Poster number: B169

Retrospective baseline biomarker analyses in a first-in-human Phase 1 trial of the PKMYT1 inhibitor lunresertib (RP-6306) in patients with advanced solid tumors harboring *CCNE1* amplification and/or deleterious alterations in *FBXW7* or *PPP2R1A*

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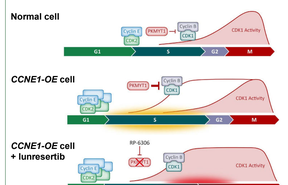
Introduction

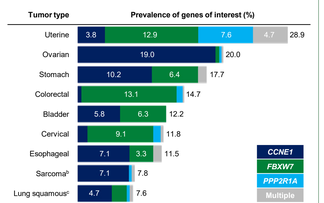
- Lunresertib (RP-6306) is a first-in-class PKMYT1 inhibitor that disrupts the G2/M checkpoint leading to premature mitosis and catastrophic DNA damage in cells harboring synthetic lethal genomic alterations
- The safety and tolerability of lunresertib alone and in combination with camonsertib (RP-3500) is being investigated in the Phase 1 MYTHIC study in patients with advanced solid tumors with either *CCNE1* amplifications or deleterious alterations in *FBXW7* or *PPP2R1A* (NCT04855656)
- Preliminary results from the MYTHIC study show that lunresertib is safe and well-tolerated as a monotherapy or in combination with camonsertib
- Robust PK/PD proof-of-mechanism and antitumor responses or durable clinical benefit were observed at biologically active doses of lunresertib + camonsertib, providing the first clinical proof-of-concept for synthetic lethal targeting of PKMYT1 in cancer medicine
- Here, we present a comprehensive retrospective biomarker analysis aimed at understanding concordance between local vs. central and tissue vs. plasma-based NGS results. The correlation between *CCNE1* copy number assessed by NGS and FISH and the relationship between *CCNE1* amp and cyclin E1 protein levels were investigated

Lunresertib mechanism of action and biomarker prevalence

Lunresertib disrupts the G2/M checkpoint leading to premature mitosis and catastrophic DNA damage



Top tumor types with highest prevalence of *CCNE1* amplification or inactivating mutations in *FBXW7*/*PPP2R1A*[a]



[a]Based on estimated lesion prevalence in The Cancer Genome Atlas (TCGA). [b]Soft-tissue sarcoma only. [c]Squamous subtype of non-small cell lung cancer only.

Methods

PKMYT1 inhibition for the treatment of cancers: MYTHIC

Inclusion criteria:

- Patients aged ≥ 12 y with solid tumors resistant or intolerant to standard therapy
- Measurable disease or high CA-125
- Local NGS report (tissue- or plasma)[a]
- Tumors with ***CCNE1*** amplification[b], deleterious ***FBXW7*** or ***PPP2R1A*** alterations
- ECOG PS of 0–2 (Module 1) or 0–1 (Module 2)
- Hgb ≥ 9 g/dL (Module 1) or ≥ 10 g/dL (Module 2)
- Platelets ≥ 100 K/uL
- ANC ≥ 1.5 K/uL

Module 1:
Single agent lunresertib
67 patients

Module 2:
Lunresertib with camonsertib
59 patients

Study is ongoing: **NCT04855656**

Primary endpoints:

- Safety and tolerability
- RP2D and schedule

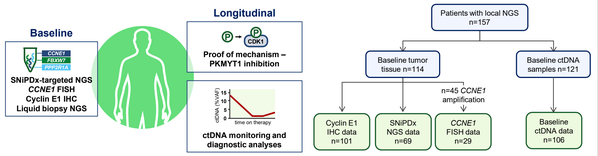
Other endpoints:

- PK
- PD in paired tumor biopsies
- Preliminary antitumor activity
- Kinetics of ctDNA

[a]NGS report centrally reviewed and annotated by the Precision Oncology Decision Support group at MD Anderson Cancer Center. [b]*CCNE1* amplification (CN ≥ 6).

Methods

MYTHIC study: Summary of sample collection and data availability

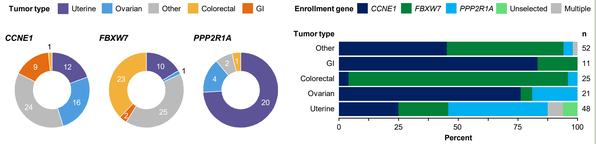


Baseline analyses – key translational questions:

- Are local NGS assays a reliable method to identify patients for RP-6306 Phase 1 studies?
- What is the most accurate method to detect *CCNE1* amplifications? – NGS vs. FISH
- Is liquid biopsy a sensitive method to identify patients with *CCNE1* amplifications and mutations in *FBXW7*/*PPP2R1A*?
- What is the relationship between *CCNE1* amplifications and cyclin E1 protein levels?

Results

Phase 1 population matches expected prevalence for each biomarker



Enrolled cancer types agree with expectation for each biomarker:

- Higher frequency of uterine and ovarian tumors for *CCNE1* and *PPP2R1A*
- Higher frequency of colorectal tumors for *FBXW7*

***TP53* co-mutation is frequent in all biomarker groups:**

- 77% (48/62) *CCNE1* patients, 57% (34/60) *FBXW7* patients, and 81% (22/27) *PPP2R1A* patients have *TP53* mutations

DDR pathway co-mutations were observed in four patients in the lunresertib + camonsertib cohort:

- One patient each (*BRCA1* rearrangement, *BRCA2* biallelic loss, *ATM* biallelic loss, and *ATM* monoallelic mutation)

Figure 1: (**A**) Distribution of indications observed in MYTHIC patients screened per each biomarker. "Other" includes cancers of brain, skin, sinus, lung, bladder, head and neck, bile duct, vulva, gallbladder, pancreas, eye, liver, cervix, kidney, anus, bone, and soft tissue. (**B**) Frequency of lunresertib biomarkers in top five most prevalent indications screened.

Pre-approved local NGS assays were a reliable patient identification method in the MYTHIC phase 1 study

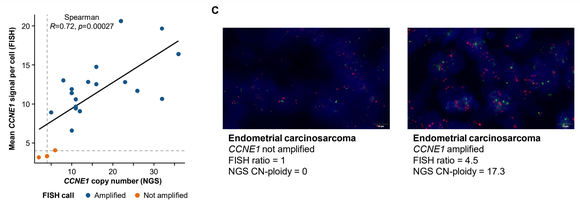
	Enrollment biomarker (detected by local NGS[a])			
Central NGS result	***CCNE1* amplification** (n=23)	***FBXW7* mutation** (n=27)	***PPP2R1A* mutation** (n=12)	**Total (n=62)**
Amplified[b] or mutated, n	19	25	12	**56 (90%)**
Not amplified or mutated, n	4[c]	2	0	**6 (10%)**

[a]Local NGS included 25 different tests, with the top five most common being FoundationOne CDx (Foundation Medicine, Cambridge, MA), MSK-IMPACT (Memorial Sloan Kettering, New York, NY), MI Profile (Caris Life Sciences, Irving, TX), Oncopanel (Brigham and Women's Hospital, Boston, MA) and Solid Tumor Genomic Assay 2018 (MD Anderson Cancer Center). [b]For central NGS, amplification was defined as CN ≥ ploidy + 4 and gain was defined as CN ≥ ploidy + 2. [c]Includes 2 gains.

- Retrospective central analysis by SNiPDx confirms 56/62 (90%) of enrollment alterations

Results

CCNE1 amplification calls are highly concordant between FISH and tissue NGS



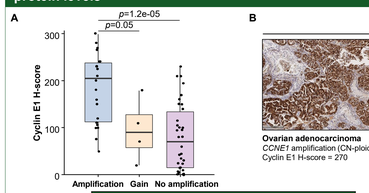
B

	FISH *CCNE1* CN call	
Central NGS *CCNE1* CN call	**Amplified[a] (n=18)**	**Not amplified (n=3)**
Amplified or gain[b] (n=19)	18	1
Not amplified (n=2)	0	2

[a]For FISH, amplification was defined as *CCNE1*/control ratio ≥ 2. [b]For central NGS, amplification was defined as CN ≥ ploidy + 4 and gain was defined as CN ≥ ploidy + 2.

Figure 2: (**A**) Correlation between mean *CCNE1* signal observed by FISH and *CCNE1* copy number values from tissue NGS. Spearman's rank-order correlation coefficient and two-sided *p*-value are reported. (**B**) Concordance table demonstrates 95% overall percentage agreement on *CCNE1* amplification calls between FISH and NGS assays. (**C**) Representative images of *CCNE1* FISH in amplified and non-amplified ovarian tumors. Orange foci represent *CCNE1* target probe and chr19 subtelomeric control probe is represented in green.

Patient selection based on *CCNE1* amplification enriches for tumors with high cyclin E1 protein levels

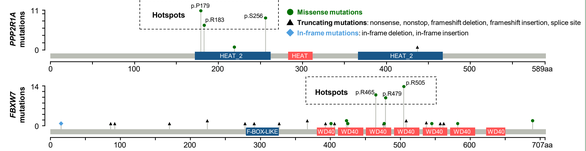


	***CCNE1* genomic status**		
	***CCNE1* amplification[a] (n=22)**	***CCNE1* gain (n=4)**	**No amplification (n=34)**
Median cyclin E1 H-score	205	90	70

[a]For central NGS, amplification was defined as CN ≥ ploidy + 4 and gain was defined as CN ≥ ploidy + 2.

Figure 3: (**A**) Cyclin E1 protein H-score distributions from IHC for *CCNE1*-amplified, gain, and nonamplified tumors, estimated by central NGS assay. Two-sided *p*-values were calculated using the Wilcoxon test. (**B**) Representative cyclin E1 IHC images in gynecological tumors with *CCNE1* amplification (magnification 10x).

Hotspot mutations are most prevalent in *FBXW7* and *PPP2R1A* patients screened for MYTHIC



B

Biomarker	Hotspot	Missense	Truncation
FBXW7	32[a] (50.8%)	10 (15.9%)	21 (33.3%)
PPP2R1A	27[b] (93.2%)	1 (3.4%)	1 (3.4%)

[a]*FBXW7* hotspots: R465, R479, R505. [b]*PPP2R1A* hotspots: P179, R183, S256.

Figure 4: (**A**) Lollipop representation of *PPP2R1A* (upper panel) and *FBXW7* mutations (lower panel) screened for MYTHIC. Hotspot mutations are highlighted. (**B**) Frequency of *FBXW7* and *PPP2R1A* mutations grouped by alteration type.

Results

Liquid biopsy NGS tests confirm majority of *FBXW7*/*PPP2R1A* mutations, but demonstrate high false negative rate for *CCNE1* amplifications

A

	Enrollment biomarker (detected by local NGS)			
Liquid biopsy NGS test	***CCNE1* amplification** (n=46)	***FBXW7* mutation** (n=40)	***PPP2R1A* mutation** (n=10)	***FBXW7*/ *PPP2R1A* mutation** (n=3)
Confirmed, n	21	32	8	2
Not confirmed, n	25	8[a]	2	1[b]

[a]Three patients did not have variants detected in plasma. [b]Patient did not have any variants detected in plasma.

B

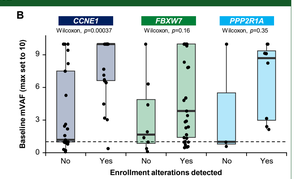


Figure 5: (**A**) Plasma ctDNA NGS confirms ~80% of *FBXW7* and *PPP2R1A* mutations and ~46% of *CCNE1* amplifications. (**B**) Detection of *CCNE1* amplifications by liquid biopsy NGS is dependent on mean variant allele frequency of variants detected at baseline. Baseline plasma ctDNA samples were analyzed using tempus xF (Module 1) and xF+ (Module 2) NGS panels. Baseline mVAF distribution comparison between cases where the enrollment alteration was detected vs. not detected by ctDNA assay for each lunresertib biomarker. mVAF was set to 10% for samples with mVAF > 10%. Two-sided *p*-values were calculated using the Wilcoxon test. The dotted line represents the 1% threshold used to filter out samples below the limit of quantification.

Detection of *FBXW7* and *PPP2R1A* mutations in plasma ctDNA is not confounded by CHIP

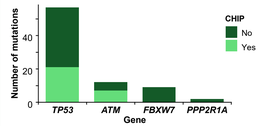


Figure 6: *FBXW7* and *PPP2R1A* mutations detected in plasma ctDNA were not found in PBMCs, confirming tumor tissue origin. PBMCs derived from the same blood samples used for ctDNA analysis were analyzed by SNiPDx. Counts of tumor- and CHIP-derived mutations observed in MYTHIC baseline ctDNA for common CHIP genes such as *TP53* and *ATM*, and lunresertib biomarkers *FBXW7* and *PPP2R1A*.

Conclusions

- Pre-approved local NGS tests were a reliable method to identify biomarker-defined patients for MYTHIC Phase 1 trial
- FISH and NGS are both appropriate methods to evaluate *CCNE1* copy number. Cyclin E1 protein overexpression is strongly enriched in *CCNE1* amplified tumors
- Plasma ctDNA detected 80% of *FBXW7* and *PPP2R1A* enrollment alterations and is not confounded by CHIP
- *CCNE1* amplification had a high false-negative detection rate in ctDNA; therefore, tissue testing is preferrable
- This retrospective analysis of lunresertib baseline biomarkers (*CCNE1*, *FBXW7*, and *PPP2R1A*) provides an understanding and framework for interpretation of clinical data from MYTHIC and informs future patient selection strategies

Acknowledgments and Disclosures

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Abbreviations

ANC, absolute neutrophil count; CA-125, cancer antigen-125; CDK, cyclin-dependent kinase; CHIP, clonal hematopoiesis of indeterminate potential; chr, chromosome; CN, copy number; ctDNA, circulating tumor DNA; DDR, DNA damage response; ECOG PS, Eastern Cooperative Oncology Group performance status; FISH, fluorescence *in situ* hybridization; GI, gastrointestinal; Hgb, hemoglobin; IHC, immunohistochemistry; MYTHIC, PKMYT1 inHIbition for the treatment of Cancers; mVAF, mean variant allele frequency; NGS, next-generation sequencing; OE, overexpression; PBMC, peripheral blood mononuclear cells; PD, pharmacodynamics; PK, pharmacokinetics; PKMYT1, membrane-associated tyrosine- and threonine-specific Cdc2-inhibitory kinase; PSA, prostate-specific antigen; RP2D, recommended phase 2 dose; SNiPDx, SyNthetic lethal Interactions for Precision Diagnostics; y, years.