Myoferlin targeting primes ferroptosis in pancreatic cancer cells

Yasmine Boumahd[1], Gilles Rademaker[1,2], Raphaël Peiffer[1], Sandy Anania[1], Ferman Agirman[1], Naïma Maloujahmoum[1], Akeila Bellahcène[1], Olivier Peulen,[1]

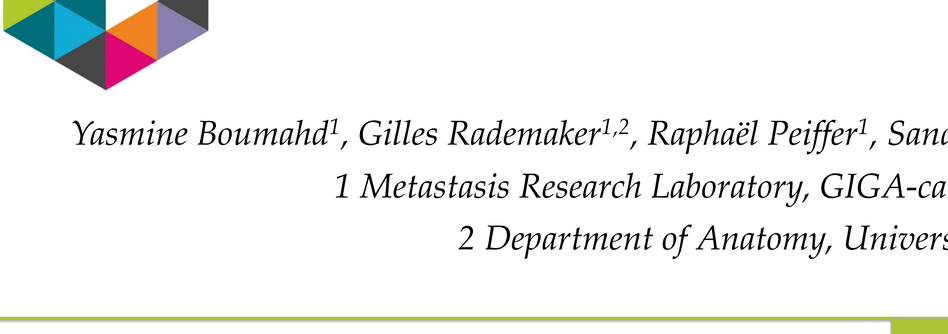
1 Metastasis Research Laboratory, GIGA-cancer, University of Liège, Pathology Institute B23, B-4000 Liège, Belgium

2 Department of Anatomy, University of California, San Francisco, San Francisco, CA 94143, USA

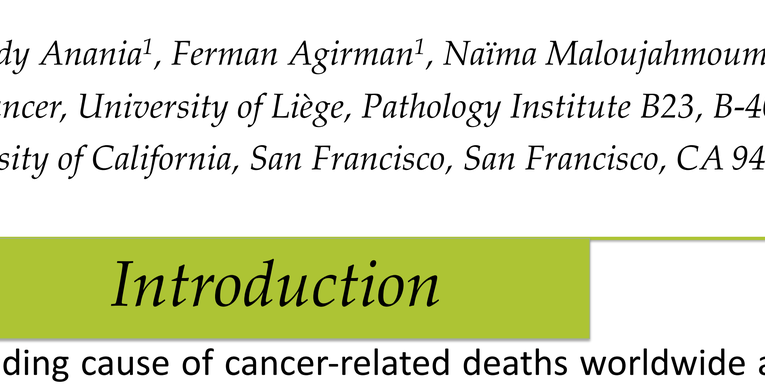
Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the seventh leading cause of cancer-related deaths worldwide and it is estimated that PDAC will become the second leading cause of cancer-related deaths by 2030. Myoferlin is a protein implicated in membrane fusion and in receptors recycling. It allows the formation of vesicles for the trafficking in cells. In PDAC, myoferlin is overexpressed in high grades in comparison to low grades. We used an innovative small compound (WJ460) to target myoferlin in PDAC cell lines and reported the triggering of an iron-dependent cell death, namely ferroptosis, considered as an alternative to apoptosis in cancer cells.

Results



****P<0.0001, ***P<0.001, **P<0.01, *P<0.05



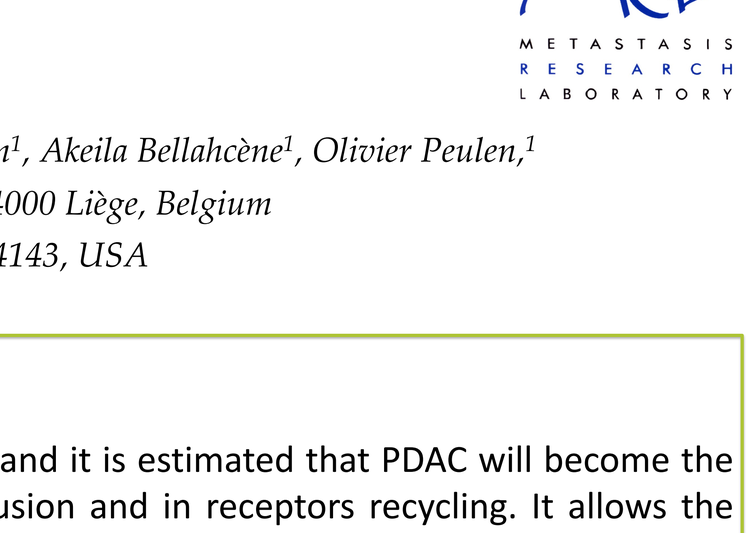


Figure 1: Pharmacological targeting of myoferlin exhibits the same effects than myoferlin gene silencing.
(A) Cell growth assessment during treatment of PDAC cell line (Panc-1) and normal pancreatic cell line (HPNE) with WJ460 ranging from 1 to 100 nM. **(B)** Kinetic oxygen consumption rate (OCR) in PDAC cell line (BxPC-3) and HPNE in response to oligomycin (O), FCCP (F), rotenone and antimycin A (RA). **(C)** Reactive oxygen species (ROS) accumulation in PDAC cell lines and HPNE cell line treated for 24h with 50 nM of WJ460. **(D)** Visualisation of mitochondrial network shape in PDAC cell line (Patu8988T) and HPNE after 50 nM WJ460 treatment during 24h. Scale bars represent 10 µm (PaTu8988T) or 7.5 µm (HPNE).

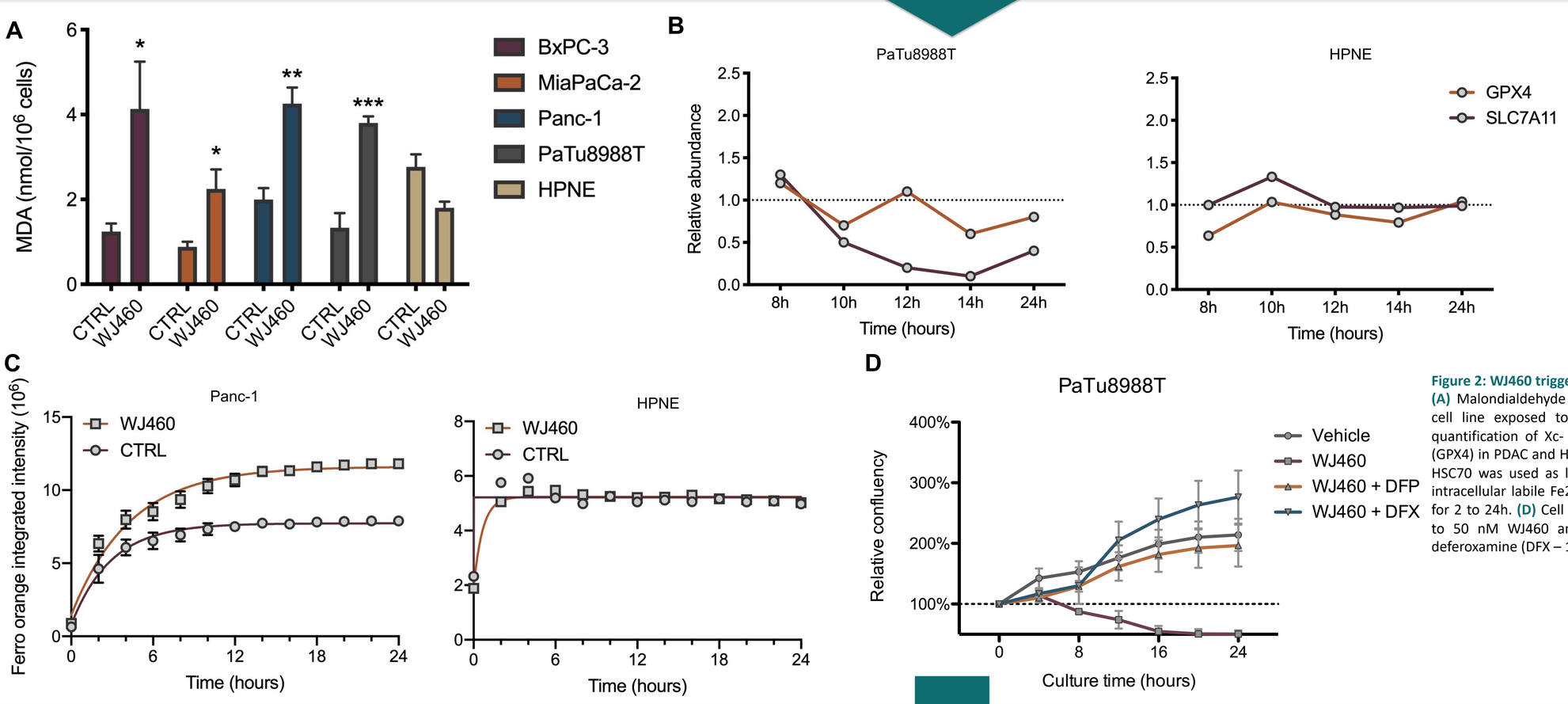


Figure 2: WJ460 triggers an iron-dependant cell death in PDAC cell lines.
(A) Malondialdehyde (MDA) concentration in PDAC cell lines and HPNE cell line exposed to 50 nM WJ460 for 24h. **(B)** Western-blot-based quantification of Xc- transporter (SLC7A11), and glutathione peroxidase (GPX4) in PDAC and HPNE cell lines exposed to 50 nM WJ460 for 8 to 24h. HSC70 was used as loading control. **(C)** Live-cell fluorescent imaging of intracellular labile Fe2+ in Panc-1 and in HPNE exposed to 50 nM WJ460 for 2 to 24h. **(D)** Cell growth rescue in PaTu8988T PDAC cell line exposed to 50 nM WJ460 and iron chelators, deferiprone (DFP – 1 mM) or deferoxamine (DFX – 1 mM).

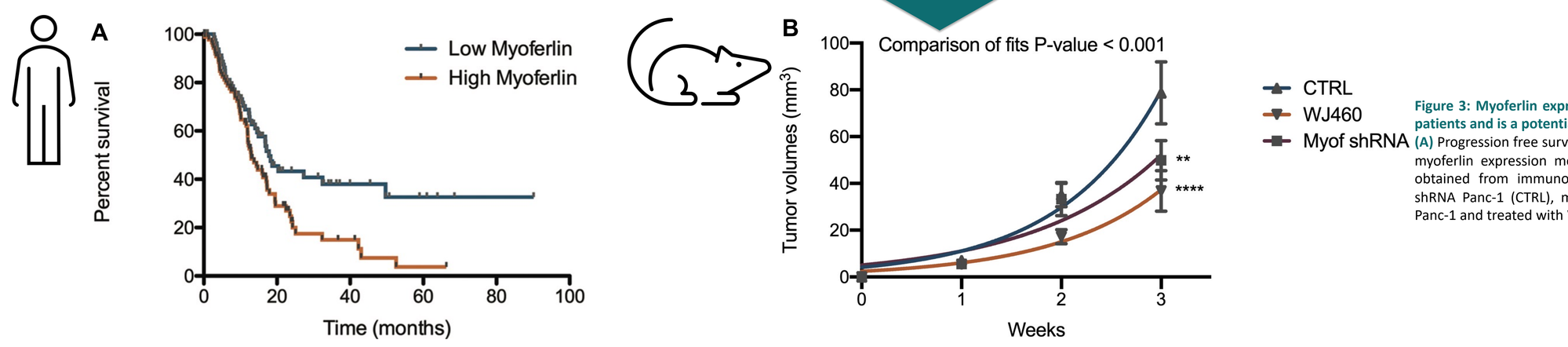


Figure 3: Myoferlin expression correlates with low survival in PDAC patients and is a potential therapeutic target.
(A) Progression free survival of PDAC patients segregated according to myoferlin expression median. **(B)** Volume of subcutaneous tumors obtained from immunocompromized mice grafted with irrelevant shRNA Panc-1 (CTRL), myoferlin shRNA Panc-1 or irrelevant shRNA Panc-1 and treated with WJ460 during 3 weeks every two days.

Conclusions and potential clinical relevance

The mortality rate of PDAC is currently almost equal to its incidence and its 5-year survival rate is the lowest among all cancers. In this study, we described that WJ460, a small compound binding to myoferlin, induced the same main biological effects than myoferlin gene silencing in several PDAC cell lines. Important iron pool is naturally present within tumors. Interestingly, erastin and RSL3, two ferroptosis inducers, were reported to be particularly efficient in KRAS-mutated cancer cells. More than 90% of PDACs carry mutated KRAS alleles, as such, ferroptosis has been proposed as an alternative to treat patients with KRAS mutation. Our results demonstrate that myoferlin targeting induces an iron-dependent cell death in PDAC cell lines.

Contact

YASMINE BOUMAHD
yasmine.boumahd@uliege.be