Anti-cancer activity of “Pyr1”, a new LIM-Kinases inhibitor

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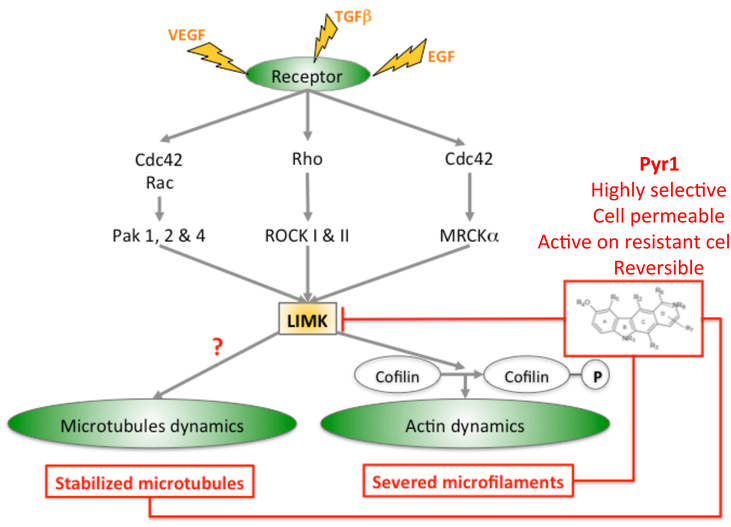
LIMK : a signaling node that controls both actin and microtubules dynamics



Pyr1
Highly selective
Cell permeable
Active on resistant cell lines
Reversible

Analysis of therapeutic efficacy : Methodology

① *In vitro*

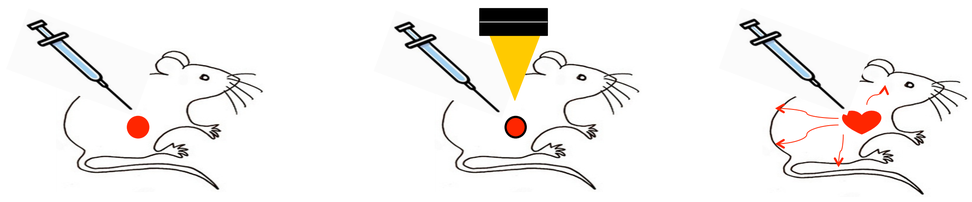
Effect on migration
Effect on invasion
Effect on spheroids

Breast cancer cell lines
TS/A-pGL3, MDA-MB-231, MDA-MB-231-ZNF217

② *In vivo*

Antitumor activity
Comparison with Paclitaxel (PTX)

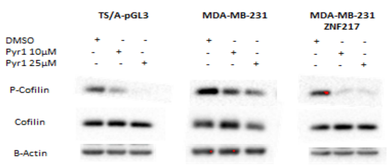
1) TS/A-pGL3 cells Luciferase
2) MDA-MB-231 cells
3) MDA-MB-231 Dendra2 cells + intravital microscopy
4) MDA-MB-231-ZNF217 cells luciferase



Intravital imaging and effect on metastasis

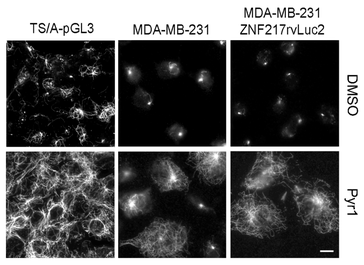
Pyr1 is active *in vitro* on breast cancer cell lines resistant to paclitaxel

Pyr1 inhibits cofilin phosphorylation



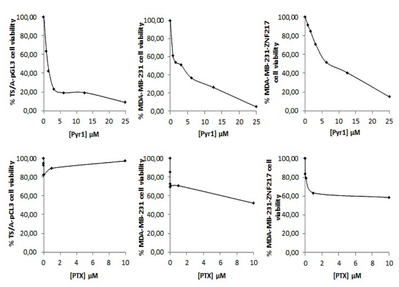
Western Blot analysis of cofilin phosphorylation in breast cancer cell lines

Pyr1 stabilizes microtubule network



Immunofluorescence analysis of microtubule resistance to nocodazole-induced depolymerization, bar=10µm

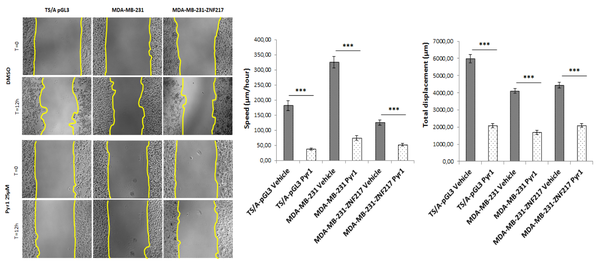
Pyr1 is toxic for resistant cell lines



MTT analysis of Pyr1 and PTX effect on breast cell lines

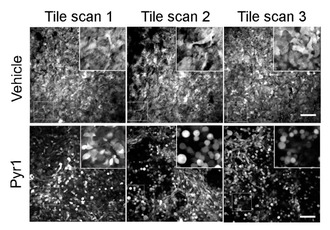
Pyr1 impacts cell migration differentially *in vitro* and *in vivo*

In vitro, Pyr1 slows down cell mobility

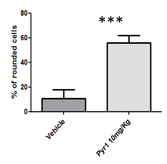


*Representative images and quantification of Pyr1 effect on breast cancer cell movement: velocity, total displacement and persistence, ***p<0,01*

Intravital analysis shows that Pyr1 affects tumor cell morphology and mobility

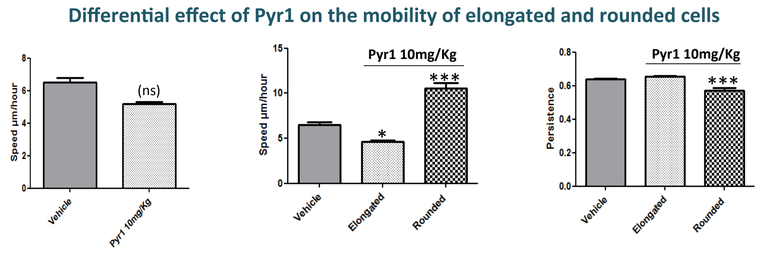


Representative intravital images of MDA-MB-231 Dendra2 tumors from mice treated with vehicle or 10 mg/Kg Pyr1 for at least 8 days. Three tile scans obtained from 3 different mice are shown. Upright inserts are 4X magnifications of regions of interest. bar= 100µm.



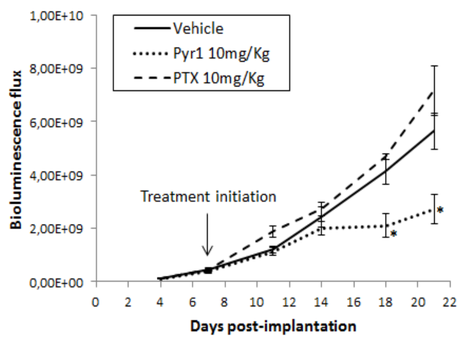
*Quantitative analysis of Pyr1 effect on cell morphology, ***p<0,01*

Differential effect of Pyr1 on the mobility of elongated and rounded cells



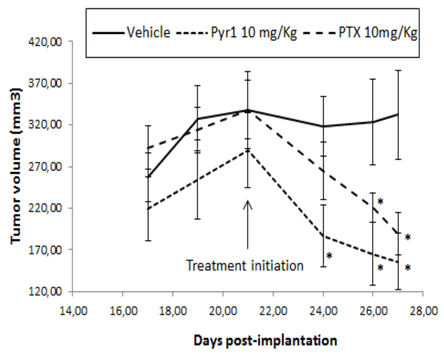
Pyr1 slows down tumor growth and decreases tumor size

Effect of Pyr1 and PTX on tumor growth rate

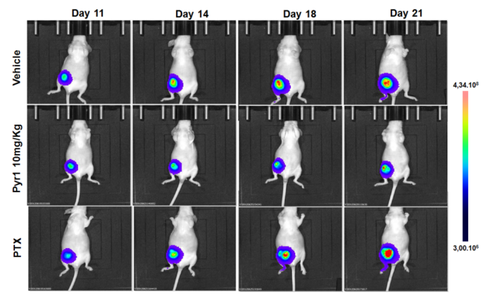


*Bioluminescent flux analysis of primary TS/A-pGL3 tumors over days, *p<0,05*

Effect of Pyr1 and PTX on tumor size

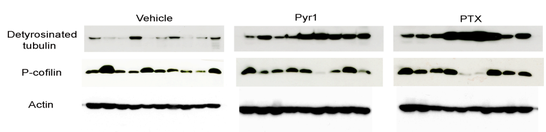


*Tumor volume analysis of primary MDA-MB-231 tumors over days, *p<0,05*

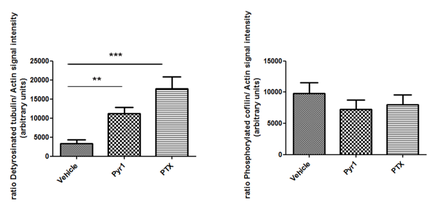


Representative images of the bioluminescent flux in mice bearing TS/A-pGL3 tumors over days

Pyr1 anti-tumor effect involves microtubule stabilization



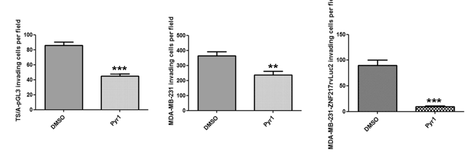
Lysates of MDA-MB-231 tumor xenografts (30µg) treated with vehicle, Pyr1 or PTX were blotted for detyrosinated tubulin, phosphorylated cofilin (P-cofilin) and actin as indicated.



*Signal intensities were quantified using ImageJ software and the ratios of detyrosinated tubulin/ actin (left) and phosphorylated cofilin/actin (right) were calculated. Bars = SEM, ** p <0.01, *** p <0.001.*

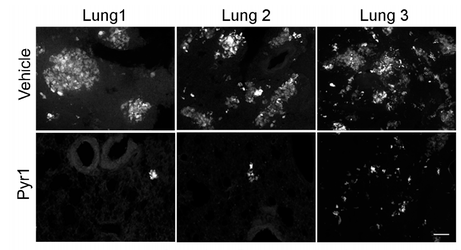
Pyr1 impairs the formation of macrometastasis

In vitro, Pyr1 inhibits cell migration through matrigel



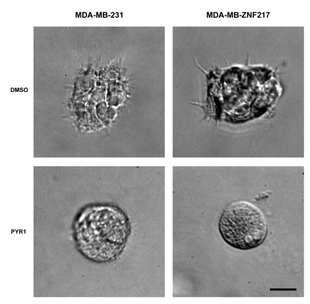
Matrigel invasion assay. Histograms represent quantification (mean ± SEM) of invasion of TS/A-pGL3, MDA-MB-231 and MDA-MB-231-ZNF217rvLuc2 cells treated with 25µM Pyr1 or 0.25% DMSO
*** p < 0.01, ***p < 0.001*

In vivo analysis of the presence of tumor cells in lungs at the end of intravital imaging experiment indicates that Pyr1 reduces the colonization of distant organs

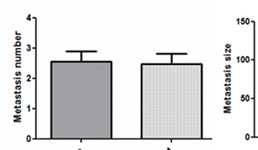


Representative images of Dendra2 fluorescence in lung sections, of mice bearing MDA-MB-231 Dendra2 mammary tumors, treated (Pyr1) or not (vehicle). bar= 100µm.

In vitro, Pyr1 inhibits the formation of filipode-like protrusions (FLPs)



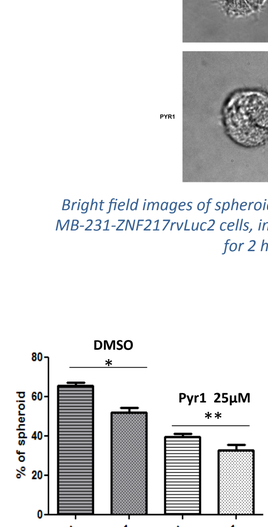
Bright field images of spheroids derived from MDA-MB-231 and MDA-MB-231-ZNF217rvLuc2 cells, incubated with 0.25% DMSO or 25µM Pyr1 for 2 hours. bar= 20µm.



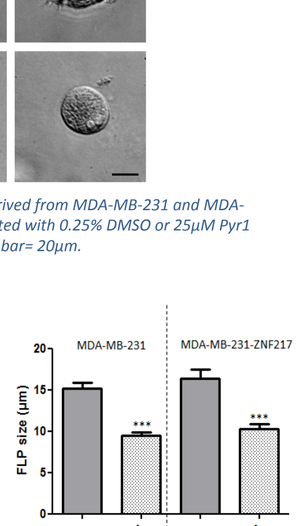
Quantification of metastasis number. Histograms represent the average number ± SEM of metastasis nodules in the lung of mice treated with vehicle or 10 mg/Kg Pyr1. n = 6 fields per lung, 3 mice per group .

Effect of Pyr1 on MDA-MB-231-ZNF217rvLuc2 metastatic colonization

MDA-MB-231-ZNF217rvLuc2 cells stably expressing luciferase were injected in the blood stream. Metastases colonization was followed by bioluminescence

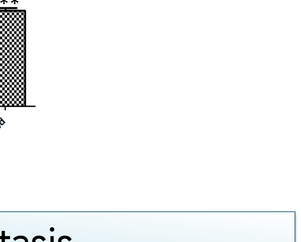


Average percentage of spheroids with FLPs in the absence (DMSO) or in the presence of 25 mM Pyr1, as indicated
* p < 0.05, ** p < 0.01



Average length of FLPs. The length of FLPs was measured on 25 spheroids per treatment, in 3 independent experiments
* p < 0.05, ** p < 0.01, *** p < 0.001

Average number ± SEM of metastatic sites

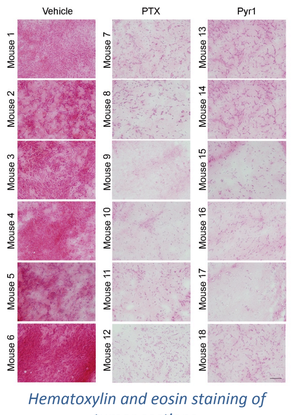


Effect of the treatment on the total metastatic load, over days n = 10 mice for each group.
** p < 0.01*

Representative bioluminescence images (one mouse per condition)

Pyr1 decreases cell proliferation and induces apoptosis in tumors

Reduction of the cellular density of Pyr1- and PTX- treated MDA-MB-231 tumors



Hematoxylin and eosin staining of tumor sections.
bar= 100µm.

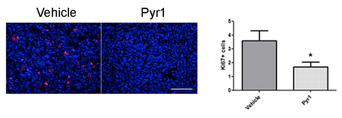
Intravital imaging shows that Pyr1 decreases the number of tumor cells

Quantification of MDA-MB-231 cells in tumors

Examples of images analyzed; red lines separate the green fluorescent areas from the non-fluorescent areas.
bar= 100µm

Ratios of Dendra2 fluorescent areas over non-fluorescent areas
*(mean ± SEM of at least 3 tumors analysis, 5 fields per tumor). * p < 0.05.*

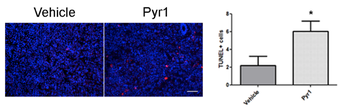
Proliferation analysis



Ki67 staining after 8 days of treatment.
bar=50µm

Quantification of Ki67 positive cells
(mean ± SEM of 3 tumors analysis)
* p < 0.05.*

Apoptosis analysis



TUNEL assay tumor staining after 8 days of treatment.
bar= 50µm

Quantification of TUNEL positive cells
(mean ± SEM of 3 tumors analysis)
* p < 0.05*

Conclusions

- Pyr1 has a potent anti-tumor effect on primary mammary tumors in breast cancer models.
- Pyr1 does not inhibit metastases spreading, but leads to a drastic inhibition of metastases growth.
- LIMK inhibitors may represent a pharmacological alternative for the treatment of taxane resistant tumors.