



HAL
open science

A translational study for biomarker identification of PEP-010, a pro-apoptotic peptide restoring apoptosis in cancer models

D. Germini, R. Farhat, L. Dadon, A. Lacroix, F. Nemati, A. Rebollo, D. Decaudin, J. Wiels, Catherine Brenner

► To cite this version:

D. Germini, R. Farhat, L. Dadon, A. Lacroix, F. Nemati, et al.. A translational study for biomarker identification of PEP-010, a pro-apoptotic peptide restoring apoptosis in cancer models. *Biochimica et Biophysica Acta - Molecular Basis of Disease*, 2025, 1871 (1), pp.167492. 10.1016/j.bbadis.2024.167492 . hal-04740800

HAL Id: hal-04740800

<https://hal.science/hal-04740800v1>

Submitted on 17 Oct 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License



A translational study for biomarker identification of PEP-010, a pro-apoptotic peptide restoring apoptosis in cancer models

D. Germini^{a,*}, R. Farhat^a, L. Dadon^a, A. Lacroix^a, F. Nemati^b, A. Rebollo^c, D. Decaudin^{b,d}, J. Wiels^e, C. Brenner^{e,*}

^a PEP-Therapy, 111 Avenue de France, 75013 Paris, France

^b Translational Research Department, Laboratory of Preclinical Investigation, PSL University, Institut Curie, 26 rue d'Ulm, Paris 75248, France

^c Unité de Technologies Chimiques et Biologiques pour la Santé (UTCBS), INSERM U1267 - CNRS UMR8258, Université de Paris, Faculté de Pharmacie, France

^d Department of Medical Oncology, Institut Curie, 26 rue d'Ulm, Paris 75248, France

^e Université Paris-Saclay, CNRS, Institut Gustave Roussy, Aspects métaboliques et systémiques de l'oncogénèse pour de nouvelles approches thérapeutiques, 94805 Villejuif, France

ARTICLE INFO

Keywords:

Apoptosis
Biomarkers
In vivo efficacy
Translational study
Breast cancer

ABSTRACT

- PEP-010 is a pro-apoptotic peptide based on a proprietary cell-penetrating and interfering peptide technology for the treatment of advanced solid tumors.
- PEP-010 acts by disrupting the interaction between caspase-9 and PP2A, two key proteins involved in apoptosis, a physiological process frequently altered in cancers.
- PEP-010 efficiently induces apoptosis in cancer cells.
- PEP-010 demonstrates antitumor activity by inhibiting the growth of breast cancer patient-derived xenografts (PDX).
- This study establishes cleaved caspase-3 and Ki67 protein expression as two candidate pharmacodynamic (PD) biomarkers for monitoring PEP-010-induced apoptosis *in vitro* and *in vivo*.

Apoptosis is a physiological cell death modality. Its alteration or the inhibition of its main actors is often associated with several human pathologies, particularly with cancers [1]. Thus, “re-teaching the cell to die” is a promising strategy for innovative anticancer therapies [2].

Two main pathways can induce a caspase-dependent apoptosis, namely the extrinsic and the intrinsic pathways. The first is initiated by stimulation of transmembrane receptors [3] while the intrinsic pathway is induced by mitochondrial outer membrane permeabilization (MOMP) triggered by the BH3-only members of Bcl-2 family (e.g. Bax, Bim, Bad and/or Bid). These proapoptotic proteins act as sentinels for cell stress and are mobilized *via* subcellular relocalization and/or post-translational modifications [1]. They can be regulated by protein phosphatase 2A (PP2A) a major serine/threonine phosphatase in eukaryotic cells, both indirectly at transcriptional level and directly at post-translational level [4]. MOMP leads to the release of mitochondrial cytochrome *c* into the cytosol followed by its assembly with caspase-9, a

cysteine-aspartic protease, and APAF1, thus forming a caspase-activating complex called the apoptosome, which in turn activates effector caspases (including caspase-3). Of note, failure to activate caspase-9 has been linked with pathophysiological consequences, such as cancers [5]. PP2A is a member of the serine-threonine phosphatase family and is responsible for the majority of this activity in eukaryotic cells. It maintains cellular homeostasis by counteracting most of the intra-cellular signaling pathways induced by kinases and is involved in several key cellular pathways as cell proliferation, DNA damage response and apoptosis [4,6]. PP2A is genetically modified or functionally inactivated in many solid cancers and numerous studies show that inhibition of expression and/or function of PP2A may contribute to cancer development [7,8]. Inhibition of PP2A activity is essential to promote cell transformation, tumor progression, and angiogenesis, indicating that PP2A has a tumor suppressor role [9,10]. Recent reports show that pharmacological restoration of PP2A tumor suppressor

* Corresponding authors.

E-mail addresses: diego.germini@pep-therapy.com (D. Germini), catherine.brenner@universite-paris-saclay.fr (C. Brenner).

<https://doi.org/10.1016/j.bbadis.2024.167492>

Received 18 March 2024; Received in revised form 31 July 2024; Accepted 22 August 2024

Available online 30 August 2024

0925-4439/© 2024 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

activity effectively antagonizes cancer development and progression [11,12]. PP2A can interact with different proteins, one of them is the caspase-9 and, when this interaction takes place, it can prevent these proteins to play their physiological role in apoptosis [13].

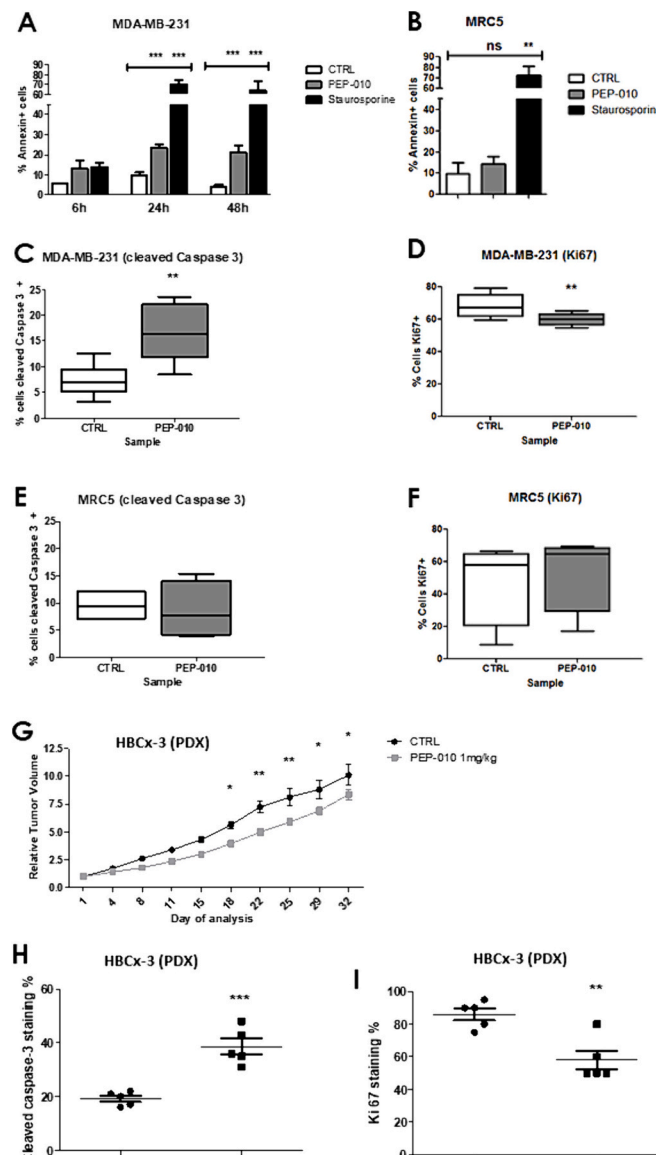
Based on this knowledge, a peptide drug candidate, PEP-010, has been designed, which dissociates the caspase-9/PP2A complex, thereby restoring their physiological roles and apoptosis in cancer cells [13]. In a previous study, the direct interaction between caspase-9 and the catalytic domain of PP2A has been identified and characterized [13,14]. This resulted in the design of the first version of the drug candidate (DPT-C9h). DPT-C9h sequence was optimized to improve its stability and bridging studies *in vitro* and *in vivo* were performed confirming a comparable efficacy between DPT-C9h and the optimized version of the peptide. Only the penetrating part of the peptide (DPT) was modified, the interfering part (C9h) is the same in both versions. These data are described in [15] and led to PEP-010 development.

PEP-010 is a pro-apoptotic peptide-based molecule developed as an anti-cancer drug candidate. It is bi-functional, comprising a Cell Penetrating Peptide (CPP), allowing cell penetration and an Interfering Peptide (IP) to target and disrupt protein-protein interaction. Both

functionalities are thus present in one single peptide sequence named “Cell Penetrating and Interfering Peptide” (CP&IP) (patent number WO2013098337A1).

To the aim of defining candidates pharmacodynamic (PD) biomarkers to monitor the antitumor effect of PEP-010, we first show that PEP-010 efficiently induces cell death in different cancer cell models, and inhibits tumor growth *in vivo* in a patient-derived xenograft (PDX) mouse model. These data served as a preclinical proof of efficacy of PEP-010 in different models of solid tumors before moving to the ongoing clinical trial. Exploiting *in vivo* and different *in vitro* models we found that the increased cleaved caspase-3 and decreased Ki67 protein expression correlates with PEP-010-induced apoptosis and we propose them as candidates PD biomarkers.

First, we measured the proapoptotic effect of PEP-010 by Annexin V/PI staining and flow cytometry analysis in different cell models. Twenty-four hours post-treatment, PEP-010 induce apoptosis in cell lines of different solid tumor origin: MDA-MB-231 (triple negative breast cancer, percentage of total Annexin positive cells \pm SEM: 23.5 % \pm 1.7 PEP-010 vs 9.9 % \pm 1.3 CTRL, $***, p < 0.0001$) (Fig. 1A); BxPC-3 (pancreatic adenocarcinoma, 23.4 % \pm 1.3 PEP-010 vs 11 % \pm 0.7 CTRL) (Fig. S1A);



(caption on next page)

Fig. 1. (A, B) PEP-010 induces apoptosis in cancer cells but not in non-cancer cells. Percentages of cells expressing Annexin-V after 50 μ M PEP-010-treatment of MDA-MB-231 for 6, 24 or 48 h (A) and MRC5 cells for 24 h (B). Staurosporine was used as positive control at the concentration of 1 μ M. Analysis of cell death was performed by Annexin-V/PI staining and flow cytometry analysis in at least three independent experiments. *** $p < 0.001$; ** $0.01 < p < 0.05$; ns, non significant. (C, D, E, F) PEP-010 increases cleaved caspase-3 and decreases Ki67 expression in PEP-010 sensitive cells MDA-MB-231 but not in PEP-010 non-sensitive cells MRC5. Cells were fixed in 4 % paraformaldehyde at the indicated timepoints. Upon permeabilization in 2 % Triton X-100 and subsequent saturation in 0.5 % BSA cells were immunostained for 2 h at room temperature with a primary antibody specific for the cleaved form of caspase-3 as a marker of apoptosis (Anti-caspase-3 (Cleaved Asp175) Rabbit Polyclonal Antibody, 1:200 from Invitrogen #PA5-114687) (D, F) or for the marker of proliferation Ki67 (Anti-Ki67 (8D5) Mouse Monoclonal Antibody, 1:500, Cell Signaling #9449) (E, G). Appropriate secondary antibody (Alexa Fluor 546 goat anti-rabbit IgG Invitrogen #A11010 or Alexa Fluor 488 goat anti-mouse IgG Invitrogen #A11034, diluted 1:200) were then used for 1 h incubation. Coverslips were mounted on microscopy glass slides using a mounting medium containing DAPI. Images were acquired with a fluorescence microscope Zeiss Observer Z1. Images were analyzed by means of ImageJ software. Percentages of cells expressing the cleaved caspase-3 or Ki67 were calculated over the total number of cells. The box-plots represent results obtained in at least two biological replicates at the time of the peak of the expression in MDA-MB-231 (C, D) or MRC5 (E, F). At least 60 cells and 3 images were analyzed in each condition. ** $0.01 < p < 0.05$.

(G, H, I) PEP-010 decreases tumor volume in luminal breast cancer PDX mice, activates caspase-3 and decreases Ki67 expression.

Tumor model used, the HBCx-3, was obtained from primary luminal breast cancer tumor of patient grafted into immunodeficient mice and established by serial passage in Swiss Nude mice. The histology HBCx-3 is the same as corresponding patient. Female Swiss nude mice, 6 to 8 weeks old (Charles River Laboratories) were maintained under specific pathogen-free conditions and used as xenograft recipients. Mice were housed in group cages of 5 mice each at the Animal Platform CRP2-UMS 3612 CNRS-US25 Inserm-IRD (Faculty of Pharmacy, Paris Descartes University, Paris). Food and water were sterilized and provided *ad libitum*. Study was performed in compliance with the recommendations of the French Ethical Committee (Agreement 01020.03, France) and under the supervision of authorized investigators. This study was conducted according to the standard operating procedures of the Test Facility, in reference with the principles of Good Laboratory Practice standards, and in compliance with applicable animal health regulations.

PDX were maintained by serial transplantations and all the mice of the same experiment were implanted the same day from the same donor. Tumor fragments were implanted into the interscapular fat pad, under xylazine-ketamine anesthesia. All treatment started at day 1 as the tumors reached a volume comprised between 60 mm^3 and 150 mm^3 . Only healthy mice were included in the study.

Two groups of ten mice were treated with PEP-010 or vehicle (Glucose 5 %) (control group). Mice were injected intraperitoneally with PEP-010 at 1 mg/kg once a day for the indicated times (5 days/week, for 3 weeks). Tumors from the 2 groups were measured twice weekly. Tumor volume (TV) was calculated by measuring 2 perpendicular diameters with a caliper, 2 times/week as $TV = a \times b^2/2$ where a, is the longest diameter and b the perpendicular axis. (G) The mean and the standard deviation of the relative tumor volume (RTV, calculated as the ratio of the volume at the selected time divided by the initial volume at day 1) are represented. ** $0.01 < p < 0.05$, * $p < 0.05$ (H, I) Tumor bearing mice were euthanized, tumor material was cut into pieces of 14–20 mm^3 and maintained into culture media. Tissue sections of 3 μm of thickness were fixed in formal and paraffin embedded. They were first deparaffined, rehydrated, endogen peroxidase activity blocked and a specific staining was inhibited. Slides were incubated 60 min at room temperature with the following antibodies: anti-caspase-3 (Cleaved Asp175) (H), or anti-Ki67 (I). After incubation, tissue cuts were washed with PBS and incubated with a binding reagent for IgG. IgG Poly-HRP reagent allowed to localize the primary antibody. Immunosignals were detected using a substrate of hydrogen peroxide and 3,3'-diaminobenzidine which forms a brown precipitate. Sections were then counterstained using hematoxylin of Mayer for 5 min and observed. Graph represents the percentage of active caspase-3 (apoptosis) clusters obtained in the different samples. *** $p < 0.001$ (H) or the percentage of Ki67 clusters obtained in the different samples. ** $0.01 < p < 0.05$ (I).

Statistical analysis was performed using the Student *t*-test (two-tail distribution) to compare means between two samples. Relative tumor volumes are compared using the 2-way ANOVA test. All tests were performed using the GraphPad Prism 5 software (GraphPad software).

H1299 (non-small cell lung carcinoma, 37.2 % \pm 7.2 PEP-010 vs 8.7 % \pm 3.8 CTRL, *, $p = 0.015$) (Fig. S1B); RH41 (rhabdomyosarcoma, 27 % \pm 2 PEP-010 vs 9.7 % \pm 1.9 CTRL) (Fig. S1C) and Cov318 (ovarian epithelial serous carcinoma, 42.4 % \pm 4.2 PEP-010 vs 10.6 % \pm 1.6 CTRL) (Fig. S1D) while no increase in apoptotic cells was revealed in non-tumoral MRC5 embryo lung fibroblasts (Fig. 1C) (14.4 % \pm 3.5 PEP-010 vs 9.9 % \pm 5.2 CTRL, ns, $p = 0.51$). The cell penetrating part of the peptide Mut3-DPT which penetrates cells but do not disrupt the PP2A-caspase-9 interaction or the interfering part C9h which cannot penetrate into cells, do not induce apoptosis on MDA-MB-231 (Fig. S1E) confirming the specificity of PEP-010 effect. Moreover, PEP-010 showed a long term effect in inhibiting cell growth by clonogenic assay (colony intensity PEP-010 vs CTRL set as 1 = 0.4 \pm 0.1) (Fig. S1F).

To confirm that the apoptotic cascade efficiently took place upon PEP-010 treatment, we quantified the percentage of cells expressing the active cleaved form of the caspase-3, a major effector caspase, by immunofluorescence in the tested cell lines. A specific antibody detecting only the caspase-3 cleaved form was used in PEP-010-treated and untreated cells. PEP-010 significantly increased the percentage of cells expressing the active form of caspase-3 (cleaved) compared to the untreated cells at 24 h post treatment in MDA-MB-231 cells (16.5 % \pm 2.4 vs 7.3 % \pm 1.3, **, $p = 0.007$) (Figs. 1C and S2A). An increase of cleaved caspase-3 expression has been found also in the other cell lines sensitive to PEP-010 treatment (BxPC-3, 27.5 % \pm 1.4 vs 3.1 % \pm 0.4, ***, $p < 0.0001$; H1299, 34.9 % \pm 2 vs 20.3 % \pm 1.8, ***, $p = 0.0003$; RH41, 42.4 % \pm 1.6 vs 32.4 % \pm 2.1, **, $p = 0.0042$; Cov318 26.6 % \pm 2.3 vs 16 % \pm 1.9, **, $p = 0.0048$) (Fig. S2B, C, D, E). In parallel with the pro-apoptotic events, we observed a significant decrease (*, $p = 0.04$) of proliferation of MDA-MB-231 upon PEP-010 treatment (Fig. S2F) by

means of the Carboxyfluorescein Diacetate Succinimidyl Ester (CFSE) assay. Thus, we checked, by immunofluorescence, the percentage of cells expressing Ki67, a well-known marker of proliferation, after PEP-010 treatment. Percentage of cells expressing Ki67 was significantly decreased in PEP-010-treated MDA-MB-231 cells compared to the untreated control (59.8 % \pm 1.2 vs 68.3 % \pm 2.4, **, $p = 0.0064$) (Figs. 1D and S2G) at 24 h post-treatment. A decrease of Ki67 expression has also been observed in the other cell lines sensitive to PEP-010 treatment (BxPC-3 61.3 % \pm 3.3 vs 48.6 % \pm 3.3, ***, $p < 0.0001$; H1299 92.7 % \pm 0.7 vs 82.4 % \pm 3.2, **, $p = 0.009$; RH41 93.2 % \pm 1.1 vs 88.4 % \pm 1, **, $p = 0.0053$; Cov318 90.6 % \pm 1.3 vs 83 % \pm 2.3, *, $p = 0.01$) (Fig. S2H, I, J, K).

Moreover, changes of expression of both cleaved caspase-3 (Fig. 1E) and Ki67 (Fig. 1F) were absent in cells where PEP-010 is not effective as the MRC5 cell line (8.7 % \pm 2.6 vs 9.6 % \pm 1.4, ns, $p = 0.78$ cleaved caspase-3; 54.2 % \pm 12.3 vs 47.8 % \pm 13.22, ns, $p = 0.73$ Ki67).

All these results taken together, suggest that the cleaved caspase-3 and Ki67 expression levels represent two candidates as pharmacodynamic (PD) biomarkers to monitor PEP-010 activity.

To further evaluate cleaved caspase-3 and Ki67 expression as potential PD biomarkers *in vivo*, we subsequently analyzed the effects of PEP-010 *in vivo* using luminal breast cancer HBCx-3 PDX mice. PEP-010 was previously tested on these cells showing a good efficacy [15]. Two groups (PEP-010-treated and untreated) of ten mice each were used. Mice were treated intraperitoneally once a day, 5 days/week with 1 mg/kg PEP-010, for 3 weeks and the tumor volume (TV) was calculated by measuring 2 perpendicular diameters with a caliper, 2 times/week as $TV = a \times b^2/2$ where a, is the longest diameter and b the perpendicular axis. Relative tumor volume (RTV) was calculated as the ratio of the

volume at the selected time divided by the initial volume at day 1 and compared between the two groups. PEP-010 treated mice showed a significant decrease in RTV starting from day 18 compared to the untreated group (Fig. 1G) and a tumor growth inhibition (TGI) of 31.2 % when the effect is peaking (day 22, Fig. S3A). PEP-010 did not show any toxicity, estimated notably by loss of body weight (Fig. S3B).

At mice euthanasia, tumors were excised, fixed, paraffin embedded and tissue sections were prepared for immunohistochemistry using anti-cleaved caspase-3 or anti-Ki67-specific antibodies for staining. Percentages of expression of these candidate PD biomarkers were calculated in the different sections and compared in treated and untreated mice samples. Percentage of expression of active caspase-3 was significantly increased in PEP-010 treated mice as compared to the control ($38.6 \% \pm 3$ vs $19.2 \% \pm 1.2$; $***, p = 0.000007$) (Fig. 1H) while Ki67 staining was significantly decreased ($58 \% \pm 5.8$ vs $86 \% \pm 3.7$; $** , p = 0.0036$) (Fig. 1I). These *in vivo* data showed that PEP-010 is an effective agent to reduce breast tumor size. Moreover they support the reliability of the cleaved caspase-3 and Ki67 expression as PD biomarkers of this drug candidate, confirming *in vitro* data.

In this study, we show that PEP-010 induces apoptosis specifically in cancer cell lines of different origins and *in vivo* in a luminal breast cancer PDX mouse model. We have observed that cell lines of triple negative breast cancer (MDA-MB-231), pancreatic adenocarcinoma (BxPC-3), non-small cell lung carcinoma (H1299), rhabdomyosarcoma (RH41) and ovarian epithelial serous carcinoma (Cov318) respond to PEP-010 treatment while non-cancer cells (MRC5) are not sensitive to treatment. Data obtained *in vitro* are also substantiated by *in vivo* efficacy of PEP-010 on a PDX mouse model of luminal breast cancer. We hypothesize that, thanks to its CPP, PEP-010 penetrates efficiently into tumor cells and reaches its target, the caspase-9/PP2A complex; this leads to caspase-9 activation, possibly through PP2A activity [16–18], thus inducing activation of caspase-3 and cell death. The described data supported the approval of PEP-010 for the ongoing Phase Ia/b clinical trial where the safety, pharmacokinetics and preliminary antitumor activity of PEP-010 in monotherapy and in combination with standards-of-care are under investigation on different solid tumors (NCT04733027).

In 2020, around thirty-nine clinical trials involving pro-apoptotic drugs were ongoing in oncology showing the medical need. A Bcl-2-inhibitor, Venetoclax, was approved by the FDA in 2016 for the treatment in monotherapy of chronic lymphocytic leukemia, or in combination with azacytidine for acute myeloid leukemia [19]. This approval strongly increased the interest in the apoptotic pathway re-activation as a new approach to develop anti-cancer drugs. Therapeutic peptides also acquired a great interest in the recent years as a novel approach of cancer therapy. Peptides offer advantages over small molecules, such as target specificity and low toxicity. Additionally, they can specifically target pathological protein-protein interactions, which are challenging to address with small molecules [20,21]. The development of biomarkers is essential in guiding the research, clinical development, and clinical use of new drugs, especially in the field of oncology. We focused our attention in finding candidates PD biomarkers for PEP-010 activity monitoring. These kinds of biomarkers are an important tool for the clinical practice as they can indicate the drug-related molecular effect and activity even in the absence of macroscopic features (as, for example, the reduction of tumor size assessed *via* imaging). To this aim, we present the cleaved caspase-3, effector protein fundamental for PEP-010 action and apoptosis induction, and the Ki67, a marker of cell proliferation, as candidate PD biomarkers. Indeed, expression of these candidate biomarkers is affected upon PEP-010 treatment only in cells sensitive to PEP-010. *In vitro* data were further confirmed *in vivo* in a PDX mouse model, which showed significant tumor growth inhibition upon treatment, without side-effects. As the experiment was conducted in a single PDX model, it will be important to extend this observation to other PDX models. To exploit a biomarker in clinical practice, widely diffused and rapid techniques should be used and in this study IHC and

immunofluorescence are exploited, this could ease their future development as biomarkers for PEP-010. The next validation step will be to assess these biomarkers in tumor biopsies from patients treated with PEP-010 in the ongoing Phase Ia/b clinical study. We anticipate that this will significantly contribute to the development of this first-in-class clinical-stage peptide restoring apoptosis.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbadis.2024.167492>.

CRediT authorship contribution statement

D. Germini: Writing – original draft, Supervision, Project administration, Investigation, Data curation, Conceptualization. **R. Farhat:** Writing – review & editing, Investigation, Data curation. **L. Dadon:** Writing – review & editing, Data curation. **A. Lacroix:** Writing – review & editing, Investigation, Data curation. **F. Nemati:** Writing – review & editing, Methodology, Investigation. **A. Rebollo:** Writing – review & editing, Resources. **D. Decaudin:** Writing – review & editing, Resources, Investigation, Data curation. **J. Wiels:** Writing – review & editing, Supervision. **C. Brenner:** Writing – original draft, Supervision, Project administration, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Diego Germini reports financial support was provided by PEP-Therapy SAS. Rayan Farhat reports financial support was provided by PEP-Therapy SAS. Laura Dadon reports financial support was provided by PEP-Therapy SAS. Aline Lacroix reports financial support was provided by PEP-Therapy SAS. Fariba Nemati reports financial support was provided by PEP-Therapy SAS. Angelita Rebollo reports financial support was provided by PEP-Therapy SAS. Didier Decaudin reports financial support was provided by PEP-Therapy SAS. Diego Germini reports a relationship with PEP-Therapy SAS that includes: employment. Rayan Farhat reports a relationship with PEP-Therapy SAS that includes: employment. Laura Dadon reports a relationship with PEP-Therapy SAS that includes: employment. Aline Lacroix reports a relationship with PEP-Therapy SAS that includes: employment. Fariba Nemati reports a relationship with PEP-Therapy SAS that includes: equity or stocks. Angelita Rebollo reports a relationship with PEP-Therapy SAS that includes: equity or stocks. Didier Decaudin reports a relationship with PEP-Therapy SAS that includes: equity or stocks. Angelita Rebollo has patent issued to WO2013098337A1. Fariba Nemati has patent issued to WO2013098337A1. Didier Decaudin has patent issued to WO2013098337A1. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We wish to thank the Animal Platform CRP2-UMS 3612 CNRS-US25 Inserm-IRD (Faculty of Pharmacy, Paris Descartes University, Paris) for the *in vivo* studies (Agreement 01020.03, France). This study was supported by the region Ile de France and by French government within the framework “Plan France 2030”.

References

- [1] M.C. Maiuri, E. Zalckvar, A. Kimchi, G. Kroemer, Self-eating and self-killing: crosstalk between autophagy and apoptosis, *Nat. Rev. Mol. Cell Biol.* 8 (2007) 741–752, <https://doi.org/10.1038/nrm2239>.

- [2] C. Pfeffer, A. Singh, Apoptosis: a target for anticancer therapy, *Int. J. Mol. Sci.* 19 (2018) 448, <https://doi.org/10.3390/ijms19020448>.
- [3] S. Elmore, Apoptosis: a review of programmed cell death, *Toxicol. Pathol.* 35 (2007) 495–516, <https://doi.org/10.1080/01926230701320337>.
- [4] X. Deng, F. Gao, W.S. May, Protein phosphatase 2A inactivates Bcl2's antiapoptotic function by dephosphorylation and up-regulation of Bcl2-p53 binding, *Blood* 113 (2009) 422–428, <https://doi.org/10.1182/blood-2008-06-165134>.
- [5] M.I. Avrutsky, C.M. Troy, Caspase-9: a multimodal therapeutic target with diverse cellular expression in human disease, *Front. Pharmacol.* 12 (2021) 1728, <https://doi.org/10.3389/fphar.2021.701301/BIBTEX>.
- [6] S. Mazhar, S.E. Taylor, J. Sangodkar, G. Narla, Targeting PP2A in cancer: combination therapies, *Biochim. Biophys. Acta, Mol. Cell Res.* 2019 (1866) 51–63, <https://doi.org/10.1016/j.bbamcr.2018.08.020>.
- [7] J. Sangodkar, C.C. Farrington, K. McClinch, M.D. Galsky, D.B. Kastrinsky, G. Narla, All roads lead to PP2A: exploiting the therapeutic potential of this phosphatase, *FEBS J.* 283 (2016) 1004–1024, <https://doi.org/10.1111/febs.13573>.
- [8] D. Perrotti, P. Neviani, Protein phosphatase 2A: a target for anticancer therapy, *Lancet Oncol.* 14 (2013), [https://doi.org/10.1016/S1470-2045\(12\)70558-2](https://doi.org/10.1016/S1470-2045(12)70558-2).
- [9] C. Lambrecht, L. Libbrecht, X. Sagaert, P. Pauwels, Y. Hoorne, J. Crowther, J. V. Louis, W. Sents, A. Sablina, V. Janssens, Loss of protein phosphatase 2A regulatory subunit B56 δ promotes spontaneous tumorigenesis in vivo, (2018). doi:<https://doi.org/10.1038/onc.2017.350>.
- [10] L. Cucinotta, A. Filippone, G. Casili, M. Lanza, V. Bova, A.P. Capra, R. Giuffrida, C. Colarossi, D. Sciacca, I. Paterniti, S. Cuzzocrea, M. Campolo, E. Esposito, The pivotal role of protein phosphatase 2A (PP2A) in brain tumors, *Int. J. Mol. Sci.* 23 (2022), <https://doi.org/10.3390/IJMS232415717>.
- [11] H. Johnson, S. Narayan, A.K. Sharma, Altering phosphorylation in cancer through PP2A modifiers, *Cancer Cell Int.* 24 (2024) 1–17, <https://doi.org/10.1186/s12935-023-03193-1>.
- [12] C.M. O'Connor, A. Perl, D. Leonard, J. Sangodkar, G. Narla, Therapeutic targeting of PP2A, *Int. J. Biochem. Cell Biol.* 96 (2018) 182–193, <https://doi.org/10.1016/j.biocel.2017.10.008>.
- [13] I. Arrouss, F. Nemati, F. Roncal, M. Wislez, K. Dorgham, D. Vallerand, N. Rabbe, N. Karboul, F. Carlotti, J. Bravo, D. Mazier, D. Decaudin, A. Rebollo, Specific targeting of caspase-9/PP2A interaction as potential new anti-cancer therapy, *PLoS One* 8 (2013) e60816, <https://doi.org/10.1371/JOURNAL.PONE.0060816>.
- [14] K. Dorgham, S. Murail, P. Tuffery, E. Savier, J. Bravo, A. Rebollo, Binding and kinetic analysis of human protein phosphatase PP2A interactions with caspase 9 protein and the interfering peptide C9h, *Pharmaceutics* 14 (2022) 2055, <https://doi.org/10.3390/pharmaceutics14102055>.
- [15] J. Fominaya, J. Bravo, D. Decaudin, J.Y. Brossa, F. Nemati, A. Rebollo, Enhanced serum proteolysis resistance of cell-penetrating peptides, *Ther. Deliv.* 6 (2015) 139–147, <https://doi.org/10.4155/tde.14.100>.
- [16] L. Liu, Z. Huang, J. Chen, J. Wang, S. Wang, Protein phosphatase 2A activation mechanism contributes to JS-K induced caspase-dependent apoptosis in human hepatocellular carcinoma cells, *J. Exp. Clin. Cancer Res.* 37 (2018) 142, <https://doi.org/10.1186/s13046-018-0823-2>.
- [17] H. Sun, Y. Wang, Novel Ser/Thr protein phosphatases in cell death regulation, *Physiology (Bethesda)* 27 (2012) 43–52, <https://doi.org/10.1152/PHYSIOL.00034.2011>.
- [18] P.P. Ruvolo, X. Deng, W.S. May, Phosphorylation of Bcl2 and regulation of apoptosis, *Leukemia* 15 (2001) 515–522, <https://doi.org/10.1038/sj.leu.2402090>.
- [19] B.A. Carneiro, W.S. El-Deiry, Targeting apoptosis in cancer therapy, *Nat. Rev. Clin. Oncol.* 17 (2020) 395, <https://doi.org/10.1038/S41571-020-0341-Y>.
- [20] U. Anand, A. Bandyopadhyay, N.K. Jha, J.M. Pérez de la Lastra, A. Dey, Translational aspect in peptide drug discovery and development: an emerging therapeutic candidate, *Biofactors* 49 (2023) 251–269, <https://doi.org/10.1002/BIOF.1913>.
- [21] C.M. Li, P. Haratipour, R.G. Lingeman, J.J.P. Perry, L. Gu, R.J. Hickey, L.H. Malkas, Novel peptide therapeutic approaches for cancer treatment, *Cells* 2021, Vol. 10, Page 2908 10 (2021) 2908. doi:<https://doi.org/10.3390/CELLS10112908>.