Identification of potent ALK2 inhibitors as potential treatment for stone man syndrome

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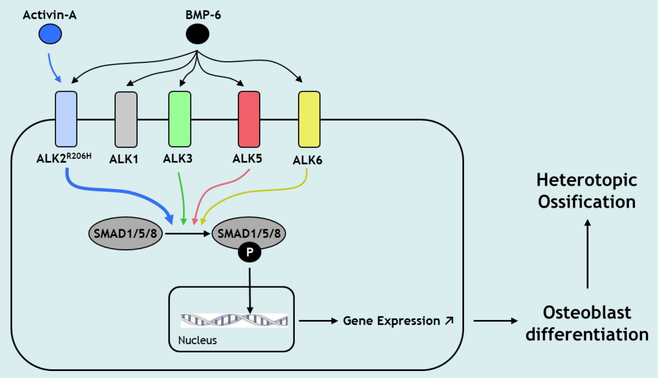
Introduction

Stone man syndrome, also known as Fibrodysplasia Ossificans Progressiva (FOP), is a genetically inherited rare disease, which classical form has been related to an activating mutation of ACVR1 gene (coding for ALK2 protein kinase). From childhood, patients are developing a progressive ossification of fibrous tissues, which ultimately leads to a significantly reduced life expectancy (about 40 years). This disabling disorder is not only transforming tissues during the so-called flare-up periods, but also worsen symptoms in case of injury, vaccination or surgery.

We identified OD36 probe as a potent ALK2 inhibitor with interesting selectivity within BMP kinase receptor family. Through a first phase of optimization, we focused on the improvement of potency and physico-chemical properties by modulation of the linker composition as well as substitutions on the aryl moiety. This exercise enabled to identify OD52 with enhanced kinase profile and better activities.

In-vitro proof-of-concept studies with our compounds, using LDN-193189 (a known pan-ALKi) as benchmark, demonstrated sub-micromolar cellular activity through the inhibition of Smad signaling and highlighted significant functional efficacy in a model of pre-osteoblastic differentiation assay as well as in *ex-vivo* osteoblastic cells from FOP donors.



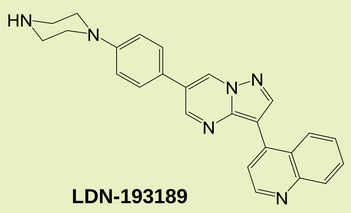
RESULTS

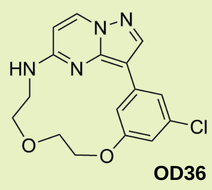
Biochemistry

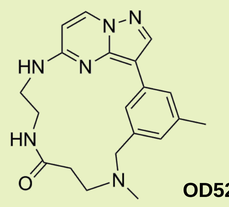
Broad kinase screening of the Nanocyclix® library led to the identification of OD36, our initial probe on ALK2. The compound displays low molecular weight, interesting physicochemical properties but was lacking kinase selectivity with 4.3% of the kinases hit in a full profiling (386k) with more than 50% inhibition at 100nM (S50@100nM). Through a first round of optimization, we discovered that modulation of the linker enhanced physicochemical properties and kinase selectivity while maintaining ALK2 potency.

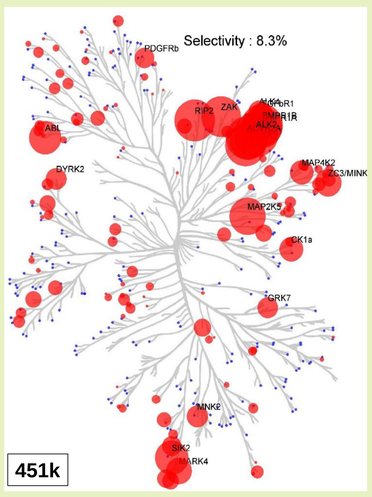
The determination of biochemical IC_{50}s on kinases from BMP family in comparison with LDN-193189 as reference compound highlighted a very high selectivity. Furthermore, we observed a slightly stronger inhibition of the ALK2 FOP mutant by the determination of biochemical IC_{50}s. Recently we identified OD138, a potent ALK2 inhibitor without ALK1 affinity.

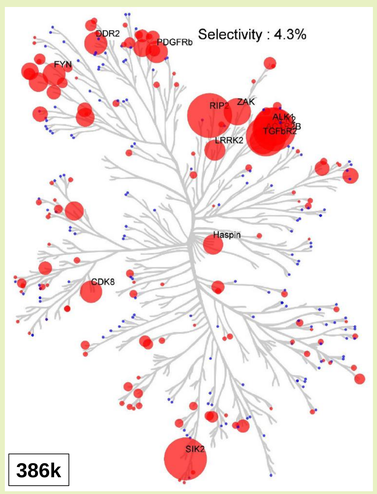
Compound ID	Kd in nM (DiscoverX)								IC50 in nM (RBC)	
	ALK1	ALK2	ALK3	ALK4	ALK5	ACVR2A	ACVR2B	TGFBR2	ALK2	ALK2 (R206H)
LDN-193189	4.2	1.2	3.8	91	77	12	11	26	18	8
ODS36	90	37	3000	3000	3000	3000	90	680	47	22
ODS52	13	9.6	1300	260	850	590	220	2500	72	32
OD138	3000	33	3000	ND	ND	ND	ND	ND	ND	ND

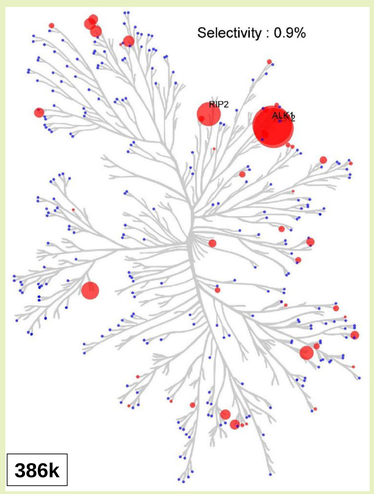

LDN-193189


OD36


OD52

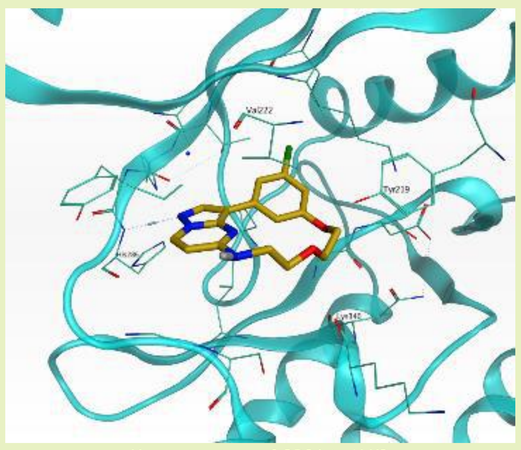


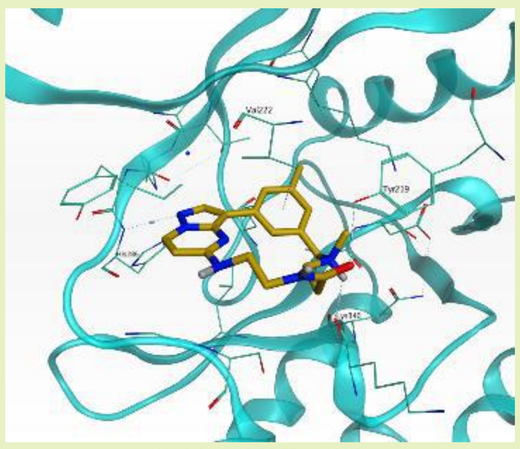




These compounds we used to perform an *in-vitro* proof-of-concept study prior to initiate a lead optimization program with FOP as primary therapeutic indication.

X-ray crystallography and modeling


X-ray structure of OD36 in ALK2

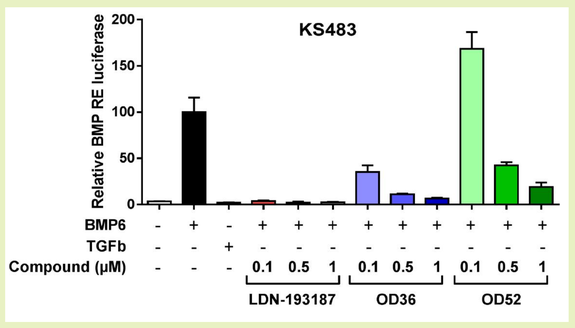

Modeling of OD52 in ALK2

Through a collaboration with Structural Genomic consortium, the X-ray crystallography of OD36 in ALK2 was obtained with a resolution of 2.56Å (to be published in pdb). The analysis of the X-ray structure enabled to confirm the binding mode of OD36 as type 1 ATP competitor as shown on the left picture. The compound is displaying a single H-bond interaction located on the hinge region with His286.

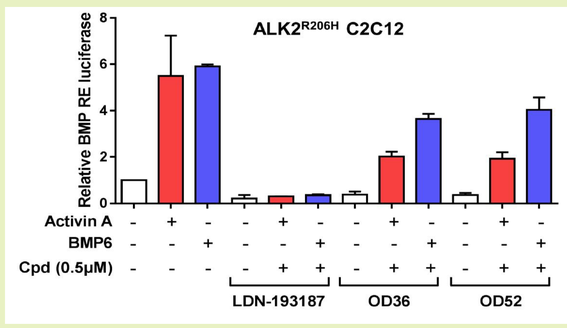
To our knowledge, the shape complementary is mainly responsible for the affinity and the selectivity. We used this model to perform structural-based drug design based on our lead compound OD52 to develop the next generation of ALK2 inhibitors using MOE as modeling tool.

Signaling / *in-vitro*

OD compounds prevent BMP6- or Activin A-induced ALK2 activation in preosteoblasts (KS483) and in C2C12 ALK2^{R206H} expressing cell line



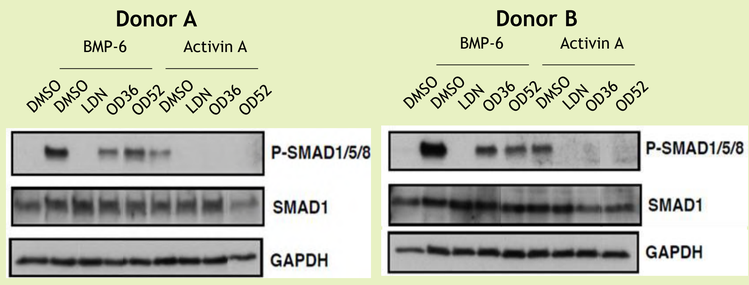
KS483 pre-osteoblast cell line was stimulated with BMP6 to activate ALK2 signaling pathway. OD compounds dose-dependently inhibited BMP6-induced ALK2 activation as observed by the decrease of BMP reporter signal.



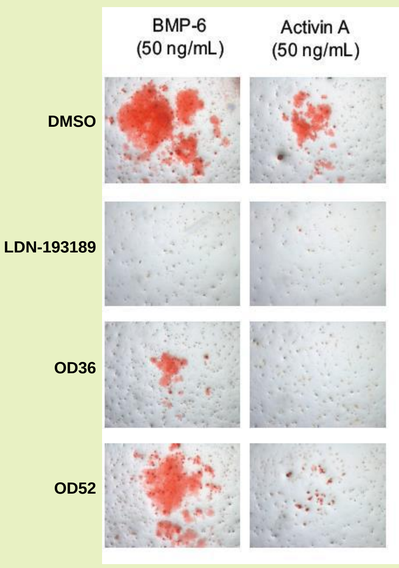
C2C12 muscle cells were transfected with FOP related R206H ALK2 then stimulated either with BMP6 or Activin A. OD compounds displayed stronger inhibitory effect when Activin A, a specific ALK2^{R206H} ligand, was used for cell stimulation as compared with BMP6.

Ex-vivo

***Ex-vivo* assay using Endothelial Colony Forming Cells (ECFCs) from FOP donors**



ECFCs from 2 FOP donors were activated either with BMP6 or Activin A then treated either with DMSO, LDN193189 or OD compounds. At 0.5uM, LDN193189 completely inhibited Smad phosphorylation upon BMP6 or Activin A stimulation. OD36 and OD52 displayed a weak effect on BMP6-stimulated ECFCs, whereas they displayed a total inhibition of Smad 1/5/8 phosphorylation upon Activin A stimulation.



ECFC clones were used in osteogenic differentiation assay with calcium deposit as readout. Upon treatment with 0.5uM of LDN193189, BMP6- and Activin A-induced calcification was completely inhibited. At the same concentration, OD compounds displayed a significantly higher efficacy on clones stimulated with AA than BMP6.

The evaluation of OD compounds in *ex-vivo* cellular models from FOP donors confirmed the results obtained from ALK2^{R206H} C2C12 cells, strengthening the rationale to further investigate the potential of small molecules inhibiting ALK2 to discover an efficient therapy for FOP patients.

Conclusions and perspectives

- **A series of ALK2 inhibitors with low nanomolar affinities and improved selectivity was identified,**
- **X-ray crystallography confirmed the binding mode of type 1 inhibitors to support optimization of the lead compound OD52,**
- **Mechanistic studies highlighted a specific inhibition of ALK2^{R206H} activated by Activin A,**
- **Ex-vivo efficacy was demonstrated on ECFCs from FOP patients,**
- **These encouraging results warranted further investigation and enabled the initiation of lead optimization effort to identify a preclinical candidate with enhanced DMPK properties together with a clean safety profile. We aim to discover a chronic oral treatment as an alternative to Activin A Antibody currently under clinical investigation.**

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