TOS-358, a first-in-class covalent PI3Kα inhibitor, demonstrates superior efficacy and does not induce significant hyperglycemia at efficacious doses in multiple animal models

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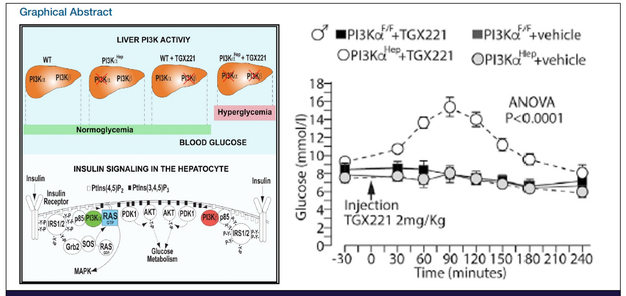
Abstract

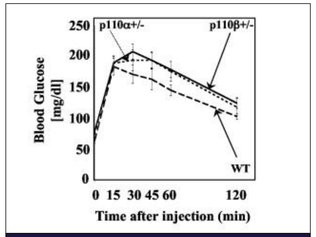
PI3Kα is frequently mutated in a variety of cancer types, and the PI3K-AKT signaling axis also plays a role in insulin signaling and glucose homeostasis. TOS-358 is a highly selective first-in-class covalent inhibitor of PI3Kα and is currently in clinical development in multiple solid malignancies. Interestingly, TOS-358 potently and specifically inhibits PI3Kα deeply and durably, but does not induce significant hyperglycemia in a variety of animal models. TOS-358 has consistently demonstrated superior efficacy compared to reversible PI3Kα inhibitors (ATP-competitive and Allosteric) across 30+ different PDX and CDX mutant PI3Kα dependent cancer models. Detailed metabolic studies also revealed TOS-358 does not induce significant hyperglycemia effects in mice, rats and dogs at efficacious doses, which mirrors previous finding that show that PI3Kα knockout does not induce significant hyperglycemia. Furthermore, we elucidate that previous reversible PI3Kα inhibitors lead to dramatic hyperglycemia due to their potent inhibition of multiple PI3K isoforms at effective concentrations of the molecules in a cellular setting. This data reveals that highly specific and potent covalent inhibition of PI3Kα leads to dramatically superior efficacy and an improved safety profile.

Inhibition of both PI3Kα and PI3Kβ is required to induce significant hyperglycemia

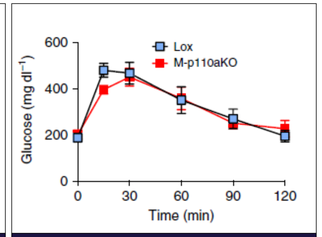
Graphical Abstract



PI3K-alpha liver knockout does not induce significant hyperglycemia.
Dual PI3K-alpha/beta inhibition are required for significant hyperglycemia.[1]



Both PI3Kα and PI3Kβ contribute to increase glucose levels in whole tissue knockouts.[2]



PI3K-alpha muscle knockout does not induce significant hyperglycemia compared to control.[3]

A. Inhibition of both PI3Kα and PI3Kβ activities are necessary to induce significant hyperglycemia (Molinaro *et al.*, 2019). **B.** PI3Kα and PI3Kβ contribute to increased glucose levels in mice (Brachmann et al., 2005). **C.** Selective knockout of PIK3CA in skeletal muscle does not induce systemic glucose intolerance (Ella Li *et al.,* 2020).

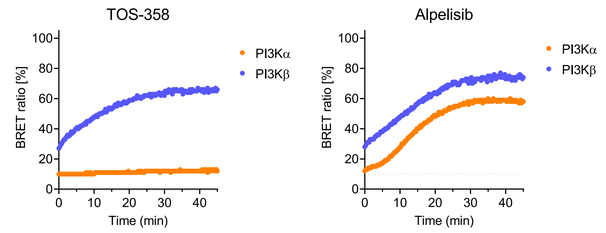
Results

TOS-358 displays a highly unique profile as the only clinical covalent inhibitor of PI3Kα

	PI3Kα-mutant IC_{95}	Average Cellular pAKT inhibition (pAKT/tAKT, n=7, 1uM)	Cellular Anti-Proliferative EC90 (n=10)	Predicted C_{trough} Requirement[d]	PI3Kβ Binding IC_{50}	PI3Kα IC_{50}/ PI3Kβ IC_{50} Ratio	Glucose Uptake Impact at Cellular EC90[e]
TOS-358	120nM[a]	96%	251nM	0nM[d]	2418nM	20X	10%
Alpelisib	3857nM[a]	85%	3585nM	3857nM[a]	10008nM	3X	84%
Pan-Mut Specific ($PI3K\alpha^{PAN}$ inh)	1015nM[b]	61%	6490nM	1015nM[b]	11798nM	10X	72%
H1047X-Mut Specific ($PI3K\alpha^{H1047R}$ inh)	8701nM[b]	-[c]	-[c]	8701nM[b]	-[c]	-[c]	43%

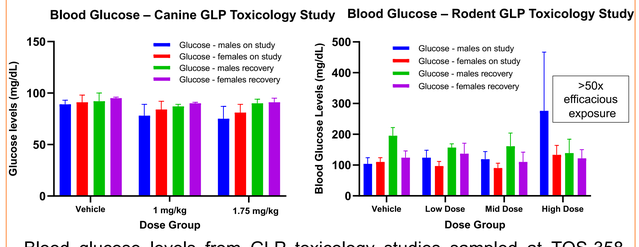
a-IC_{95} measured using the ATP-competitive NanoBRET assay
b-IC_{95} measured using Nanosyn kinase assay.
c-Not measured as molecule can only impact H1047R cell lines and no PI3Kβ activity was measured. 70% of PI3K
d- Predicted C_{trough} minimum concentration in patients for single agent activity and retained PI3Kα IC_{95}. TOS-358 achieves continuous inhibition in absence of drug.
e- Zenbio assay was performed per standard protocol (link).

TOS-358 does not result in significant or sustained inhibition of PI3Kβ



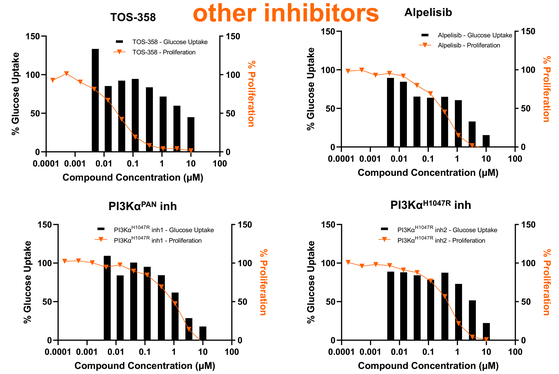
We performed residence time experiment using NanoBRET in Expi293 suspension cells expressing NanoLuc fused to PI3Kα (orange) and PI3Kβ (blue). The cells were incubated with 10 μM concentration of each inhibitor for 24 hours, followed by washing out free inhibitor. After exposure to TOS-358 for 24 hours, the cells expressing PI3Kα did not show a BRET signal, yet the BRET signal restored rapidly in PI3Kβ. The prolonged residence time was not observed in Alpelisib against either PI3Kα or PI3Kβ. The prolonged residence time demonstrated therefore that TOS-358 selectively and irreversibly react with PI3Kα, but not with PI3Kβ.

TOS-358 does not induce significant hyperglycemia in GLP toxicology studies



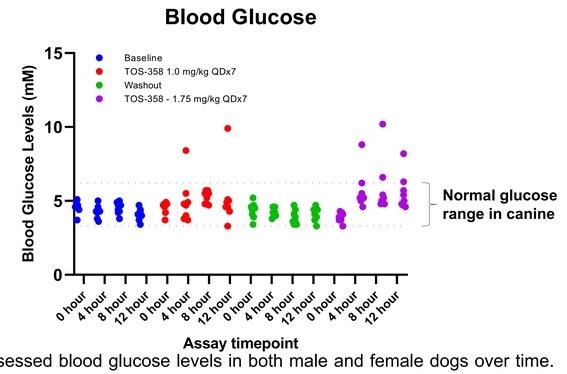
Blood glucose levels from GLP toxicology studies sampled at TOS-358 steady-state. Note: exposure of rodent high dose group is >50x higher than required for efficacy.

TOS-358 uniquely shows no effects on glucose uptake at effective concentrations compared to other inhibitors



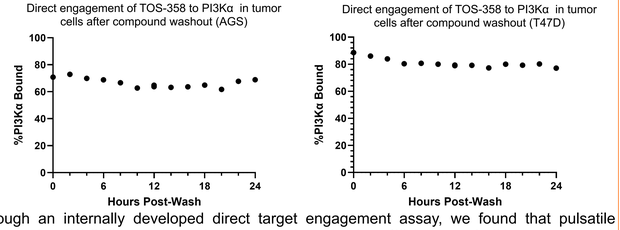
In vitro assessment of glucose uptake was assessed in human adipocytes and performed by Zenbio. Compounds were incubated for 2 hours and the proliferative impact on cancer cells has been overlayed on this data (T47D cells).

TOS-358 *does not* induce hyperglycemia in dogs at exposures for superior efficacy



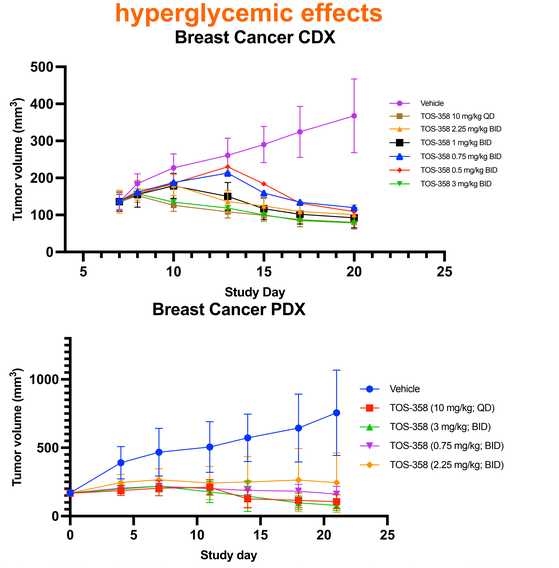
We assessed blood glucose levels in both male and female dogs over time. Glucose levels were measured in samples at baseline, following 7 daily TOS-358 administrations at 1.0 mg/kg, following 7 daily TOS-358 administrations at 1.75 mg/kg, and after a 7 day washout period.

A single pulsatile dose of TOS-358 can achieve sustained inhibition of PI3Kα for over 24 hours



Through an internally developed direct target engagement assay, we found that pulsatile dosing with TOS-358 leads to continuous inhibition of active PI3Kα for up to 24 hours. To note, drug was pre-incubated with cells and then washed away at time 0.

TOS-358 accumulates on PI3Kα over time to induce significant tumor regressions with no hyperglycemic effects



In both the CDX and PDX models of breast cancer, low doses of TOS-358 can yield superior efficacy with no hyperglycemic effects. TOS-358 was found to accumulate on target over time promoting regressions as late as day 13 at a 0.5mg/kg BID dose.

TOS-358 demonstrates superior PI3Kα targeting properties

	Efficacy across PI3Kα-mutant Subtypes	Inhibition of Pathway Feedback	Manageable Hyperglycemia Effect	Low Off-target Potential
Covalent (TOS-358)	✓	✓	✓	✓
Non-covalent (ATP-comp)	✗	✗	✓	✗
Non-covalent (Allosteric)	✗	✗	✓	✓

Conclusions

- TOS-358 is a potent, irreversible inhibitor of PI3Kα
- TOS-358 does not induce significant hyperglycemia at effective doses in mouse, rat, and dog studies.
- TOS-358 demonstrated excellent efficacy at low drug doses due to the ability to covalently accumulate on target.
- Please visit Poster #CT245 on Apr. 18, 2023, 1:30 PM - 5:00 PM for a presentation of our TOS-358 clinical trial.

References

1. Molinaro et al. Insulin-Driven PI3K-AKT Signaling in the Hepatocyte Is Mediated by Redundant PI3Kα and PI3Kβ Activities and Is Promoted by RAS. *Cell Metabolism* 30, 1400 (2019).
2. Brachmann SM, Ueki K, Engelman JA, Kahn RC, Cantley LC. Phosphoinositide 3-kinase catalytic subunit deletion and regulatory subunit deletion have opposite effects on insulin sensitivity in mice. Mol Cell Biol. 2005 Mar;25(5):1596-607.
3. Ella Li et al. Role of p110a subunit of PI3-kinase in skeletal muscle mitochondrial homeostasis and metabolism. *Nature Commun* 10, 3412 (2019)

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