#4409
PB #3

Prospective identification of RB pathway alterations predict response to SY-1365, a selective CDK7 inhibitor, in a panel of high-grade ovarian cancer patient-derived xenograft models

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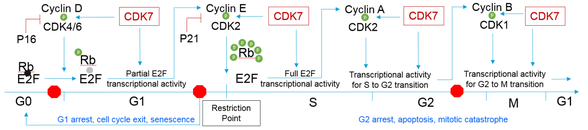
Background

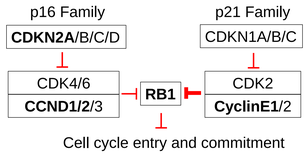
- CDK7, a key regulator of transcription and cell cycle progression (Figure 1), has been implicated in the pathogenesis of high-grade ovarian cancer (HGOC)
- CDK7 regulates transcriptional initiation and elongation through phosphorylation of the CTD domain of RNA polymerase II (RNAPOL2) and phosphorylation of the transcriptional kinase CDK9 (Glover-Cutter et al., Mol Cell Biol 2009; Larochelle et al., Nat Struct Mol Biol 2012)
- CDK7 regulates cell cycle progression through T-loop phosphorylation of the cell cycle kinases (CDK-1, -2, -4, -6), which is required for interaction with and activation by their respective cyclins (Schachter & Fisher, Cell Cycle 2013)
- SY-1365, a potent and selective inhibitor of CDK7, has been shown to induce tumor growth inhibition (TGI), including complete regressions, in HGOC PDX models (AACR Annual Meeting, 2018); SY-1365 responses were associated with oncogenic alterations in the core RB pathway (Figure 2), the master regulator of cell cycle entry and commitment
- The aims of this study were to prospectively test whether RB pathway alterations, as defined by The Cancer Genome Atlas Research Network (TCGA, Nature 2011; Table 1), predict response to SY-1365 in an independent set of HGOC PDX models, and to identify potential biomarker strategies for patient selection in SY-1365 trials

Figure 1: CDK7 regulates multiple processes critical for orderly entry into and transition through the cell cycle. CDK7 activates cell cycle CDKs by promoting cyclin binding. CDK7 regulates transcription through RNAPOL2 & CDK9 phosphorylation. Cellular responses to cell cycle disruption before or after the restriction point are shown in blue text. Rb inhibition of E2F: Black dot, full inhibition; grey dot, partial inhibition



Methods

Figure 2: Core RB Pathway



Bolded genes: Recurrently altered in HGSOC (Table 1)

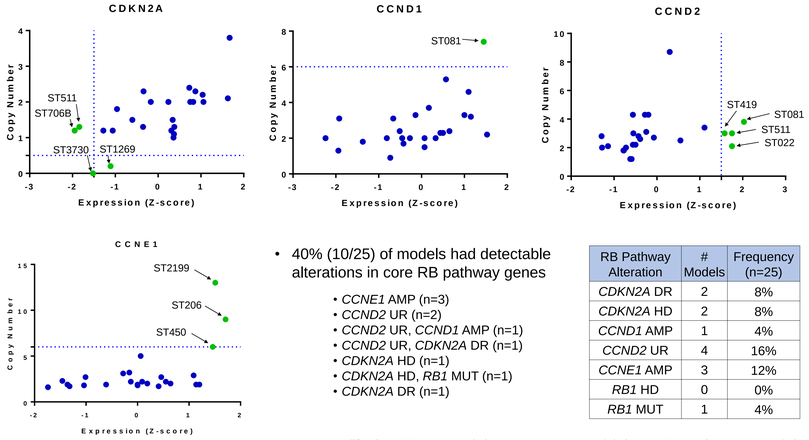
Table 1: Core RB pathway genes recurrently altered in 67% of HGSOC (TCGA, Nature 2011)

Gene	Protein	Type of alteration	Alteration Frequency
CDKN2A	P16	DR	30%
		D	2%
CCND1	Cyclin D1	AMP	4%
CCND2	Cyclin D2	UR	15%
CCNE1	Cyclin E1	AMP	20%
RB1	Rb	D	8%
		MUT	2%

AMP: Amplification; DR: Downregulation; D: Deletion; MUT: Mutation UR: Upregulation

- **Molecular analysis of PDX tumors:** DNA and RNA were extracted from formalin-fixed tumor tissue. Gene mutation and copy number (CN) were evaluated using the OncoCODE410 panel and NextCODE analysis pipeline (Wuxi). Genes were considered amplified or homozygously deleted if CN was ≥ 6 or ≤ 0.5, respectively. RNA expression was evaluated using the PanCancer panel (Nanostring). Genes were considered up- or down-regulated if expression was in the upper or lower 97th percentiles (Z-score ± 1.5) across all PDX models, respectively.
- **SY-1365 in vivo response studies:** Each of 25 independent HGOC PDX models (serous n=23, clear cell n=1, carcinosarcoma n=1) were randomized to 2 treatment groups: 1) SY-1365, 30-40 mg/kg twice weekly, i.v., 2) vehicle (Veh), twice weekly, i.v., with an average starting tumor volume (TV) of ~200mm^3 in each group (n=3 per group). Percent tumor growth inhibition (%TGI) was calculated at end of treatment (EOT; the last day both groups were evaluable) by comparing tumor growth between SY-1365 and vehicle groups as follows:1- [(Mean TV SY-1365 @ EOT – Mean TV SY-1365 @ Day 0)/(Mean TV Veh @ EOT – Mean TV Veh @ Day 0)]. Models with %TGI >50% were considered responders to SY-1365 treatment. Analysis of TGI as a function of administered dose demonstrated consistency of results across the dosing range.

Molecular Evaluation of Core RB Pathway Alterations in HGOC PDX models

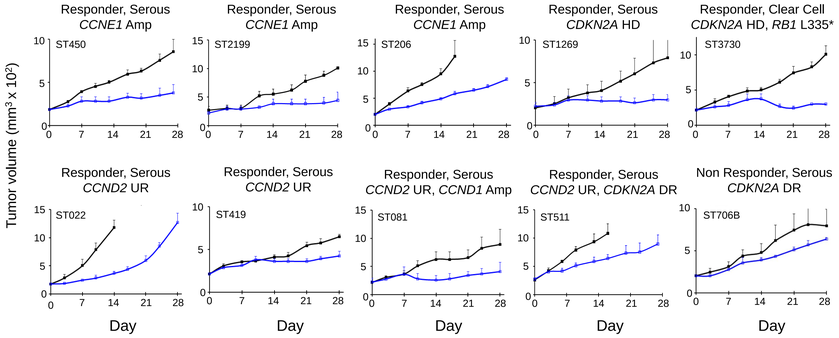


- 40% (10/25) of models had detectable alterations in core RB pathway genes
  - *CCNE1* AMP (n=3)
  - *CCND2* UR (n=2)
  - *CCND2* UR, *CCND1* AMP (n=1)
  - *CCND2* UR, *CDKN2A* DR (n=1)
  - *CDKN2A* HD (n=1)
  - *CDKN2A* HD, *RB1* MUT (n=1)
  - *CDKN2A* DR (n=1)

RB Pathway Alteration	# Models	Frequency (n=25)
CDKN2A DR	2	8%
CDKN2A HD	2	8%
CCND1 AMP	1	4%
CCND2 UR	4	16%
CCNE1 AMP	3	12%
RB1 HD	0	0%
RB1 MUT	1	4%

AMP: Amplification; DR: Downregulation; HD: Homozygous deletion; MUT: Mutation UR:Upregulation

TGI Responses in PDX Models with Core RB Pathway Alterations



- 90% (9/10) of PDX models with core RB pathway alterations (RB-incompetent) responded to SY-1365 (true positives)
- Similar responses observed in 40% (6/15) of models without alterations in the core RB pathway (false negatives)

Core RB Pathway Alterations Predict Response to SY-1365 in HGOC PDXs

PDX Model	Ovarian Subtype	*CDKN2A*	*CCND1*	*CCND2*	*CCNE1*	*RB1*#	Core RB Status	Response Class	% TGI
ST3730	Clear Cell	HD	-	-	-	p.L335*	Incompetent	Responder	86
ST1269	Serous	HD	-	-	-	-	Incompetent	Responder	84
ST022	Serous	-	-	UR	-	-	Incompetent	Responder	81
ST2199	Serous	-	-	-	AMP	-	Incompetent	Responder	75
ST081	Serous	-	AMP	UR	-	-	Incompetent	Responder	74
ST450	Serous	-	-	-	AMP	-	Incompetent	Responder	74
ST206	Serous	-	-	-	AMP	-	Incompetent	Responder	62
ST511	Serous	DR	-	UR	-	-	Incompetent	Responder	55
ST419	Serous	-	-	UR	-	-	Incompetent	Responder	51
ST706B	Serous	DR	-	-	-	-	Incompetent	Non Responder	34
ST3210	Serous	-	-	-	-	-	Competent	Responder	87
ST024	Serous	-	-	-	-	-	Competent	Responder	78
ST2044	Serous	-	-	-	-	-	Competent	Responder	77
ST1301	Serous	-	-	-	-	-	Competent	Responder	68
ST2476	Serous	-	-	-	-	-	Competent	Responder	57
ST004	Serous	-	-	-	-	-	Competent	Responder	52
ST036	Serous	-	-	-	-	-	Competent	Non Responder	50
ST270	Serous	-	-	-	-	-	Competent	Non Responder	43
ST2418	Carcinosarcoma	-	-	-	-	-	Competent	Non Responder	37
ST2054	Serous	-	-	-	-	-	Competent	Non Responder	34
ST663	Serous	-	-	-	-	-	Competent	Non Responder	12
ST2072	Serous	-	-	-	-	-	Competent	Non Responder	11
ST103	Serous	-	-	-	-	-	Competent	Non Responder	-20
ST409	Serous	-	-	-	-	-	Competent	Non Responder	-37
ST1162	Serous	-	-	-	-	-	Competent	Non Responder	-52

AMP: Amplification; DR: Downregulation; HD: Homozygous deletion; UR: Upregulation; # RB1 mutation in ST3730 results in premature stop codon (99.7% allele frequency)

	Responder	Non Responder	Total	
Core RB Incompetent (Predicted Responder)	9	1	10	Positive Predictive Value 90% (9/10)
Core RB Competent (Predicted Non-Responder)	6	9	15	Negative Predictive Value 60% (9/15)
Total	15	10	25	
	Sensitivity 60% (9/15)	Specificity 90% (9/10)		Balanced Accuracy 75% (p<0.01)

Conclusions

- In a prospectively defined study, core RB pathway alterations predict response to SY-1365 in HGOC PDX models with high positive predictive value (true positives: 90%, 9/10), providing a potential biomarker-driven patient enrichment strategy for clinical development of SY-1365
- SY-1365 responses observed in models without detectable core RB pathway alterations (false negatives: 40%, 6/15) suggest the presence of RB pathway alterations undetected in this analysis and/or alternate mechanisms driving SY-1365 activity including transcriptional regulation
- The results support the ongoing development of SY-1365 in patient populations enriched for RB pathway alterations and evaluation of core RB pathway alterations as biomarkers of SY-1365 clinical activity in patients with high grade ovarian cancer
- SY-1365 is being evaluated in the expansion phase of a phase 1 trial (NCT03134638), including in cohorts of patients with relapsed high grade serous ovarian cancer, clear cell ovarian cancer and CDK4/6 inhibitor resistant HR+ breast cancer with planned exploratory analyses of RB pathway alterations

AACR Annual Meeting, Atlanta GA. Mar 29 – Apr 5, 2019