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SCHADOC
Societies, Climate, Health, Artificial Intelligence Doctorate program

0000000246– APO-SWITCH

Structure-guided modulation of mitochondrial apoptosis via VDAC2–BAX/BAK interactions for Drug Discovery

- **Abstract (350 words max, here 340):**

Apoptosis is essential for tissue homeostasis, development, and immune regulation. Its dysregulation underlies a wide range of diseases, from cancer—where apoptosis is suppressed—to degenerative pathologies such as stroke, heart failure, and neurodegeneration, which are characterized by excessive cell loss. Therapeutic modulation of apoptosis has therefore become a major focus in oncology, exemplified by venetoclax, which indirectly activate the pro-apoptotic effectors BAX and BAK by inhibiting anti-apoptotic BCL-2 family members. Although clinically effective in certain cancers, responses to venetoclax are frequently transient, with resistance emerging rapidly, highlighting the need for alternative strategies that more directly control BAX/BAK activation.

Recent studies have identified the mitochondrial porin VDAC2 as a central regulator of apoptotic commitment. VDAC2 controls both the recruitment and activation of BAX at the mitochondrial outer membrane and the inhibitory sequestration of BAK, positioning it as a key decision point upstream of mitochondrial outer membrane permeabilization. Targeting this regulatory node offers the possibility to fine-tune apoptotic sensitivity, either to restore cell death in apoptosis-resistant cancers or to limit pathological apoptosis in degenerative diseases. However, development of VDAC2-directed compounds has been hindered by incomplete mechanistic understanding, and the absence of a specific screening assay and high-resolution structural information on VDAC2–BAX/BAK complexes.

This PhD project aims to overcome these limitations by integrating high-resolution structural biology to enable rational targeting of VDAC2-dependent apoptosis regulation. Building on recent advances that have yielded the first structural models and near-complete cryo-EM structures of the human VDAC2–BAX and VDAC2–BAK complexes, the project will (i) elucidate the molecular basis of species-specific activity of the murine VDAC2–BAK stabilizer WEHI-9625 through comparative structural analysis of human and murine complexes, and (ii) develop robust in vitro and in cellulo assays that directly monitor VDAC2–BAX/BAK interactions. These platforms will be used to optimize existing compounds and to identify novel modulators, which will be validated in cellular models, including patient-derived leukemia samples with defined venetoclax resistance. By delivering the first integrated structural and experimental framework to directly interrogate VDAC2–BAX/BAK interactions, this project will provide a foundation for future structure-guided development of apoptosis modulators with translational relevance.

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PhD Research and Training proposal

1. EXCELLENCE (4 pages max)

1.1. Pre-proposal's context, positioning and objective(s)

Apoptosis is a programmed cell death process essential for development, tissue homeostasis, and immune regulation through the elimination of damaged or superfluous cells. Dysregulation of mitochondrial apoptosis contributes both to cancer, through inappropriate cell survival, and to degenerative pathologies characterized by excessive cell loss[1,2]. Consequently, apoptosis has become a central target of anti-cancer therapy[3]. Small molecules targeting components of the apoptotic machinery are now used clinically, most notably venetoclax (targeting BCL2) in leukemia[4], which has transformed patient outcomes without the need for conventional chemotherapy. However, clinical responses to venetoclax are frequently transient, with resistance commonly arising, underscoring the urgent need for novel therapeutic strategies.

Intrinsic, or mitochondrial, apoptosis is initiated by cellular stresses (e.g. growth factor deprivation, DNA damage) and is governed by protein-protein interactions within the BCL-2 family, which comprises both pro- and anti-apoptotic members[5]. Execution of cell death requires activation of BAX or BAK, which oligomerize to permeabilize the mitochondrial outer membrane (MOM), releasing apoptogenic factors that activate the caspase cascade and irreversibly commit the cell to death (**Figure 1**). Intensive efforts to target the apoptotic machinery in cancer have led to the development of small molecules inhibiting anti-apoptotic BCL-2 proteins and thereby unleashing BAX and BAK. The clinical success of venetoclax in leukemia and lymphoma establishes targeting the BCL-2 family as a viable and effective therapeutic strategy. However, most patients rapidly acquire resistance to venetoclax, underscoring the need for new approaches to restore BAX/BAK activation.

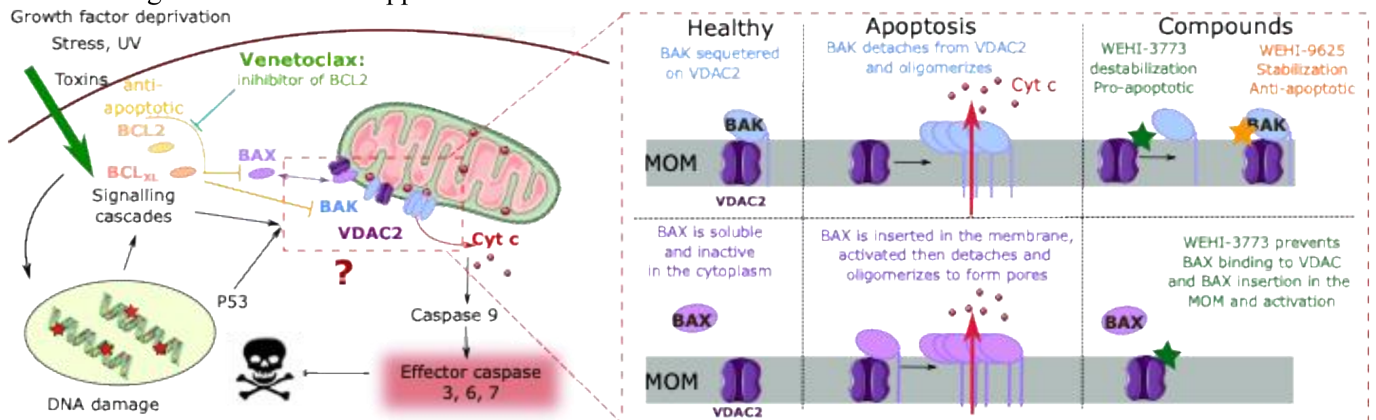

Figure 1 : Mitochondrial-mediated apoptosis. *Cellular stress activates BCL-2 family signaling, leading to BAX and BAK interaction with the mitochondrial porin VDAC2 and pore formation in the MOM, which triggers cytochrome* c *release and caspase-dependent cell death. Venetoclax inhibits the anti-apoptotic BCL-2 protein to enable BAX/BAK activation. The inset highlights VDAC2–BAX/BAK interactions in healthy cells and during apoptosis; WEHI-9625[6] and WEHI-3773[7] are small molecules that modulate this VDAC2-dependent regulation, with differential effects on BAX and BAK.*

Although the precise molecular mechanisms underlying BAX and BAK pore formation remain incompletely understood, it is generally accepted that, in healthy cells, BAX resides as an inactive soluble protein in the cytosol, whereas BAK is constitutively anchored in the MOM through its interaction with the porin VDAC2[8]. Upon apoptotic stimulation, BAX also forms a complex with VDAC2 to be recruited to and inserted into the membrane, while BAK must be released from VDAC2, enabling both proteins to undergo conformational activation and oligomerization. Over the past decade, accumulating evidence has identified VDAC2 as a central regulator of BAX and BAK activation[8–11], and a promising target for therapeutic modulation of apoptosis.

Consistent with this emerging role, two VDAC2-targeting compounds have been identified through cell-based screening as modulators of apoptosis; however, both suffer some limitations. The first (WEHI-9625) is an apoptosis inhibitor that stabilizes the VDAC2-BAK complex and prevents BAK oligomerization[6]; while potentially useful in neurodegenerative diseases, it is active exclusively in mouse systems and requires optimization for activity on the human complex. The second compound (WEHI-3773) selectively promotes BAK-, but not BAX-mediated apoptosis and, although insufficient as a standalone therapy, can overcome venetoclax resistance in cell lines when used in

combination with other drugs[7]. The lack of structural information on VDAC2-containing complexes remains a major barrier to understanding VDAC2-mediated apoptosis and to the rational development of effective modulators.

Objectives :

This PhD project aims to optimize existing VDAC2-directed molecules[6,7] and to identify new compounds that modulate apoptotic sensitivity by targeting VDAC2-dependent regulation of BAX and BAK, either to inhibit apoptosis or to restore apoptotic competence and overcome venetoclax resistance in leukemia. Recent work in Lucie Bergdoll's team (unpublished) has yielded the first structural insights and interaction models for the human VDAC2-BAK and VDAC2-BAX interfaces and is close to delivering the first high-resolution structures of these complexes (**Figure 2**). These advances open, for the first time, the possibility of structure-guided drug design to rationally optimize existing compounds and develop new modulators of VDAC2-controlled apoptosis. The central objective of this PhD project is to translate emerging structural insights into a detailed mechanistic understanding of VDAC2–ligand interactions and into robust experimental assay platforms, enabling the rational optimization of existing compounds and the identification and cellular validation of new VDAC2-directed modulators, and paving the way for future therapeutic development.

(1) Structure-guided optimization of an existing VDAC2-directed compound. We will determine the structure of the mouse VDAC2-BAK complex with and without the apoptosis inhibitor WEHI-9625 and compare it to the near-complete human complex structure. This analysis will define the molecular basis of WEHI-9625 species specificity and guide its structure-based optimization for activity on the human VDAC2-BAK complex.

(2) Identification of novel modulators of VDAC2-BAX/BAK interactions. In parallel, we will establish robust and specific screening assays to identify new compounds that modulate the VDAC2-BAX/BAK interfaces.

(3) Validation: Hits identified from the screening pipeline will be validated in cellulo using BRET-based assays[12] and further tested in primary acute myeloid leukemia patient cells.

Together, these strategies aim to deliver optimized and novel VDAC2-directed compounds with strong translational potential to modulate apoptotic sensitivity in cancer and other apoptosis-related diseases.

Methodology :

Aim 1: Structural basis of the species specificity of WEHI-9625

For the first time, Lucie Bergdoll's team managed to express and purify stable complexes of the human VDAC2-BAX and VDAC2-BAK, and high-resolution structures complexes are close to completion, supported by optimized cryo-EM grids and well-defined assemblies observed in preliminary 2D classes and low-resolution reconstructions (**Figure 2**). Building on these advances, we aim to elucidate the structural basis for the inactivity of the murine VDAC2-BAK stabilizer WEHI-9625 on the human complex and to establish a rational framework for its optimization toward human therapeutic applications.

1.a Production of the murine complex. We have murine VDAC2 and BAK constructs that express and purify with efficiencies comparable to the human proteins. Production and purification of the murine VDAC2-BAK complex are therefore expected to be straightforward. Importantly, we are able to produce full-length, functional wild-type BAK, ensuring the physiological relevance of the reconstituted complex for structural and functional analyses.

1.b Structural determination of the complex with or without the drug WEHI-9625. Cryo-EM purification and grid preparation conditions established for the human complex will be applied to the murine. High-resolution structures will be determined in the absence and presence of WEHI-9625, revealing its binding mode and enabling direct structural comparison between mouse and human complexes.

1.c Integration of Structural Data and Rational Drug Design. Structure-based modeling within the iSCB team will allow to design humanized variants of WEHI-9625. These will be synthesized by the iSCB chemistry group led by Sébastien Combes. Depending on the complexity of the required chemistry, synthesis could be achieved within the project's timeframe, allowing for timely evaluation of the optimized molecules in subsequent assays by the candidate.

Aim 2: Integrated Screening Strategies to Identify Modulators of human VDAC2:BAX/BAK Interactions

Current screens for apoptosis modulators do not directly probe VDAC2-BAX/BAK interactions and are biased toward apoptosis inhibitors[6,13]. To overcome these limitations, we will implement two complementary screening strategies to identify selective modulators of human VDAC2-BAX/BAK interactions.

2.a Unbiased Experimental Screening: An in vitro assay using purified VDAC2, BAX, and BAK will be developed to directly monitor their interactions and adapted to an HTRF (Homogeneous Time-Resolved Fluorescence) format to screen a focused chemical library with protein-protein interaction inhibitors profile as well as other libraries

available on the screening platform. This approach will enable identification of both stabilizers and disruptors of VDAC2-BAX/BAK complexes (**Figure 2**). Because the assay involves membrane protein in lipid environment (e.g. nanodiscs), its development will be supported by a collaboration with the R&D team at Cisbio-Revvity, including hands-on assay optimization by the candidate. This partnership is part of a unique collaborative laboratory framework between the iSCB team and Cisbio-Revvity, designed to foster transmobility and shared expertise to allow employees from both structures to work seamlessly together.
In parallel, the BRET assay that will be developed to provide orthogonal validation of the identified molecules (see Aim 3) will also serve as an alternative (backup) screening technology, ensuring continuity in the project regardless of the progress of the HTRF assay.
2.b Biased In Silico Screening: In parallel, we will leverage structural insights from high-resolution cryo-EM data (both VDAC2-BAX and -BAK) to perform *in silico* screening (molecular docking and pharmacophoric filtering) of larger commercial compound libraries (Figure 2 - Left), enabling rational identification and optimization of compounds targeting the interaction interface. While in silico screening will be performed by the iSCB team (lead by Philippe Roche), the PhD candidate will lead the biophysical and cellular validation and mechanistic interpretation.

Aim 3 : Validation of the compounds in cellular context and in patient cells

3.a Compounds validation in cellular context (BRET): Cellular validation of HTRF-derived hits will be performed using Bioluminescence Resonance Energy Transfer (BRET), including NanoBRET, to quantitatively monitor VDAC2-BAX/BAK interactions in live cells. This approach enables real-time assessment of protein-protein interactions in a physiological context and of the effects of compound binding. The candidate will collaborate with Laurent Maillet (CRCI2NA, Nantes Université), a recognized expert in BRET and BCL-2 family proteins[12], and will receive hands-on training in BRET assay development during a research stay in Nantes.

3.b Translational validation in patient-derived samples: Lead compounds will be tested in leukaemia cell lines with defined genetic backgrounds, including models of venetoclax resistance, and in primary cells from leukaemia patients. These experiments will assess the ability of candidate molecules to restore apoptotic responses and overcome therapeutic resistance. The candidate will benefit from the iSCB team's established expertise in cellular assays and will have the opportunity to participate to the cellular evaluations, under the supervision of Sylvain Garciaz (member of the iSCB team and associate professor at AMU in the Department of Hematology at "Institut Paoli Calmette" cancer hospital), using both cell lines and primary patient samples. Using MOLM-13 isogenic TP53 point-mutant lines, along with knockout controls, we will employ pharmacological sensitization assays, and genetic modulation (CRISPRi, in collaboration with Sandrine Roulland and the CRISPR genomic screening Platform, AMU) of both stabilizers and disruptors of the VDAC2-BAX/BAK complexes. Validation of selected compounds will be performed using primary samples from clinically and genetically annotated cohorts, including HEMATO-BIO-IPC 2013-015 (NCT02320656) and HEMATOBIO.02-IPC 2021-061 (NCT05602168). We will select only venetoclax resistant samples (n=35) and cell death assay will be done using Cell Titr Glow assay as previoulsy described[14]. To further strengthen the translational relevance of our findings, we have established a collaboration with Prof. Andrew Wei (Peter MacCallum Cancer Centre, Melbourne), an internationally recognized expert in acute myeloid leukemia (AML), with particular expertise in venetoclax resistance mechanisms involving TP53 alterations and dysregulation of BAX and BAK. This collaboration will provide the PhD student with access to complementary cellular models and an expanded cohort of patient-derived samples, reinforcing the translational impact of the project. By combining our complementary expertise, we will among other strategies, explore the synergistic potential of selected compounds in combination with other apoptotic modulators, using approaches inspired by those described in Li *et al*[13].
The most promising candidates will serve as molecular probes to study the biological impact of VDAC2-BAX/BAK complex modulation in cellular and animal models (in the future). These probes will allow us to conditionally "turn on or off" the interaction, providing critical insights into its functional role and potential therapeutic applications. Should their pharmacokinetics properties prove favorable, these compounds could also represent valuable starting points for drug development, paving the way for subsequent translational projects.

Expected outcomes:

This project targets the VDAC2–BAX/BAK complexes, a previously unexplored but highly promising regulatory node of mitochondrial apoptosis that offers the potential for more selective and less toxic therapeutic strategies than current approaches. Its originality lies in delivering the first high-resolution structural information on human and murine VDAC2–BAX/BAK complexes, providing a mechanistic framework to explain species-specific drug

responses and enabling structure-guided drug design in this pathway. Beyond compound discovery, the project will establish an integrated pipeline combining cryo-EM, biophysics, in vitro and cellular interaction assays, and rational medicinal chemistry for a new class of membrane protein–protein interactions. This pipeline will allow to tackle the issue of venetoclax resistance in leukemia and can be extended to other apoptosis-related diseases.

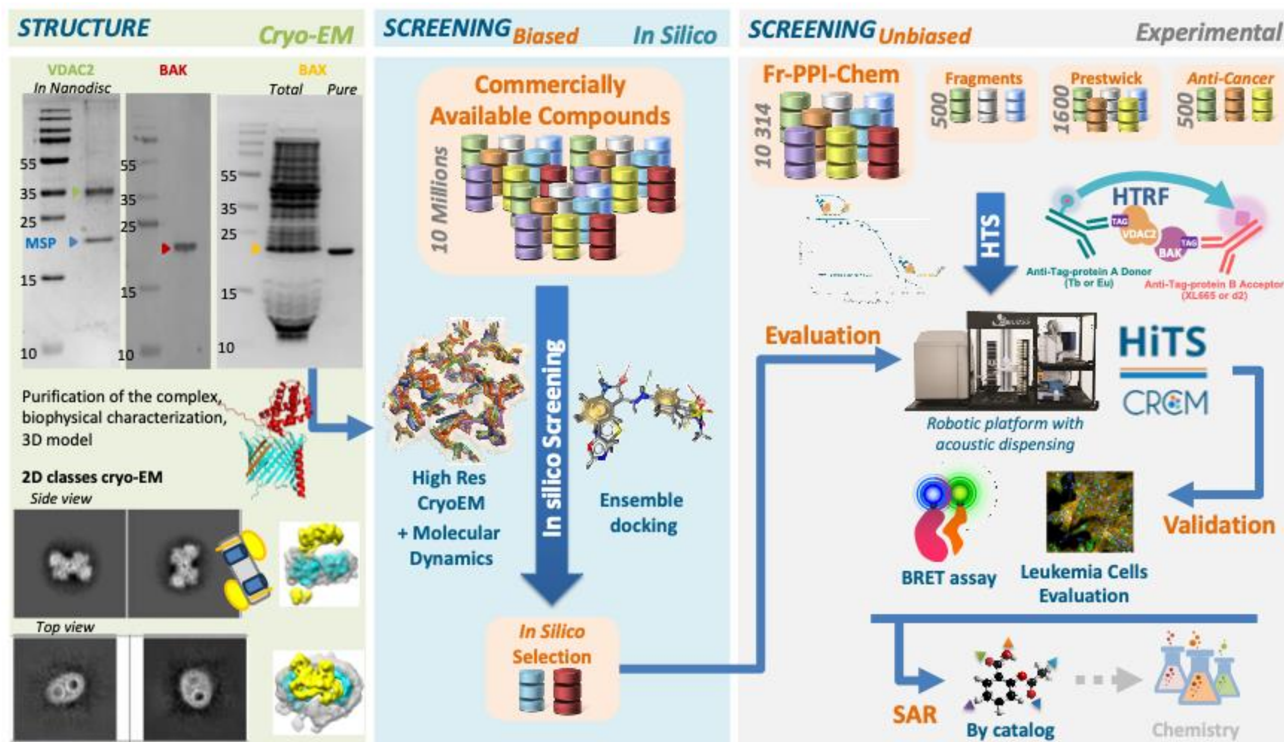

Figure 2: ***Overall work plan integrating cryo-EM, screenings, and experimental validation.*** *(Left) SDS–PAGE of the three partners; VDAC2 is reconstituted in MSP nanodiscs and the purified complex is biophysically characterized, yielding a 3D model. Cryo-EM analysis is ongoing, with well-defined 2D classes and a preliminary 3D volume from an initial dataset; the cartoon guides interpretation of side-view classes. Optimized grid data collection is planned on a 300 kV microscope. (Middle) Structure-guided (biased) in silico screening based on the cryo-EM interaction interface. (Right) Unbiased HTRF screening of small-molecule libraries, followed by cell-based validation and initial SAR analysis using commercially available analogs.*

1.2. Interdisciplinary dimension of the project

This project is inherently interdisciplinary, integrating structural biology, chemical biology, and clinical oncology—fields often pursued independently—into a unified discovery pipeline that goes beyond the current state of the art by enabling iterative feedback between structure determination, compound optimization, and functional validation.

Lucie Bergdoll, a biophysicist and VDAC specialist at the LISM (CNRS-AMU), provides the mechanistic and structural foundation by elucidating the structure-function relationships of VDAC2 and its interactions with BAX and BAK, a key yet poorly understood axis of mitochondrial apoptosis. She developed protocols for the production and purification of the expression of the 3 partners (VDAC2, BAX and BAK) functional, WT and full length, enabling the set up of selective in vitro screening assays.

Xavier Morelli (XM), head of the iSCB team at the Centre de Recherche en Cancérologie de Marseille (CRCM), contributes expertise in structure-guided drug design and screening, supported by molecular modeling and access to the HiTS high-throughput screening platform, with additional support from Cisbio-Revvity for HTRF assay development and analysis. Resources of the iSCB (in silico drug screening, chemistry) will be crucial for this project.

Clinical relevance is ensured by Sylvain Garciaz and Andrew Wei (MacCallum Cancer Centre, Melbourne), clinicians specialized in leukemia and venetoclax resistance associated with TP53 alterations (already existing TP53 international consortium), who will evaluate candidate compounds in disease-relevant cellular and patient-derived models. The originality of the project lies in the direct coupling of high-resolution structural biology with rational drug design and clinical validation focused on VDAC2, establishing a coherent pipeline from molecular mechanism to therapeutic application.

2. IMPACT (2 pages max)

2.1. Expected impact of the project on the candidate's career

This project will provide the candidate with a unique and transformative training experience that combines cutting-edge methodologies, interdisciplinarity, and direct clinical relevance. The candidate will acquire advanced technical skills in structural biology, biophysics, and cell biology, including protein production and purification, cryo-EM structure determination, high-throughput screening (HTRF), and cellular validation assays (BRET, cell viability). Such an integrated skill set—spanning fundamental structural analysis to translational drug discovery—is rarely accessible within a single PhD project and will substantially broaden the candidate's scientific expertise.

Beyond technical training, the project will foster strong interdisciplinary and transferable skills, including project management across multiple tasks, interaction with academic, industrial, and clinical partners, and adaptation to diverse research environments. These competences are highly valued both in academia and in the pharmaceutical and biotechnology sectors, positioning the candidate as a researcher capable of bridging fundamental mechanistic studies with applied therapeutic development.

A defining aspect of the project is its embedding within a cancer hospital environment (Institut Paoli-Calmettes, Marseille), where research is closely connected to patient care. Regular interactions with clinicians (Sylvain Garciaz and Andrew Wei) and exposure to patient-derived models will deepen the candidate's understanding of translational research and clinical constraints, reinforcing the societal relevance of their work. Collectively, this training will provide the candidate with a strong competitive profile for a long-term career in academia, industry, or translational research, equipped to address complex challenges in drug discovery and biomedical science. Overall, the project will train the candidate to operate as an independent and versatile researcher at the interface of structural biology, drug discovery, and clinical translation, a profile that is highly attractive for a future career in both academia and industry.

2.2. Expected impact for the thematic axis

This project directly addresses the "Health and Well-Being" thematic axis by tackling a central regulatory node of mitochondrial apoptosis, the VDAC2:BAX and VDAC2:BAK. By structurally and functionally characterizing VDAC2-BAX/BAK interactions, the project will elucidate fundamental mechanisms that govern apoptotic commitment and contribute to resistance to current apoptosis-based therapies. This knowledge will advance the field by shifting the focus from indirect modulation of apoptosis toward direct, mechanism-based targeting of a central regulatory node, thereby opening new therapeutic avenues for apoptosis-resistant cancers such as venetoclax-resistant leukemias, as well as novel strategies to limit pathological cell loss in neurodegenerative diseases or heart failure through controlled inhibition of apoptosis.

By determining high-resolution structures of the human and murine VDAC2-BAX/BAK complexes, the project will uncover the molecular basis of the species-specific inactivity of WEHI-9625, a compound with demonstrated efficacy in mouse models. For the first time, structure-based drug design will be applied to these complexes, providing a rational framework to adapt existing molecules to the human system. This approach addresses a major bottleneck in the translation of apoptosis modulators from preclinical animal models to patients.

In addition, this project overcomes a major limitation in the field—the lack of reliable assays to directly probe VDAC2-BAX/BAK interactions. Previous efforts relied primarily on cell-based apoptosis readouts[6,13], which do not specifically interrogate this regulatory node and bias discovery toward apoptosis inhibitors. By developing dedicated high-throughput and cellular assays (HTRF and BRET) that directly monitor VDAC2-BAX/BAK interactions, the project will establish robust and transferable methodological tools for screening and optimizing new modulators. The resulting compounds will serve both as molecular probes for the research community and as potential lead molecules for innovative anti-cancer therapies. More broadly, the ability to fine-tune apoptotic sensitivity may have implications for diseases characterized by either excessive or insufficient cell death.

Finally, the project will reinforce the translational pipeline of the CRCM by strengthening links between structural biology, drug discovery, and clinical research. By fostering durable inter-laboratory collaborations and establishing reusable experimental platforms, it will contribute to long-term advances within the thematic axis and generate societal impact through improved strategies to treat cancer and potentially other apoptosis-related diseases.

2.3. Dissemination, exploitation and communication activities planned

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The knowledge generated by this project will be disseminated and exploited through a multi-level strategy targeting the scientific community, industry stakeholders, and the general public.

At the scientific level, the structural and mechanistic insights into VDAC2-BAX/BAK interactions will be disseminated through publication in a high-visibility, generalist journal with strong translational reach and open access, and will be deposited in HAL-AMU. These publications will report the first high-resolution structures of these complexes and define structure-based strategies to adapt compounds such as WEHI-9625 for activity on the human target, independently of whether full optimization is achieved within the project timeframe. Together, these outputs will advance fundamental understanding of apoptosis regulation while providing concrete frameworks for drug development in cancer and other apoptosis-related diseases.

The candidate will actively disseminate results through oral and poster presentations at national meetings (e.g. GDR APPICOM during the first and second years) and major international conferences (e.g. Biophysical Society Meeting, USA, during the second and third years, European Hematology Association (EHA) and American Society of Hematology (ASH). In addition, the development of innovative screening assays (HTRF and BRET) and the identification of small-molecule modulators will be presented in specialized workshops (Ecole Thématique de Criblage, ETC) and industry-oriented seminars (Drug Discovery Chemistry, San Diego), targeting biotechnology and pharmaceutical stakeholders interested in apoptosis-based therapies.

Regarding exploitation, the project has strong potential for downstream valorization. Structural insights, validated assays, and identified modulators of VDAC2-BAX/BAK interactions constitute transferable assets (patents) for drug discovery pipelines. In collaboration with institutional innovation and technology-transfer offices, opportunities for intellectual property protection will be explored, following established practices successfully implemented by the iSCB team in previous CNRS-supported projects. This strategy will facilitate the translation of project outcomes toward preclinical development and future industrial partnerships.

Finally, public engagement will be promoted through the development of accessible and visually compelling communication tools, including infographics and short 3D animations derived from structural data. These materials will be disseminated via laboratory and institutional websites and social media following publication. The candidate will also participate in outreach activities such as the *Fête de la Science* and educational events, using the project as a concrete example to illustrate how structural biology contributes to cancer research and therapeutic innovation, thereby fostering public awareness and interest in scientific careers.

3. IMPLEMENTATION (2 pages max)

3.1 Work plan, task interaction, and mobilized resources

The project is organized into three interconnected yet independently executable aims, forming a coherent workflow from structural elucidation to compound validation. **Aim 1** establishes the structural framework for understanding VDAC2–BAX/BAK regulation and species-specific drug responses; **Aim 2** combines unbiased experimental and structure-guided *in silico* screening to identify modulators; and **Aim 3** provides rigorous biophysical, cellular, and translational validation. Structural data from Aim 1 guide compound identification and optimization, while validation in Aim 3 feeds back into mechanistic interpretation and refinement. Importantly, Aim 1 alone guarantees robust, publishable outcomes and standalone translational opportunities, ensuring significant impact regardless of progress in downstream aims.
The project benefits from a highly complementary environment: Lucie Bergdoll (LISM) brings expertise in membrane protein biophysics and cryo-EM of VDAC2-BAK complexes, while the iSCB team at CRCM (led by Xavier Morelli, with Stéphane Betzi coordinating screening activities) provides access to the HiTS high-throughput screening platform and an integrated academic drug-discovery pipeline. Medicinal chemistry support is available in-house within the iSCB team, ensuring seamless integration between structure determination, screening, validation, and compound optimization. Sylvain Garciaz (iSCB team) and Andrew Wei (Peter MacCallum Cancer Centre, Melbourne) will evaluate candidate compounds in disease-relevant cellular and patient-derived models. The resources required to carry out this project are available across the participating laboratories, supported by existing funding (ANR JCJC, LB; ANR PRME, XM), with additional funding applications planned (Canceropôle, INCa, ARC, ERC).

3.2 Feasibility and timeline within the 36-month PhD duration

AIM	Tasks	Subtask (Year / Month)	1	2	3
1	Structure	Expression + Purification			
		Grid screening + Data acquisition			
		Data analysis			
		Chemistry *(S. Combes)*			
2	Screening	Assay optimization *(CISBIO)*			
		Primary screening *(libraries)*			
		Secondary screening (IC50)			
		In Silico Screening *(P. Roche)*			
		SAR by catalog *(each hit)*			
3	Validation	HTRF *(repurposing + in silico)*			
		BRET cell lines & plasmids			
		BRET setup *(Nantes)*			
		BRET evaluation			
		Cell assays			
		Cell assays *(Melbourne)*			
		Patient cells			
Training		ED courses + SCHADOC modules			
Thesis		Writing & defense			

The objectives of the project are achievable within the 36-month PhD timeframe owing to the advanced state of the preliminary work and the parallelization of key activities. Once initial hits are identified in Year 1 through primary screening, follow-up screening and optimization steps (e.g. SAR exploration and HTRF testing of new analogs) can be carried out progressively and in parallel with other ongoing tasks.

Year 1 will focus on completion of the high-resolution structures of the human and murine VDAC2-BAK complexes (**Aim 1**), alongside initial development of the HTRF interaction assay and in silico screening pipelines (**Aim 2**). During this period, the candidate will receive training in cryo-EM data processing and analysis.

Aim 1. Building on protocols already optimized for the human VDAC2–BAK and VDAC2–BAX complexes, the murine VDAC2–BAK complex will be produced, purified, and screened for optimal cryo-EM grid preparation. Access to the Marseille cryo-EM platform (AFMB) and to European facilities through the Instruct program will enable grid screening and high-resolution data collection on 300 kV microscopes. These steps require limited hands-on time from the candidate and can be performed in parallel with activities in Aim 2. Cryo-EM data processing and analysis will be carried out within Lucie Bergdoll's team, with dedicated training for the candidate. Given the expertise already acquired on the human complex, analysis of the murine dataset is expected to be straightforward.

Aim 2. Plasmids for the expression of VDAC2, BAX, and BAK are already available in the laboratory. The corresponding proteins will be produced to develop the in vitro interaction assay. To account for the presence of lipids surrounding VDAC2 (nanodisc), the candidate will undertake a short secondment (2-3 weeks) at the industrial

partner Cisbio to optimize the HTRF assay. Following this optimization, the assay will be implemented at the iSCB platform for screening activities, in parallel with ongoing structural analyses from **Aim 1**. A focused library of 10,314 protein-protein interaction-oriented compounds[15], together with additional libraries available on the screening platform, will be screened to identify both stabilizers and disruptors of VDAC2–BAX/BAK complexes. In parallel, structure-guided in silico screening will be performed by members of the iSCB team.

Aim 3. In the last trimester, plasmids encoding BRET-compatible fusion constructs of VDAC2, BAX, and BAK will be generated for cellular interaction assays. In parallel, appropriate cellular models will be established, including the generation or acquisition of VDAC2- and BAX/BAK-deficient (knockout) cell lines (some already available).

Year 2 will center on experimental and computational screening, hit identification, and early validation (**Aim 2**), together with implementation of cellular BRET assays. A secondment to the University of Nantes will provide hands-on training in BRET assay development.

Aim 2. After the primary screening and a counter screen assay, all active molecules from the *in silico* and HTRF screens will be evaluated in dose response experiments during a secondary screening. The candidate will then travel to Nantes to learn the BRET technology, setup and optimize an assay that will be brought back to the iSCB as an orthogonal validation of the selected molecules. The most promising compounds will be subjected to a first round of Structure Activity Relationship (SAR) by catalog to explore commercially available analogs of the best molecules that might be more active/soluble. These compounds will be purchased and evaluated by the candidate.

Year 3 will be dedicated to advanced translational validation in leukemia cell lines and patient-derived samples (**Aim 3**), and integration of the results into a mechanistic and therapeutic framework. Part of this work will be conducted at the CRCM, while complementary experiments will be performed at the Peter MacCallum Cancer Centre (Melbourne), where the candidate will undertake a 3-month secondment at the beginning of Year 3.

The project management structure relies on close co-supervision between structural biology and drug-discovery experts, facilitated by the physical proximity of the host laboratories. Regular joint meetings will ensure coordination between aims and rapid decision-making. The candidate will benefit from a comprehensive training environment in basic science complemented by translational exposure through collaborations with clinicians at the Institut Paoli-Calmettes and international partners. Mandatory doctoral training (ED courses and SCHADOC modules, cryo-EM data analysis) will be integrated throughout the project. Dissemination activities will include participation in international conferences such as the Biophysical Society Meeting and the Gordon Research Conference on Cell Death, Drug Discovery Chemistry (DDC, San Diego), European Hematology Association (EHA) and American Society of Hematology (ASH).

3.3 Risk management:

The main scientific and technical risks have been identified and mitigated through built-in alternatives.

Aim 1 (structural determination). Structural studies are supported by validated constructs, optimized purification protocols, and preliminary cryo-EM data. The use of a cell-free expression system ensures production of full-length, non-toxic BAK. While minor re-optimization may be required for the murine complex, the risk is low given the team's expertise and existing high-resolution data. WEHI-9625 is commercially available via custom synthesis, and, if needed, can also be synthesized in-house.

Aim 2 (screening). The primary screening strategy relies on the development of an HTRF-based assay. Should this assay require additional optimization, a robust backup strategy is in place: BRET-based interaction assays, already functional and validated in the laboratory of Laurent Maillet (CRCI2NA, Nantes Université), can be used directly for screening. This redundancy ensures continuity and efficiency of compound identification regardless of assay development timelines.

Aim 3 (cell validation): Validation in primary patients cells relies on the availability of samples and difficulties in cell thawing, culture and heterogeneity in drug response. Multiple samples will be collected throughout the project (approximately 40 venetoclax-resistant AML per year, included in biobanking cohorts at IPC and at Peter MacCallum Cancer centre) allowing mulitple testing and validations. Moreover, several mice models including patient-derived xenografts (PDX) of venetoclax resistant cells have been set up in our labs both in Marseille and Melbourne, and can provide cellular material for validations.

Overall, the combination of advanced preliminary data, complementary expertise, methodological redundancy, and integrated training ensures the feasibility of the work plan and the successful completion of the project within the PhD duration.

4. ETHICS SELF-ASSEMENT

Use of human cells or tissues : This project involves the use of human cells and tissues in the context of in vitro cellular assays to evaluate candidate modulators of VDAC2–BAX/BAK interactions. These materials will consist exclusively of established human cell lines and patient-derived cellular samples obtained through existing collaborations. No human embryos, foetal tissues, or primary human samples will be collected within the framework of this project.

All human-derived materials will be obtained from collaborators or institutional platforms that operate in full compliance with national and European regulations, including prior ethical approval, informed consent from donors, and appropriate governance procedures. The project will not involve direct interaction with human participants, nor the collection of new biological samples. The PhD candidate will not have access to any identifying information, and no personal data will be processed.

Activities involving non-EU countries and transfer of materials : Some project activities will involve collaboration with a non-EU partner (Peter MacCallum Cancer Centre, Melbourne, Australia). These activities are limited to scientific collaboration and the exchange of research materials and reagents related to apoptosis research. No human participants will be involved in these activities, and no ethical risks specific to the local context are anticipated.

Any import or export of biological materials will strictly comply with applicable national, European, and international regulations, including material transfer agreements (MTAs), biosafety regulations, and institutional authorization procedures. No endangered species, human remains, or sensitive biological resources will be used.

Other ethical considerations : The project does not involve human participants, clinical trials, personal data processing, animal experimentation, artificial intelligence, or activities that may pose risks to the environment, human health, or safety. Standard laboratory safety procedures will be followed at all participating institutions.

Overall, the ethical risks associated with this project are minimal and will be managed through strict adherence to existing regulatory, institutional, and ethical frameworks governing biomedical research.

5. REFERENCES

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