Characterization of *CCNE1* amplifications and associated genomic features in ovarian and uterine cancers

5469

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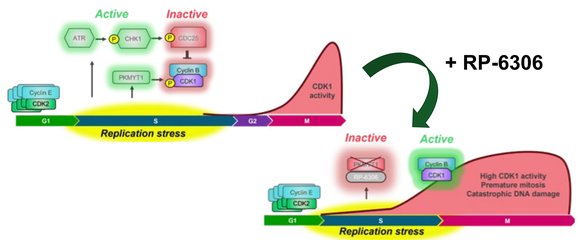
Clinical relevance of *CCNE1*

Cyclin E1 protein encoded by the *CCNE1* gene is a core component of cell cycle in normal cells. In conjunction with Cyclin Dependent Kinase 2 (CDK2), it promotes G1/S transition through phosphorylation of specific substrates. Cyclin E plays an important role in cell cycle progression and DNA replication. Oncogenic activation of the Cyclin E/CDK2 complex mediated by *CCNE1* amplification causes replication stress and DNA damage leading to genomic instability and contributing to carcinogenesis[1]. Cyclin E is not a canonically druggable target, therefore therapeutic approaches to selectively target *CCNE1*-amplified tumors are being pursued by inhibiting its close interaction partners such as CDK2, WEE1, and PKMYT1[2]. Clinical success of these therapeutic strategies would benefit from a thorough understanding of how DNA damage and replication stress caused by CCNE1 amplification alters the genomic landscape of tumors.

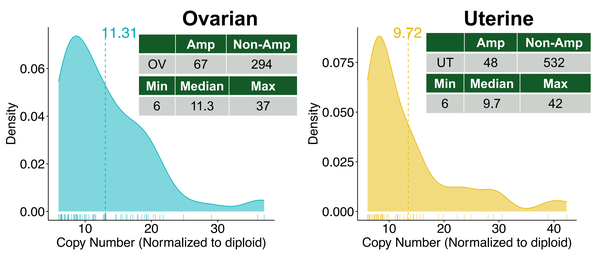
RP-6306 is a first-in-class, highly potent and selective PKMYT1 inhibitor currently being investigated in clinical trials as a single agent (NCT04855656) and in combination with other agents including; gemcitabine (NCT05147272), irinotecan (NCT05147350), or camonsertib (NCT04855656), in patients with solid tumors harboring *CCNE1* amplification[2]. In pre-clinical models, correlation between *CCNE1* copy number, cyclin E1 expression and sensitivity to PKMYT1 inhibition is imperfect. Thus, a deep understanding of genomic changes associated with *CCNE1* amplification is critical for strengthening our understanding of the mechanism of action. These learnings will be applied to clinical trials evaluating RP-6306.



Methods

In this study, we used whole exome sequencing (WES) data from TCGA and whole genome sequencing (WGS) data from the PCAWG consortium to describe the genomic landscape of *CCNE1*-amplified ovarian and uterine tumors (endometrial carcinoma and uterine carcinosarcoma). Our analysis included 361 ovarian and 580 uterine cancer exomes, and 113 ovarian and 51 uterine cancer genomes. Copy Number (CN) calls were made by ASCAT for WES (TCGA), and the Hartwig Medical Foundation pipeline for WGS (PCAWG)[3]. Tumors were considered as having a *CCNE1* amplification if (CN – Tumor Ploidy) >= 4. Structural variant (SV) signature enrichment values were used as reported in the publication by Degasperi *et al*[4]. Fold back inversion calls were made by the Hartwig pipeline, and all chromosome level plots were reviewed manually for evidence of breakage fusion bridge.

CCNE1 amplification in ovarian and uterine tumors



	Amp	Non-Amp
OV	67	294

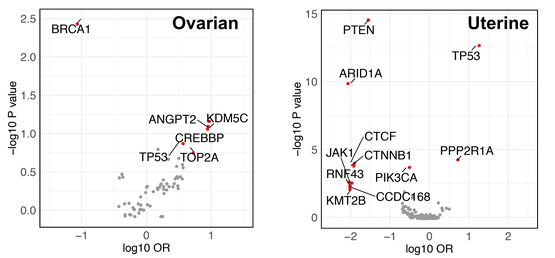
Min	Median	Max
6	11.3	37

	Amp	Non-Amp
UT	48	532

Min	Median	Max
6	9.7	42

CCNE1 amplification is a recurrent genomic alteration in ovarian and uterine tumors. *CCNE1* is amplified in about 18-20% of ovarian tumors and 8-10% of uterine tumors. In *CCNE1*-amplified tumors from TCGA, CN values ranged from 6 to 37 copies in ovarian tumors (median = 11.3), and 6 to 42 in uterine tumors (median = 9.7).

Co-mutation landscape of *CCNE1* amplifications



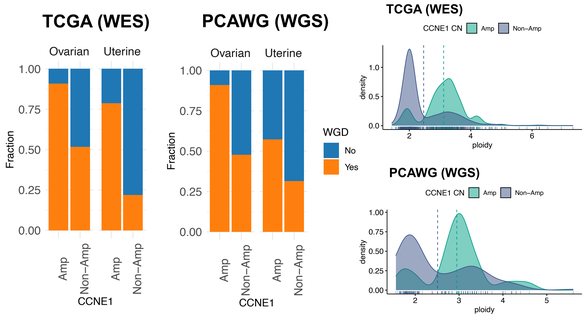
Red dots signify statistical significance by Fisher's exact test. All significant hits for ovarian cancer are shown below (left). Most prevalent significant hits (>= 90 patients) is shown below for uterine cancer (right).

Gene	CCNE1 only	Gene only	Both	Neither
TP53	**2**	**191**	**46**	**27**
TOP2A	46	2	2	216
KDM5C	46	1	2	217
CREBBP	46	1	2	217
BRCA1	**47**	**39**	**1**	**179**
ANGPT2	46	1	2	217

Gene	CCNE1 only	Gene only	Both	Neither
ARID1A	**48**	**208**	**0**	**306**
PIK3CA	40	223	8	291
PTEN	**46**	**309**	**2**	**205**
CTCF	48	98	0	416
CTNNB1	48	96	0	418
TP53	**5**	**185**	**43**	**329**

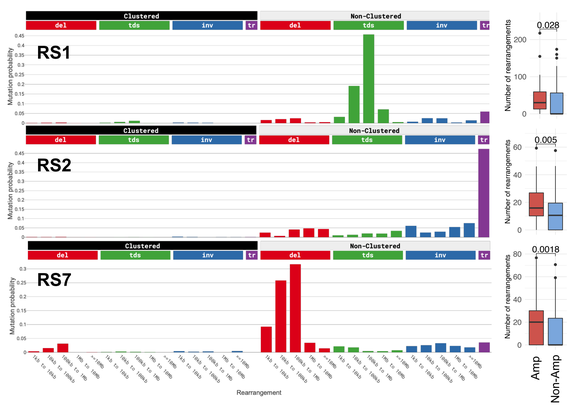
A review of the co-mutation landscape using WES revealed that *TP53* mutations frequently co-occurred with *CCNE1* amplifications in both ovarian and uterine tumors (ovarian: 95.8% vs 87.6%, OR = 3.24, p=0.1; uterine: 89.6% vs 36.0%, OR = 15.2, p =2.14e-13) and *BRCA1* mutations were mutually exclusive with *CCNE1* amplifications in ovarian tumors (2.1% vs 17.9%, OR=0.2, p = 3.1e-3). In addition, mutations in *ARID1A* (0% vs 40.5%, OR = 0, p = 1.3e-10) and *PTEN* (4.2% vs 60.1%, OR = 0.03, p =2.7e-15) were mutually exclusive with *CCNE1* amplifications in uterine tumors.

WGD in tumors with *CCNE1* amplification



Whole genome duplication (WGD) is a common genetic abnormality that occurs in about 30% of cancers and is associated with genome instability. WGD was detected significantly more frequently in ovarian and uterine tumors with *CCNE1* amplification as compared to non-amplified tumors (ovarian: 90.9% vs 48.3%, p = 6.4e-10; uterine: 78.7% vs 21.9%, p = 7.1e-15).

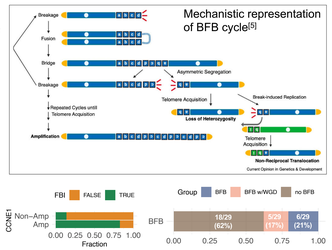
Distinct SV signatures in *CCNE1*-amplified tumors



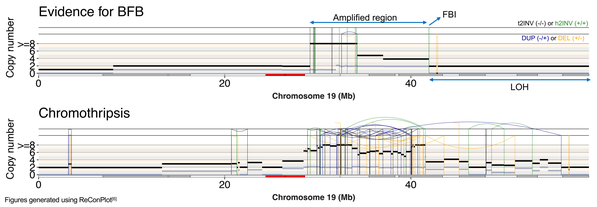
Widespread chromosomal genomic rearrangement is a hallmark of many cancers, especially cancers with high DNA replication stress and genome instability. *CCNE1*-amplified ovarian and uterine cancers were found to display an enrichment for certain SV signatures: RS1, dominated by non-clustered 100Kb-1Mb duplications (median = 30, p=0.03); RS2, dominated by non-clustered translocations (median = 16, p=0.005); and RS7, dominated by non-clustered 100Kb-1Mb deletions (median=20, p=0.002).

Evidence for BFB cycle in *CCNE1* amplification

Breakage fusion bridge (BFB) cycle is a mechanism of chromosomal instability that leads to progressive tandem duplication of chromosomal segments and eventual amplification of genes in those segments[5]. Certain genomic characteristics such as fold-back inversions (FBI) and segment copy number patterns suggest the occurrence of BFB.



FBIs were enriched in the *CCNE1* locus in patients with *CCNE1* amplification. We classified *CCNE1* amplifications as having evidence for BFB based on the presence of FBI and LOH of either chromosome arm beyond the amplification segment. Approximately 21% of cases had a strong evidence for BFB. Another 17% of patients showed signs of BFB after WGD. In many cases, the locus had more complex rearrangement architecture such as chromothripsis.



Conclusions

- *CCNE1* amplification leads to genome instability in ovarian and uterine cancer patients
- *TP53* loss frequently co-occurs with *CCNE1* amplification in ovarian and uterine cancers. *BRCA1* alterations are mutually exclusive with *CCNE1* amplifications in ovarian cancer. *PTEN* and *ARID1A* mutations are mutually exclusive with *CCNE1* amplifications in uterine cancer.
- WGD, FBIs, and specific SV signatures such as large tandem duplications, deletions and translocations are enriched in the tumors with *CCNE1* amplification.
- There is evidence for breakage fusion bridge being the mechanism of *CCNE1* amplification in about 38% of patients.

References

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