# NANOCYCLIX® ALK2 INHIBITORS TO OVERCOME CANCER-INDUCED ANEMIA

P160

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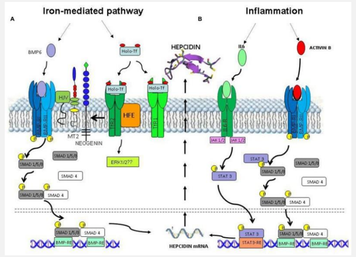
Vector of innovation.

## 1 CONTEXT & OBJECTIVES

✓ Anemia is a condition characterized by low hemoglobin levels. Anemia is observed during tumor development through the release of inflammatory cytokines, which affect iron availability, a key component of red blood cells. In recent years, consequences of anemia of cancer in patients is getting growing recognition from a quality of life perspective, as well as its impact on the treatment scheme and hospitalization duration. Cancer-induced anemia has been identified in 30% of treatment-naive cancers (ECAS study, 2011 – WHO methodology). The proportion of anemic patients reaches 70% after treatments, such as chemotherapies. Current approaches consider erythropoietin derivatives, formulated iron or transfusions, which does not address underlying chronic inflammation. Moreover, their use is limited as a consequence of toxicities (EPO, formulated iron) or availability (transfusion).

✓ Increased expression of Hepcidin, a peptidic hormone from liver regulating the storage of bioavailable iron, has been demonstrated to be a downstream effector of key inflammatory cytokines activation of ALK2/Smad axis. It has been shown in the context of anemia of inflammation/cancer that ALK2 inhibition normalizes Hepcidin expression and restores hemoglobin levels through increased iron availability. As a consequence, the selective inhibition of ALK2 has emerged as a valuable target for the treatment of Hepcidin-driven anemia of cancer to provide an alternative to current treatments.



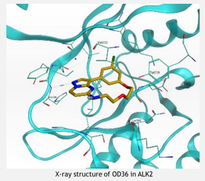

## 2 BIOCHEMISTRY / CRYSTALLOGRAPHY

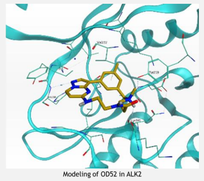
✓ From the initial hit OD36 identified from Nanocyclix® library, a first optimization enabled us to identify OD52, displaying improved affinity on ALK2 while maintaining high selectivity against ALK3 together with a high selectivity profile on a comprehensive panel of kinases (386k – S50=0.9% @ 100nM).
✓ The enhanced physicochemical properties of the compound enabled us to use it as an in vitro tool compound for initial proof-of-concept studies (Sánchez-Duffhues et al, 2019).
✓ Further optimization was performed on the series, leading to the identification of OD66, a potent compound with enhanced pharmacokinetic properties. Oncodesign's compounds have good to excellent selectivity against other members of Activin-like kinase receptor family.

| Compound ID | Kd in nM (DiscoverX) | | | | | | | | S50 @ 100nM 96k panel |
|---|---|---|---|---|---|---|---|---|---|
| | ALK1 | ALK2 | ALK3 | ALK4 | ALK5 | ACVR2A | ACVR2B | TGFBR2 | |
| LDN-193189 | 4.2 | 1.2 | 3.8 | 91 | 77 | 12 | 11 | 26 | 4.2% |
| OD36 | 90 | 37 | >3000 | >3000 | >3000 | >3000 | 90 | 680 | 4.6% |
| OD52 | 13 | 9.6 | 1300 | 260 | 850 | 590 | 220 | 2500 | 2.1% |
| OD66 | 8.6 | 3.2 | 370 | 351* | 1700 | 2405* | >3000* | 1500 | 2.1% |

* IC50 values in nM (ProQinase)

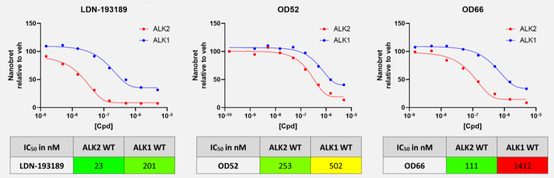
✓ Through a collaboration with the Structural Genomic consortium, x-ray crystallography of OD36 in ALK2 was performed with a resolution of 2.56Å (PDB: 5OY6). The analysis of the x-ray structure confirmed the binding mode of the compound as a type 1 ALK2 inhibitor (diagram on the left). The compound has a single H-bond interaction located on the hinge region with His286. The complementary shape of the rigid macrocyclic compound is mainly responsible for the affinity and the selectivity.
✓ We used this model to perform structure-based drug design starting from our lead compound OD52 to develop the next generation of ALK2 therapeutics.


X-ray structure of OD36 in ALK2


Modeling of OD52 in ALK2

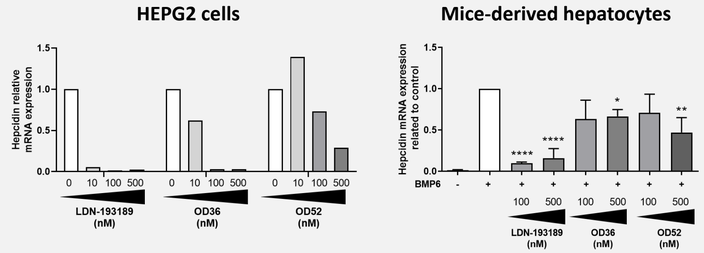
## 3 IN VITRO / EX VIVO RESULTS

✓ Evaluation of compounds using NanoBRET® technology highlighted good activities on cellular ALK2 WT assay, ranging from 23 nM to 253 nM. Surprisingly, OD66 and LDN compounds displayed 10-fold selectivity vs ALK1 assay. This result is of particular interest since it would be desirable to dial out the ALK1 component, described in the literature as being involved in angiogenesis.



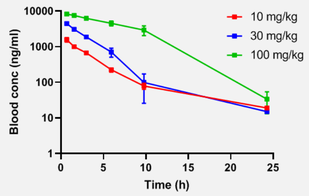

| $IC_{50}$ in nM | ALK2 WT | ALK1 WT |
|---|---|---|
| LDN-193189 | 23 | 201 |
| OD52 | 253 | 502 |
| OD66 | 111 | 1412 |

✓ Potency of OD36 and OD52 compounds were tested in vitro on the HEPG2 cell line and ex vivo on mice hepatocytes, using LDN-193189 as a benchmark. HEPG2 cells, derived from a hepatocellular carcinoma, were stimulated with BMP6 (2.5 ng/mL) and treated with 10, 100 or 500 nM of compounds. After 2h, Hepcidin expression was assessed by qPCR and showed a dose-dependent effect of compounds. Hepatocytes from C57BL/6 mice were retrieved after collagenase perfusion, then stimulated with BMP6 (50 ng/mL) and treated with 100 or 500 nM of compounds. After 4h, Hepcidin expression was assessed by qPCR and showed that its expression was inhibited by tested compounds.




## 4 PHARMACOKINETIC PROFILE

✓ In addition to good physico-chemical properties (ChromlogD=3.2, Kinetic solubility >200uM, mice Fu=0.7%), OD66 is highly bioavailable (>96%) with dose-dependent exposure in mice pharmacokinetic studies.




| Parameter | Unit | OD66 | | |
|---|---|---|---|---|
| Dose | mg/ kg | 10 | 30 | 100 |
| $DNAUC_{0\text{-}inf}$ | (h.ng/ mL)/(mg/ kg) | 780 | 789 | 1173 |
| $C_{max}$ | ng/ mL | 2570 | 5760 | 19600 |
| F* | % | 96 | 97 | > 100 |

✓ In complement to a clean profile in a safety pharmacology panel (CEREP safety screen 44), in vivo studies on mice have shown that OD66 was tolerated several weeks at pharmacological doses without adverse events.

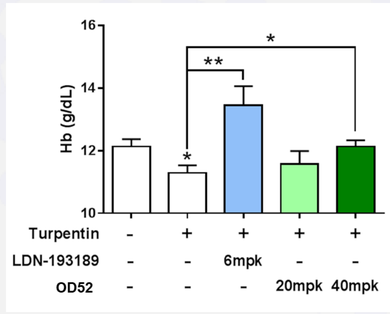
## 5 IN VIVO RESULTS

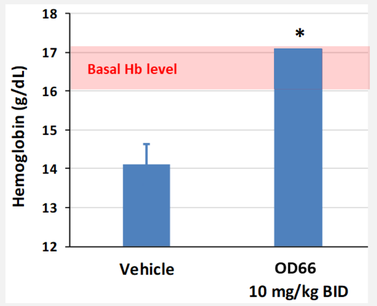
### Anemia of Inflammation model

✓ Anemia of inflammation was induced in C56BL/6 mice following turpentine (5 mL/kg) injection.

✓ LDN-193189 (IP) and OD52 (PO) were administered BID for 48h before assessment of hemoglobin (Hb) levels.

✓ Results show that LDN-193189 (pan-ALK inhibitor) increases Hb concentration to a level above normal, while OD52 normalizes Hb levels at 40 mg/kg.




### Anemia of Cancer model

✓ Anemia of cancer was induced in SCID Beige mice by xenografting A498 cells.

✓ Mice were randomized once tumor volumes reached 100-200 $mm^3$ and were then treated PO BID with 10 mg/kg of OD66 over a period of 28 days.

✓ Mice were then euthanized and Hemoglobin levels were assessed.

✓ Results indicate that OD66 restores Hemoglobin levels to normal ranges

## 6 CONCLUSION

✓ A series of ALK2 inhibitors with low nanomolar affinities and improved selectivity were identified – Activity differentiation has been observed in NanoBRET® on ALK2 vs ALK1

✓ X-ray crystallography confirmed the binding mode of type 1 inhibitors to support optimization of the lead compound OD52

✓ Mechanistic studies highlighted the potency of compounds to inhibit Hepcidin expression in vitro and ex vivo.

✓ The optimized PK profile / toxicity allows the use of OD66 in vivo

✓ OD66 restores in vivo the hemoglobin levels in tumor-bearing mice.

✓ Oncodesign seeks a partner to advance its ALK2 program towards drug candidate selection with therapeutic potential in Anemia of cancer and rare disease IRIDA

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