BIOINFORMATIC, DISEASE LINKAGE AND STRUCTURAL ANALYSIS OF CYCLIN T2 (CCNT2) Y223A/F AND Y224A/F MUTATION AS A PROMISING ANTI-CANCER DRUG TARGET INHIBITION: A COMPUTATIONAL CRYSTALLOGRAPHIC & EXPERIMENTAL APPROACH

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CANCER RESEARCH UK | NEWCASTLE DRUG DISCOVERY UNIT

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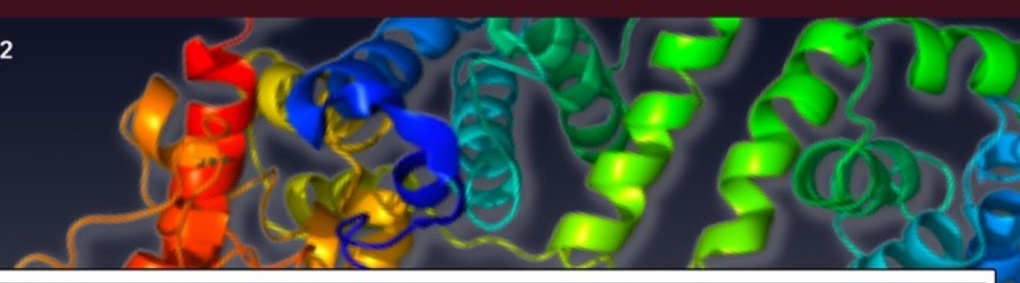
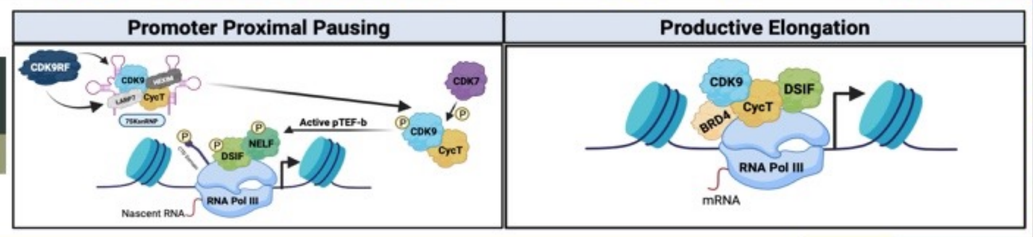


1. INTRODUCTION

• **Cancer** is one of the greatest challenges to human health. It is the world's leading cause of death, accounting for almost 10 million deaths in 2020. One way to address this challenge is through molecularly targeted therapies. Various targets for such therapies have been discovered, including Cyclin-dependent kinases (CDKs).

• **CDKs** are regulatory enzymes that drive orderly transitions through various points of the cell cycle to ensure complete mitotic division. The **delicate balance and close cooperation between cyclins, CDKs, & CKIs** are essential to safeguard the orderly cell cycle. Other CDKs are involved in regulation of transcription. One such transcriptional regulator is CDK9 which, when complexed with a cyclin T isoform, forms **Positive Transcription Elongation Factor b (P-TEFb)**. P-TEFb plays a role in promoting transcription from certain promoters, including those of antiapoptotic factors such as MIC-1.

CYCLIN T ROLE IN TRANSCRIPTION PROCESS

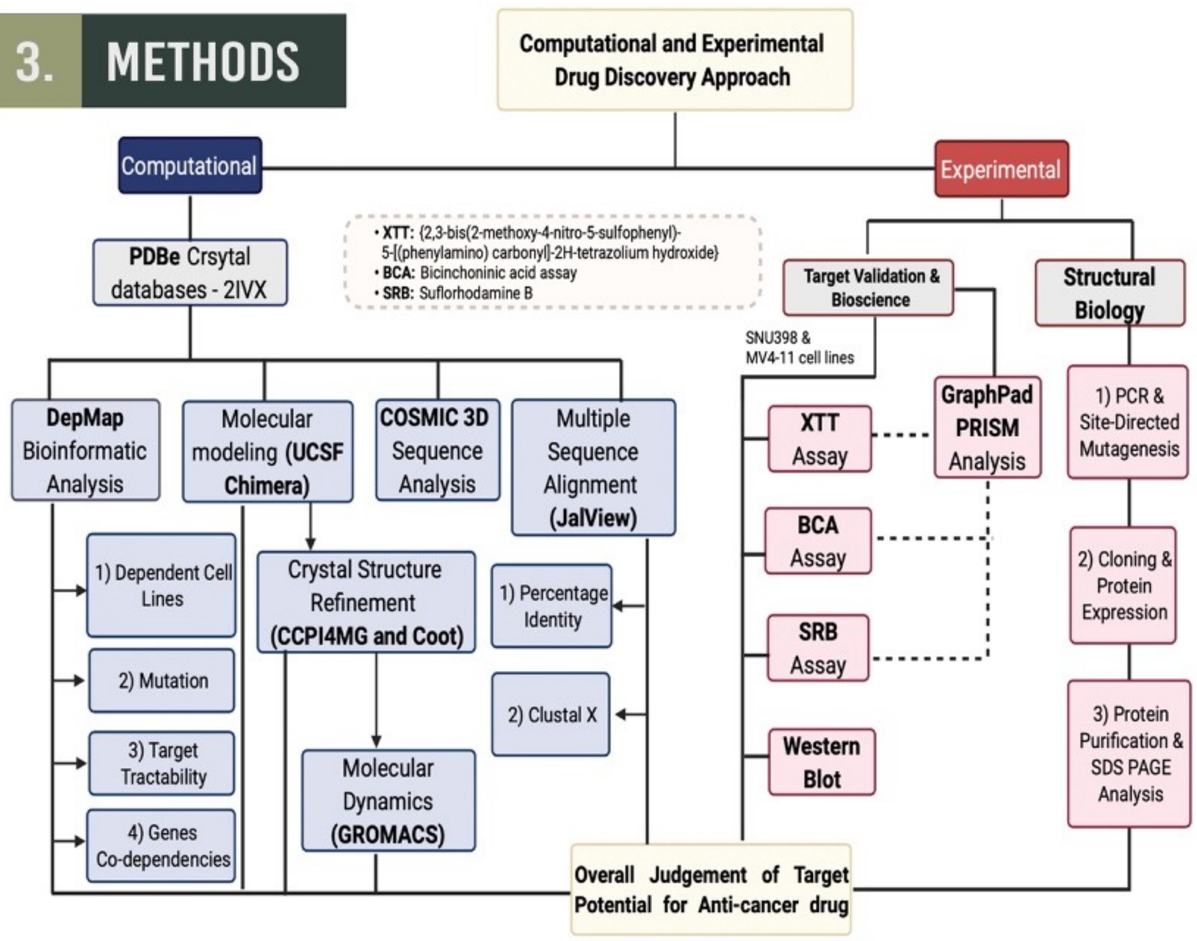


2. AIM & OBJECTIVES

• To investigate whether cyclin T2 has good potential as a target for a novel anti-cancer drug through computational and experimental approach drug discovery

1. **Application of bioinformatics tools** to investigate the rationale for targeting cyclin T2
2. **Analysing X-ray crystallographic** data to solve the structure of cyclin T2 bound to fragment "hits"
3. **Simulation using molecular dynamics** to analyze protein behavior and to predict the stability of protein-ligand complexes outside a crystalline environment
4. **Conducting protein expression** in the laboratory to produce site directed mutations to probe the functional importance of surface-exposed residues on cyclinT2
5. **Validating the target protein** through bioscience experiment by testing the effect of CDK9 inhibitors in cell-based assays

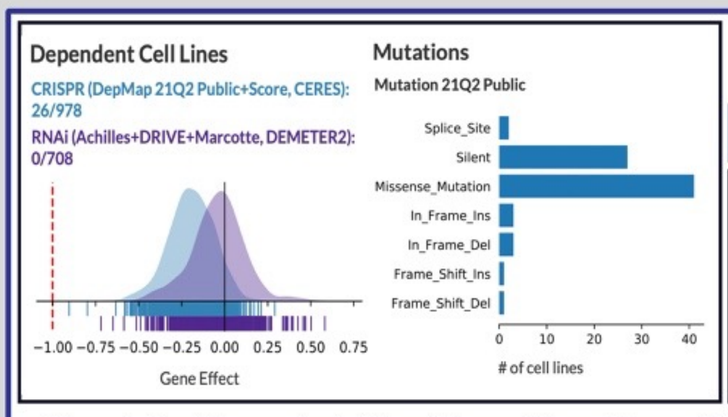
3. METHODS



• In the **computational approach**, various applications and programs were used to investigate structure and ligand interaction of cyclin T2 using programs and resources that include DepMap, Chimera, CCP4i2 and Coot, JalView, and GROMACS.

• In the **experimental approach**, two different laboratory experiments were conducted, which constitute steps towards **structural biology** and **target validation & bioscience laboratory.**

4. RESULTS: COMPUTATIONAL



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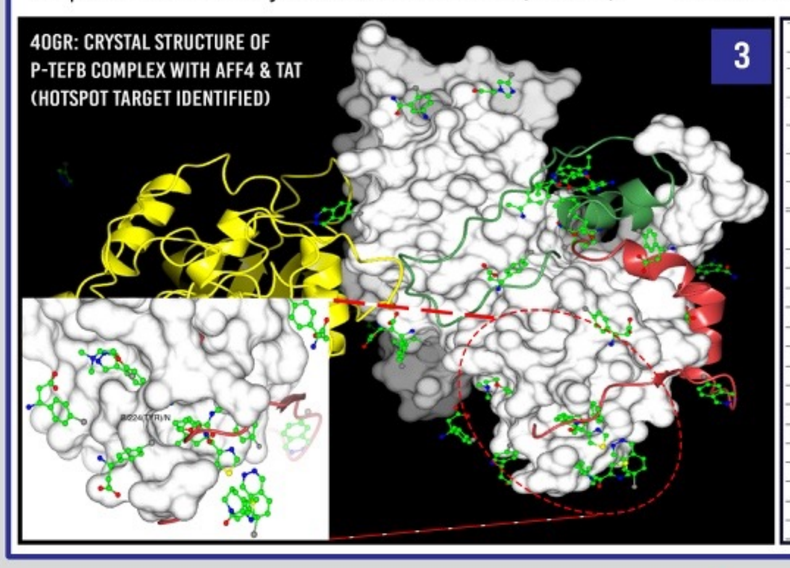
No.	Genes (CRISPR)	Pearson Correlation	Genes (RNAi)	Pearson Correlation
1	HEXIM1	-0.26	MRPL28	-0.27
2	DBI	0.25	SGSM3	0.26
3	TMEM177	0.24	C5orf24	0.26
4	SLC35F5	0.24	ASTN1	-0.26
5	MARCO	0.23	CCNY	0.26

• Through **DepMap** analysis, 26 cell lines with positive CCNT2 gene effects (dependent) & top 5 gene co-dependencies were identified (table 1). The types of CCNT2 mutation that predominate in cell lines are missense (>40 cell lines). **COSMIC 3D** was utilized to perform deep mutational analysis with results listed (table 2)

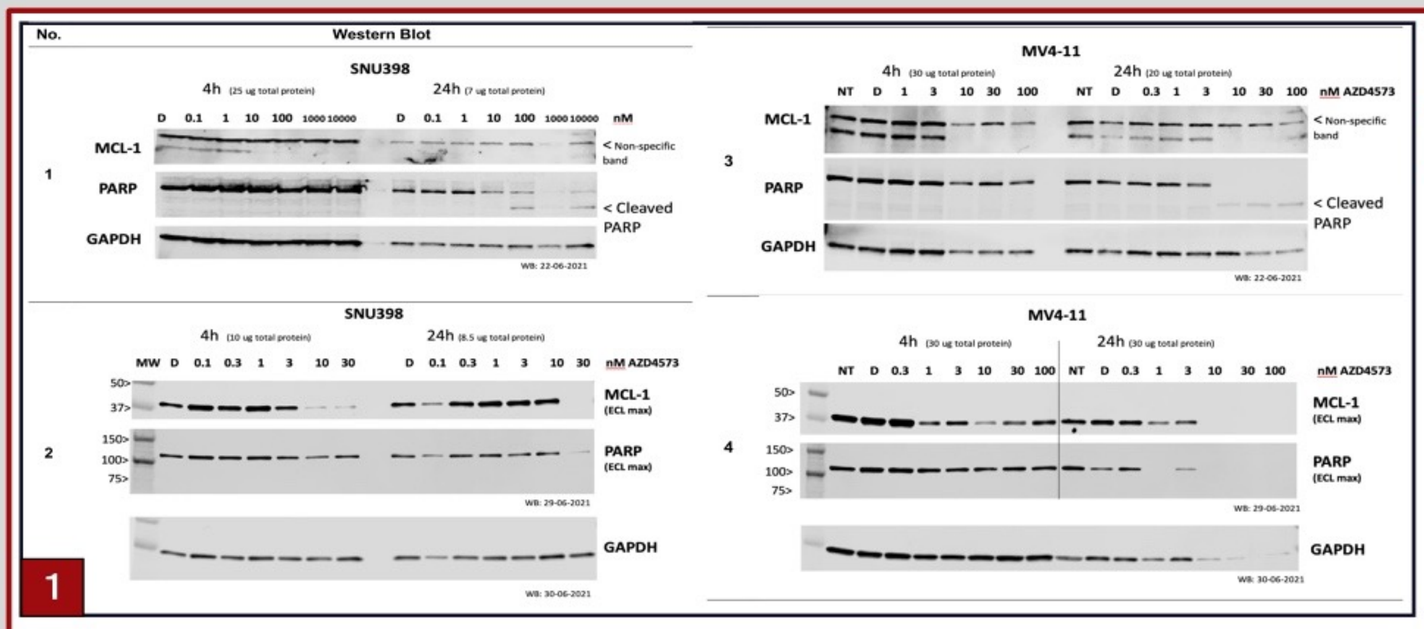
• 15 different datasets were used to determine ligand-bound structures of cyclin T2. A subset of these were further explored using **MD simulation** as shown in table 2. Lastly, through **CHIMERA & CCP4MG**, several hotspot target were identified and investigated (Figure 3).



No.	Position (Bp)	Count	Name	Code	Mutation
1	77	2	PHE 77 A,B	F	COSV56063489
2	81	2	ILE 81 A,B	I	COSV56063303
3	116	2	PRO 116 A, B	P	COSV56063892
4	120	2	THR 120 A, B	T	COSV56063543
5	149	2	ILE 149 A, B	I	COSV56064443
6	201	2	HIS 201 A,B	H	COSV56064130

2

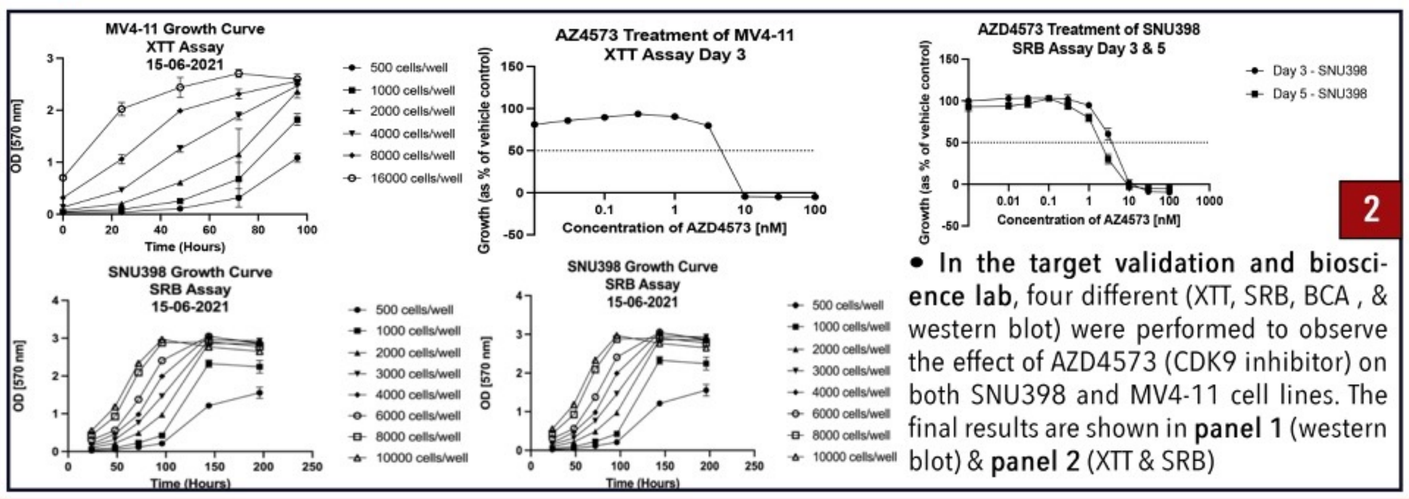
No.	Cyclin T2 Data Subset	R-factor	R-free
1	Cyclin T2_34	0.190	0.215
2	Cyclin T2_127	0.192	0.223
3	Cyclin T2_129	0.255	0.280
4	Cyclin T2_131	0.182	0.209
5	Cyclin T2_x0030	0.192	0.215
6	Cyclin T2_x0413	0.210	0.234
7	Cyclin T2_x0415	0.190	0.230
8	Cyclin T2_x0484	0.261	0.290
9	Cyclin T2_x0557	0.193	0.230
10	Cyclin T2_x0609	0.198	0.215
11	Cyclin T2_93	0.191	0.222
12	Cyclin T2_96	0.211	0.255
13	Cyclin T2_97	0.189	0.215
14	Cyclin T2_106	0.238	0.305
15	Cyclin T2_122	0.425	0.472



5. RESULTS: EXPERIMENTAL

A: FLAG T2 Y223A, **B:** FLAG T2 Y224F, **C:** T2 Y223 A, **D:** T2 Y224A - **SDS PAGE analysis**

• **In the structural biology laboratory**, site-directed mutagenesis for specific mutants Flag T2 Y223A/F, Flag T2 Y224 A/F, T2 Y223 A/F, and T2 Y224 A/F were successfully cloned. The final result of protein expression was confirmed through by SDS Page Analysis shown in Panel 3.



• **In the target validation and bioscience lab**, four different (XTT, SRB, BCA , & western blot) were performed to observe the effect of AZD4573 (CDK9 inhibitor) on both SNU398 and MV4-11 cell lines. The final results are shown in **panel 1** (western blot) & **panel 2** (XTT & SRB)

6. CONCLUSION

Functionally relevant sites on the **surface of cyclin T2** exist and can be targeted by small molecules (fragments) as **starting points for inhibitor development**

7. FUTURE WORKS

1. **To construct** a specific small molecule that can bind to cyclin T2 surface to inhibit its role in cancer development
2. **To search** for an optimal lead compound that further can be developed as the final compound for cyclin T2 inhibitor drug
3. **To assess** the clinical benefit of a constructed lead compound in inhibiting cancer progression

FULL RESULT & MANUSCRIPT

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