP Research Team

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.

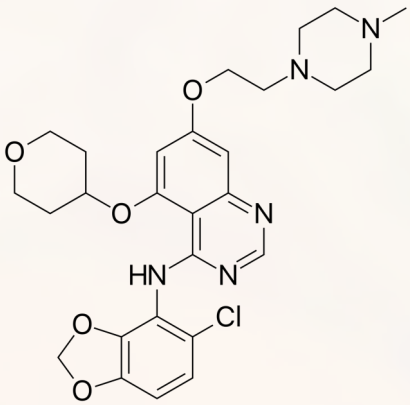
One complication with trying to find an inhibitor to bind to the main site of ACVR1, is finding something that will stop, or inhibit, ACVR1 from working but not affect any of the very similar proteins that exist in your body. These other proteins are responsible for things like muscle formation and normal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

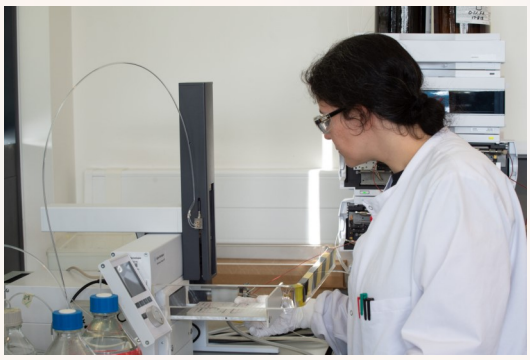
Crucially this means that this is an inhibitor which seems to stop FOP bone formation in our experiments that has already been shown to be safe when taken by people. This means that saracatinib would be able to go straight into phase 2 trials in FOP patients to see if it actually works in treating the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

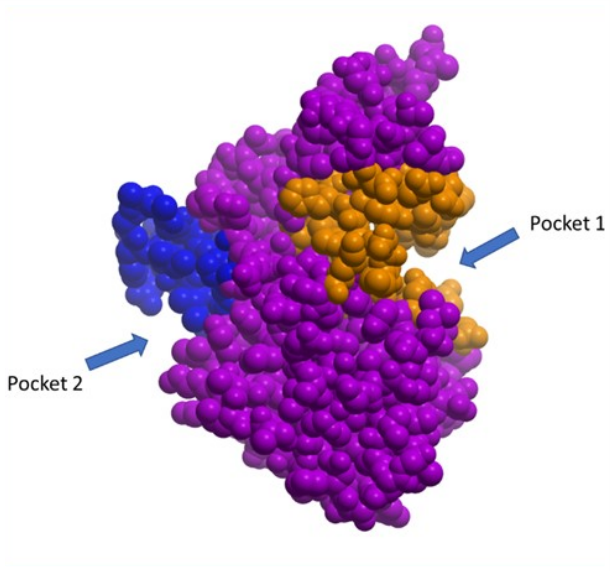


Figure shows a 3D structural model of the ACVR1 kinase domain. Each little sphere represents an atom in the protein molecule.

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.