RÉGION NORMANDIE

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A NOVEL STRATEGY TO OVERCOME PARPI RESISTANCE TARGETING UBE2N WITH NON-COVALENT INHIBITORS

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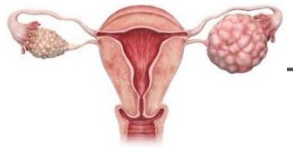
Background



Leading Cause of Death → 5-Year Survival Rate → Below 40%

5300 new cases/year
3500 deaths

205,000 new cases/year
140,000 deaths

Factors Contributing to Low Survival Rate in Ovarian Cancer

First-line maintenance therapy for advanced epithelial ovarian cancer

Homologous recombination deficiency

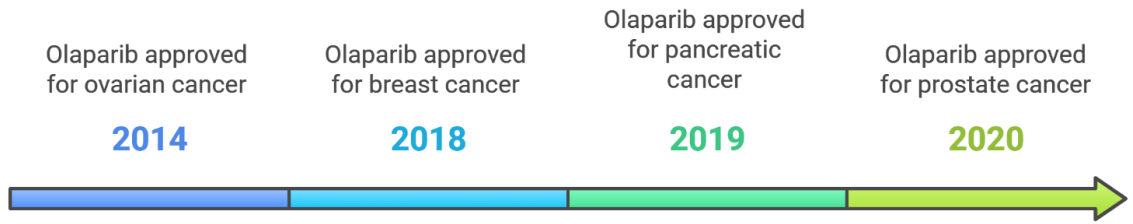
PARPi Alone

Effective as a standalone treatment option for many patients.

PARPi with Bevacizumab

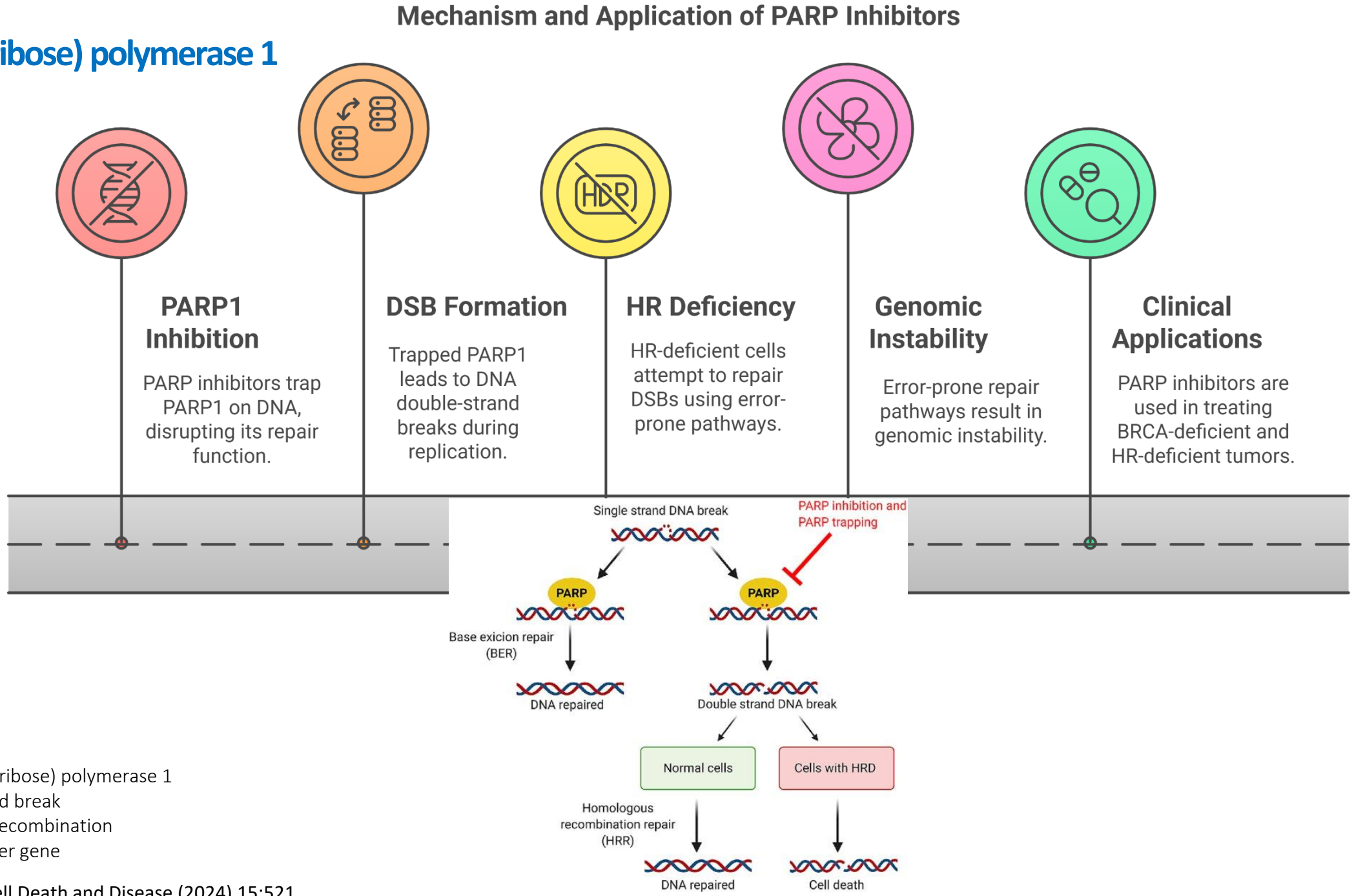
Combines PARPi with an anti-angiogenic agent for enhanced efficacy.

The Evolution of PARP Inhibitor Approvals



M. Chiappa et al. Cell Death and Disease (2024) 15:521

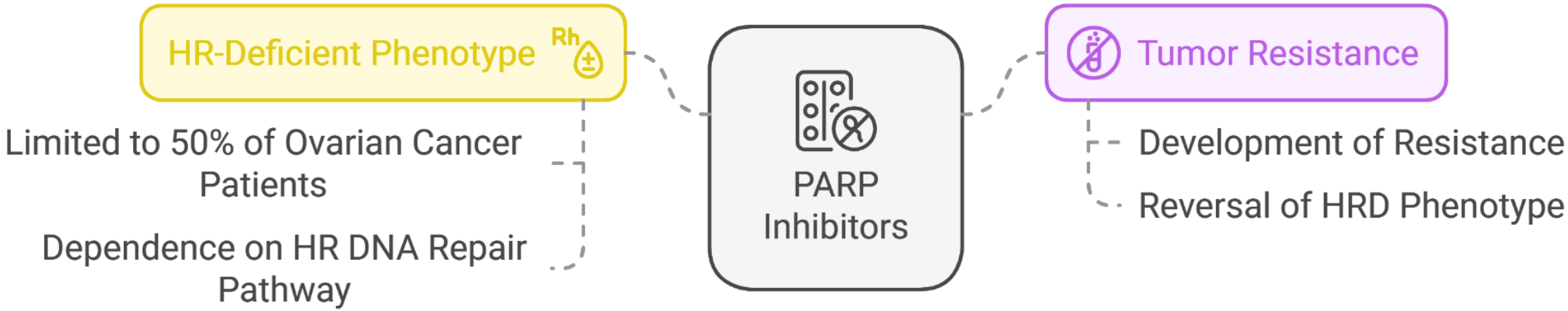
PARP1: Poly (ADP-ribose) polymerase 1



PARP1: Poly (ADP-ribose) polymerase 1
DSB: Double-strand break
HR: Homologous recombination
BRCA: Breast cancer gene

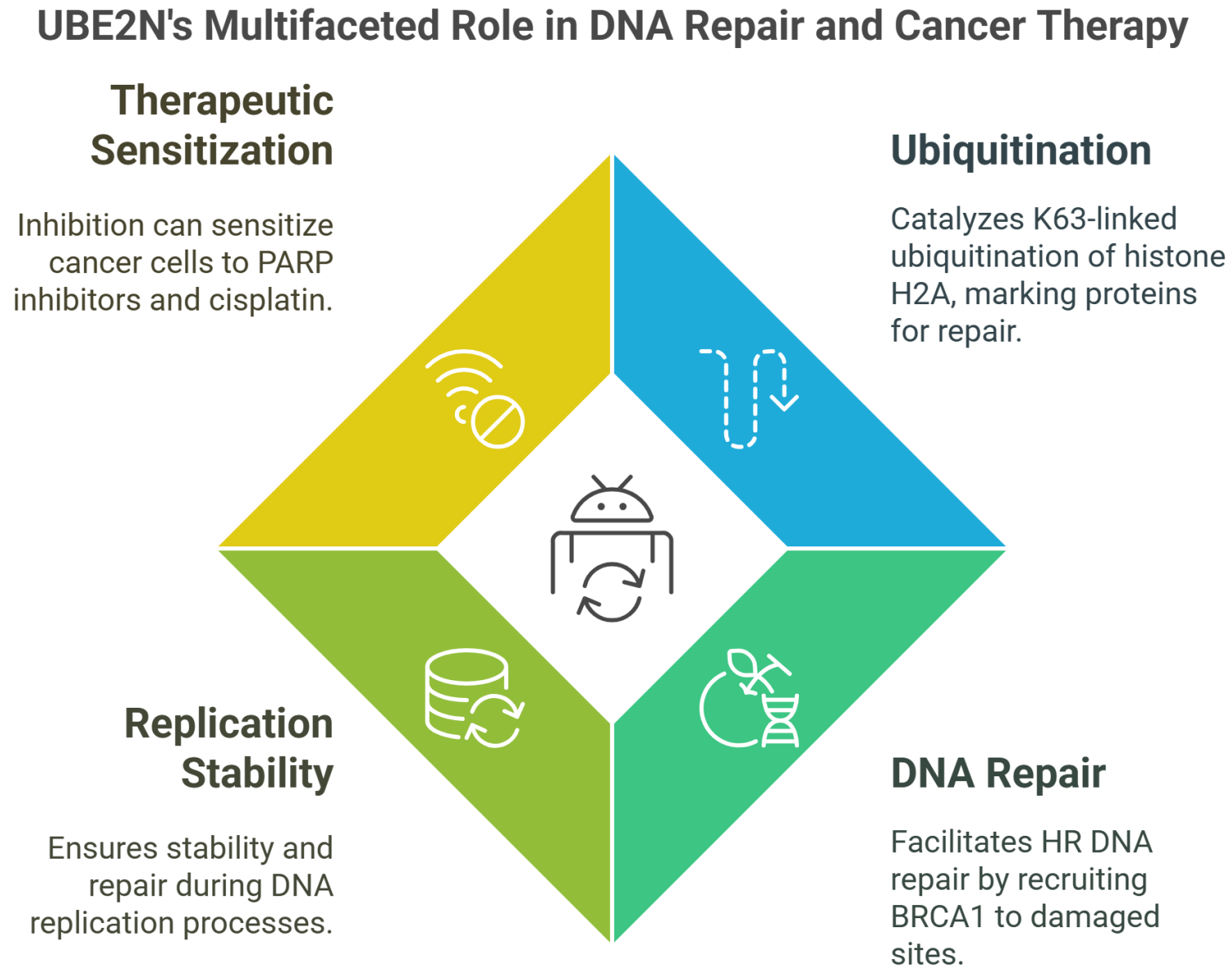
M. Chiappa et al. Cell Death and Disease (2024) 15:521

PAPRi Challenges



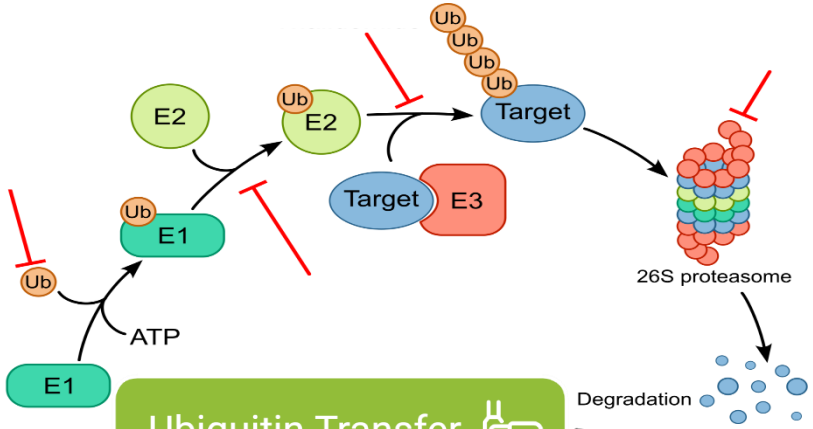
PARP1: Poly (ADP-ribose) polymerase 1
DSB: Double-strand break
HR: Homologous recombination

Rational for UBE2N Selection



UBE2N: Ubiquitin-conjugating enzyme 2N
HR: Homologous Recombination (a type of DNA repair)
PARP: Poly(ADP-ribose) polymerase
BRCA1: Breast Cancer type 1 susceptibility protein

Hodge, Curtis D., Oncotarget 7, no. 39 (2016): 64471



- Ubiquitin Transfer
  - E1 Enzyme
  - E2 Enzyme
  - E3 Ligase
- Types of Ubiquitin Chains
  - K48 Linked
  - K63 Linked
- UBE2N's Role in Ubiquitination
- NF-κB Signaling
  - IKK Activity
  - TRAF6
- DNA Repair
  - Histone H2A
  - RAP80 and BRCA1
- Therapeutic Implications
  - Inhibition in Cancer Models
  - Monotherapy and Nuclear Functions

- UBE2N: Ubiquitin-conjugating enzyme 2N
- HR: Homologous Recombination (a type of DNA repair)
- PARP: Poly(ADP-ribose) polymerase
- BRCA1: Breast Cancer type 1 susceptibility protein
- NF-kB: Nuclear Factor kappa-light-chain-enhancer of activated B cells
- IKK: IκB kinase
- TRAF6: Tumor necrosis factor receptor-associated factor 6
- K48: Lysine 48
- K63: Lysine 63

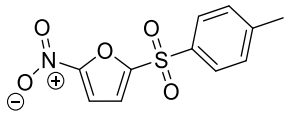
Hodge, Curtis D., Oncotarget 7, no. 39 (2016): 64471

Limiitation of existing UBE2N inhibitors

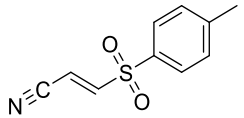
Covalent Inhibitor

NSC697923

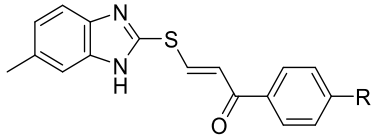
Poor stability in aqueous solvents limits its viability.



NSC697923



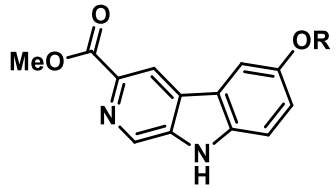
BAY 11-7082



R = CH_2-CH_3 UC-764864
R = OCH_3 UC-764865

Variabines

Derived from marine sponges, low potential for further optimization

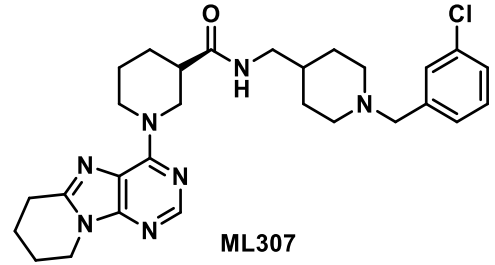


variabine A, R = SO_3H - B, R = H

Non-covalents Inhibitors

ML307

Poor microsomal stability limits clinical viability.



ML307

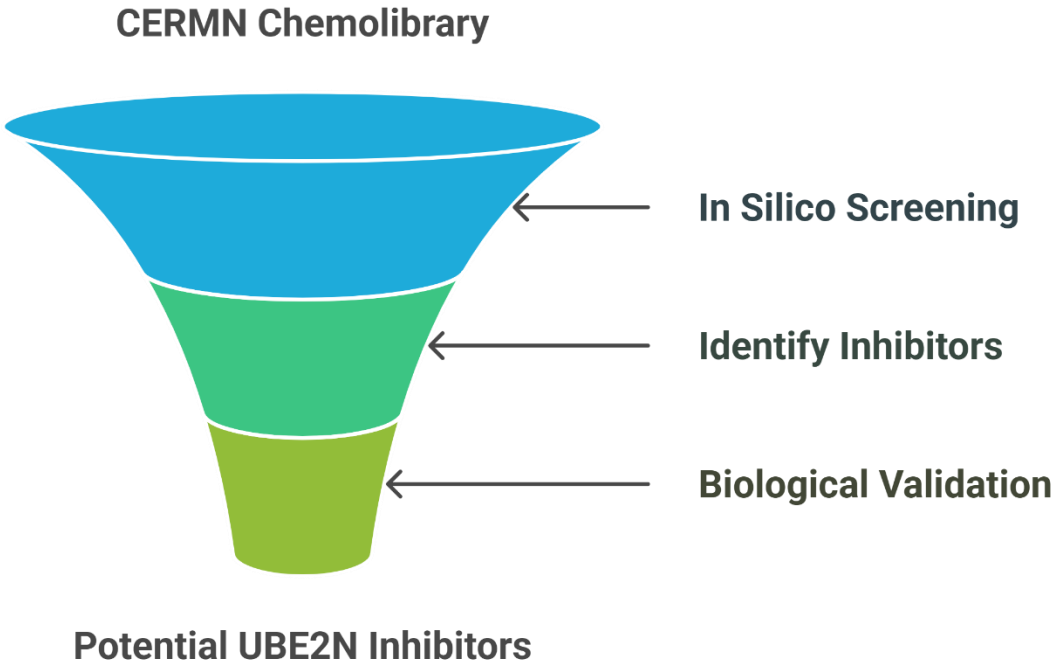
Schwalen F. et al. J Med Chem. 2025 23;68(2):915-928

Project objectives

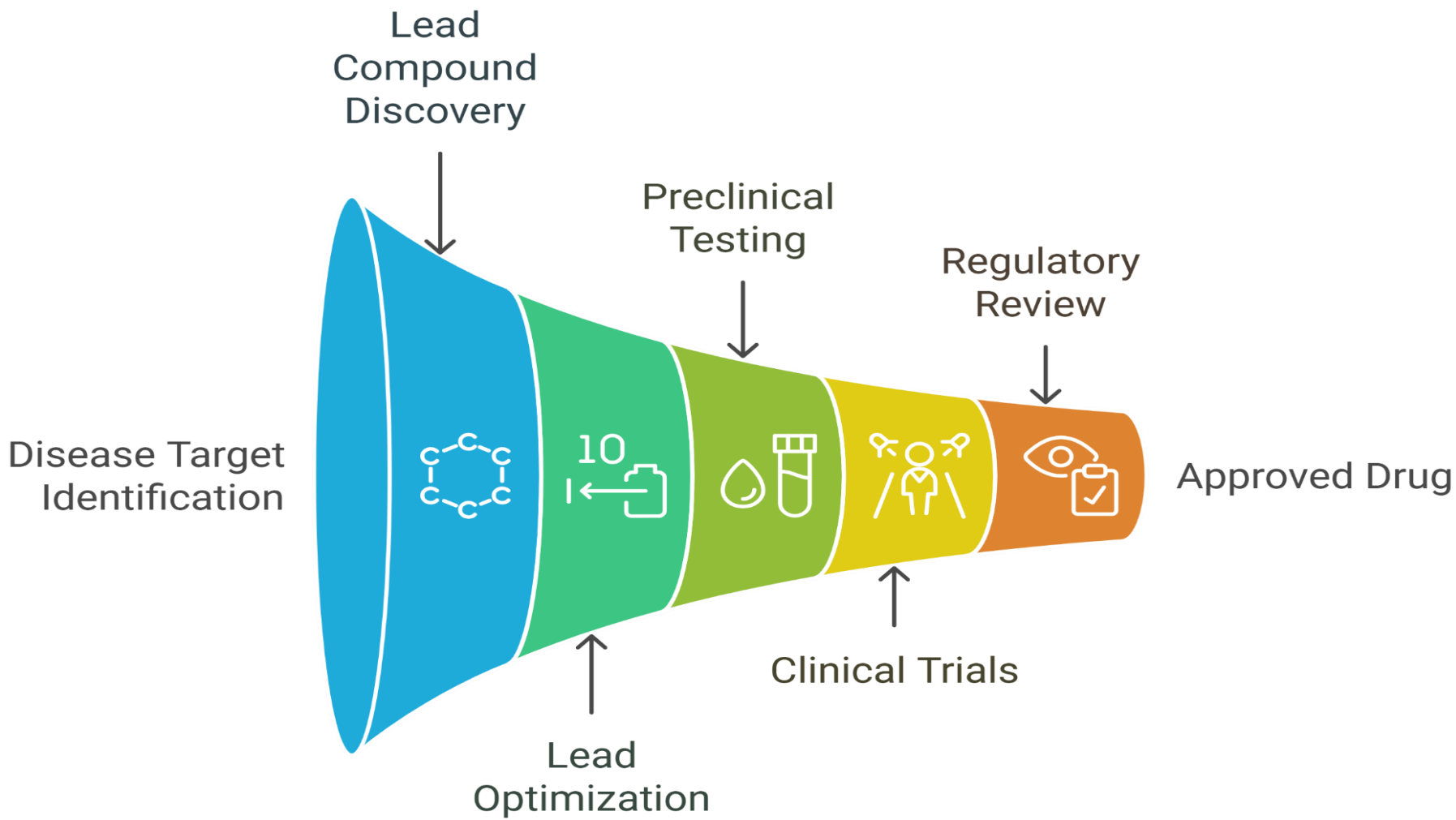
1. **Validating UBE2N as a therapeutic target in ovarian cancers**
   - ❖ **Inhibition of UBE2N (=Ubc13) sensitizes to the action of platinum salts** Wambecke A. et al., *Mol Oncol* (2021)
   - ❖ **Inhibition of UBE2N (=Ubc13) sensitizes to the action of PARPi**

2. **Developing novel clinically relevant UBE2N inhibitors**

CENTRE D'ÉTUDES ET DE RECHERCHE SUR LE MÉDICAMENT DE NORMANDIE

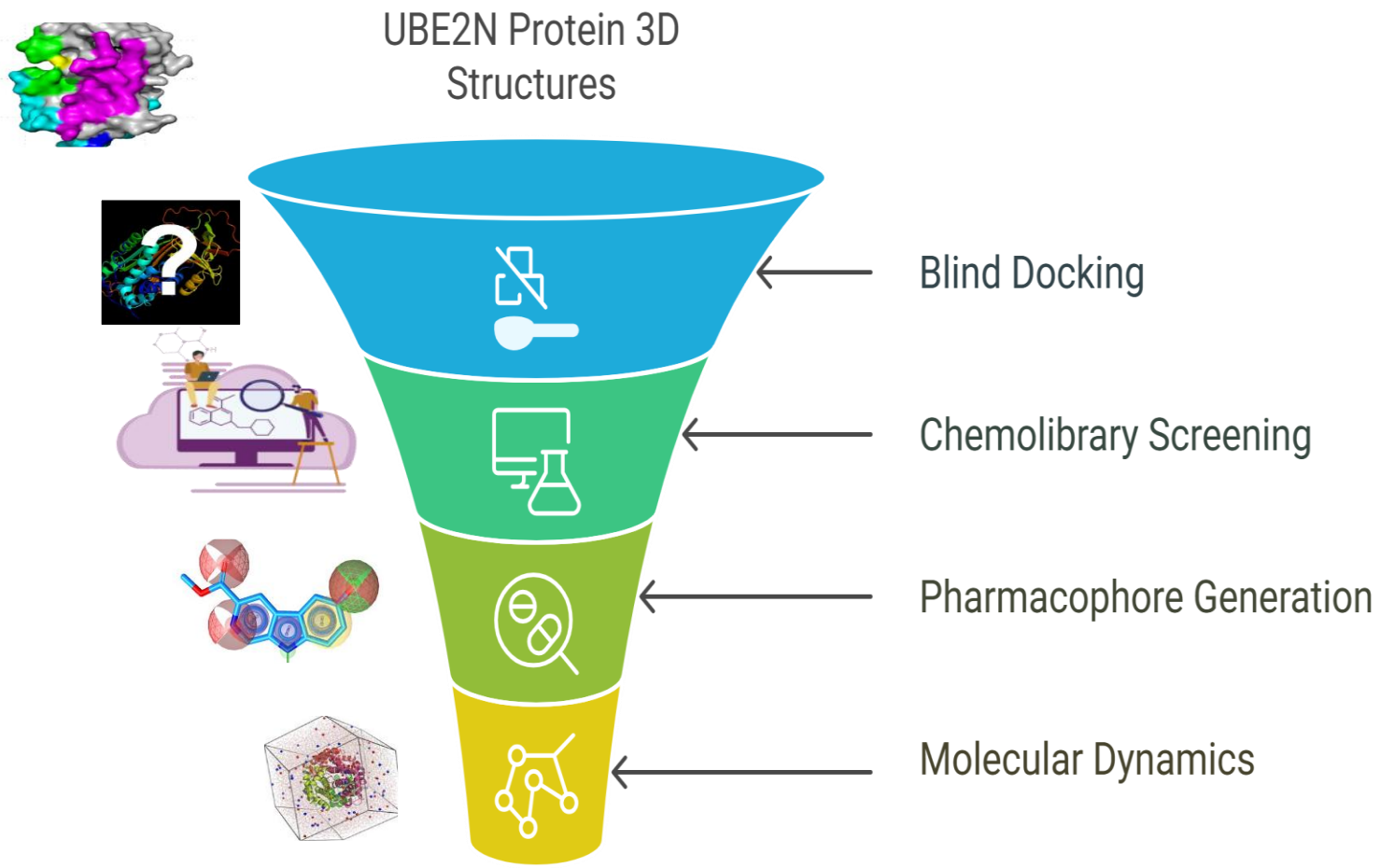


Drug Discovery Process Funnel



2 New UBE2N inhibitors

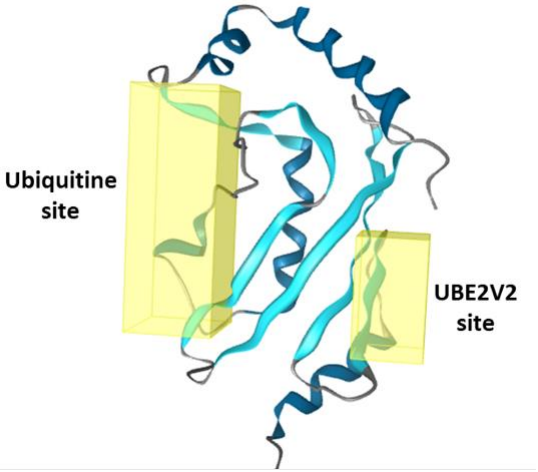
Molecular Modelling Process for UBE2N Inhibitors



2 *Insilico* Based New UBE2N Inhibitors

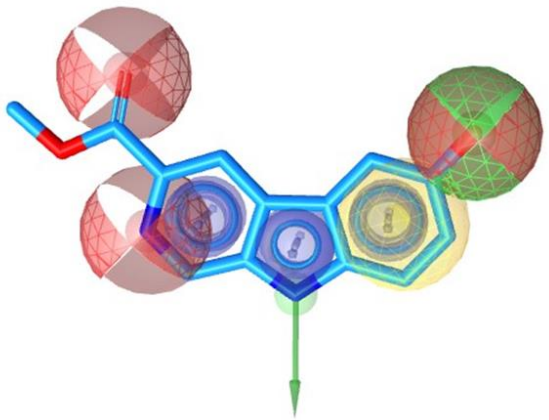
CENTRE D'ÉTUDES ET DE RECHERCHE SUR LE MÉDICAMENT DE NORMANDIE

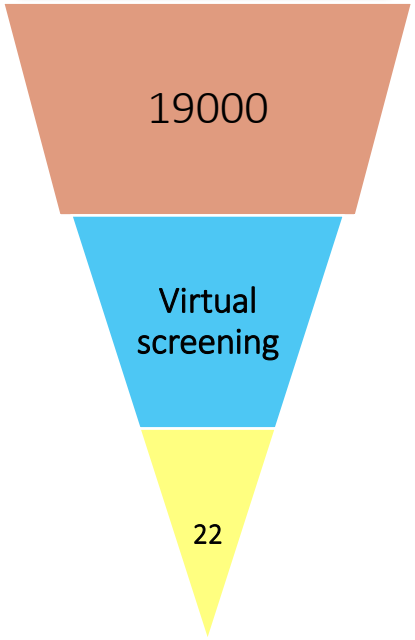
UBE2N and interaction with its Protein partners → Construction of a 3D pharmacophore screening model from Variabine B → Virtual screening of the CERMN chemical library (approximately 19,000 compounds) → 22 compounds selected for in vitro testing on cellular models



Structures available in PDB

Variabine's 3D Pharmacophore

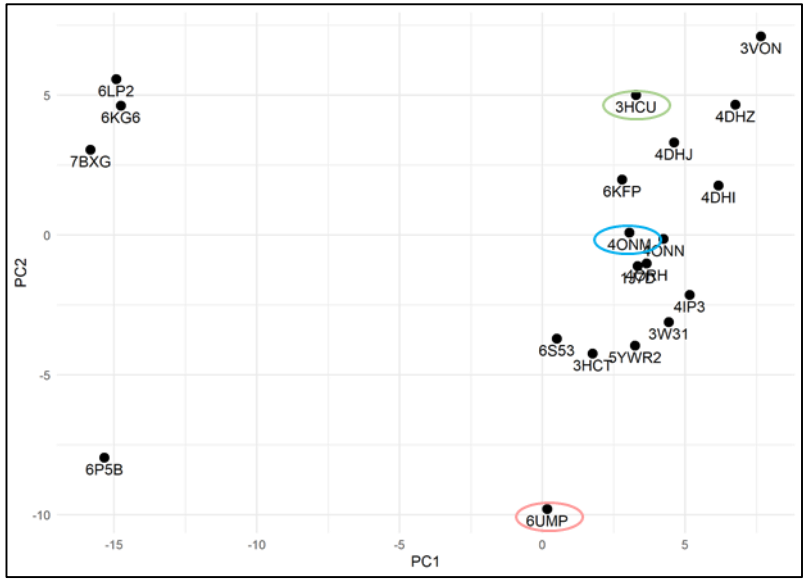




Manuscript Submiited

Modulation of the ubiquitin-binding site shape.

PCA Analysis for the selection of PDBs



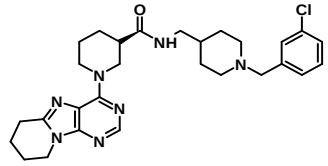
Challenge: Limited information on non-covalent binding sites on UBE2N:
Blind Docking to Identify Non-Covalent UBE2N Inhibitors

Blind docking of two known non-covalent inhibitors, ML307 and Variabine B, was performed.

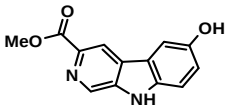
Three distinct binding modes were identified:

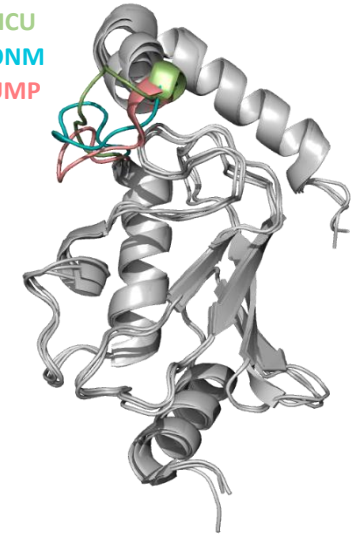
- Binding exclusively to the ubiquitin site
- Binding exclusively to the UBE2V2/UBE2V1 site
- Binding simultaneously to both sites

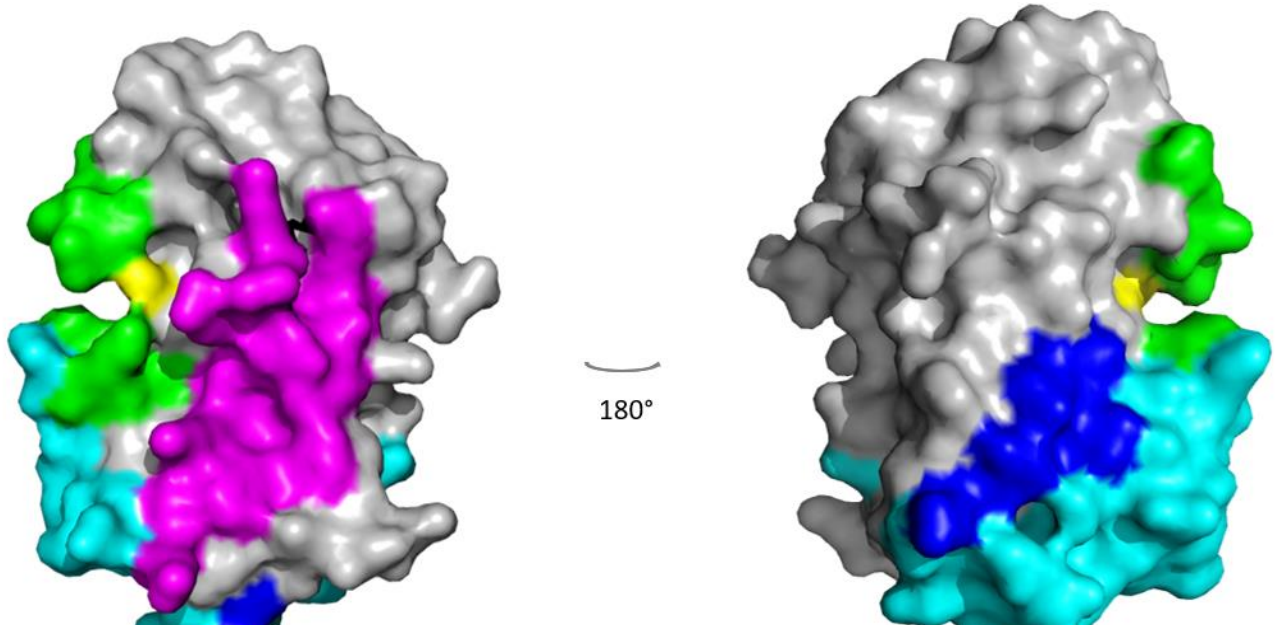
ML307



Variabine B



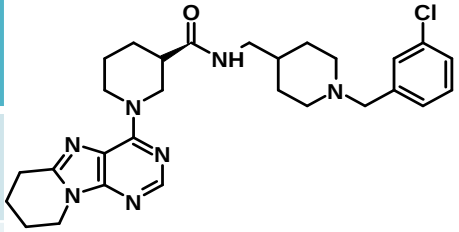




Binding of Non-Covalent UBE2N Inhibitors

ML307

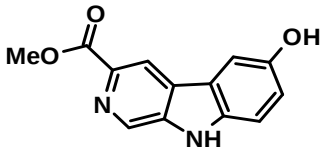
	Ubiquitin site		UBE2V2/UBE2V1		Ubiquitin and UBE2V2/UBE2V1 sites simultaneously		Another Sites	
	Number of poses	Docking scores [kcal/mol]	Number of poses	Docking scores [kcal/mol]	Number of poses	Docking scores [kcal/mol]	Number of poses	Docking scores [kcal/mol]
3HCU	4	from -7.0 to -7.6	8	from -6.6 to -8.2	4	from -6.6 to -7.2	4	From -6.7 to -7.2
4ONM	0		17	from -7.9 to -10.1	3	from -8.7 to -9.2	0	
6UMP	10	from -6.6 to -8.3	3	from -7.3 to -7.8	1	from -7.2 to -7.2	6	From -6.6 to -7.9



ML307

Variabine B

	Ubiquitin site		UBE2V2/UBE2V1		Ubiquitin and UBE2V2/UBE2V1 sites simultaneously		Another Sites	
	Number of poses	Docking scores [kcal/mol]	Number of poses	Docking scores [kcal/mol]	Number of poses	Docking scores [kcal/mol]	Number of poses	Docking scores [kcal/mol]
3HCU	11	from -4.7 to -5.9	2	from -4.8 to -5.4	0		7	from -4.7 to -5.8
4ONM	2	from -5.0 to -5.5	10	from -4.8 to -6.2	1	-5.5	7	from -4.6 to -5.5
6UMP	7	from -4.8 to -6.1	0		4	from -4.8 to -5.9	9	from -4.9 to -5.5

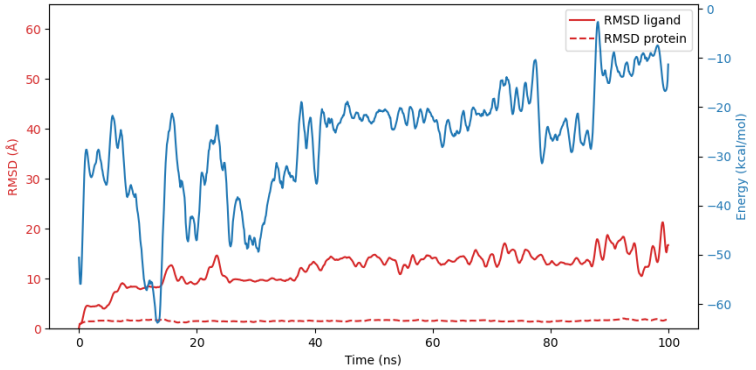


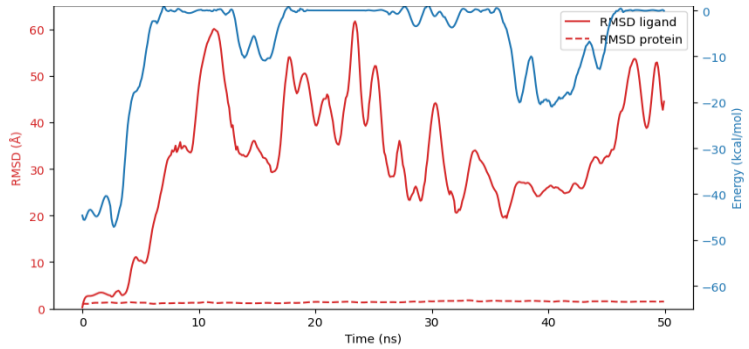
Variabine B

The blind docking results suggested that both ML307 and Variabine B have the potential to bind to ubiquitin and/or the UBE2V2/UBE2V1 sites. However, no clear preference for either compound was identified.

MD Simulation

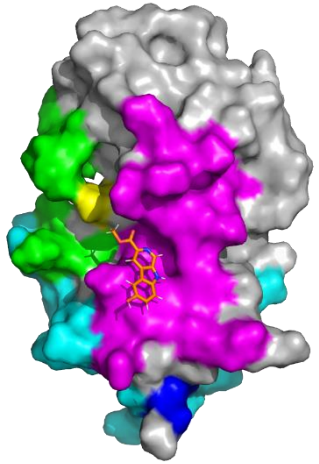
For ML307**, none of the five tested complexes** - three bound at the ubiquitin site, one to the cofactor site, and one spanning both sites - exhibited stable binding during simulations



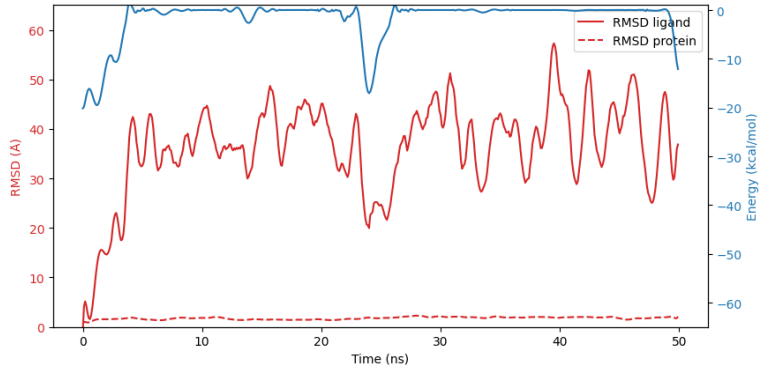


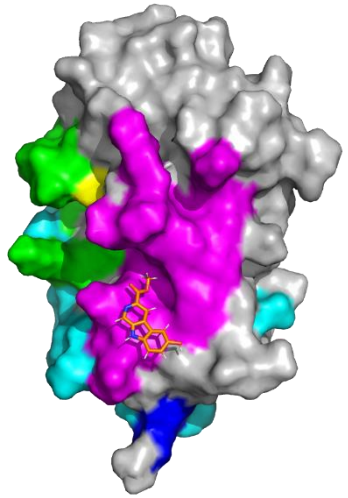
ML307 Unstable MD

Highest-scoring Variabine B pose at the ubiquitin site proved to be unstable. More, the two highest-scoring poses at the cofactor-binding site were submitted to the MD simulations.
While the highest-scoring pose was unstable, **the second highest-scoring pose** remained stably bound throughout 100 ns of the simulation

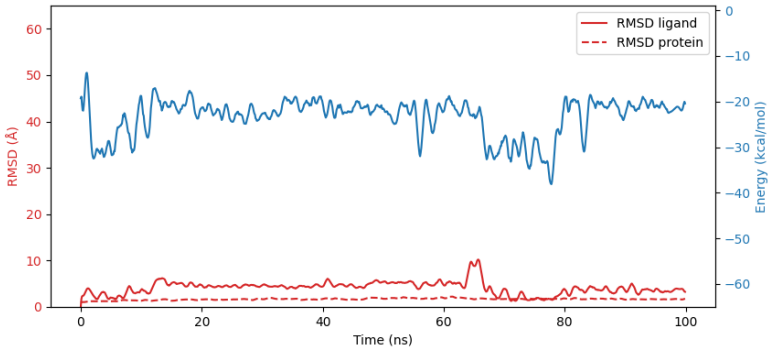


Pose 1





Pose 2

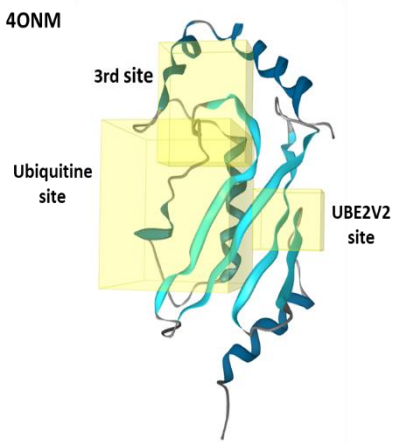


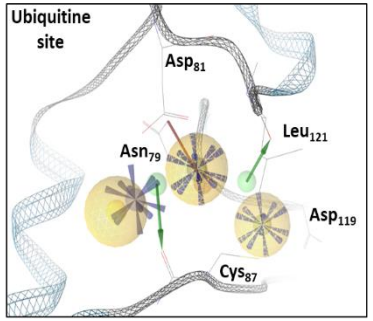
Docking Based-Screening of the CERMN chemolibrary

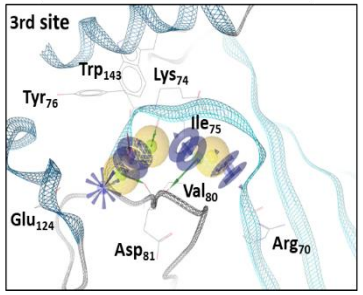
	Ubiquitin Site						UBE2V2/UBE2V1 Site					
	Docking scoring criterion [kcal/mol]	Number of selected compounds	Number of compounds submitted to MD	Number of compounds validated by MD	Number of tested compounds	Name of selected compound	Docking scoring criterion [kcal/mol]	Number of selected compounds	Number of compounds submitted to MD	Number of compounds validated by MD	Number of tested compounds	Name of selected compounds
3HCU	< - 8.0	96	17	4	0		< -7.5	54	4	0	0	
4ONM	< - 8.0	193	16	4	2	CERMN-1 CERMN-6	< -12.0	161	27	8	7	CERMN-4 CERMN-5 CERMN-7 CERMN-9 CERMN-10 CERMN-12 CERMN-13
6UMP	< - 8.0	167	26	5	3	CERMN-2 CERMN-3 CERMN-11	< -12.0	92	22	3	1	CERMN-8

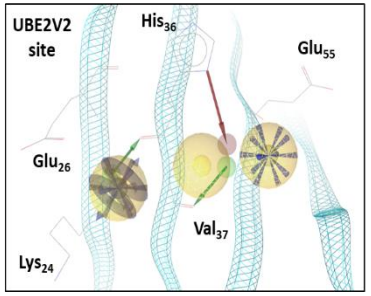
3D pharmacophore screening campaigns of the CERMN chemolibrary-Method

4ONM

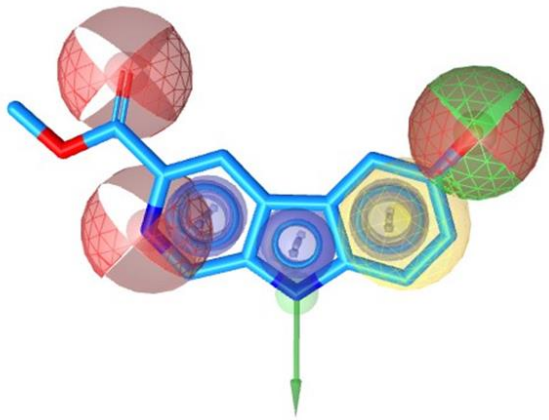




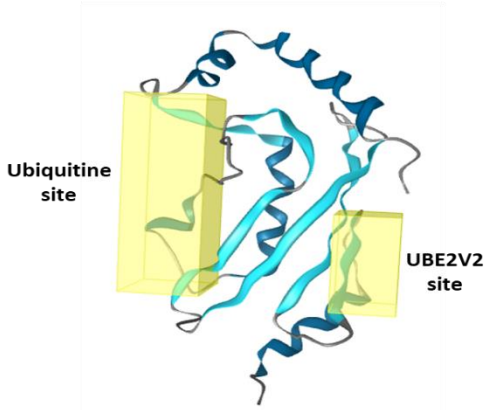


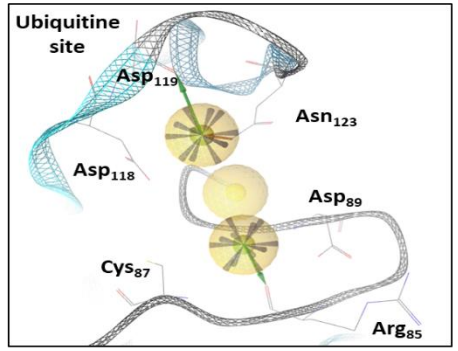


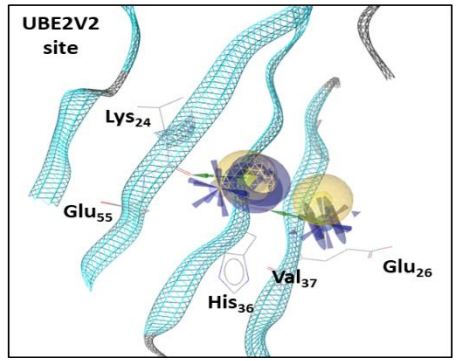
Variabine's 3D Pharmacophore



6UMP



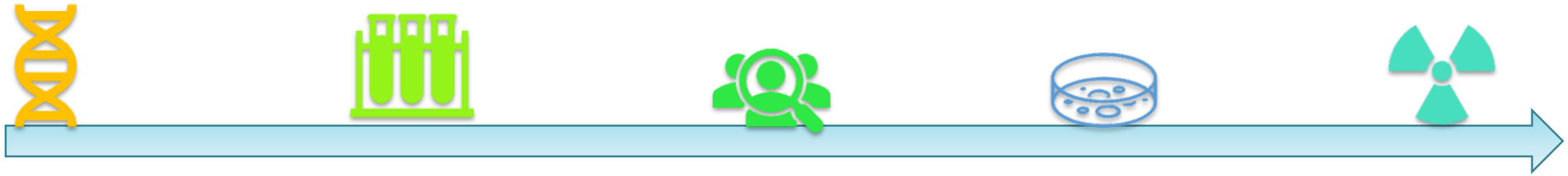




3D pharmacophore screening campaigns of the CERMN chemolibrary-Result

Site		Number of features	Maximal Number of omitted features	Number of aligned compounds to the pharmacophore	Number of compounds submitted to MD	Number of compounds validated by MD	Number of tested compounds	Name of selected compounds
4ONM	Ubiquitine site	9	5	172	24	1	1	CERMN-22
	UBE2V2/UBE2V1 site	8	3	3	1	0	0	
	3rd site	11	5	17	4	0	0	
6UMP	Ubiquitine site	7	2	154	10	3	0	
	UBE2V2/UBE2V1 site	8	3	699	43	3	1	CERMN-19
Variabine B		9	5	58	20	15	7	CERMN-14 CERMN-15 CERMN-16 CERMN-17 CERMN-18 CERMN-20 CERMN-21

3 Cell based Evaluation of Hits



SKOV-3, a human ovarian carcinoma cell line, was used for screening selected CERMN Chemolibrary Compounds

Preliminary screening and hit validation of selected compounds from virtual screening were performed.

Sensitization of SKOV-3 cells to Olaparib was assessed during the screening.

Colony forming assay was performed on selected compounds to evaluate efficacy

Toxicity studies were conducted for top hit compounds.

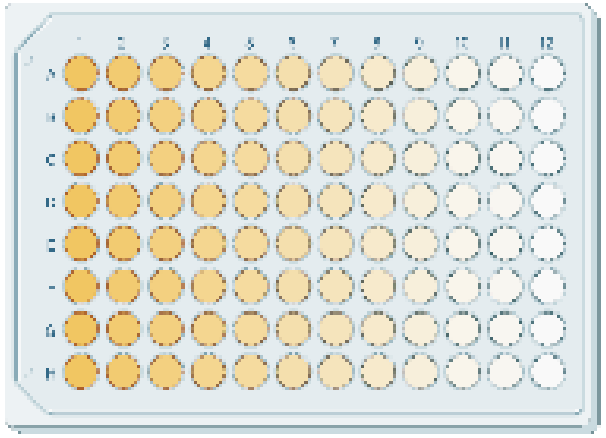
3 Assessment of Existing UBE2Ni (NSC697923 and ML307) and PARPi (Olaparib)

Existing UBE2Ni

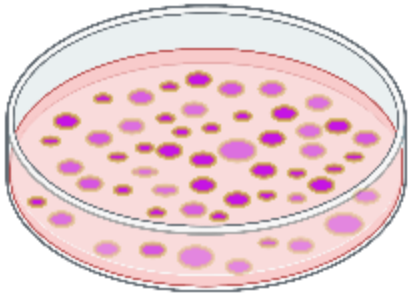
Proliferation and sensitizing SKOV3 cells treated with existing UBE2N inhibitor (ML307 a non-covalent UBE2Ni and NSC697923, a covalent) UBE2Ni) and co-treated with existing PARPi (Olaparib).

3 Cell based validations of Newly Identified Compounds

Viability test Using SKOV3 Cell line



Colony-forming
Assay Using SKOV3 Cell line



Manuscript Submiited

4 Toxicity assessment

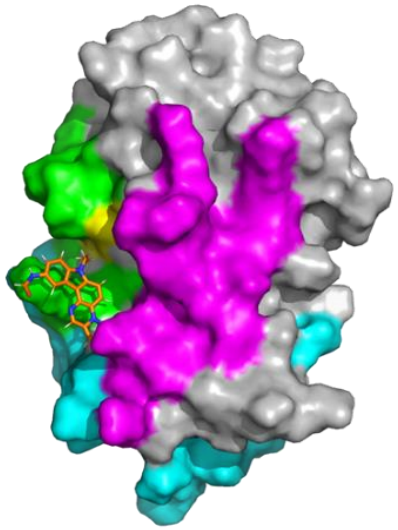
Toxicity assessment of CERMN-2 and CERMN-16 in Normal T1074 Ovarian Epithelial Cells

MD simulations based mechanisms of action:

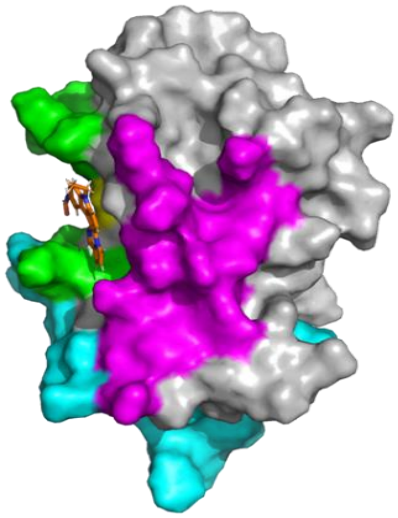
CERMN-2

CERMN-2 targets the **ubiquitin-binding site**, effectively obstructing access to the catalytic Cys87 residue via structural complementarity (van der Waals forces).

Docking pose



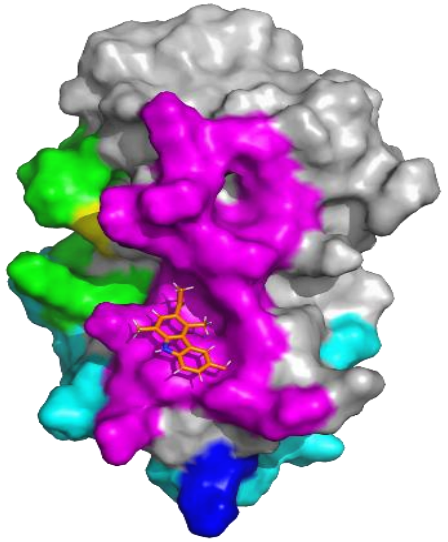
Final MD pose



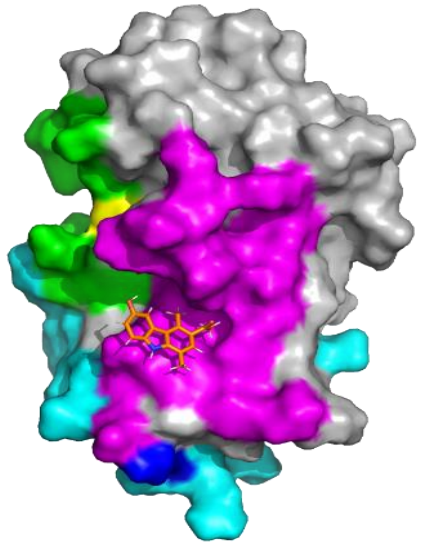
CERMN-16

CERMN-16 targets the **cofactor interface**, predicted to disrupt the UBE2N/cofactor complex (similar to Variabine B).

Docking pose



Final MD pose



Affinity Validation

Quantification of Hit Compound Binding to UBE2N via Microscale Thermophoresis (MST)

Summary

- **Dual In Silico Discovery:** A systematic strategy combining **structure-based docking** and **ligand-based 3D pharmacophore modeling** was executed against UBE2N's key functional interfaces (ubiquitin-binding and cofactor sites).
- **Novel Lead Identification:** Screening a of 19,000 compound library identified two novel, non-covalent inhibitors, **CERMN-2** and **CERMN-16**, validated by Molecular Dynamics (MD) simulations.
- **Crucial Functional Advance:** Both compounds significantly **sensitized chemoresistant SKOV-3 ovarian cancer cells** to the PARP inhibitor **Olaparib**.
- *Significance:* This sensitization was achieved where previous non-covalent inhibitors (ML307 and Variabine B) failed, highlighting a superior therapeutic profile.
- **Favorable Safety Profile: CERMN-2** exhibited low cytotoxicity in normal T1074 ovarian epithelial cells at effective concentrations.
- **Distinct Mechanisms of Action (MD Confirmed):**
- **CERMN-16** targets the **cofactor interface** (disrupting UBE2N/cofactor binding).
- **CERMN-2** targets the **ubiquitin-binding site**, physically obstructing the catalytic Cys87 residue.
- **Biophysical validation** (e.g., MST) confirmed direct binding affinity.

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Thème 4 : Precision medicine for ovarian cancers

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