

PROPRANOLOL, AN ACCESSIBLE, AFFORDABLE, AND AVAILABLE ORPHAN DRUG FOR THE VON HIPPEL-LINDAU DISEASE

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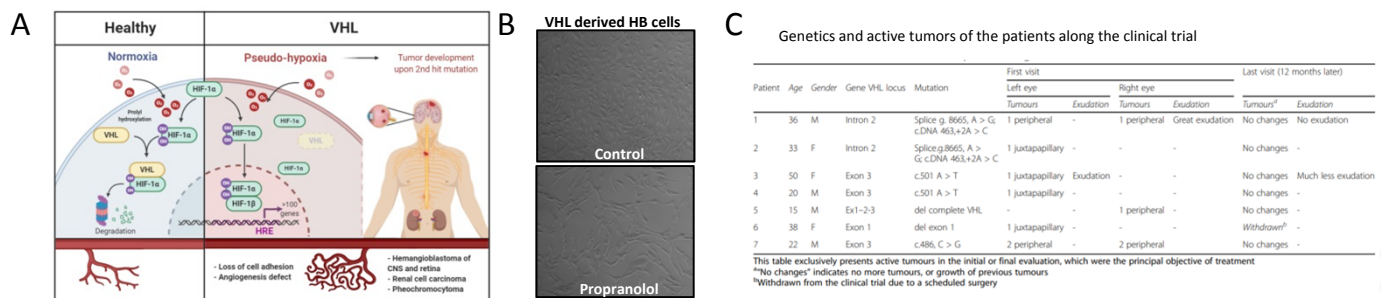
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Von Hippel-Lindau disease (VHL) research in Spain has a notably translational character with direct networking between clinicians, VHL Spanish Alliance patients, and CSIC researchers. Propranolol, an antihypertensive, non-specific β -blocker, was re-discovered as a cure for Infantile Hemangioma and, later, different publications showed that Propranolol has antiangiogenic and proapoptotic properties. Different *in vitro* assays using propranolol in VHL primary cultures demonstrated that Propranolol triggered apoptosis in these cells. Moreover, Propranolol reduced the levels of Hypoxia Inducible Factor (HIF), constitutively expressed in VHL-derived tumors due to the lack of pVHL protein targeting HIF to proteasomal degradation under normoxia. A reduction of HIF led to downregulation of HIF targets, remarkably, VEGF (Vascular Endothelial Growth Factor), EPO (Erythropoietin), or SOX2. *In vitro* results and the known safety profile of Propranolol, allowed to organize a Phase III Clinical Trial (EudraCT Number: 2014-003671-30) evaluate the therapeutic effect and safety of Propranolol on 7 VHL patients suffering from retinal hemangioblastomas with no therapeutic option. Patients were treated with 120 mg/day Propranolol for a year. Retinal hemangioblastomas and VEGF plasma levels were followed-up along the trial. The results of the clinical trial showed a stop in the retinal hemangioblastoma growth, and no new tumors arose. In addition, 2 patients had a significant reduction of eye exudates detectable before the clinical trial and a significant reduction of the VEGF plasma levels was detected. Due to *in vitro* and *in vivo* results, Propranolol was designated by EMA as orphan drug for VHL treatment (EU/3/17/1841).



A. VHL disease schema. The protein VHL (component of the Ubiquitin ligase complex) is mutated in this disease. VHL regulates the degradation of HIF1 α and HIF2 α . In the presence of oxygen (normoxia) PHD proteins hydroxylate certain proline residues of HIF, which will be recognized by VHL, that mark them for degradation. In the case of mutated VHL, it is similar to what happens in hypoxia, either because PDHs are not active due to lack of oxygen, or because VHL is mutated and does not recognize HIF, in both cases HIF is not degraded and remains in the cell increasing the expression of its target genes involved in certain processes such as angiogenesis, migration or proliferation of tumor cells. The main clinical manifestations are CNS and retinal HB and also clear cell renal carcinoma or pheochromocytomas. **B. VHL derived HB cells** treated with Propranolol show a decrease in proliferation, promoting cell death. **C. Clinical results** are shown; the number and size of all the tumors present on the retina at the beginning of the clinical trial remained stable without any other treatment. Two of the 7 patients had a decrease of the exudate present near to the HB.

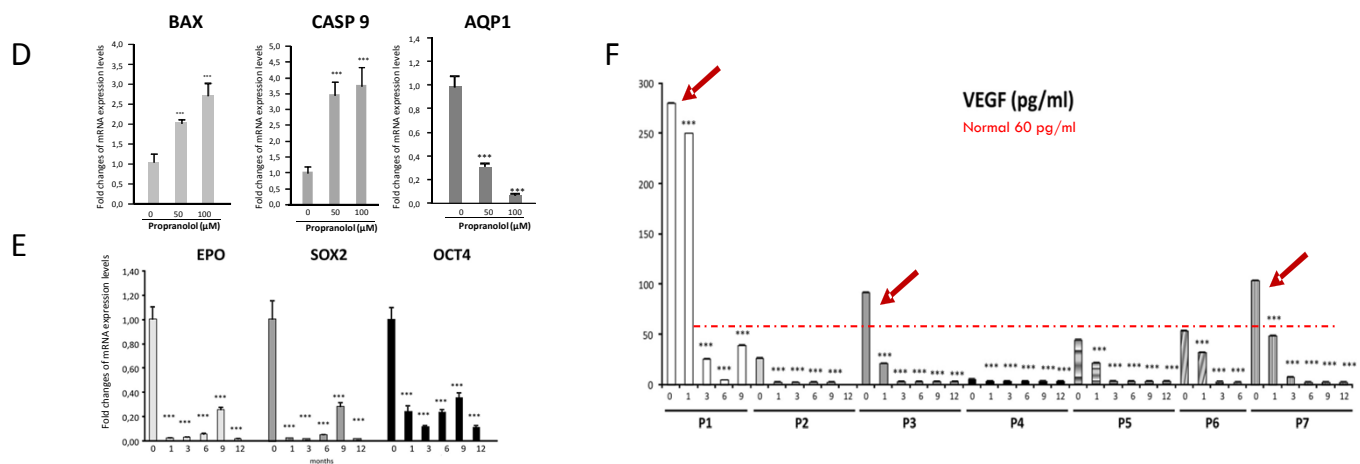
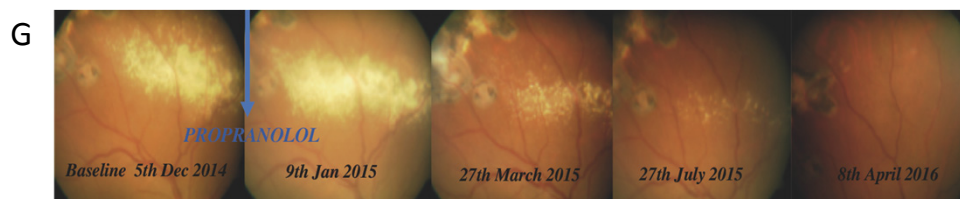


Figure D, E and F. RT-PCR results of the relative expression levels of different HIF target genes, following propranolol treatment. **D.** The proapoptotic genes Bax and Casp9, are upregulated in VHL derived HB cells (from surgery) while the expression of the transmembrane water channel AQP1 decreases (involved in the formation of the cyst or exudate linked to the formation of the tumor). **E.** Sox, Oct 4 and Epo expression (dependent genes for the HB growth and angiogenesis, respectively) are downregulated. RNA was obtained from the PBL fraction of patients included in the clinical trial. The results are representative of one of the patients. Each RT-PCR was repeated at least three times and in triplicate. **F.** Evolution of VEGF plasma levels of the different patients (P1-P7) at the different visits during the clinical trial. Arrows indicate three cases wherein VEGF levels were initially greater than the normal threshold, and the decrease was significant, reaching normal levels following propranolol treatment.



G. Reabsorption of exudation in Patient 1. The first image corresponds to the baseline visit and the last one after 1 year of treatment. This patient had the highest baseline levels of VEGF in peripheral blood and experienced the highest reduction during the follow-up, decreasing to those found in general population.

Propranolol shows properties as therapeutic drug for retinal hemangioblastomas and capabilities for CNS hemangioblastomas, and should be considered in the treatment of VHL patient. Orphan drug designation for Propranolol by EMA to treat von Hippel Lindau disease was achieved in 2017 (EU/3/17/1841).