A successful high-throughput screening campaign for new small molecule inhibitors of BLM Helicase

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Abstract

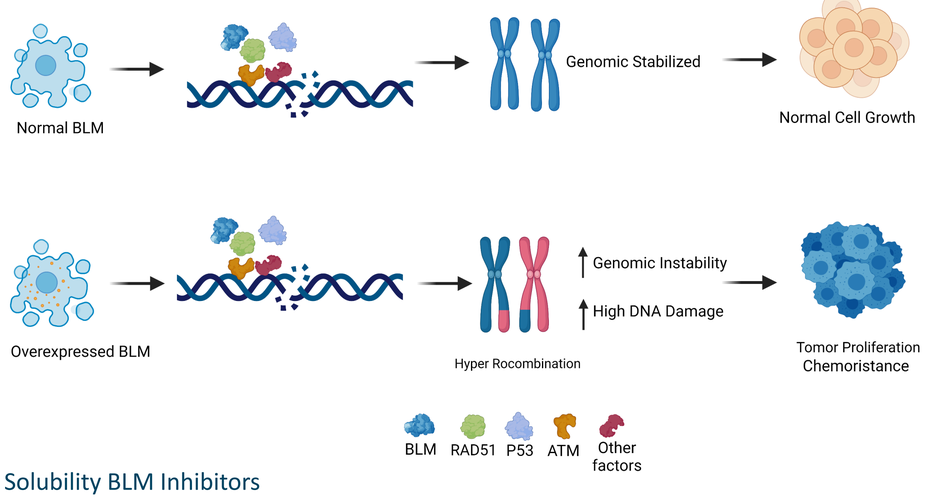
BLM (Bloom syndrome protein) is an essential RECQ-family helicase involving DNA replication and repair via homologous recombination repair pathways. Synthetic lethality study showed BLM as a promising target in a range of cancers with defects in the DNA damage response. Unfortunately, selective small molecule inhibitors are still lacking.

We have established easy, cheap, fast and robust assays to measure duplex DNA unwinding and ATPase activity in a high-throughput fashion. The primary assay employs förster resonance energy transfer (FRET) methods utilised a labelled forked DNA with a fluorophore and a quencher. The assay carried out in a 1536-well plate format and provided robust and good quality (Z prime = 0.85) for large-scale and high-throughput applications.

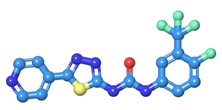
In this study, approximately 333,000 small molecules were screened to measure inhibition of duplex DNA unwinding by a catalytically active BLM helicase domain. In addition, selected molecules are screened using a bioluminescent approach to validate their ATPase activity. The second assay employs firefly luciferase to detect ATP turnover. As a result, we observed 277 compounds have a dose-response profile against BLM, while 87 compounds showed a selective inhibition on BLM compared to another member helicase (RECQ1). Hence, this paired fluorescence and bioluminescence methods are suitable for screening against helicase family members.

Scientific Background

- Overexpressed BLM cause hyper-recombination, thus promoting tumor growth and chemoresistance



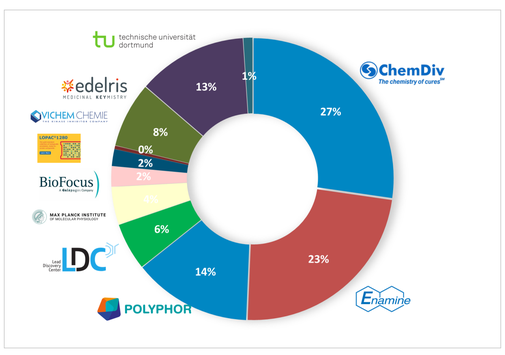
- Poor Solubility BLM Inhibitors



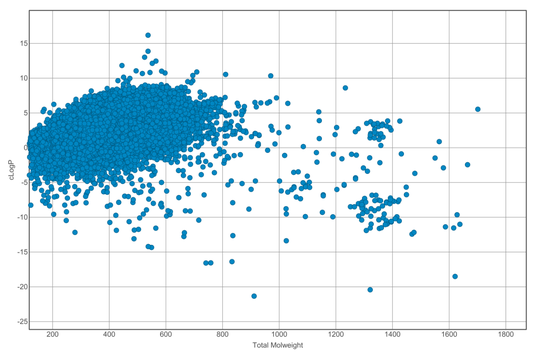
We tested reference inhibitor, ML216, has solubility 1 of 500 micromolar Hepes Buffer pH 7.5.

Compounds and Target Profile

❑ Description of LDC chemical Library



The percentage of source chemical vendors

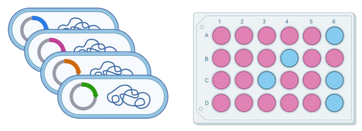


Scatter plot of molecular weight against cLogP

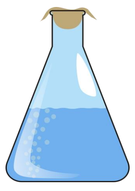
❑ Protein Expression and Purification

Codon optimization

Gene Synthesis

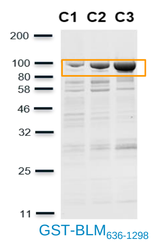


Expression Test Conditions; Media, Tag, IPTG, Celsius



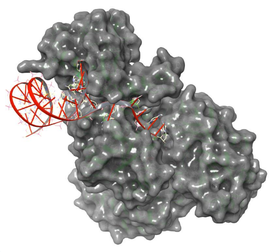
2 Liter Expression

GST-Trap Column



GST-BLM$_{636-1298}$

SDS-PAGE profile

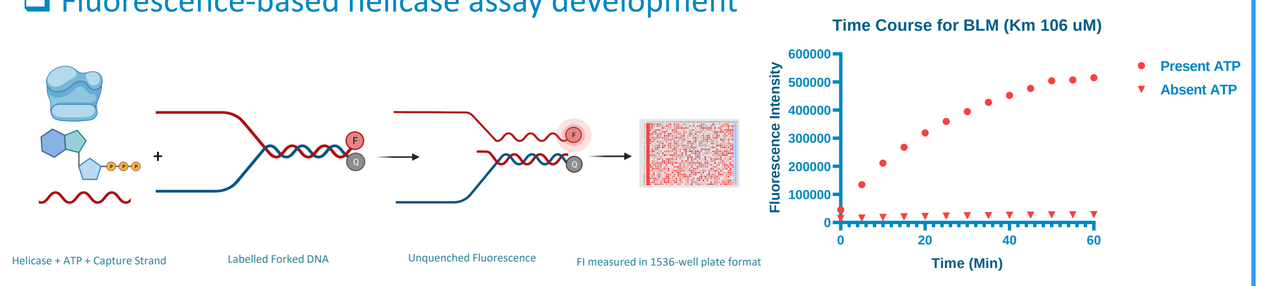


PDB ID: 4CGZ

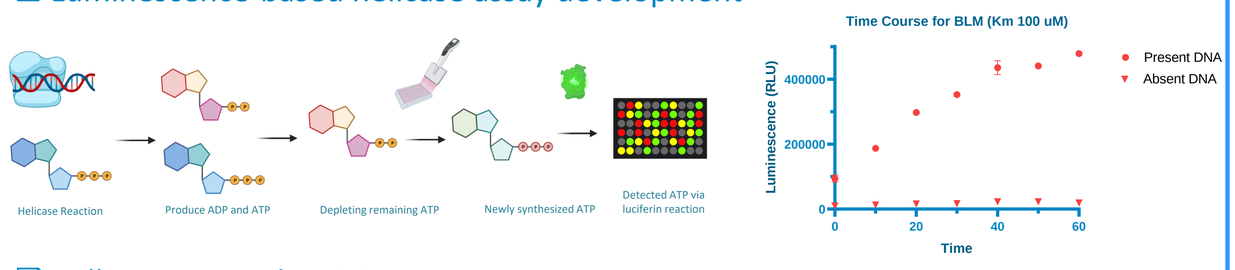
- 58 milligrams of interested protein obtained from 2-liter expression
- DNA and ATP dependent activity was observed using both Fluorescence and Luminescence helicase assay
- An inhibition of unwinding and turnover ATP activity was shown by some references inhibitors

Methods

❑ Fluorescence-based helicase assay development



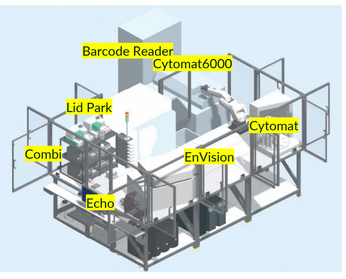
❑ Luminescence-based helicase assay development



❑ Fully Automated HTS Screening

Arm's F5 Robot

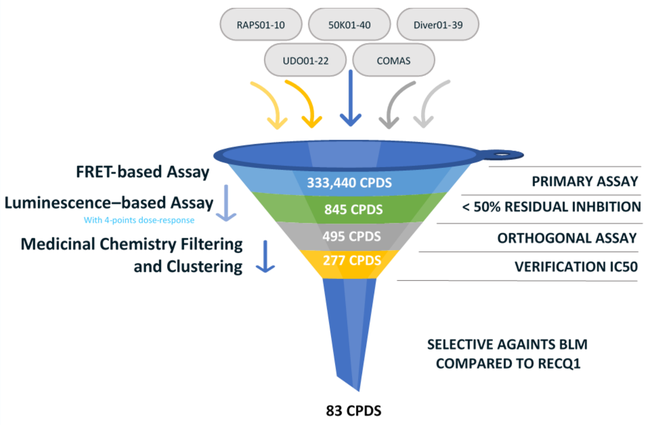
Thermo Scientific F5 Robot



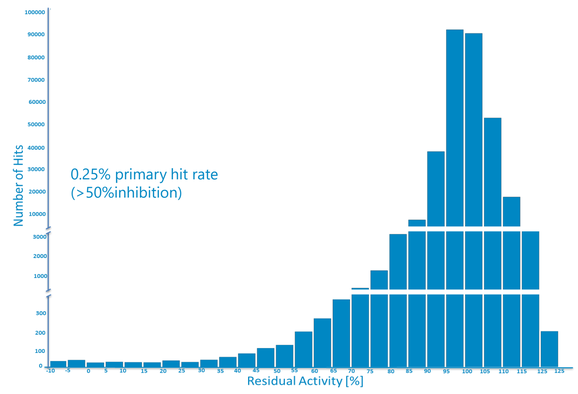
Room design

Results

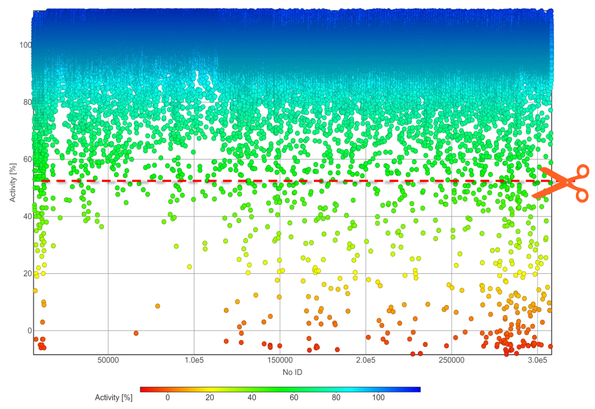
- Diagram for the identification of specific BLM inhibitors



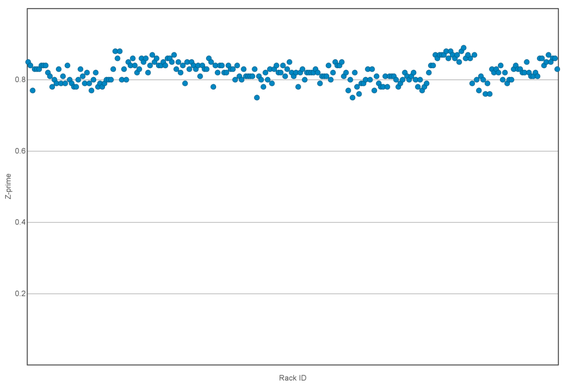
- Distribution of Primary Hits



- The scatter plot represents the percentage of activity of BLM for each compound



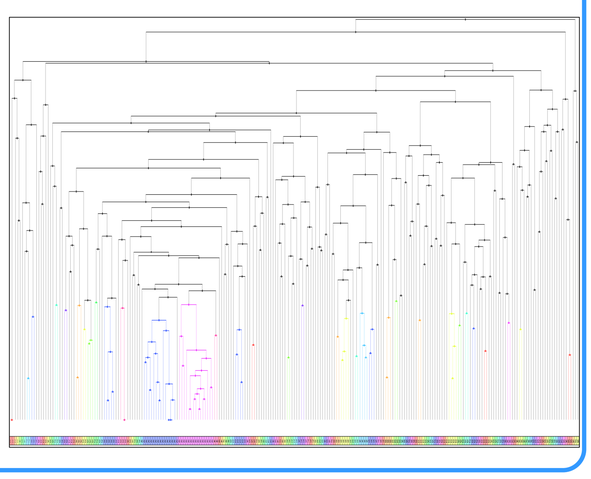
- The Z' Prime of intraplate controls



- Potency and Selectivity Profile

Compound ID	DNA Unwinding Assay: IC_{50} on BLM [µM]	DNA Unwinding Assay: IC_{50} on RECQ1 [µM]	ATP Turnover Assay: IC_{50} on BLM [µM]	ATP Turnover Assay: IC_{50} on RECQ1 [µM]	Solubility Rank: HEPES pH 7.4 (uM)	Cluster	MW	PSA	LE
LDC088619:02	0.3	>30	4.6	>50	204	123	415.3	91.8	0.45
UDO016926:02	0.7	16.3	3.7	13.4	228	24	431.5	94.1	0.41
LDC000488:01	2.1	>30	7.5	>30	457	102	395.4	211.7	0.44
LDC047852:01	4.1	>30	14.3	>30	490	155	218.2	69.9	0.69
UDO030968:02	24.8	>50	11.7	>30	527	143	345.2	92.3	0.44
Compound 29	>30	17.8	>30	40	305	35	462.6	47.9	0.29
Compound 1	37.2	>30	6.6	>30	31.7	127	387.4	138.7	0.400
ML219	37.2				1	75	383.3	108.0	0.385

- The hierarchical relationship of 243 compounds



Conclusion

- **We established easy, cheap, fast and robust assays to measure duplex DNA unwinding and ATPase activity in a high-throughput fashion.**
- **We discovered selective BLM inhibitors with good solubility and selective profile compared to reference inhibitors.**
- **We are co-crystalizing top inhibitors with BLM to establish a structure-activity relationship (SAR) with LDC's medicinal chemistry department.**