ETX-197/BG-68501, a Potential Best-in-class Potent, Selective, Oral, Small Molecule CDK2 Inhibitor, has Anti-Tumor Activity in Cancer Models with Cyclin E Amplification or Deficiency in the Retinoblastoma 1 Gene.

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SABCS December 10-13, 2024
Presentation ID: P4-12-29

SUMMARY

In normal cells, there is redundancy in the role of the cell-cycle dependent kinases (CDKs) in regulating G1/S phase transition[1,2]. In cancer cells, the regulation of G1/S transition can be subverted by (a) amplification and elevated expression of Cyclin E1 (CCNE1), or (b) mutation/loss of the Retinoblastoma 1 (RB1/RB) gene[5-8]. Cancer cells with these genomic alterations have been shown to exhibit profound sensitivity to CDK2 depletion, validating CDK2 as a potential therapeutic target.

- A broad range of aggressive cancers overexpress CCNE1 including gynecological (e.g., ovarian, endometrial), gastric, triple negative breast cancer (TNBC), and other cancers
- CCNE1 amplification correlates with poor survival (ovarian)
- Up-regulation of CCNE1 or loss of RB1 confers CDK4/6i- resistance in ER$^+$HER2$^-$ breast cancer
- CRISPR screening data from DepMap portal shows that cancer cells with CCNE1 amplification or RB1 mutation are highly sensitive to CDK2 depletion[3,4]

Thus, selective targeting of CDK2 will inhibit cancer growth and may limit off-target CDK-driven toxicities. Here, we report the discovery and preclinical characterization of ETX-197, a potent and selective, orally bioavailable small molecule inhibitor of CDK2 activity.

BACKGROUND

Figure 1. Targeting Dysregulated CDK2 Activity in CCNE1 Amplified or RB1 Mutant Tumors

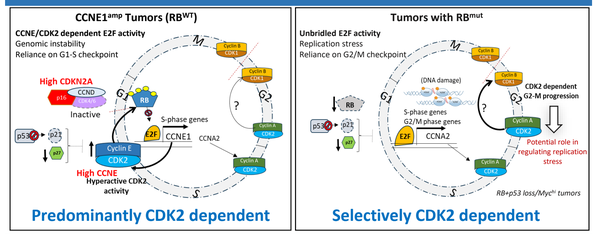
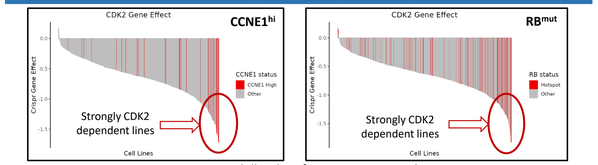


Figure 2. Functional Genomics Screens Confirm CCNE1 and RB1 Status as Predictors of Strong CDK2 Dependency in Cancer Cell Lines



Project Achilles data from DepMap portal

RESULTS

Figure 3. ETX-197 is a Potent and Selective, Orally Bioavailable Inhibitor of CDK2 Kinase Activity

ETX-197 shows improved potency and selectivity over PF07104091 in biochemical, biophysical and cellular assays

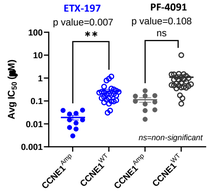
Kinome S(10)[a]	Biochemical activity[b] (IC_{50}; nM)							
	CDK2:CycE1	CDK2:CycA2	CDK1:CycB1	CDK4:CycD1	CDK6:CycD3	CDK7:CycH	CDK9:CycT1	GSK3β
0.047	0.93	1.9	73.6	247.9	567.6	255.4	116.03	213.7

Compound	Biophysical (SPR)			Biochemical K_i	Cellular activity (IC_{50}; nM)		
	k_{on} ($M^{-1}S^{-1}$)	k_{off} (S^{-1})	K_D (nM)	K_i (nM)	OVCAR3 (pRB-S780; nM)[c]	TOV21G (pNPM1-T199; nM)*	Selectivity
ETX-197	1.48E+07	1.06E-03	7.18E-02	0.11	3	100	33 x
PF-07104091	1.43E +07	6.30E-03	4.42E-01	1.45	40	450	11 x

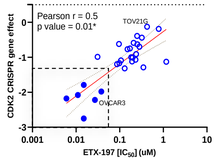
[a]Kinome S(10): Kinase hits with 90% efficiency as that of control at 2 μM, Competition binding assays @ KinomeScan with 403 non-mutant kinases
[b]Enzyme activities IC_{50} were measured at varying concentration of ATP using canonical CDK/Cyclin pairs as indicated
[c] Phosphorylated RB1 (pRb-S780) protein was assessed by western blot in asynchronized OVCAR-3 cells to reflect CDK2 cellular potency
*p-NPM1(T199) is a measure of CDK1 activity in Nocodazole arrested TOV21G cells

Figure 4. ETX-197 Shows Selectivity for CCNE1^{amp} Cancer Cells and Growth Inhibition Correlates with CDK2 Dependence

(a) ETX-197 shows selectivity towards CCNE1 amplified human cancer cell lines

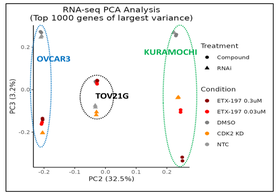


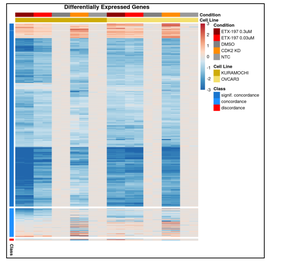
(b) Cell lines most sensitive to ETX-197 have a CRISPR DepMap score <-1.5



(A few CCNE1^{amp} lines were excluded for which Depmap scores were not available)

Figure 5. Bulk RNA-Seq Data Confirm ETX-197 Downregulated Genes Recapitulate CDK2 KD Profile in CDK2i Sensitive Cells



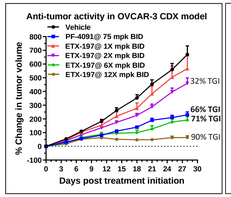


Geneset Enrichment of PC3 Genes (top 3 terms)

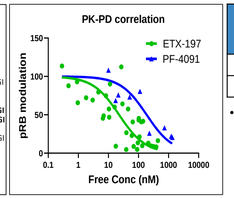
GeneSet	FDR
E2F targets	1.70E-19
G2/M Checkpoint	1.7 E-19
Cell cycle Checkpoints	3.6 E-11

Figure 6. ETX-197 is Efficacious *In Vivo* and Demonstrates Dose-Dependent PD Response

(a) ETX-197 shows anti-tumor activity in OVCAR-3 CDX-model



(b) *In vivo* potency of ETX-197 in modulation of phospho-RB-S780 in OVCAR3 tumor



	Free IC_{50} (nM)	Cellular potency IC_{50} (nM)
ETX-197	22.7	30
PF-4091	187	200

- ETX-197 has ~8x higher intrinsic potency *in vivo* than PF-4091

(c) PD: Inhibition of pRB/RB in tumor lysates Day 4 post last dosing

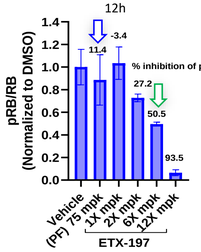


Figure 7. ETX-197 is Efficacious in CCNE^{hi} TNBC PDX models

- ETX-197 is more potent than PF-4091, achieving equivalent efficacy at a lower dose.
- Tumor PD biomarker (phospho-RB) shows dose-dependent inhibition, consistent with tumor growth inhibition.
- Inhibition of pRB is sustained for at least 12 h at 12X mpk dose level.

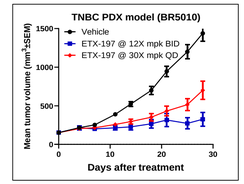
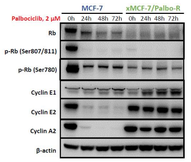


Figure 8. ETX-197 Shows Anti-Tumor Activity in Palbociclib-Resistant MCF-7 CDX model

(a) RB loss is a possible cause of resistance in Palbociclib-resistant MCF-7 CDX model (WB data)



(b) ETX-197 is efficacious both as a single agent and in combination with Palbociclib in Palbociclib-resistant MCF-7 CDX model

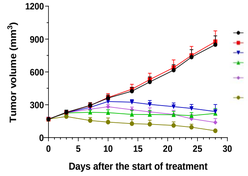
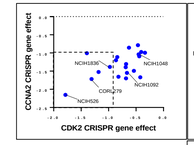
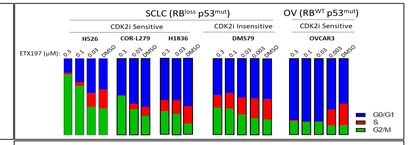


Figure 9. ETX-197 Treatment Correlates with Induction of G2/M Cell-cycle Arrest, DNA Damage and Apoptosis in CDK2i Sensitive RB^{mut} SCLC

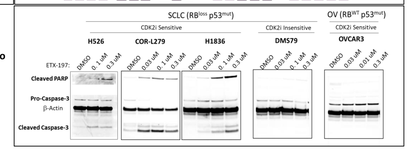
(a) CDK2 CRISPR dependency data against Cyclin A2 (CCNA2) in SCLC



(b) CDK2i sensitive SCLC undergo G2/M arrest in response ETX-197 treatment



(c) CDK2i sensitive SCLC cells undergo apoptosis in response to ETX-197 treatment



(d) SCLC cells sensitive to CDK2 inhibition accumulate DNA damage in response to ETX-197 treatment

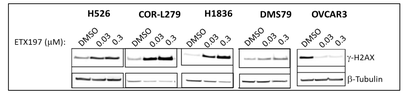
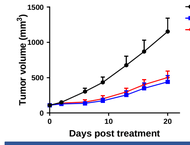
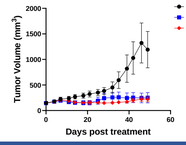


Figure 10. ETX-197 Shows Anti-tumor Activity in RB^{mut} SCLC CDX and PDX models





CONCLUSION

- ETX-197 is a selective and potent, oral, small molecule CDK2 inhibitor
- ETX-197 demonstrates single agent anti-tumor efficacy in both RB^{WT} CCNE1-amplified and RB1 deficient CDX and PDX mouse models
- ETX-197 shows anti-tumor activity in a breast cancer xenograft model with acquired resistance to a CDK4/6 inhibitor due to loss of RB1 expression.
- ETX-197 is being clinically developed by BeiGene as BG-68501, in a first-in-human, Phase 1a/1b in patients with advanced, nonresectable, or metastatic solid tumors (NCT06257264; Poster # P4-08-20)

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