Evaluating Immune Checkpoint Inhibitors for Liver Toxicity in a Biomimetic Liver Microphysiology Model

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BACKGROUND & PURPOSE

- The prominence of biologic drugs has rapidly gained traction and has delivered life-changing therapies for cancer patients
- Some biologics are reported to cause hepatotoxicity, i.e., biologics-induced liver injury (BILI)
- Comparison of drug toxicity between biologics and small molecules is an active field of research
- Two immune checkpoint inhibitor (ICI) biologics, ipilimumab (ipi) and nivolumab (nivo), have demonstrated synergistic effects in cancer immunotherapy at the cost of increased toxicity [1]
- Although immune-mediated hepatic injury by activated T cells is suspected, mechanisms underlying liver-specific toxicity by ICIs remain unknown
- In this study, effects of ipi, nivo, and cabozantinib (cabo, a tyrosine kinase inhibitor that has interplay in immunoregulation) mono- and combination treatments on the liver microenvironment were evaluated in the Liver Acinus MicroPhysiology System (LAMPS) biomimetic model [2,3]

METHODS

- Compounds were administered at doses based on clinical C_{max} ranges for 10 days under continuous perfusion in the 4-cell type LAMPS
- Toxicity signals from the LAMPS were measured using a combination of fluorescence microscopy and immunohistochemistry
- Statistical comparisons of assay outputs were completed using regression-based and non-parametric tests comparing treated groups to untreated groups
- Significant findings are reported as mean percentages relative to the untreated group

RESULTS

		Nivolumab		Ipilimumab		Nivo + Ipi	
Biomarker Measured	Day	0.920 µM	3.30 µM	0.557 µM	2.06 µM	0.920 µM Nivo 0.557 µM Ipi	3.30 µM Nivo 0.557 µM Ipi
HMGB1	3	—	·	*	***, †	—	†
Lactate Dehydrogenase	1	·	—	—	—	*	**, †
	3	—	—	—	·	—	—
	5	—	—	—	—	—	*, †
	7	—	—	·	—	***, ·	***, ·
	9	—	—	*	**, †	**	**, †
ROS	10	***, †††	***, †††	—	—	***, †††	***, †††
Bile Efflux	10	—	—	—	**, ††	***, ††	***, †††
Mitochondrial Function	2	NA	NA	***, †††	*	—	**, ††

Table 1: Summary of LAMPS data for ipilimumab, nivolumab, and co-administered ipilimumab and nivolumab. Significance levels of Bonferroni-adjusted p values are denoted by asterisks (regression: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$) and obelisks (Dunn's test: † $p < 0.05$, †† $p < 0.01$, and ††† $p < 0.001$). Pink and red represent significant decrease in one test and two tests, respectively. Light and dark green represent significant increase in one test and two tests, respectively. Purple represents p-value between 0.05 and 0.1. NA: not available.

- **Co-administration of nivo and ipi led to significant increases in LDH compared to monotreatment, concordant with clinical reports**
- **Intrinsic hepatocyte stress signals were observed with nivo monotreatment (↑ROS), ipi monotreatment (↓bile efflux, ↓mitochondrial function), and co-administration (↑ROS, ↓bile efflux, ↓mitochondrial function)**
- **HMGB1, a damage-associated molecular pattern (DAMP), increase may indicate the compound(s) is directly or indirectly driving an inflammatory response**

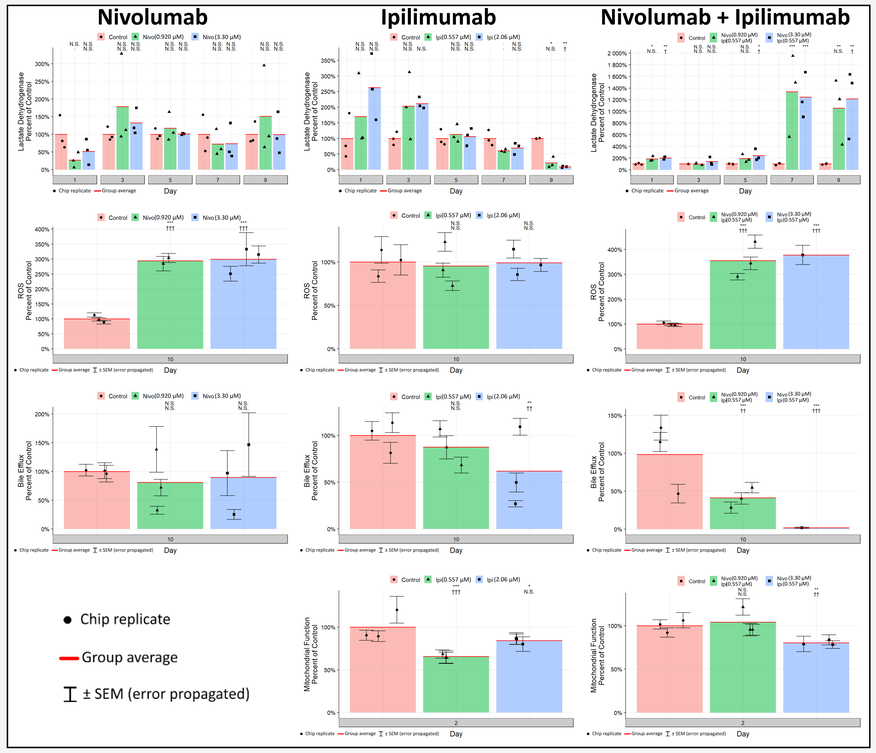

Fig. 1: Replicate data and summary statistics for LAMPS outputs of LDH (top row), day 10 ROS (second row), day 10 bile efflux (third row), and day 2 mitochondrial function (fourth row) from experiments with nivolumab (left column), ipilimumab (middle column), and co-administered nivolumab with ipilimumab (right column). LAMPS data for mitochondrial function during nivolumab administration in-progress.

		Cabozantinib		Cabo + Nivo	
Biomarker Measured	Day	1.20 µM	4.39 µM	1.20 µM Cabo 2.92 µM Nivo	4.39 µM Cabo 2.92 µM Nivo
HMGB1	3	***, †	**	—	—
Lactate Dehydrogenase	1	—	†	**, †	—
	3	***, †	·	†	—
	5	**, †	—	**, †	·
	7	†	—	—	—
	9	·, †	—	—	—
ROS	10	—	—	—	—
Bile Efflux	10	—	—	—	—
Mitochondrial Function	2	—	—	***, †††	*, †

Table 2: Summary of LAMPS data for cabozantinib and co-administered cabozantinib and nivolumab.

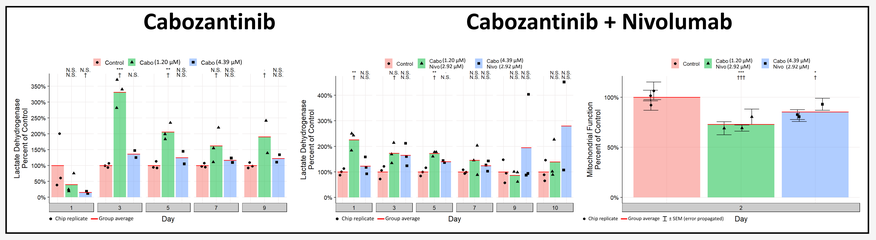

Fig. 2: Replicate data and summary statistics for LAMPS outputs of LDH during cabo administration (left), LDH during cabo+nivo administration (middle), and day 2 mitochondrial function during cabo+nivo administration (right).

- **Cabo alone did not significantly affect bile efflux, ROS, or mitochondrial function, suggesting that there may be an alternate mechanism of injury during cabo administration that is not captured in the current LAMPS outputs, to account for the increase in LDH and HMGB1**
- **Co-administration of cabo and nivo significantly decreased mitochondrial function, but significantly increased LDH only at the low dose cabo; further studies are in-progress**

CONCLUSION

- These findings demonstrate the capacity for ipi and nivo to induce intrinsic hepatocyte stress signals in a well-established biomimetic model of the liver, that may contribute to liver-specific adaptive immune responses
- Mechanisms of cabo-mediated hepatotoxicity warrants further investigation
- The Microphysiology Systems Database will manage, archive, and disseminate the meta, raw, and analytical data including experimental reproducibility analysis and BILI predictive outcomes in our ongoing studies
- In addition, the results from this study will be used in the novel quantitative systems toxicology platform, BIOLOGXsym™, to predict BILI in populations by combining clinically relevant drug exposure predicted by physiologically-based pharmacokinetic modeling and mechanistic representation of liver responses [4]
- This integrated approach may set the stage for more efficient development of novel biologics for cancer immunotherapies

REFERENCES

[1] Peeraphatdit et al. (2020) Hepatology. 72:315-329.
[2] Vernetti et al. (2016) Exp Biol Med. 241:101-114.
[3] Lee-Montiel et al. (2017) Exp Biol Med. 242:1617-1632.
[4] Beaudoin et al. (2023) Int. J. Mol. Sci. 24(11):9692.

ACKNOWLEDGMENTS

Supported by National Institute of Health R44TR003535.

CONFLICTS OF INTEREST

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