Selective Targeting of Cyclin E1 Using Molecular Glue Degraders in *CCNE1* Amplified Solid Malignancies

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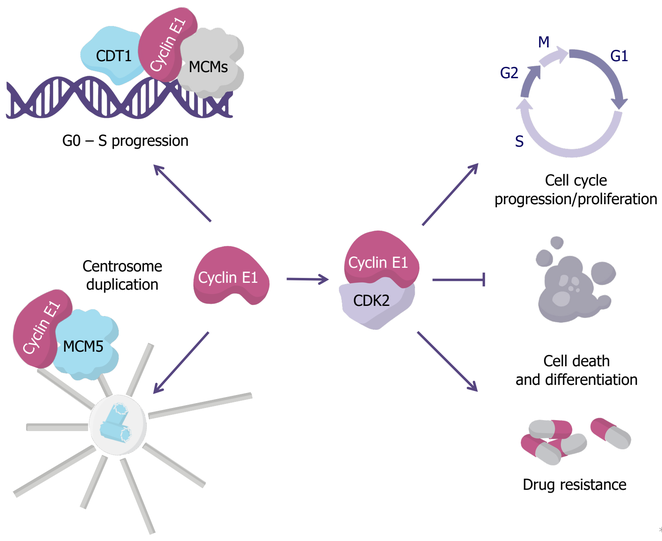
LBA 511

Cyclin E1 drives multiple hallmark cancer mechanisms



Therapeutic hypothesis:

Cyclin E1 (*CCNE1**) is a well-recognized human oncogene that drives multiple hallmarks of cancer, and has been considered undruggable

Selective degradation of cyclin E1 can target tumors with deregulated cyclin E1 (*CCNE1* amplification or overexpression)

Clinical opportunity:

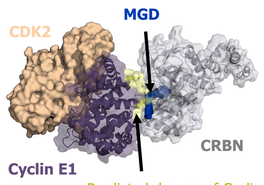
First-in-class cyclin E1 degrader for cyclin E1 amplified cancers

- Ovarian, endometrial, gastric, breast cancers

* On this poster, Cyclin E1 denotes the protein, *CCNE1* the gene or mRNA.

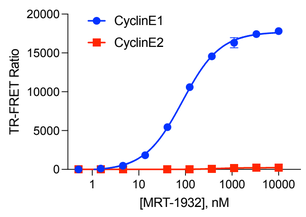
Cyclin E1 can be engaged by cereblon (CRBN)-based molecular glue degraders (MGD) through a cryptic pocket

Novel degron in CCNE1 predicted with fAIceit™ surface matching algorithm



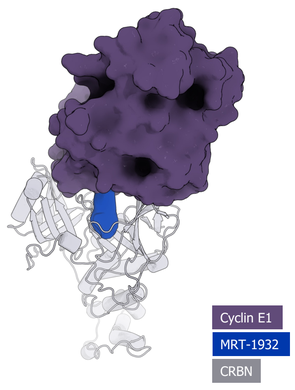
Predicted degron of Cyclin E1

Hit from library TR-FRET screen engages Cyclin E1 but not Cyclin E2



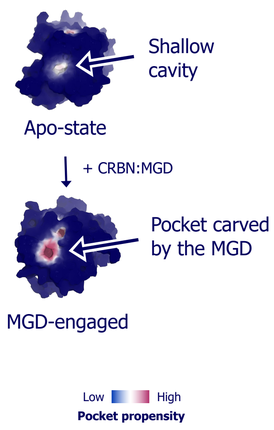
TR-FRET, time-resolved fluorescence resonance energy transfer

Cyclin E1 binds CRBN through a novel binding mode



Cryo-EM structure of the ternary complex comprising CRBN, Cyclin E1 and the MGD MRT-1932

CRBN: MGD induces a cryptic pocket on the Cyclin E1 surface



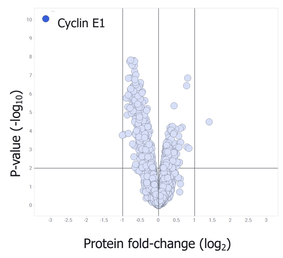
MRT-50969 is a potent and highly selective cyclin E1-directed MGD

MRT-50969 is a potent cyclin E1 MGD

In vitro data	
CRBN binding, IC_{50}	0.15 μM
Ternary complex, EC_{50}	3 nM
Degradation, DC_{50}/D_{max}	3 nM / 94 %

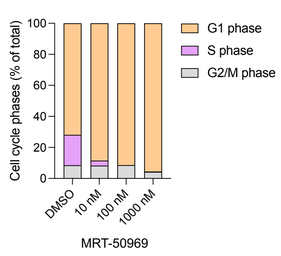
CRBN binding (thalidomide displacement) and ternary complex formation: TR-FRET assay; degradation: NanoBiT assay in CAL51 cells

MRT-50969 is highly selective for Cyclin E1



TMT Proteomics, MDA-MB-157 RB KO, 1μM MRT-50969, 24h

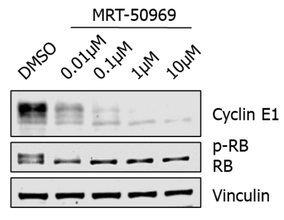
MRT-50969 induces robust G1/S cell cycle arrest



Kuramochi cells, FACS (EdU incorporation), 48h

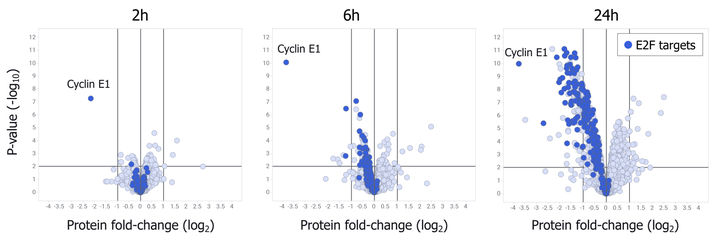
Cyclin E1 degradation inhibits cell cycle progression in an RB-dependent manner

MRT-50969-induced cyclin E1 degradation leads to loss of phospho-RB



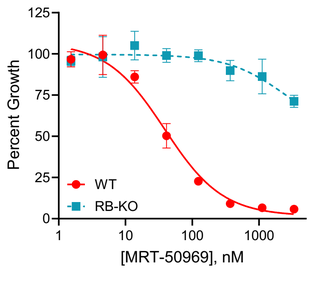
Western blot, OVISE (*CCNE1* amplified ovarian cancer cell line), 24h treatment with MGD

MRT-50969-induced Cyclin E1 degradation suppresses E2F targets

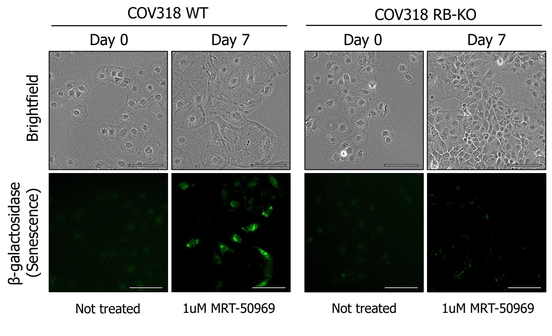


Tandem mass tag (TMT) proteomics, HCC1569 cells, 1μM MRT-50969, proteins encoded by E2F target genes in blue

Cyclin E1 degradation inhibits growth of, and induces senescence in, RB-proficient but not RB-deficient cells

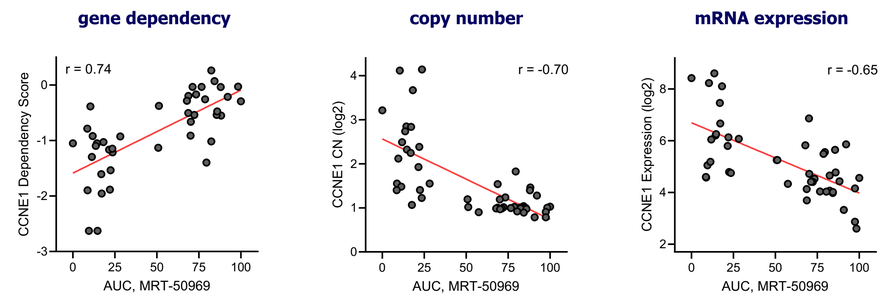


COV318 cells, CyQuant, 5 days



COV318 cells, CellEvent Senescence Green; images: 10X; scale bar: 300μm

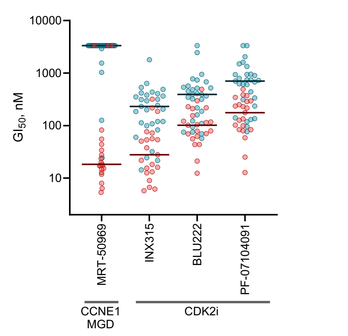
Cyclin E1 MGD sensitivity is highly correlated with *CCNE1* gene dependency, copy number, and expression



5 Day CyQuant assay, 50 cancer cell line panel;
Gene dependency and genomics data from DepMap/Broad Institute

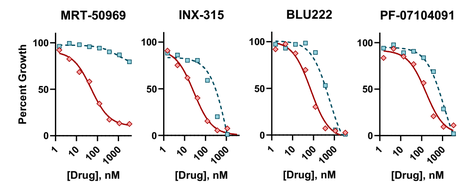
MRT-50969 shows superior differential activity in *CCNE1* dependent cell lines compared to clinical-stage CDK2 inhibitors

Panel of 50 ovarian, endometrial, and breast cancer cell lines

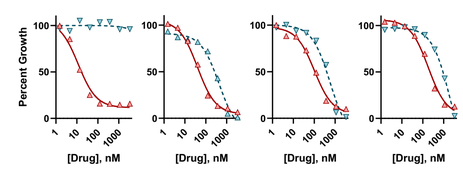


5 Day CyQuant Assay, black bars indicate median GI_{50} (growth inhibition 50%, the concentration of drug required to inhibit the growth of cancer cells in vitro by 50%)

Example data: ovarian cancer cell lines

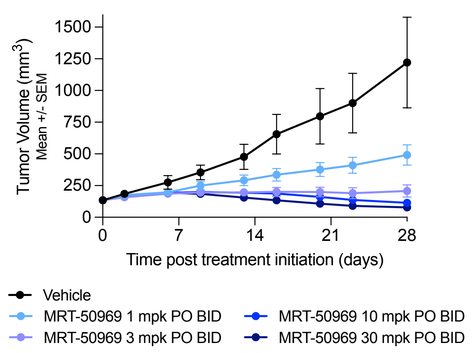


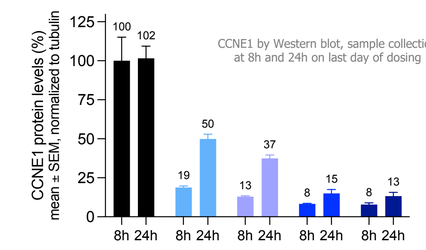
Example data: breast cancer cell lines



MRT-50969 inhibits growth of *CCNE1*-amplified cancer models *in vivo*

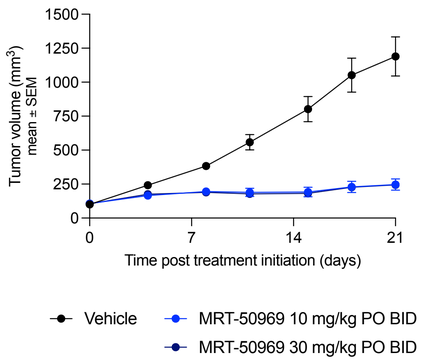
***CCNE1*-amplified breast cancer (HCC1569)**

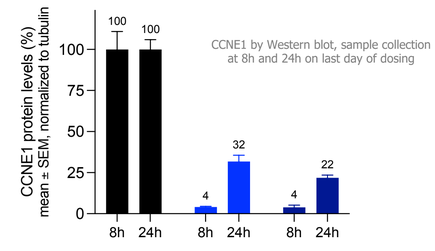




HCC1569 model: NOD/SCID mice; efficacy: n = 10 (vehicle) or n = 8 (all other groups); CCNE1 protein: n = 3 per time point; BID dosing, alternating 7h and 17h intervals

***CCNE1*-amplified gastric cancer (MKN1)**





HCC1569 model: BALB/c nude mice; efficacy: n = 7 (vehicle) or n = 8 (all other groups); CCNE1 protein: n = 4 per time point; BID dosing, alternating 7h and 17h intervals

Summary and discussion

- Using our MGD discovery engine QuEEN™ encompassing biochemical and cellular assays as well as *in silico* modelling, we identified and optimized MGDs that engage and selectively degrade cyclin E1.
- Cryo-EM structures of the ternary complex containing CRBN, cyclin E1 and a cyclin E1 MGD revealed a novel degron that consists in part of a cryptic pocket induced by the MGD.
- MRT-50969 is a cyclin E1-directed MGD that was optimized to induce highly selective and potent degradation.
- Cellular effects of cyclin E1 degradation in *CCNE1*-amplified cancer cell lines include suppression of RB phosphorylation and E2F targets, growth arrest associated with G1 cell cycle arrest and ablation of S phase, and induction of senescence.
- RB knockout prevents growth arrest and senescence, suggesting that the major cellular effect of cyclin E1 in *CCNE1*-amplified cell lines is mediating RB phosphorylation in complex with CDK2.
- When profiled across a larger panel of ovarian, endometrial and breast cancer cell lines, MRT-50969 activity recapitulates genetic *CCNE1* dependencies and is associated with *CCNE1* mRNA expression and copy number.
- Unlike MRT-50969, several tested clinical CDK2 or WEE1 inhibitors did not fully recapitulate genetic dependency, potentially indicating off-target activity.
- MRT-50969 is orally bioavailable and shows anti-tumor activity in a dose dependent manner in *CCNE1*-amplified cell line-derived xenograft models.
- Cyclin E1 MGDs represent a paradigm shift due to their potential to directly target a frequently amplified non-enzymatic driver oncogene with unprecedented selectivity in a population of cancer patients with high unmet medical need.

All authors are employees of Monte Rosa Therapeutics.