#6293

GLYCOGEN SYNTHASE KINASE-3 BETA (GSK-3β) GENOMIC ALTERATIONS AND INCREASED PROGRAMMED DEATH-LIGAND 1 (PD-L1) EXPRESSION IN ADVANCED MALIGNANCIES

Brittany A. Borden[1], Joanne Xiu[2], Yasmine Baca[2], Pilar Ramos[2], Francis J. Giles[3], Andrew Mazar[4], Fabio Tavora[1,5], Howard P. Safran[1,5], Wafik S. El-Deiry[1,5], Benedito A. Carneiro[1,5]

[1]The Warren Alpert Medical School of Brown University, Providence, RI; [2]Caris Life Sciences, Phoenix, AZ; [3]Developmental Therapeutics Consortium, Chicago, IL; [4]Monopar Therapeutics, Wilmette, IL; [5]Lifespan Cancer Institute, Providence, RI

BACKGROUND

- Glycogen Synthase Kinase-3 beta (GSK-3β) is a serine/threonine kinase with regulatory activity in numerous diseases and implicated in both innate and adaptive immune responses [1,2]
- GSK-3β is involved in the pathogenesis of several malignancies [3,4,5]
- GSK-3β phosphorylates target pro-oncogenes (C-Jun and C-myc), as well as non-glycosylated forms of PD-L1 leading to its proteasome degradation [6]
- GSK-3β inhibitors have advanced to clinical trials in refractory malignancies [7]
- Genomic alterations in *GSK-3β* have been described, yet a comprehensive analysis of these alterations is lacking [8]

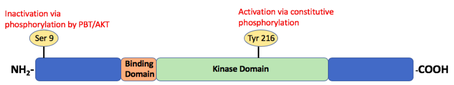

Figure 1. *GSK-3β* gene schematic.

METHODS

- Publicly-available tumor genomic data was accessed using cBioPortal
- All tumor samples with a *GSK-3β* alteration were included for analysis
  - For each tumor, histology and *GSK-3β* residue change were assessed
- A second dataset was obtained from Caris Life Sciences Precision Oncology Alliance (CLSPOA)
- In *GSK-3β* mutated tumors, Microenvironment Cell Population (MCP)-counter was used to quantify immune and stromal cell populations
  - Median MCP values were compared across cancer types using Wilcoxon/Kruskal-Wallis tests
- *GSK-3β* expression data was obtained from the CLSPOA database
  - Median transcripts per million (TPM) were compared across cancer types using Wilcoxon/Kruskal-Wallis tests
- PD-L1 expression was assessed via SP-142 antibody, using a cutoff of 5%
  - Chi-square test was used to assess significance between *GSK-3β* mutated tumors and *GSK-3β* wild type tumors

CHARACTERIZATION OF ALTERATIONS

- **cBioPortal: Of 46,237 tumor samples, 430 (1%) tumors had *GSK-3β* alterations**
  - 227 tumors had mutations (183 unique mutations)
  - 217 had copy number alterations
  - 58% of mutations located in the kinase domain
  - Two of the top mutated loci comprise a binding pocket for GSK-3β substrate
- **CLSPOA: Of 73,324 tumor samples, 819 (1%) had *GSK-3β* mutations**

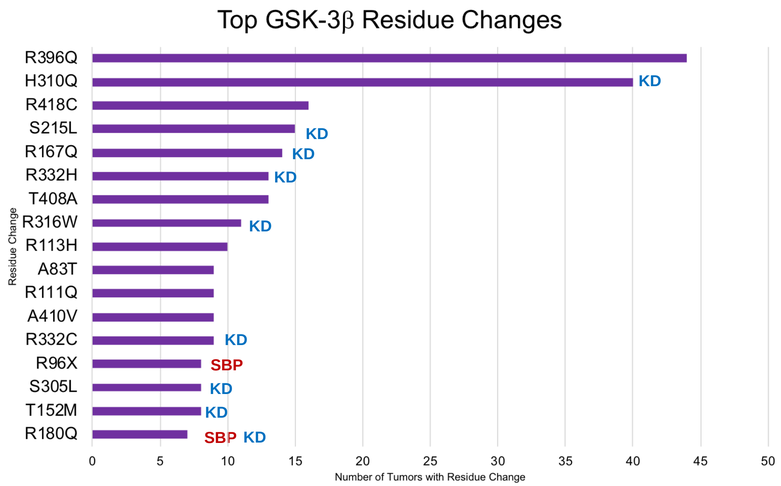

Figure 2. Top *GSK-3β* residue changes (combined cBioPortal and CLSPOA data). **KD: kinase domain; SBP: substrate binding pocket**

TOP HISTOLOGIES WITH *GSK-3β* MUTATIONS

Histology	GSK3B mutant	Total samples for given histology	Percent mutated
Non-Melanoma Skin Cancer	17	618	2.8%
Uterine Neoplasms	171	6564	2.6%
Melanoma	70	3205	2.2%
Non-Small Cell Lung Cancer	203	16590	1.2%
Cervical Squamous Cell Carcinoma	17	1480	1.1%
Bladder Cancer	29	2528	1.1%
Colorectal Adenocarcinoma	141	12424	1.1%
Prostatic Adenocarcinoma	33	3367	1.0%
Head and Neck Cancer	13	1736	0.7%
Small Cell Lung Cancer	7	960	0.7%
Breast Carcinoma	58	8179	0.7%
Esophageal Cancer	17	2492	0.7%
Serous Ovarian Carcinoma	60	10001	0.6%
Pancreatic Cancer	21	4564	0.5%
Renal Cancer	8	1947	0.4%
Hepatocellular Carcinoma	4	1335	0.3%

Table 1. Top histologies with *GSK-3β* mutations. Histologies were included if present in both cBioPortal and Caris. Combined cBioPortal and Caris cohorts reveal non-melanoma skin cancer, uterine neoplasms, and melanoma as top mutated histologies. **The subtype of uterine endometrioid carcinoma was mutated at a rate of 4%.**

GSK-3β EXPRESSION ACROSS CANCER TYPES

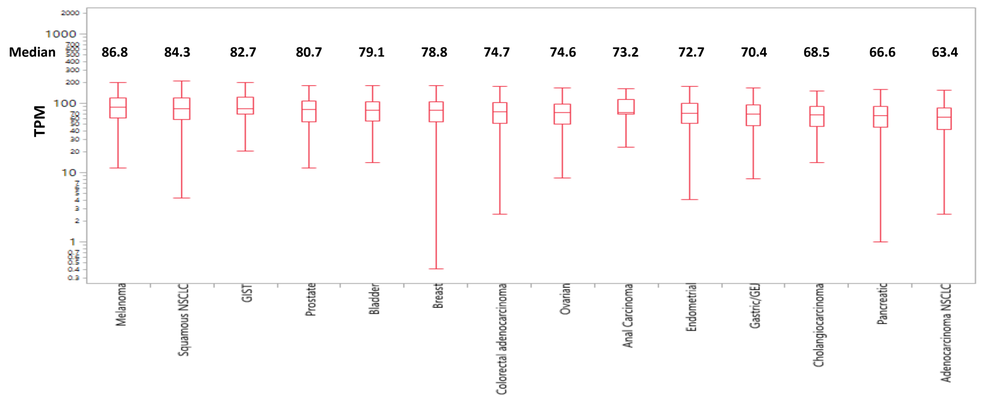

Figure 3. GSK-3β expression by RNA-Seq across tumor types using CLSPOA data. A statistically significant difference of expression among multiple histologies was observed (**p<0.0001**). Notably, when comparing squamous cell and adenocarcinoma subtypes of NSCLC, a significant difference in TPM values was also observed (**p<0.0001**).

GSK-3β MUTATIONS ARE ASSOCIATED WITH HIGHER FREQUENCY OF PD-L1 EXPRESSION

Histology	Frequency of PD-L1 Positive Tumors (GSK3B Wild Type)	Frequency of PD-L1 Positive Tumors (GSK3B Mutant)	P-value
Colorectal Adenocarcinoma	3.5% (330/9437)	8.1% (8/99)	0.02
Endometrial Cancer	6.7% (369/5541)	11.2% (14/125)	0.05
Melanoma	22.5% (319/1417)	41.9% (13/31)	0.01
Ovarian Surface Epithelial Carcinoma	7.0% (638/9087)	19.6% (11/56)	0.001
Uterine Sarcoma	7.5% (57/756)	40.0% (4/10)	0.005

Table 2. Differences in PD-L1 expression between GSK-3β mutated tumors and GSK-3β wild-type tumors were assessed using the CLSPOA database. 38 total histologies were assessed, and those with significant results are shown in the table.

GSK-3β MUTATED TUMORS DISPLAY INCREASED B CELL INFILTRATION IN THEIR MICROENVIRONMENT

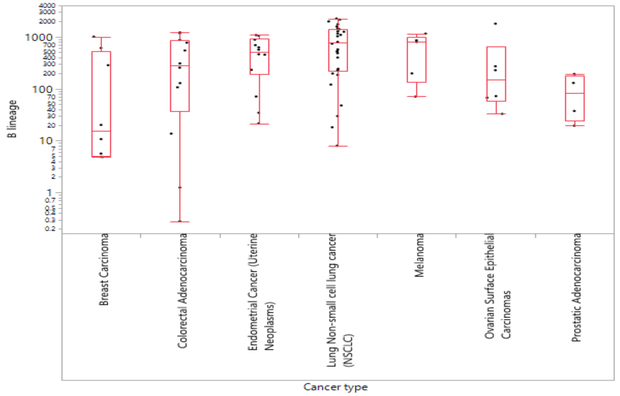

Figure 4 Cell populations in the microenvionment calculated by MCP counter for immune and stromal cell populations in GSK-3β mutated tumors were analyzed across tumor types. The distribution was statistically significant for B cells (shown; **p=0.018**), in addition to monocytes (**p=0.002**), dendritic cells (**p=0.005**), neutrophils (**p=0.0003**), and endothelial cells (**p=0.014**). The highest MCP counts were observed in **melanoma** for B cells, monocytes, dendritic cells, and endothelial cells. **Of note, no significant difference was observed for T cells.**

CONCLUSIONS

- Top *GSK-3β* mutated residues are often part of relevant binding pockets or kinase domain
- The most commonly mutated histologies include uterine neoplasms, non-melanoma skin cancers, and melanoma
- *GSK-3β* is differentially expressed across cancer types, with highest expression seen in melanoma
- In *GSK-3β* mutated melanoma, B cells, monocytes, dendritic cells and endothelial cells are significantly higher than other GSK-3β mutated tumors
- *GSK3β* mutations were associated with a higher frequency of PD-L1 expression in selected tumors

REFERENCES

1. Reddy, P.H. *BBA* 2013;1832(12): 1913-1921.
2. Beurel, E at al. *Trends Immunol* 2010; 31(1): 24-31.
3. Domoto, T. et al. *Cancer Sci.* 2016;107(10):1363-72.
4. Walz, A et al. *Clin Cancer Res* 2017;23:1891-1897.
5. Sahin I et al. *Cancer Biol Ther* 2019;12:1-10.
6. Li, CW et al. *Nat Commun* 2016; 7:12632
7. Carneiro, BA et al. *J Clin Oncol* 2020: 38 (suppl; abstr 3507)
8. Weinstein, IN et al. *Nat Genet* 2013; 45(10):1113-20..