Tumor heterogeneity of CCNE1 copy number assessed by fluorescence in situ hybridization (FISH) in ovarian and uterine cancers

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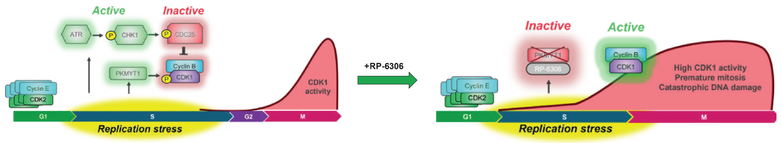
Introduction

CCNE1 is a gene that encodes cyclin E1 and is responsible for promoting entry and progression into S phase in tandem with CDK2[1]. *CCNE1* amplification is a recurrent genetic alteration that has been associated with chemoresistance and poor prognosis in gynecological malignancies. Specifically, in ovarian and endometrial cancers, *CCNE1* amplification is linked to platinum resistance and poor outcomes, representing a critical area of unmet clinical need[2,3,4]. Recent advances in the field of synthetic lethality have identified Protein Kinase Membrane-associated Tyrosine/-Threonine 1 (PKMYT1) as synthetically lethal in tumors harboring *CCNE1* amplifications. Increased cyclin E1 pushes tumor cells into S phase which drives replication stress, requiring PKMYT1 to postpone entry into mitosis. Inhibition of PKMYT1 in *CCNE1* amplified cells causes premature mitotic entry, mitotic catastrophe and, in turn, cell death[5].

RP-6306 is a first-in-class, highly potent and selective PKMYT1 inhibitor currently being tested in clinical trials as a single agent (NCT04855656) and in combination with gemcitabine, irinotecan, or the ATR inhibitor camonsertib (RP-3500) (NCT05147272, NCT05147350 and NCT04855656 respectively) in solid tumors harboring *CCNE1* amplification. In pre-clinical models, correlation between *CCNE1* copy number, cyclin E1 expression and sensitivity to PKMYT1 inhibition is imperfect. Thus, a deep understanding of *CCNE1* amplification as a predictive biomarker for PKMYT1 inhibition is critical for refining patient selection strategies and strengthening our understanding of the mechanism of action. These learnings will be applied to correlative biomarker analyses in clinical trials evaluating RP-6306.



Methods

Tumor Survey Cohort (n = 450)
Ovarian (n = 100), TN Breast (n = 100), Gastric (n = 100), CRC (n = 100), Uterine (n = 50)

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FISH Validation Cohort (n = 51)
Ovarian (n = 3), TN Breast (n = 1), Gastric (n = 2), CRC (n = 5), Uterine (n = 31), Normal Ovary (n = 5), Normal Colon (n = 5)

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Gynecological Exploratory Cohort (n = 104)
Ovarian Tumors (n = 54), Normal Ovarian Tissue (n = 5), Uterine Tumors (n = 45)

Cyclin E1 levels were evaluated across tumor tissue microarrays (TMA) spanning five tumor indications: colorectal (n=100), gastric (n=100), uterine (n=50), ovarian (n=100) and triple negative breast (n = 100) cancers. To explore *CCNE1* amplification on a single cell level, we established a novel FISH assay using a subtelomeric q-arm control probe. Human cancer FFPE samples (n = 41) consisting of ovarian (n = 3), uterine (n = 30), colon (n = 5), gastric (n = 2), and triple-negative breast (n = 1) tumors in addition to normal ovarian (n = 5) and colon (n = 5) tissue were used as a validation cohort to establish the assay. Comparison of FISH results to NGS (SNIPDx)[6] was used to determine assay sensitivity and specificity. Additional human tumor FFPE samples (n = 99) and normal human tissue (n = 5) were stained using a validated *CCNE1* FISH assay to explore amplification and heterogeneity.

q-arm subtelomeric region is more frequently diploid than other Chr19 segments in gynecological cancers

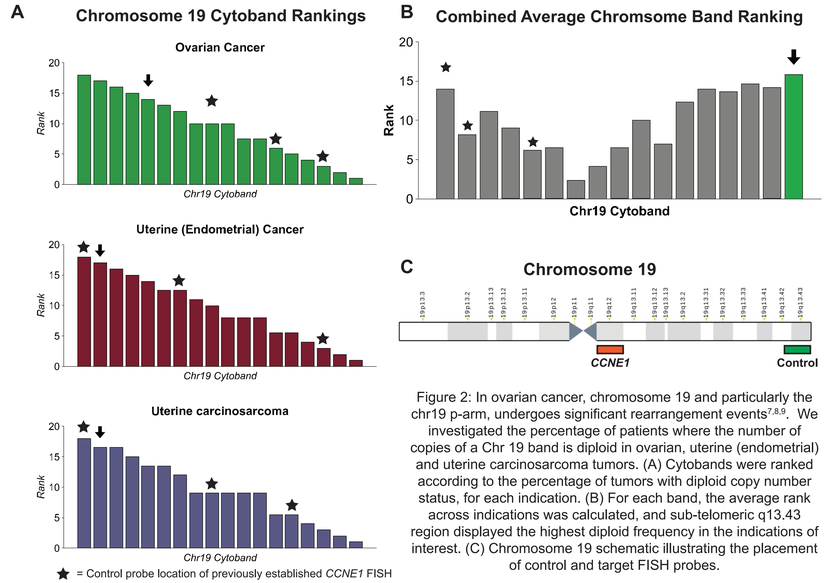

Figure 2: In ovarian cancer, chromosome 19 and particularly the chr19 p-arm, undergoes significant rearrangement events[7,8,9]. We investigated the percentage of patients where the number of copies of a Chr 19 band is diploid in ovarian, uterine (endometrial) and uterine carcinosarcoma tumors. (A) Cytobands were ranked according to the percentage of tumors with diploid copy number status, for each indication. (B) For each band, the average rank across indications was calculated, and sub-telomeric q13.43 region displayed the highest diploid frequency in the indications of interest. (C) Chromosome 19 schematic illustrating the placement of control and target FISH probes.

Intratumoral heterogeneity of *CCNE1* copy number in gynecological cancers

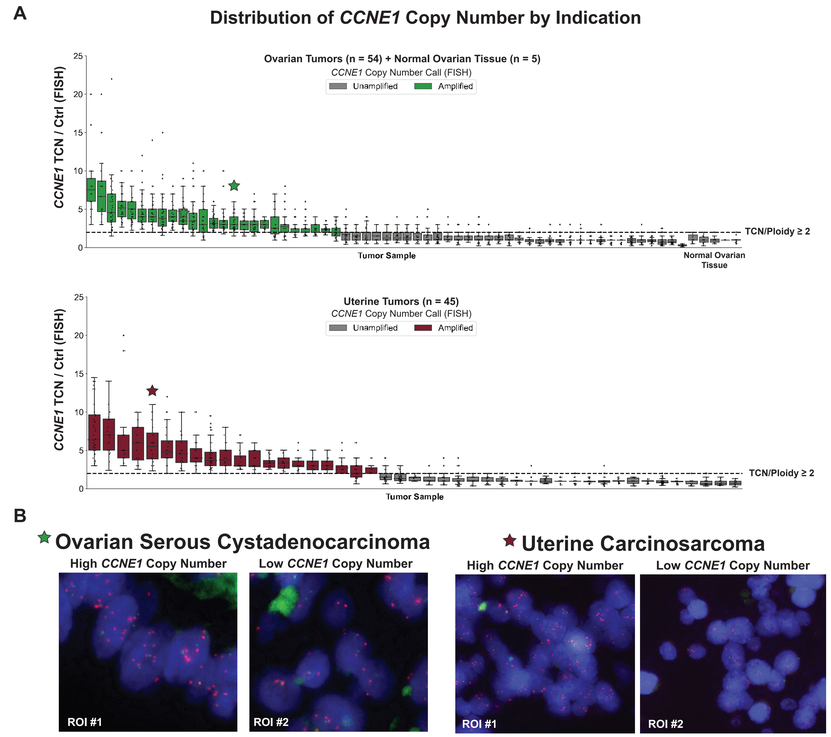

Figure 4: (A) Distribution of *CCNE1* copy number was evaluated by FISH across several tumor regions within the exploratory cohort. Distribution of *CCNE1*/ploidy ratio per cell in ovarian cancer, normal ovarian tissue (upper panel) and uterine tumors (lower panel) are displayed. Each box represents a different tumor sample. The gray dashed line denotes the *CCNE1* amplification cutoff, with amplified samples colored in green or red respectively and unamplified samples in gray. (B) Representative images of different tumor regions illustrating intra-tumoral heterogeneity in select ovarian and uterine tumor samples.

CCNE1 amplifications are enriched in gynecological and gastrointestinal cancers

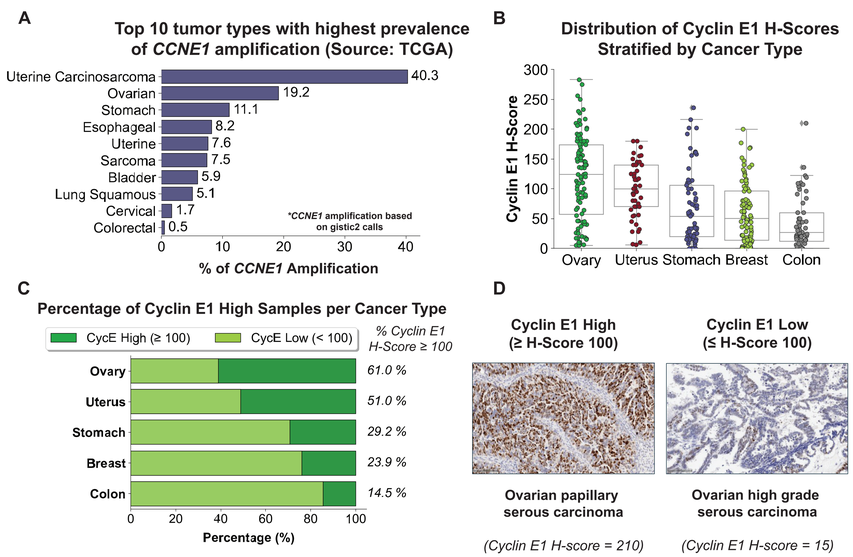

Figure 1: (A) Prevalence of *CCNE1* amplification (TCGA database) reveals enrichment of this genomic alteration in gynecological and gastrointestinal cancers. (B) Cyclin E1 levels were evaluated by IHC in tumor tissue arrays spanning 5 tumor indications: triple-negative breast (n=100), colorectal (n=100), gastric (n=100), uterine (n=49) and ovarian (n=100) cancers. The highest cyclin E1 protein levels were found in ovarian and uterine malignancies, consistent with indications with high prevalence of *CCNE1* amplification (median H-score ovarian = 125; uterine = 100; gastric = 45; TNBC = 48 and CRC = 24). (C) Frequency of tumors with high cyclin E1 protein levels, defined as H-score ≥ 100, across indications. Ovarian and uterine malignancies display the highest proportion of tumors with high cyclin E1 (61% and 51% respectively) (D) Representative IHC images (magnification 20x) of ovarian tumors with high and low cyclin E1 protein levels.

CCNE1 FISH assay demonstrates strong concordance with NGS

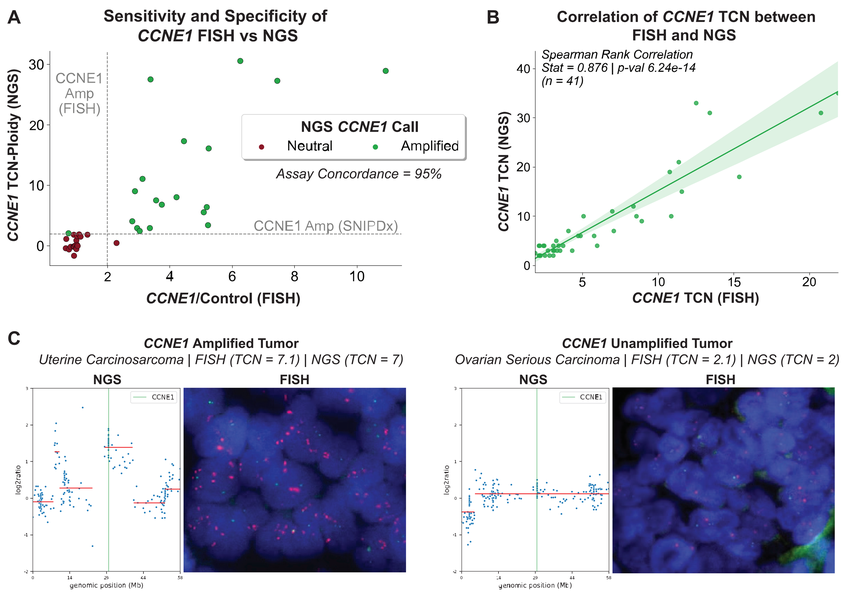

Figure 3: *CCNE1* amplification status was determined by targeted NGS (SNIPDx panel) and FISH in a cohort of uterine and ovarian tumors (n=41). A threshold of [*CCNE1* total copy number (TCN)-ploidy] ≥ 2 was used as amplification cutoff for NGS (SNIPDx), and a ratio of *CCNE1*/Control ≥ 2 was utilized for FISH. (A) *CCNE1* amplification calls were concordant between both assays in 95% (39/41) samples, with 95% sensitivity (19/20) and 95.2% specificity (20/21) (B) *CCNE1* total copy number values estimated by FISH are highly correlated with NGS-based copy number calculations. (C) Representative FISH images and FACETS plots from a *CCNE1* amplified ovarian tumor (left panel) and a unamplified uterine tumor (right panel).

Conclusions

- In this study, multiple features of *CCNE1* were examined using a combination of targeted NGS (SNIPDx panel), FISH, and IHC.
- SNIPDx targeted NGS panel provides accurate *CCNE1* copy number estimation with correction for tumor purity and ploidy.
- A novel FISH assay was developed to interrogate the copy number state of *CCNE1* at a single cell resolution. The results from validation experiments indicate robust assay performance and confirm that the selected amplification cutoffs lead to consistent results across all methodologies.
- Intra-tumoral heterogeneity in *CCNE1* copy number was observed in ovarian and uterine cancers.

References

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