Poster #1068

SDX-7320, a novel inhibitor of methionine aminopeptidase 2 (MetAP2), inhibits MCF-7 tumor growth and mechanisms of resistance in combination with palbociclib (Ibrance®)

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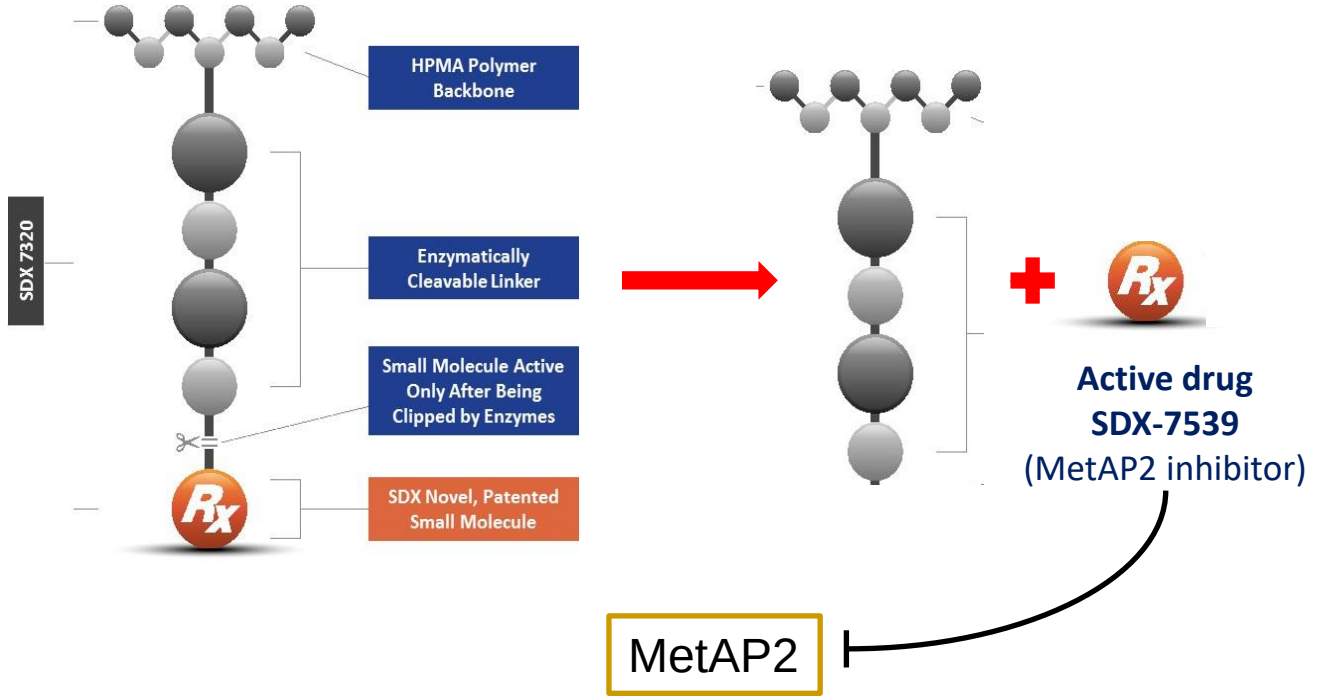
The Metabo-Oncology Company™

SDX-7320: A Novel, Clinical-Stage Polymer-Drug Conjugate

A methionine aminopeptidase 2 (MetAP2) inhibitor conjugated to a polymer backbone

- SDX-7320 is a pro-drug of a small molecule MetAP2 inhibitor (fumagillin-class)
- The prodrug is a polymer-drug conjugate and consists of:
  1. *A hydroxypropylmethacrylamide (HPMA) – methacrylamide (MA) co-polymer carrier*
  2. *A specific peptide linker (enzyme-cleavable)*
  3. *The pharmacologically active fumagillol derivative SDX-7539*
- Metabolism *in vivo* yields the pharmacologically active drug SDX-7539 (MetAP2 inhibitor)
- Polymer-conjugation:
  1. *limits CNS penetration of small molecule (prevents CNS toxicity seen with other MetAP2 inhibitors)*
  2. *solubilizes the hydrophobic small molecule*
  3. *enzyme-mediated release minimizes small molecule concentrations in general circulation*
- SDX-7320 has completed a Phase I trial, in late-stage, heavily pretreated cancer patients with solid tumors



- Cell cycle arrest (increased p21, decreased CDK2, Cyclin E1, Rb in ER+ tumors)
- Inhibits signaling downstream of WNt5a (blocks non-canonical wnt pathway)
- Decreases levels of pro-angiogenic growth factors (VEGF-C, bFGF)
- Modifies immune-suppressive tumor immune micro-environment (↓Arg-1, ↓Treg)
- Improves insulin sensitivity in states of insulin resistance
- Impacts adipose tissue to decrease leptin, increase adiponectin in circulation

Study Design: SDX-7320 +/- CDK4/6 Inhibitor Palbociclib

CDK4/6 inhibitors are standard-of-care in 1st line treatment for metastatic ER+/Her2- breast cancer

Objectives:

1. To evaluate the anti-tumor activity of SDX-7320 and the CDK4/6 inhibitor palbociclib alone and in combination in orthotopic MCF-7 xenografts.
2. To measure the changes in known and reported mechanisms of treatment resistance to CDK4/6 inhibitors.

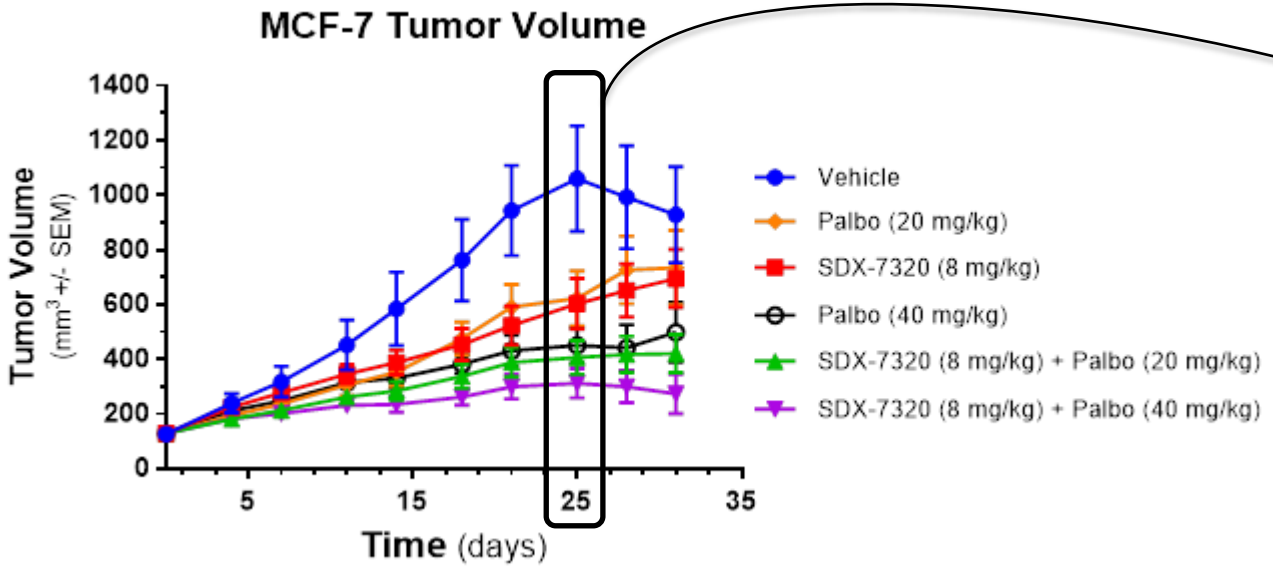
Methods:

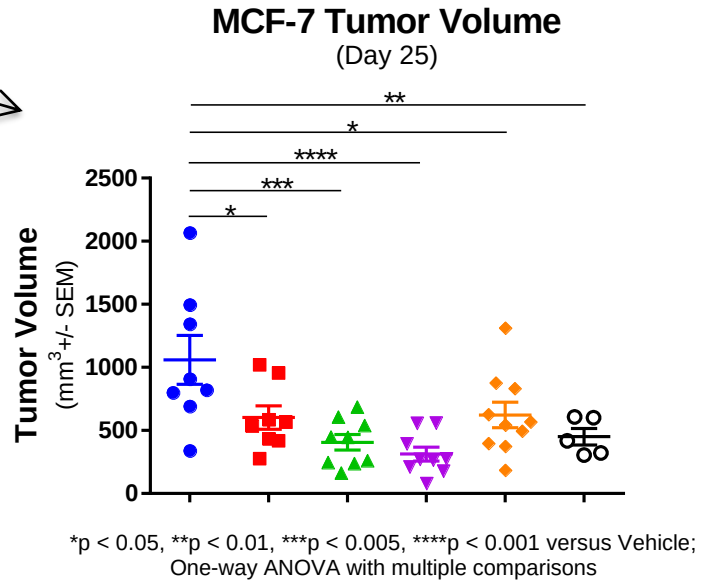
- Female nude mice (Charles River) were implanted with one 90-day (0.72 mg) β-estradiol pellet 3 days prior to cell inoculation.
- MCF-7 cells (5 x 10^6 in 1:1 PBS/Matrigel) were injected into the fourth mammary gland.
- Treatment with test agents began when group mean tumor volume was 125-175 mm^3 with no tumors <100 mm^3.
- Tumor volume and body weight were measured twice weekly; gross observations were made daily.
- At end of study, whole blood collected by cardiac puncture (under ether anesthesia) for CBC, tumors dissected (n=3): half of each tumor was placed in RNALater (for analysis of gene expression) and the other half was homogenized in RIPA buffer containing protease and phosphatase inhibitors then frozen at -70ºC.

Group	**N**	**Vehicle** (QD to End of Study Starting Day 2)	**SDX-7320** (Q4D to End of Study Starting Day 1)	**Palbociclib** (QD to End of Study Starting Day 2)
Vehicle (PO: lactic acid buffer))	10	X		
SDX-7320 8 mg/kg (SC)	10		X	
SDX-7320 8 mg/kg (SC) + Palbo 20 mg/kg (PO)	10		X	X
SDX-7320 8 mg/kg (SC) + Palbo 40 mg/kg (PO)	10		X	X
Palbociclib 20 mg/kg (PO)	10			X
Palbociclib 40 mg/kg (PO)	10			X

Anti-tumor Efficacy with SDX-7320 and Palbociclib

Both SDX-7320 and palbociclib were efficacious alone; additional activity with combinations





*p < 0.05, **p < 0.01, ***p < 0.005, ****p < 0.001 versus Vehicle;
One-way ANOVA with multiple comparisons

	T_0	T_t	TGI (%)
Vehicle	129	1060	-
SDX (8)	129	602	49
SDX (8) + Palbo (20)	129	406	70
SDX (8) + Palbo (40)	129	313	80
Palbo (20)	129	623	47
Palbo (40)	129	451	65

Significant inhibition of tumor growth both in response to SDX-7320 alone, and to palbociclib alone.

Additional efficacy was observed with the combination of SDX-7320 and palbociclib (20 and 40 mg/kg).

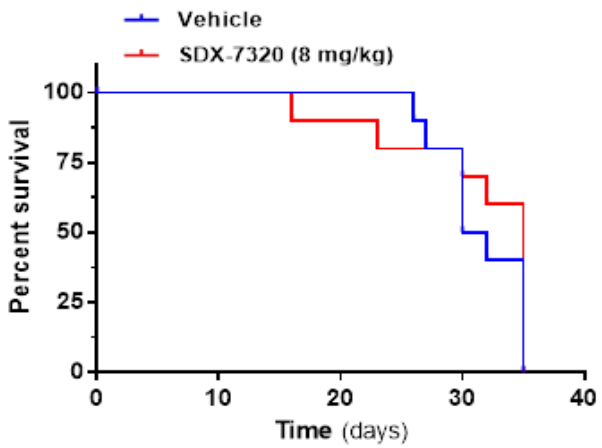
Tumor growth in vehicle group was variable

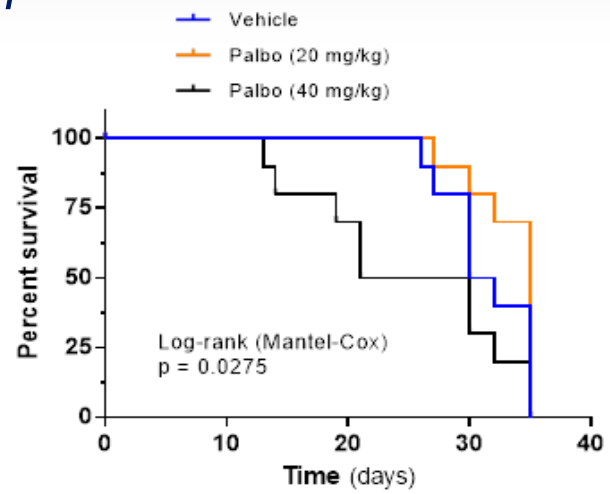
- *One animal removed from analysis due to poor tumor growth*
- *Tumor volume decreased after day 25 due to death of two mice with large tumors, therefore day 25 data was used for TGI% and statistical analysis*

Source: SDX internal data

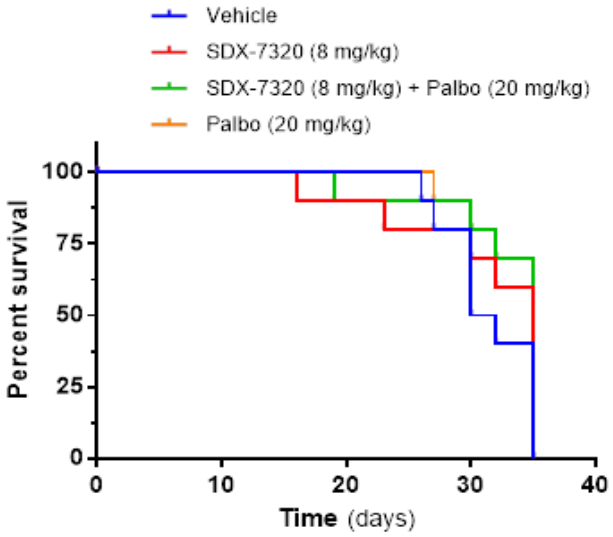
Survival Trend Favored SDX-7320 Plus Lower Dose Palbociclib

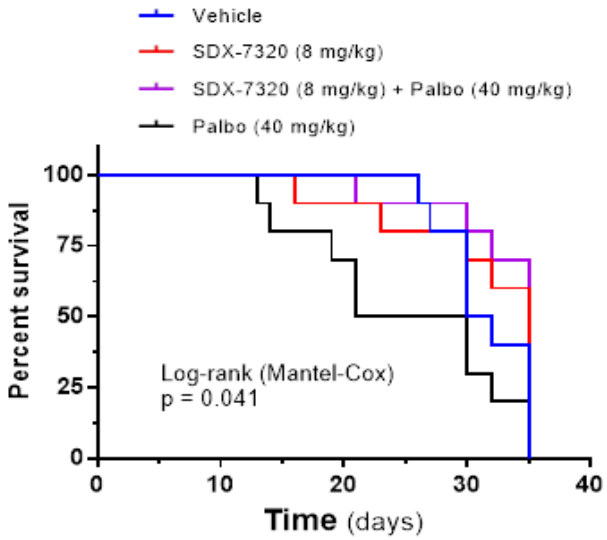
Palbociclib at 40 mg/kg was poorly tolerated in this model





Palbociclib @40 mg/kg was not well tolerated, leading to multiple premature events.



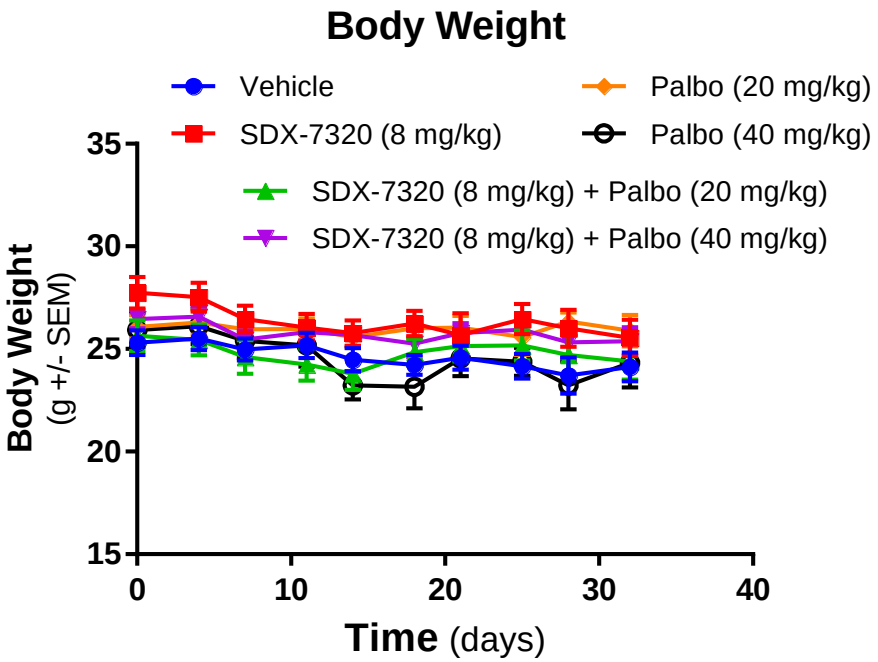


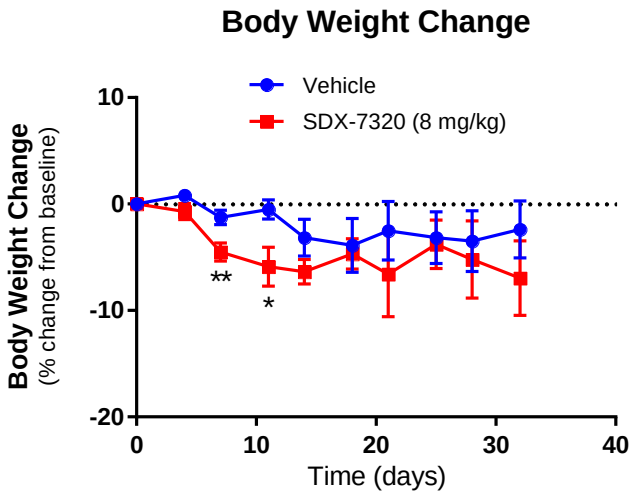
Combination of SDX-7320 with palbociclib (40 mg/kg) improved survival compared to Palbociclib (40 mg/kg) alone.

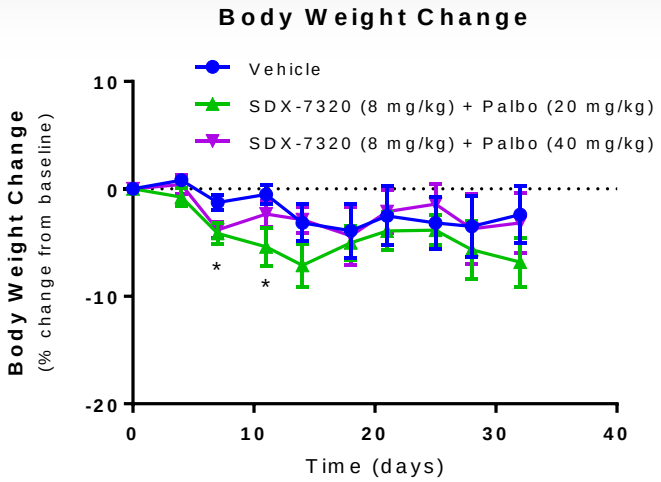
Source: SDX internal data

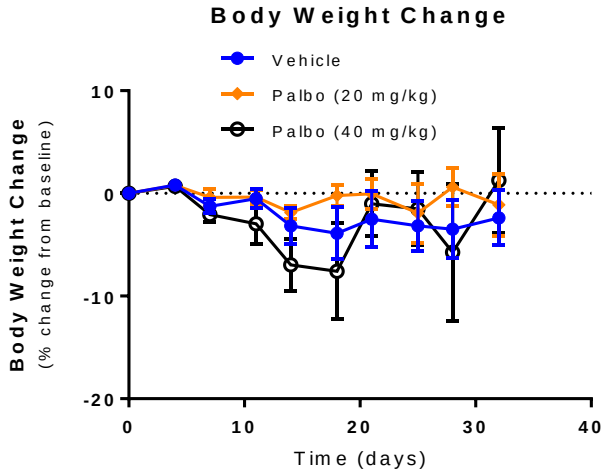
Safety: Changes in Body Weight

SDX-7320 typically induces loss of fat mass in normal animals









No additional weight loss seen with combination.

*p < 0.05, **p < 0.01 versus vehicle; multiple t-tests (Holm-Sidak method)

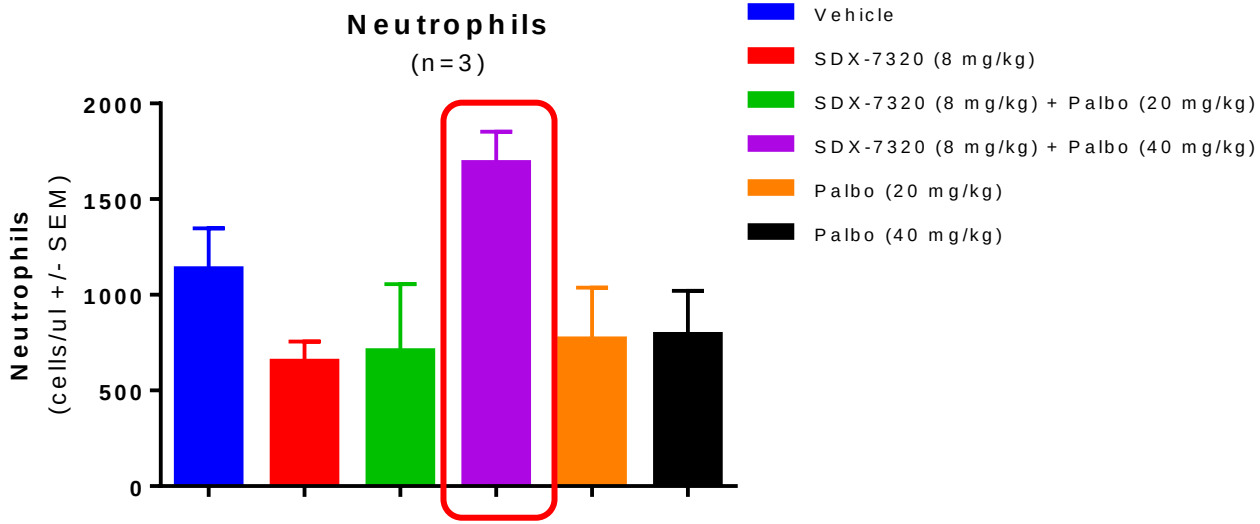
Source: SDX internal data

Safety: Changes in Neutrophils – A Known Toxicity with Palbociclib*

Lack of significant reduction in combination suggests potential for improved hematologic safety profile

Adverse Event Summary (PALOMA-3)*

	Fulvestrant plus palbociclib (n=345)		
	Grade 1–2	Grade 3	Grade 4
Haematological			
Neutropenia	56 (16%)	189 (55%)	34 (10%)
Anaemia	86 (25%)	10 (3%)	0
Leucopenia	76 (22%)	93 (27%)	2 (1%)
Thrombocytopenia	65 (19%)	6 (2%)	2 (1%)
Lymphopenia	4 (1%)	1 (<1%)	1 (<1%)



Severe neutropenia is a major toxicity associated with palbociclib; reversed in combination of SDX-7320 and palbociclib (40 mg/kg).

*Cristofanelli, Lancet Oncol (2016), 17: 425-39; McAndrew et al., Br J Cancer 123, 912–918 (2020)

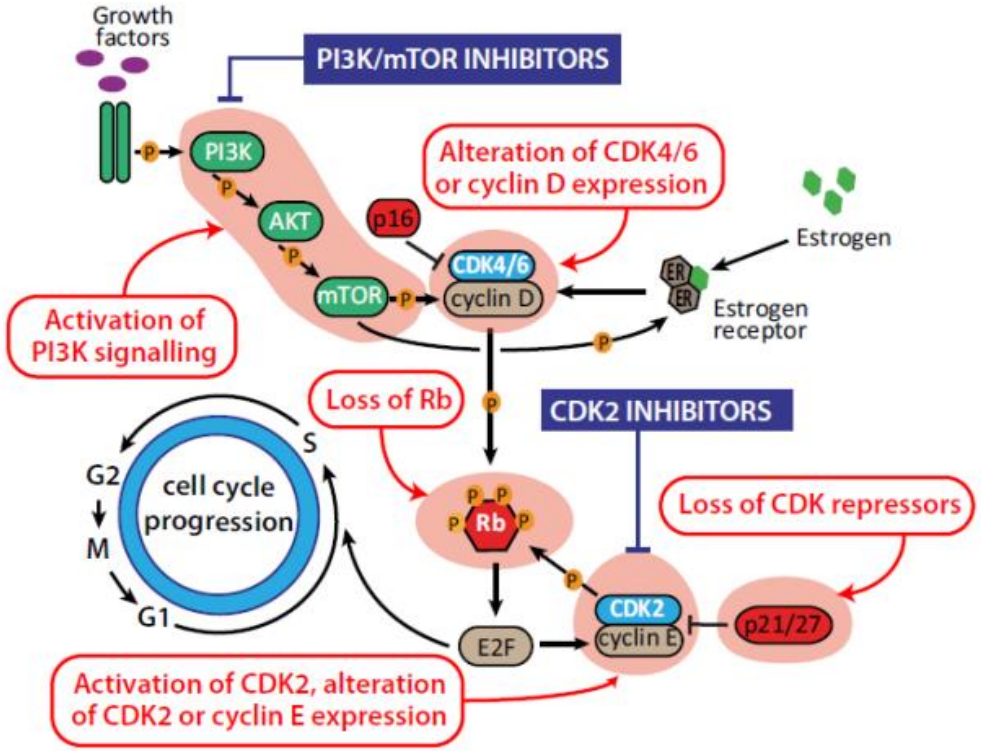
Exploring Resistance Mechanisms to CDK4/6 Inhibition

Pathways implicated in resistance may be attenuated by MetAP2 inhibition

Potential mechanisms of CDK4/6 resistance:

- Cyclin E1
- CDK 2
- Akt signaling
- Autophagy
- Cancer stem cell initiation

Methods: To assess the effect of SDX-7320 alone and in combination with palbociclib, tumor tissues (n=3/group) were dissected, homogenized then frozen, later thawed and then analyzed using an automated Western blot device (WES/ProteinSimple).

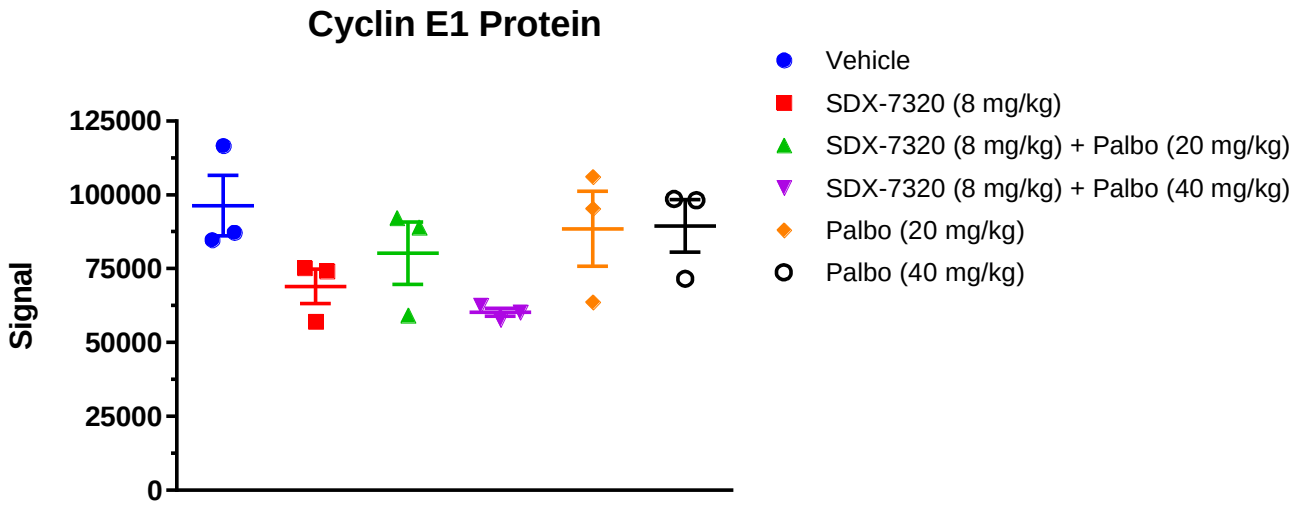


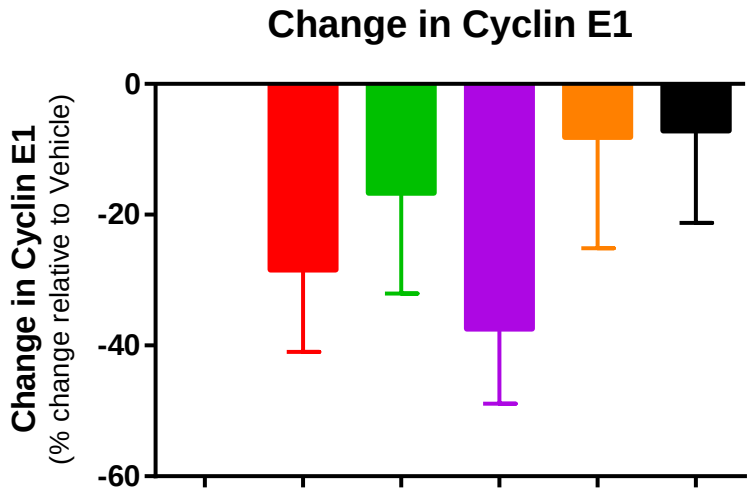
Key mechanisms implicated in the development of resistance to CDK4/6 inhibitors.

Source: Portman et al., Endocr Relat Cancer 2019 Jan;26(1):R15-R30

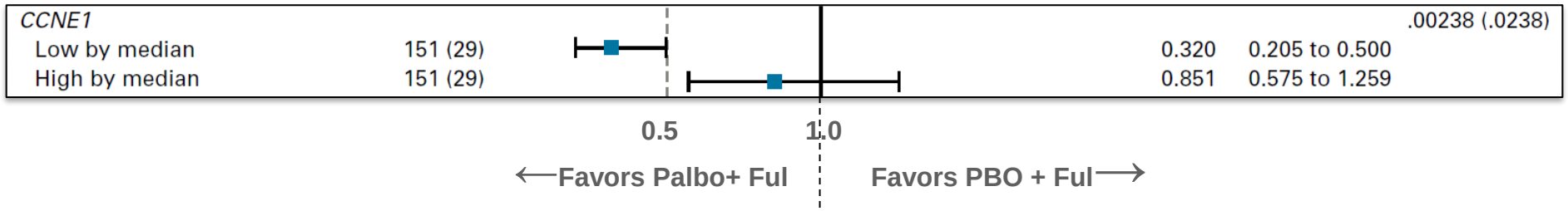
Cyclin E1: Lower Expression Associated with Improved Outcomes*

High levels associated with resistance to palbociclib, lower gene expression mapped to longer PFS





Association of cell cycle pathway gene expression and the efficacy of palbociclib (PAL) in combination with fulvestrant (FUL).*

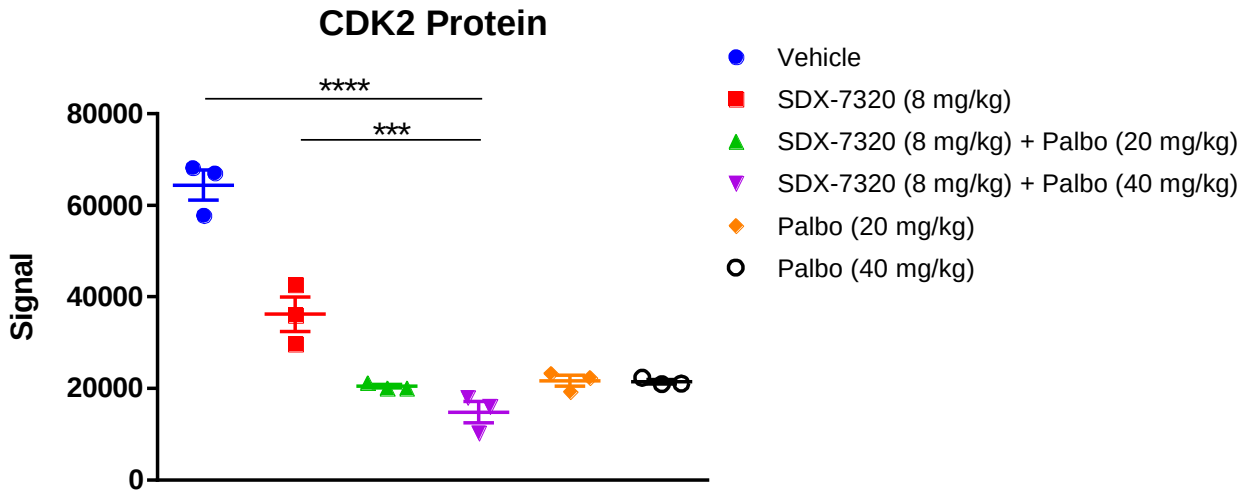


Source: SDX internal data

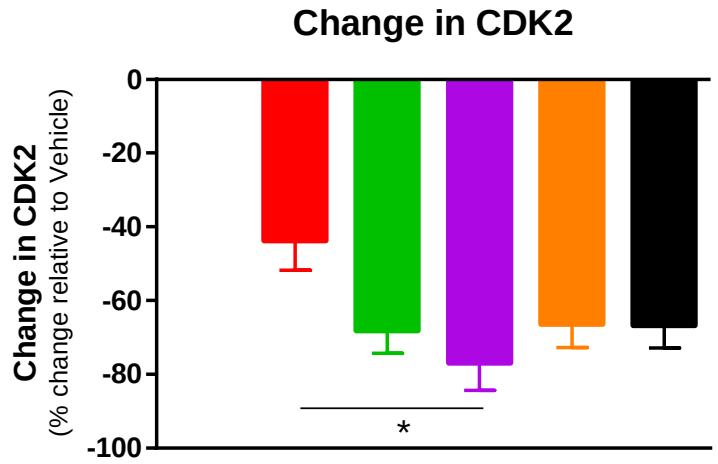
*Turner et al., J Clin Oncol (2019) 37:1169-1178

CDK2 Associated w/Progression of Breast Cancer, Resistance to CDKi

CDK2 protein decreased by SDX-7320 alone, further decrease w/combo



***p < 0.005, ****p < 0.001 versus SDX (8) + Palbo (40);
one-way ANOVA with multiple comparisons



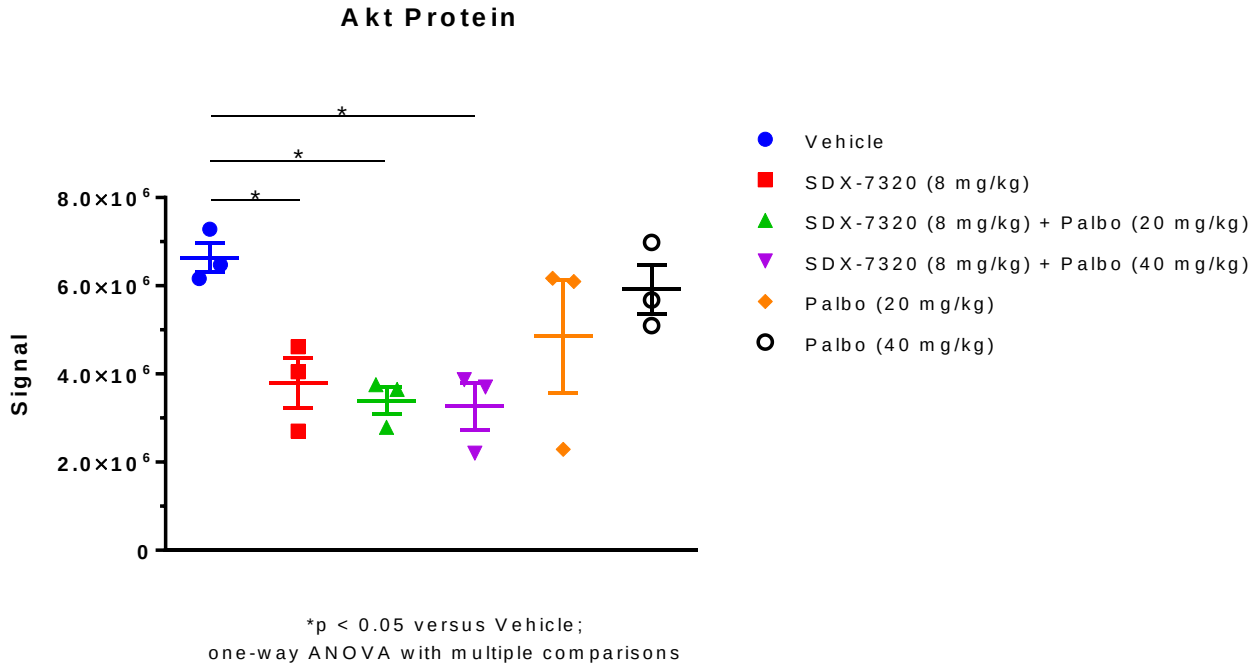
*p < 0.05 versus SDX (8);
one-way ANOVA with multiple comparisons

- Combination of palbociclib (40 mg/kg) and SDX-7320 significantly decreased the level of intra-tumoral CDK2 protein relative to both vehicle-treated controls and SDX-7320 alone
- The change in intra-tumoral CDK2 protein relative to vehicle was significantly greater in the combination of palbociclib (40 mg/kg) and SDX-7320 relative to SDX-7320 alone

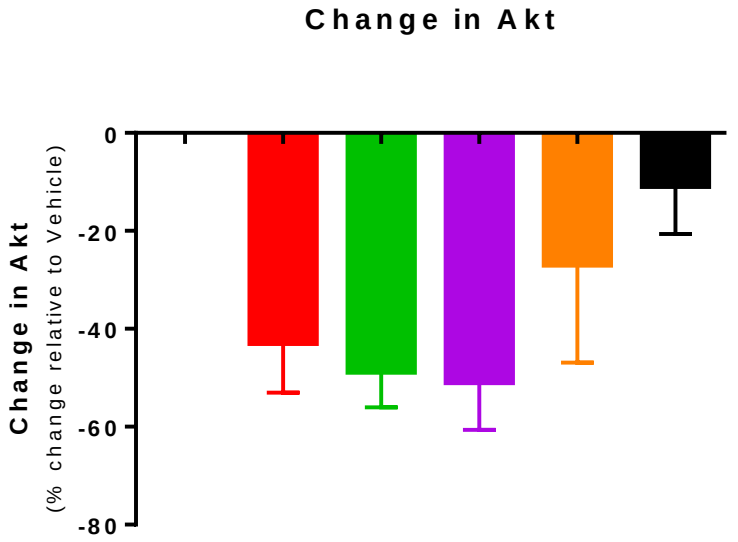
Source: SDX internal data

Akt: Common Pathway of Drug Resistance for Many Drug Classes*

Significant decreases with SDX-7320 monotherapy, and when combined with palbociclib (20, 40 mg/kg)



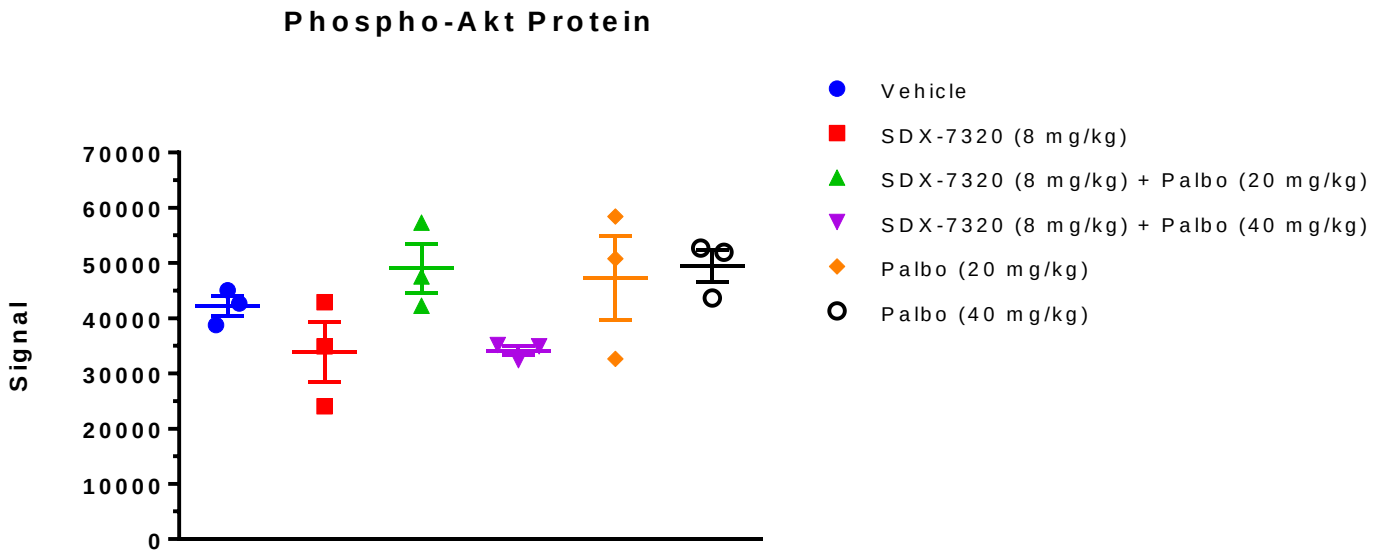
*p < 0.05 versus Vehicle;
one-way ANOVA with multiple comparisons

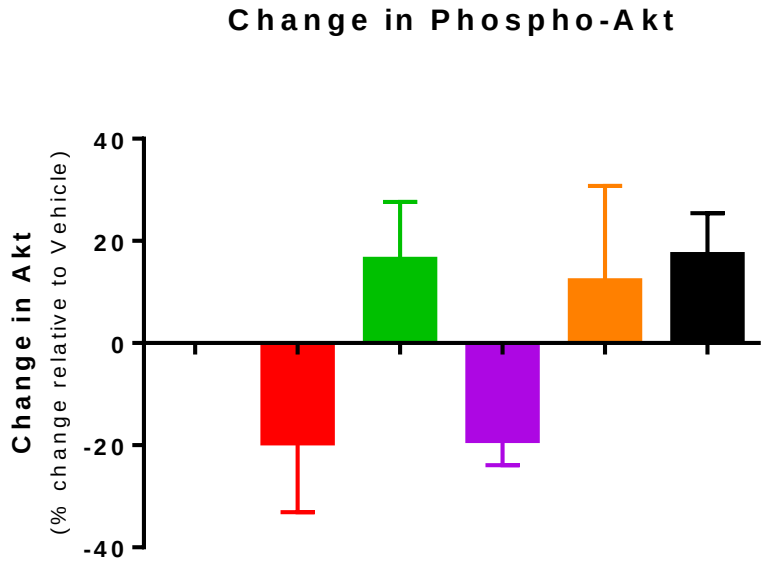


Source: SDX internal data
*Liu R, et al, Cell Death Dis. 2020;11(9):797. doi:10.1038/s41419-020-02998-6

Akt: Activation by Phosphorylation

pAkt (S473) decreased by SDX-7320 and in combination with palbociclib at 40 mg/kg

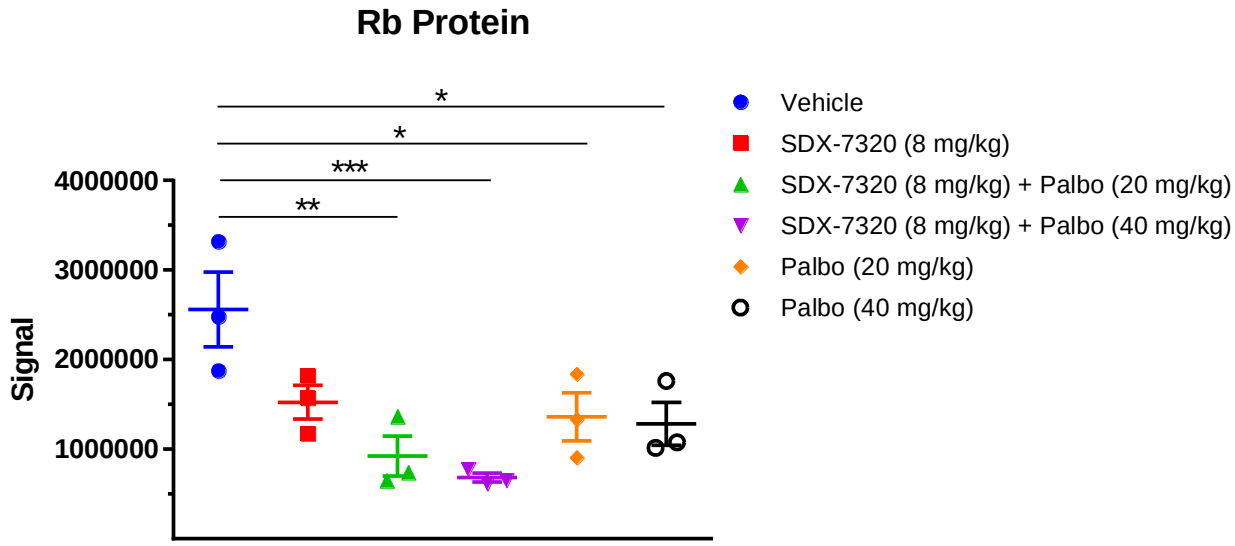




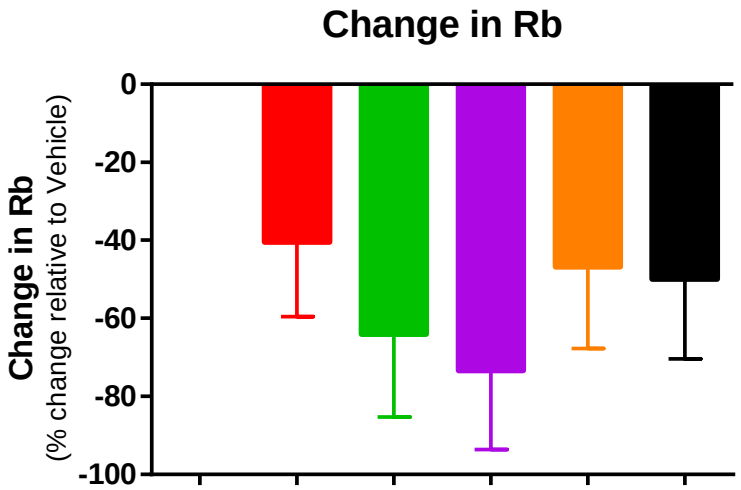
Source: SDX internal data

Rb: Reduced by Both Agents; Possible Additivity

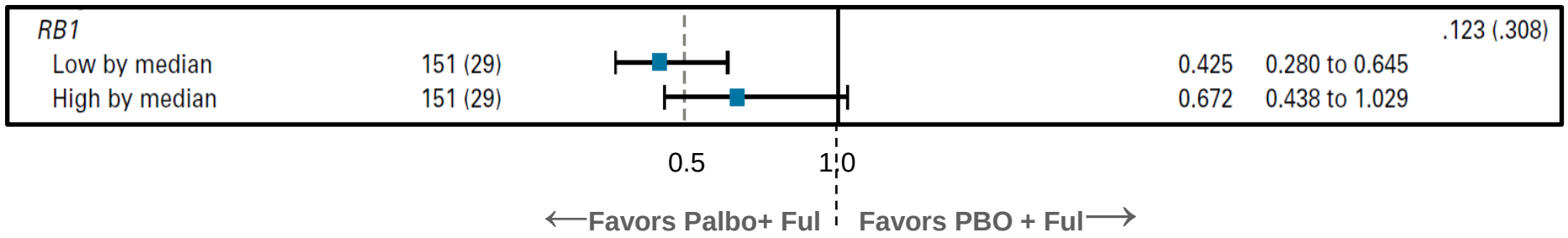
*Tumors with lower Rb protein showed a trend towards better outcomes in post-hoc PALOMA-3 analysis**



*p < 0.05, **p < 0.01, ***p < 0.005, versus Vehicle; one-way ANOVA with multiple comparisons



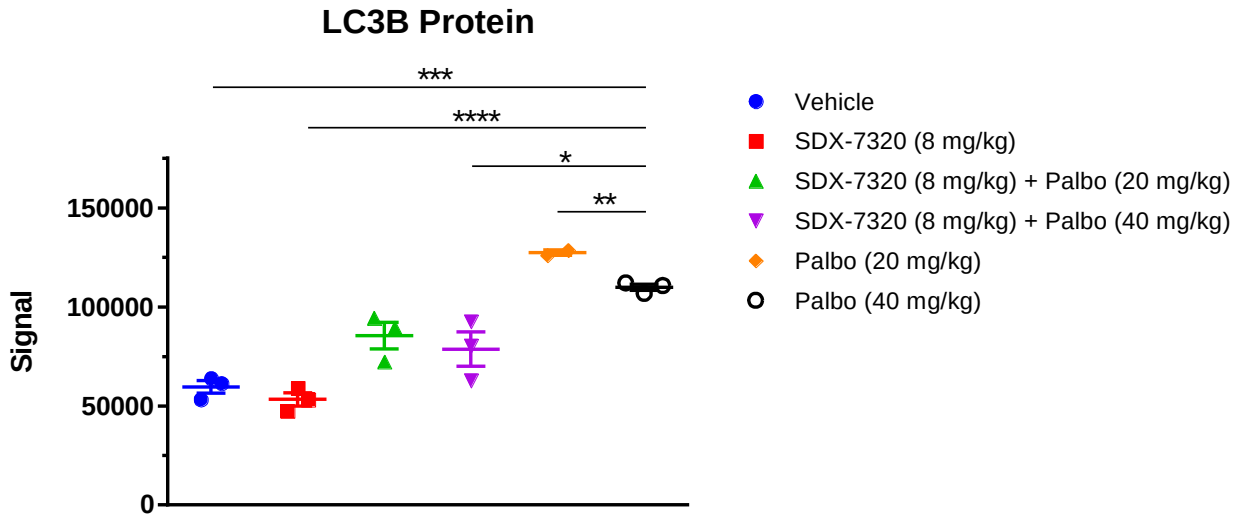
Interestingly, Turner et al* reported that lower Rb expression trended towards better PFS.



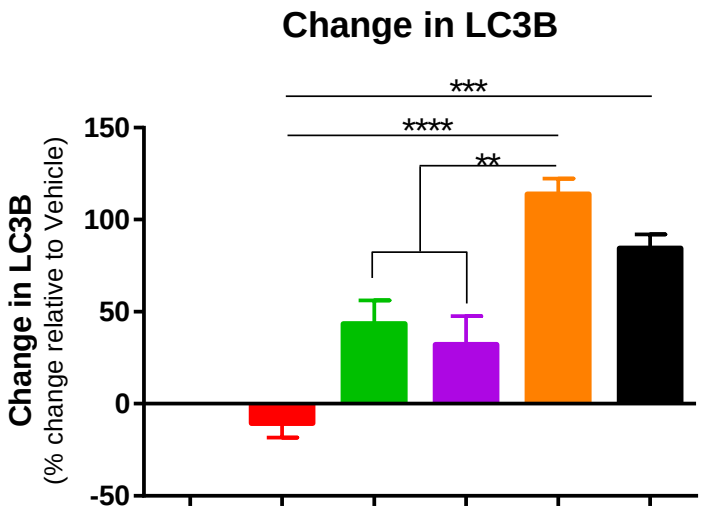
Source: SDX internal data
*Turner et al., J Clin Oncol (2019) 37:1169-1178

LC3B: Autophagy Marker; Induced by Palbociclib

Palbociclib-induced LC3B was attenuated when palbociclib was combined with SDX-7320



*p < 0.05, **p < 0.01, ***p < 0.005, ****p < 0.001 versus Palbo (40); one-way ANOVA with multiple comparisons



**p < 0.01, ***p < 0.005, ****p < 0.001 versus Palbo 20 or Palbo 40; one-way ANOVA with multiple comparisons

"Cancer cells activate autophagy in response to palbociclib, and that blockade of autophagy significantly improves the efficacy of CDK4/6 inhibition in vitro and in vivo in cancers with an intact G1/S transition."*

Source: SDX internal data

*Vijayaraghavan, et al., Nat. Commun.8, 15916 doi: 10.1038/ncomms15916 (2017).

Summary and Next Steps

Summary

- SDX-7320 alone (8 mg/kg) inhibited MCF-7 tumor growth by 49% and palbociclib alone (20, 40 mg/kg) exhibited dose-dependent efficacy (47% and 65%, respectively). This analysis is based on day 25 tumor volume data.

- When SDX-7320 was combined with palbociclib (20, 40 mg/kg) additional inhibition of tumor growth was observed (70 and 80% respectively). This analysis is based on day 25 tumor volume data.

- Both palbociclib and SDX-7320 alone suppressed levels of neutrophils by 30-40% relative to vehicle-treated mice, while the combination of SDX-7320 and palbociclib (40 mg/kg) increased neutrophils 49% relative to vehicle-treated mice.

- The combination of SDX-7320 and palbociclib attenuated the expression of proteins associated with resistance to palbociclib (i.e., cyclin E1, CDK2, Akt, Rb and LC3B).

Next Steps

- Complete the analysis of gene expression data (RNASeq)
  - *Ongoing*

- Develop palbociclib-resistant MCF-7 model
  - *Can SDX-7320 overcome acquired resistance to palbociclib?*

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