TRPML1 antagonist: Exploring new therapeutic opportunities for cancer

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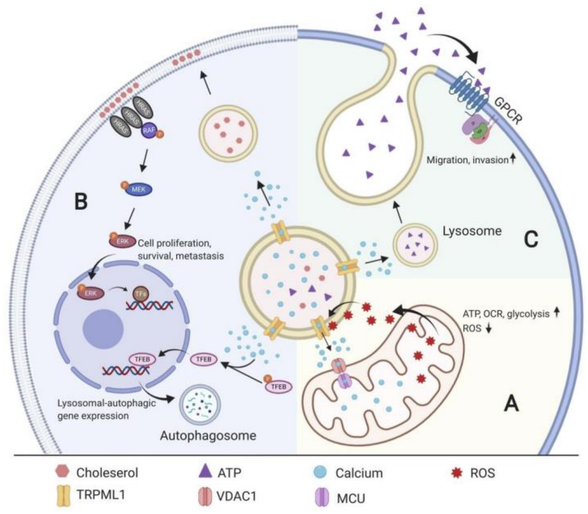
Transient Receptor Potential-Mucolipin 1

- Cation-selective channel assembled with four subunits composed by six putative transmembrane domains
- Mostly located at the lysosomal membrane, activated by the endogenous $PI_{(3,5)}P_2$
- Coordinates the synthesis and breakdown of macromolecules in lysosomes
- Plays an important role in lysosomal biogenesis, positioning, exocytosis and autophagy

Application: cancer therapy

- Due to a high energy demand of cancer cells, the function of lysosomes is often maladaptively upregulated to meet the metabolic requirement
- Cancer-related increase of mitochondrial reactive oxygen species (ROS) production triggers TRPML1-mediated lysosomal Ca^{2+} release



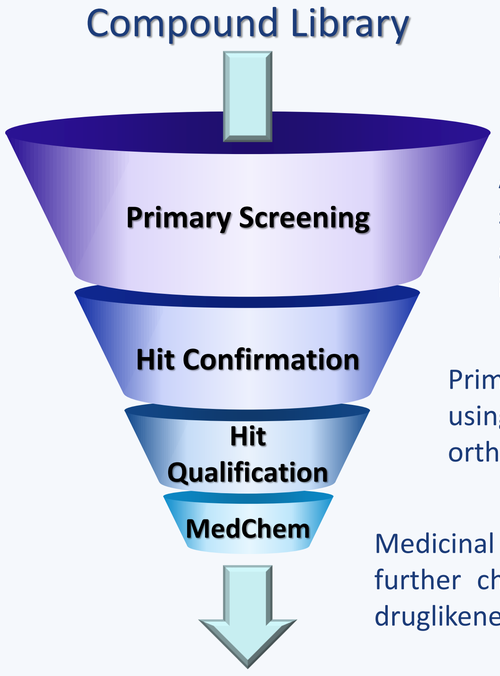
Adapted from Yang *et al.* Front. Cells, 2020

A) Ca^{2+} release facilitates the mitochondrial Ca^{2+} uptake, increasing their oxygen consumption rate (OCR) and then the overall cellular bioenergetic output

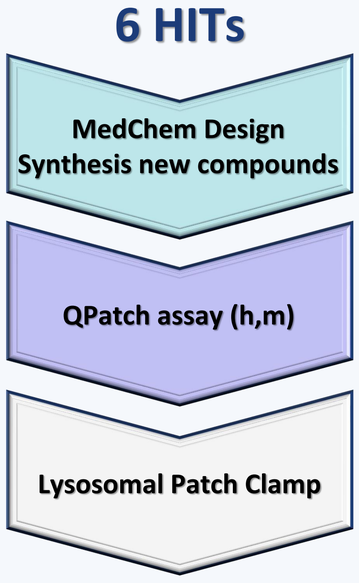
B) Ca^{2+} release facilitates the nuclear translocation of transcription factor EB (TFEB), promoting the lysosomal/autophagic gene expression and modulating the metabolic reprogramming in cancer

C) TRPML1 also mediates extracellular release of lysosomal ATP in specific cancerous cells (i. e. triple-negative breast cancer and melanoma), driving tumor migration and invasion

AXXAM Compound Library



6 HITs



Screening Funnel

Approximately 300K compounds were screened against TRPML1 in the FLIPR assay and several primary hits were identified

Primary hits were confirmed and qualified using both the FLIPR and the QPatch as orthogonal assays: 32 compounds

Medicinal Chemistry review: hit selection for further characterization based on patentability, druglikeness and chemical tractability

- TRPML1 FLIPR assay (h)
- Solubility; LogD; PPB (r/m, h), PAMPA
- Microsomal stability (r/m, h)

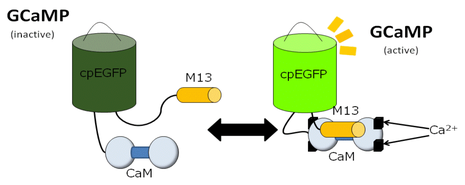
- *in vitro* cancer assay models: migration and proliferation
- Hepatocyte stability (r/m, h)
- Cyp induction/inhibition
- Cardiac safety
- Rat/mouse IV/PO PK

- TFEB translocation
- Autophagy
- Selectivity: TRPML2, TRPML3, TRPV1, TRPA1, TRPM8

PharmacoDynamic model

FLIPR (Primary Assay)

- High throughput screening assay
- Measurement of Ca^{2+} influx from FRET effect exploited by GCaMP sensor
- TRPML1 is not selective for Ca^{2+}

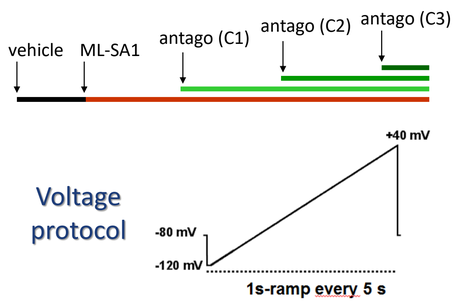


GCaMP sensor general mechanism

QPatch

- In recombinant cell lines, TRPML1 is mainly expressed in the lysosomes and in minimal part on plasma membrane
- Direct measure of channel activity expressed in the plasma membrane
- Employment of not endogenous ML-SA1 as reference agonist

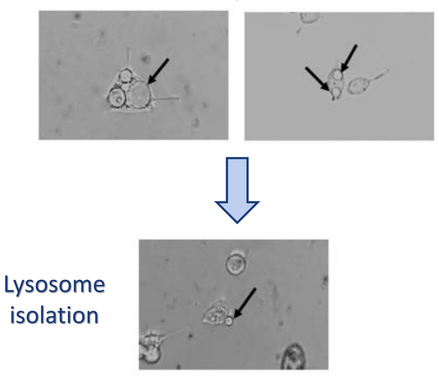
Application protocol



LysoPatch

- Direct measure of channel activity in the lysosomes
- Employment of the endogenous $PI_{(3,5)}P_2$ as reference agonist

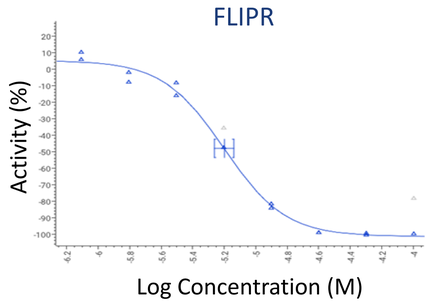
Overnight incubation of cells with vacuolin



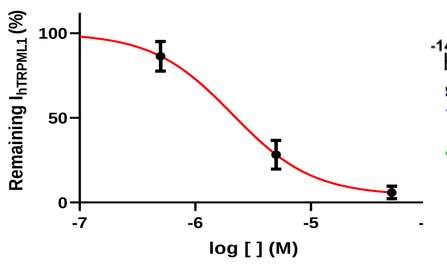
Lysosome isolation

Most interesting identified compound

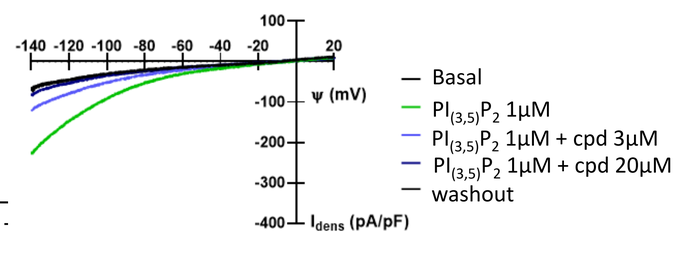
FLIPR



QPatch



LysoPatch

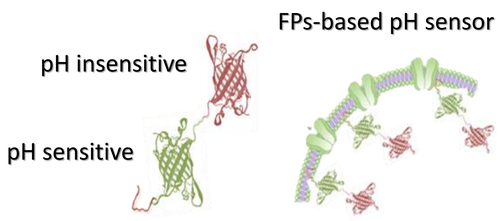


Assay	IC50 (µM)	pIC50	Imax (%)
FLIPR	6.28	5.20	-99.6
QPatch	2.44	5.61	-94.1

Compound at 3 µM reduce the channel activity of more than 50% in LysoPatch

Druglikeness ✓ IP Evaluation ✓ Synthetic feasibility ✓

Next Steps & Conclusion

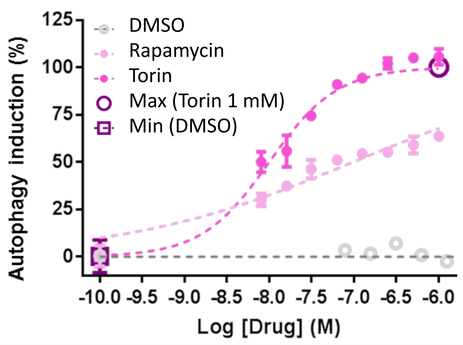


Fluorescent Proteins (FP)-based pH sensor targeted to the autophagosome by fusion with LC3

In vitro PoC: Autophagy assay

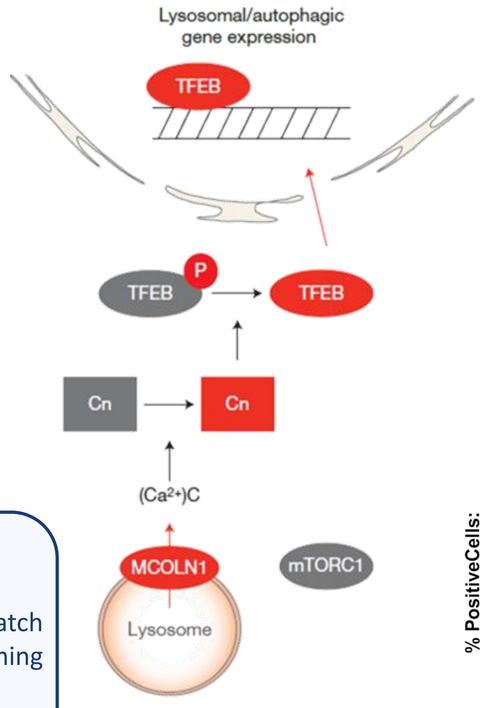
- Cells are transfected with a pH sensor and targeted to the autophagosome by means of a LC3 localization sequence.
- Cells are treated with compounds modulating the autophagy pathway. A difference of the fluorescence emission can be detected after the interaction between lysosomes and autophagosomes.

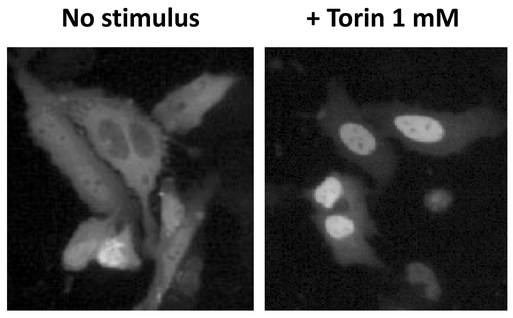
Dose-response curves of Torin and Rapamycin



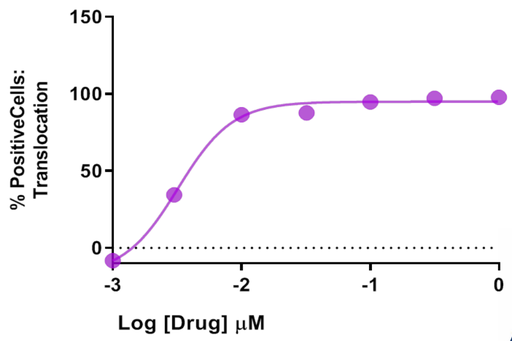
Conclusion

- 6 qualified hits prioritized
- HTS-grade FLIPR assay, medium-throughtput QPatch and LysoPatch ready to be used for the upcoming hit-to-lead development program
- 2 robust High Content assays optimized for the next in vitro Proof-of-Concept validation





Dose-response curve of Torin



In vitro PoC: TFEB assay

- The target of interested (TFEB) is mainly located in the cytosol compartment (left picture). Upon a specific stimulus (Torin), the protein translocates into the nucleus compartment (right picture).
- The localization of the target is analyzed by fluorescence intensity measurements. The percentage of cells showing the fraction of nuclear target is plotted in a dose response curve against the stimulus.

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