Inhibition of Her2+ tumor growth with SDX-7320, a novel MetAP2 inhibitor, alone and in combination with capivasertib/AZD-5363: reduced expression of hypoxia-inducible and innate-immune system genes.

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Introduction

Hyperglycemia is a common and often treatment-limiting toxicity associated with a variety of drug classes, including PI3K and Akt inhibitors. SDX-7320 is a polymer-drug conjugate of a novel, small molecule, fumagillin-derived MetAP2 inhibitor (SDX-7539) attached via a cleavable linker to a hydroxypropyl-methacrylamide (HPMA) polymer backbone. Here, we investigate the ability of SDX-7320 to attenuate Akt-inhibitor induced hyperglycemia in normal animals, and explore the mechanisms underlying SDX-7320's potentially synergistic, anti-tumor effect when combined with capivasertib/AZD-5363, which is in development for the treatment of breast cancer (Jones, 2020).

SDX-7320 is being developed to treat cancers whose growth and metastatic potential is enhanced by dysregulated metabolic hormones such as insulin, leptin and adiponectin (i.e., "metabo-oncology"). The previously reported insulin-sensitizing properties of small-molecule MetAP2 inhibitors TNP-470, beloranib (Bråkenhielm, 2004, Hughes, 2013, Kim, 2013) as well as SDX-7320, suggested that SDX-7320 might combine well with PI3K or Akt/mTOR inhibitors to attenuate on-target hyperglycemia and subsequent hyper-insulinemia. Interventions that improve insulin sensitivity, when combined with PI3Kα inhibitors in models of breast and pancreatic cancers, showed striking anti-tumor efficacy relative to PI3Kα inhibitor alone (Hopkins, 2018).

In prior studies in models of of breast cancer, SDX-7320 significantly inhibited the growth of syngeneic EO771 triple-negative breast cancers (TNBC) accelerated by obesity/metabolic dysfunction and also potentiated the anti-tumor effects of the PI3Kα inhibitor alpelisib/Piqray® in ER+/Her2- MCF-7 xenografts.

The objectives of these experiments were to determine the effects of SDX-7320 on the growth of Her2+ xenografts alone or in combination with the Akt/mTOR inhibitor capivasertib/AZD-5363 and to determine if SDX-7320 could attenuate the hyperglycemia induced by capivasertib/AZD-5363.

Materials & Methods

Insulin sensitivity was measured in obese, male C57Bl/6 mice (fed for 22 weeks with 60% fat diet; from Jackson Labs, Bar Harbor, ME) that received a single dose of SDX-7320 (8 mg/kg, SC) four days prior to an insulin-tolerance test (ITT; at Neosome Life Sciences, Lexington, MA). Male C57Bl/6 mice were pre-dosed with SDX-7320 (8 mg/kg, SC) for the indicated times (Q4D, s.c.), after which all mice (except-vehicle treated) received a single oral dose of AZD-5363/capivasertib (200 mg/kg, p.o.). Blood glucose was measured via tail vein at regular intervals using a glucometer. Insulin was measured by ELISA from a terminal plasma sample obtained 180 minutes post-AZD-5363.

At TD2 (Scottsdale, AZ) female nude mice (from Charles River, Wilmington, MA) had estrogen pellets surgically implanted and after two weeks of recovery, 5x10^6 MCF-7 cells were injected into the 4th mammary gland. When tumors became palpable (i.e., > 100 mm^3) treatment with SDX-7320 (dosed s.c. Q4D at 6 or 12 mg/kg) and/or AZD-5363/capivasertib (PO, QD at 100 or 200 mg/kg) commenced. Combinations included SDX-7320 at 6 mg/kg plus AZD-5363/capivasertib at 100 mg/kg as well as SDX-7320 at 12 mg/kg plus AZD-5363/capivasertib at 200 mg/kg. Endpoints included tumor volume and changes in body weight.

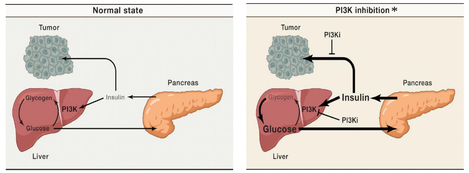
Tumor tissue samples from a subset of treatment groups (Vehicle, SDX-7320 at 12 mg/kg monotherapy, SDX-7320 at 12 mg/kg + AZD-5363 at 200 mg/kg, and AZD-5363 at 200 mg/kg) were snap frozen and stored at -80ºC. PolyA+ RNA was isolated from tumor tissue, cDNA libraries were constructed and RNASeq was conducted (range of 20-30 reads per million (RPM) base pairs (Genewiz, South Plainfield, NJ). Analysis of RNASeq data was conducted at Watershed Informatics (Cambridge, MA).

References

Bråkenhielm et al., Circ Res. 2004 Jun 25;94(12):1579-88; Hopkins et al. (2018), *Nature*, PMID 30051890; Hughes et al (2013), *Obesity,* PMID 23512440; Jones et al, *Lancet Oncol.* 2020;21(3):345-357; Kim et al (2015), *Obesity and Metabolism,* PMID 25732625.

Figure 1. Hyperglycemia, Hyperinsulinemia Induced by PI3K, Akt Inhibitors

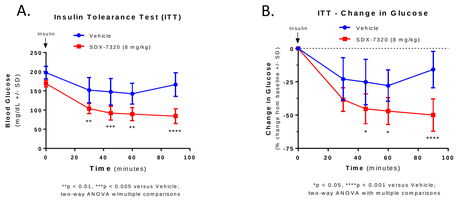
Fruman et al, (2017) *Cell*, 170: 605-635.



*Note that in addition to liver, inhibition of PI3K and Akt (and thus insulin signaling) in skeletal muscle and adipose tissue after administration of AZD-5363/capivasertib may also contributes to systemic hyperglycemia.

Figure 2. SDX-7320 Increases Insulin Sensitivity in Obese Mice

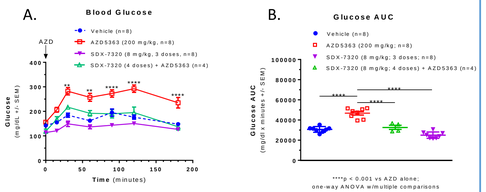
(single dose of SDX four days before insulin)



**p < 0.01, ***p < 0.005 versus Vehicle; two-way ANOVA w/multiple comparisons

*p < 0.05, ****p < 0.001 versus Vehicle; two-way ANOVA with multiple comparisons

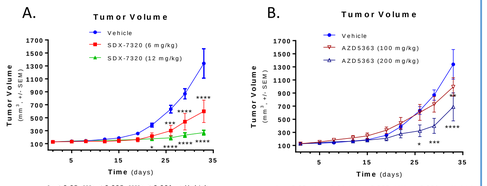
Figure 3. Pre-Treatment with SDX-7320 Attenuates AZD-5363-Induced Hyperglycemia in Normal Mice



**p < 0.01, ****p < 0.001 SDX+AZD vs AZD alone; two-way ANOVA w/multiple comparisons

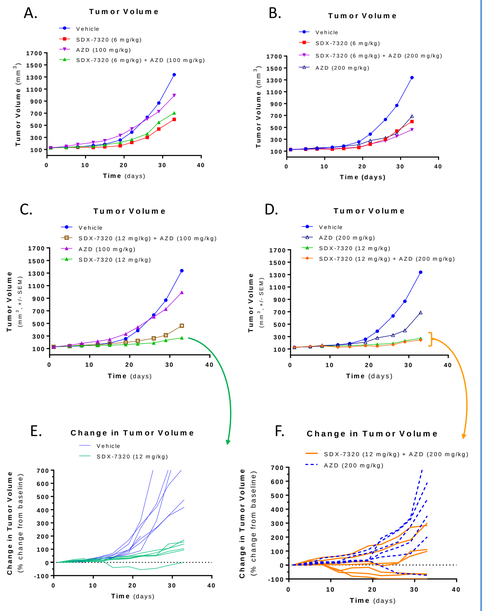
****p < 0.001 vs AZD alone; one-way ANOVA w/multiple comparisons

Figure 4. SDX-7320 and AZD-5363 Inhibit Growth of Her2+ BT-474 Xenografts



*p < 0.05, **p < 0.01 ***p < 0.005, ****p < 0.001 vs Vehicle; two-way ANOVA w/multiple comparisons

Figure 5. Effect of AZD-5363 + SDX-7320 on Growth of BT-474 Xenografts



Note that survival for all groups was >80% at the end of the study (day 32), with no statistical differences between the groups.

Table 1. Tumor Growth Inhibition (TGI)

%TGI = (1-[Tt/T0 / Ct/C0]) / 1-[C0/Ct]) X 100

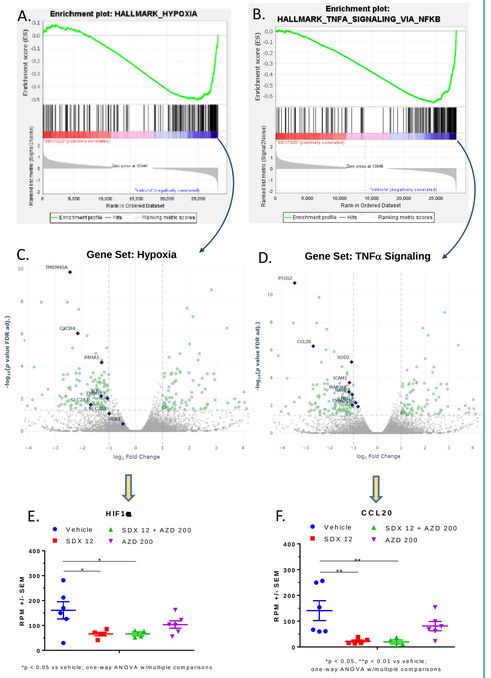
Group:	SDX-7320 (6 mg/kg)	SDX-7320 (12 mg/kg)	SDX (6) + AZD (100)	SDX (6) + AZD (200)	SDX (12) + AZD (100)	SDX (12) + AZD (200)	AZD (100 mg/kg)	AZD (200 mg/kg)
TGI (%)	*61*	*88*	*52*	*72*	*72*	*90*	*28*	*54*
# Below Baseline (%)	*1/7 (14)*	*1/6 (17)*	*1/6 (17)*	*0/7 (0)*	*1/7 (14)*	*3/7 (43)*	*0/7 (0)*	*1/6 (17)*
# of CR	*0*	*0*	*1*	*0*	*0*	*1*	*0*	*0*

AZD = AZD-5363
SDX = SDX-7320
TGI = Tumor growth inhibition
CR = Complete response

Potential synergy for tumor regression

Figure 6. Suppression of Hypoxia- and Inflammation-Related Genes by SDX-7320

Gene Set Enrichment Analysis: Vehicle vs SDX-7320 (12 mg/kg)



*p < 0.05 vs vehicle; one-way ANOVA w/multiple comparisons

*p < 0.05, **p < 0.01 vs vehicle; one-way ANOVA w/multiple comparisons

Figure 7. Decreased Expression of Genes in the Cholesterol Biosynthesis Pathway by SDX-7320

Gene Set Enrichment Analysis: Vehicle vs SDX-7320 (12 mg/kg)

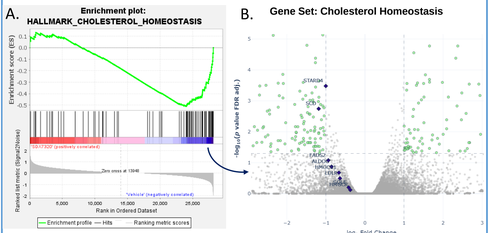
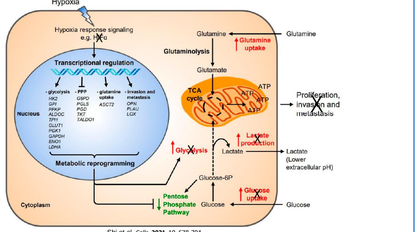


Figure 8. SDX-7320 Inhibits Tumor Responses to Hypoxia



Shi et al, *Cells*, **2021**, 10, 678-704

Summary and Conclusions

- **Pre-treatment with the MetAP2 inhibitor SDX-7320 significantly increased insulin sensitivity in DIO mice and attenuated acute hyperglycemia induced by AZD-5363/capivasertib in normal mice.**
- **SDX-7320 alone (6, 12 mg/kg, Q4D, SC) significantly and dose-dependently inhibited orthotopic Her2+ BT-474 tumor growth, while AZD-5363/capivasertib only inhibited tumor growth at 200 mg/kg.**
- **Adding SDX-7320 (12 mg/kg) to AZD-5363/capivasertib (200 mg/kg) improved the rate of tumor regression (i.e., 3/7 (43%) vs. 1/6 (17%)).**
- **Tumors from SDX-7320-treated mice had a significant reduction in the expression of HIF1α and HIF1α target genes (i.e., GLUT1, HK2, PDK1, P4HA1, SOD2, PTGS2). Other pathways/gene sets significantly improved by SDX-7320 include inflammation/TNFα signaling (e.g., CCL20, IL6, CXCR4), cholesterol homeostasis (e.g., HMGCS1, STARD1, LDLr) and fatty acid synthesis (e.g., SCD, ACSL3, FADS2).**
- **Adding SDX-7320 to the Akt inhibitor AZD-5363/capivasertib improved anti-tumor efficacy and reduced a key, on-target toxicity associated with Akt inhibitors (hyperglycemia), creating a novel and compelling clinical hypothesis for Her2+ breast cancer patients and oncologists.**

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