CPD Evidence 4 (2022)

1. Registrant Information

1.1 Full Name: Chen Liu, AMRSB
1.2 Profession: BMS
1.3 Registration Number: BS075665
1.4 CPD type: Formal and educational – Research
1.5 Date of completion: 20/06/2022
1.6 Standard(s) met:
Standard 1 – A registrant must maintain a continuous, up-to-date and accurate record of their CPD activity
Standard 4 – A registrant must seek to ensure that their CPD benefits to the service user

2. Details

Treating mice B16 melanoma with GSK-3 small molecule inhibitor

Background:

Glycogen synthase kinase 3 (GSK-3) is a ubiquitously expressed serine/threonine kinase, which plays essential roles in regulating cellular activities, including apoptosis (1), T cell activation (2), epithelia-mesenchymal transition (3). In the field of cancer immunotherapy, the interest in studying its negative regulatory effect has increased in the past decade. Previous study has shown that GSK-3 negatively mediates the DNA binding activity of nuclear factor of activated T cells 2 (NFAT2) and phosphorylation of Cbl-b, a negative regulator of T cell activation (4, 5). Our lab previously demonstrated that, the inhibition of GSK-3β via small molecule could leads to promising results in molestation of the growth of B16 and EL-4 tumour in mice models at a transcription level, via the upregulation of T-bet (6).

Methods:

<u>Tumour preparation and implantation</u>

Five groups in this experiment according to dosages: non-treatment control, 400μg (SB400), 800μg (SB800), 400μg packed by nanoparticle (NP400) and 800μg packed by nanoparticle (NP800). B16 melanoma cells were digested with trypsin, washed with 1XPBS and raw DMEM medium, at the speed of 1000rpm. Cells were passed through a 70μm strainer to purify.

Mice were anaesthetised with isoflurane inhalation prior to the implantation. Each mouse received ~4 million tumour cells via the subcutaneous route.

Drug preparation, treatment and monitoring

GSK-3 SMI (SB415286, SB) was diluted with PBS, to 400 μg/ml or 800 μg/ml. Injection started from day 4 post implantation. Tumour size was monitored prior each injection.

Results:

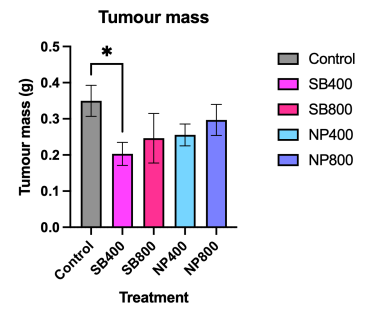
Treating mice B16 melanoma with GSK-3 small molecule inhibitor SB415286

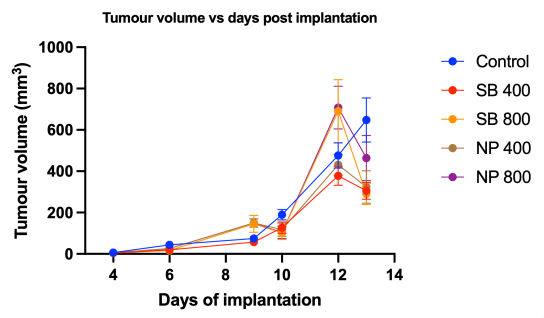
Hien, Chen

Experiment 1

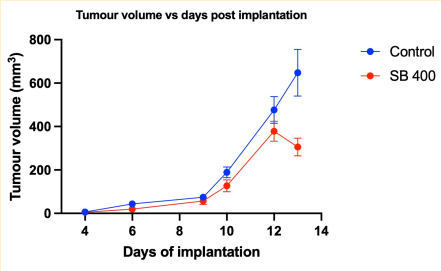
24/05/2022 – 02/06/2022

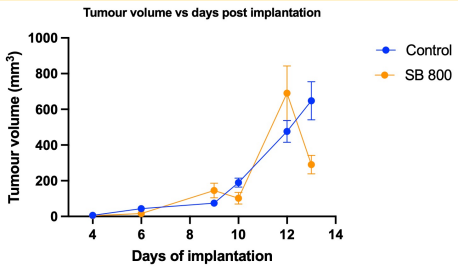
Experiment overview

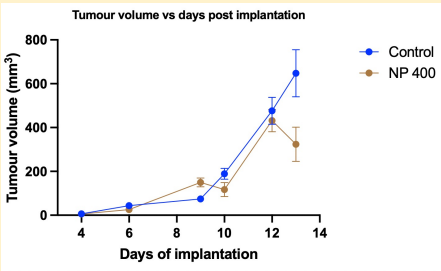


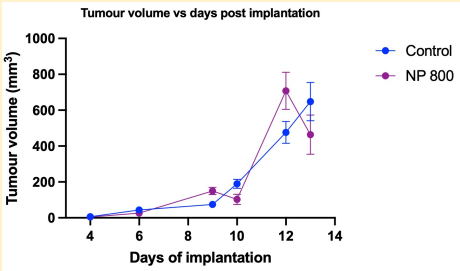


Tumour volume

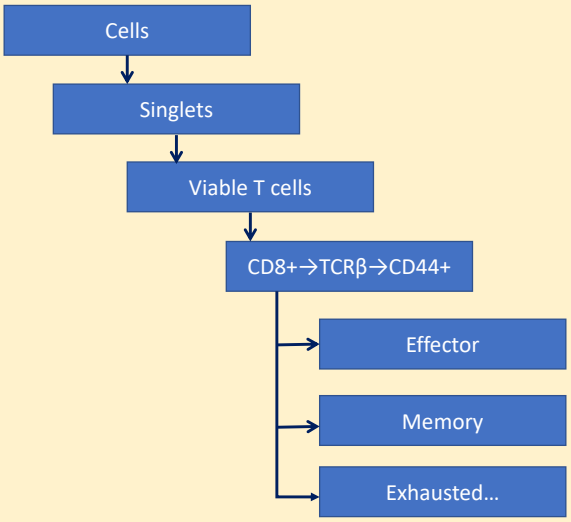




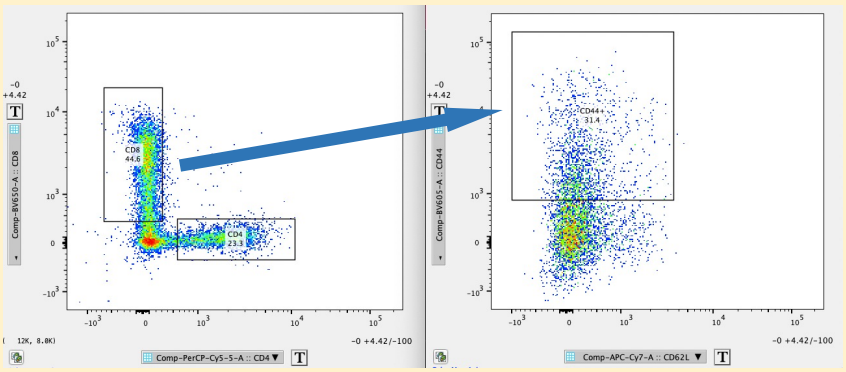




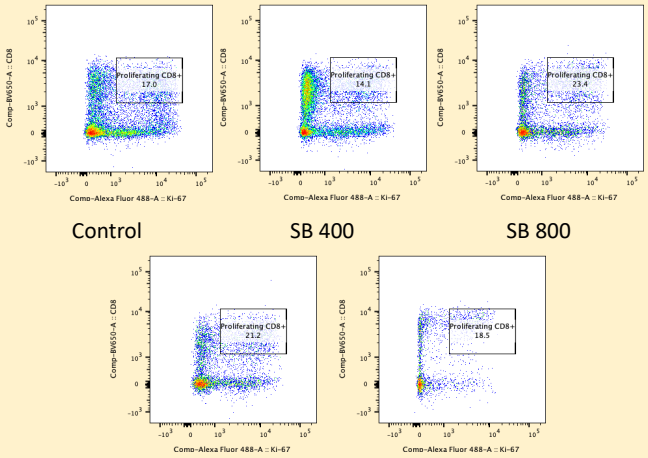
Gating strategy



Gating for CD8+ memory/terminal effectors

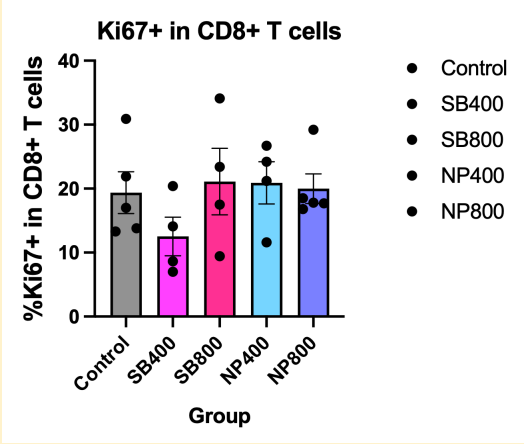


SB415286 has no significant effect on CD8+ T cell proliferation



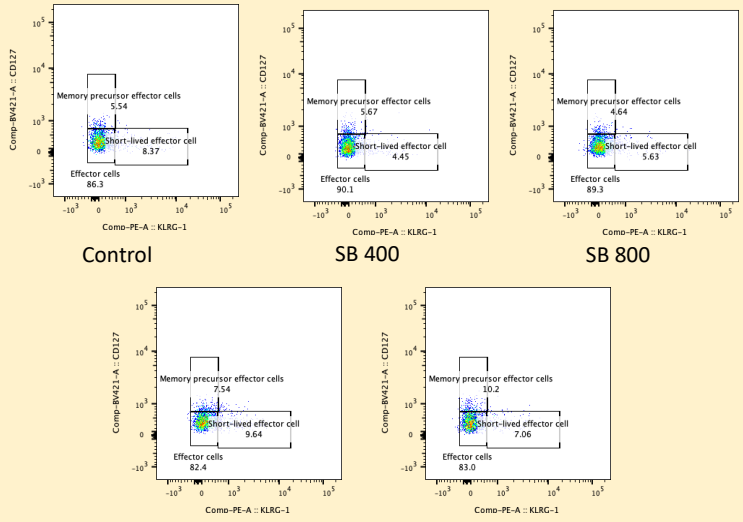
Control SB 400 SB 800

NP 400 NP 800



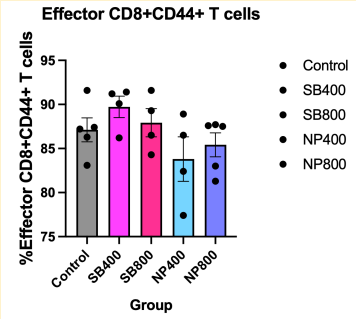
CD101, tox Tim3, PD-1, CD244

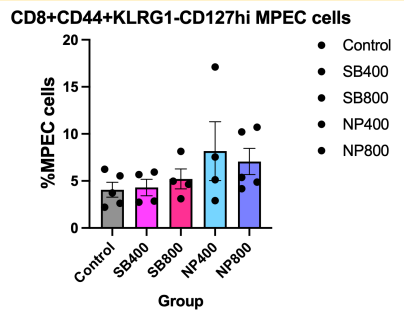
Effector, MPEC/SLEC fate decision

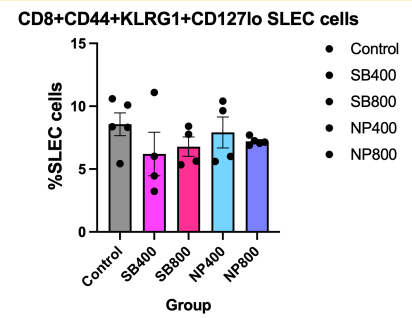


Control SB 400 SB 800

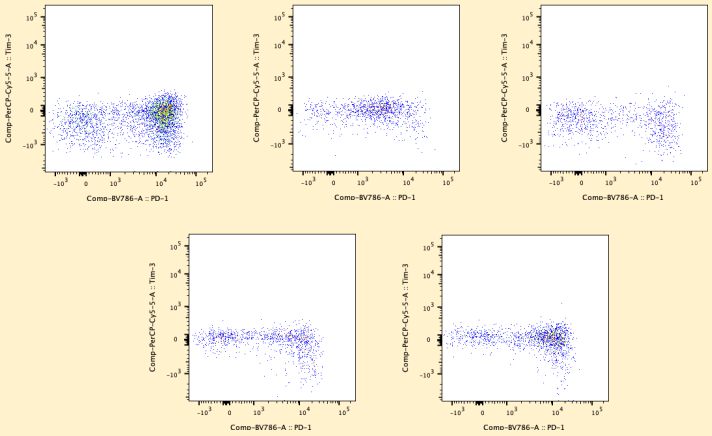
NP 400 NP 800





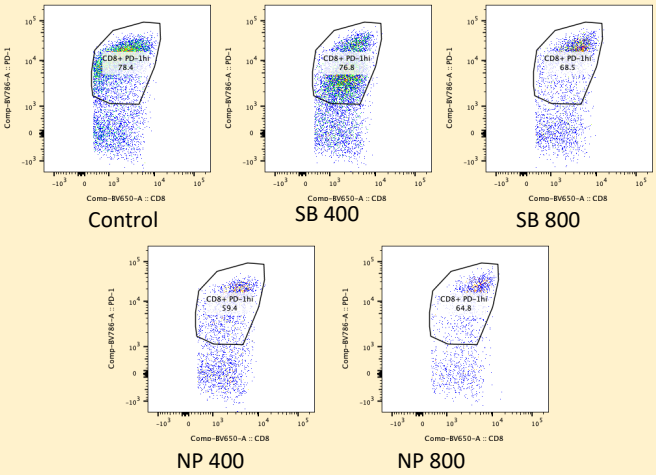


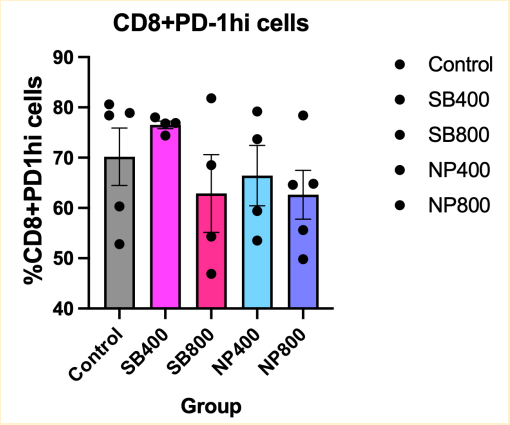
T cell exhaustion



Quality of dye?

SB415286 has no significant effect on attenuating cell exhaustion





Reference:

1. Beurel E, Jope RS. The paradoxical pro- and anti-apoptotic actions of GSK3 in the intrinsic and extrinsic apoptosis signaling pathways. Progress in Neurobiology. 2006;79(4):173-89.
2. Taylor A, Rudd CE. Glycogen synthase kinase 3 (GSK-3) controls T-cell motility and interactions with antigen presenting cells. BMC Res Notes. 2020;13(1):163-.
3. Kao SH, Wang WL, Chen CY, Chang YL, Wu YY, Wang YT, et al. GSK3β controls epithelial–mesenchymal transition and tumor metastasis by CHIP-mediated degradation of Slug. Oncogene. 2014;33(24):3172-82.
4. Neal JW, Clipstone NA. Glycogen Synthase Kinase-3 Inhibits the DNA Binding Activity of NFATc *. Journal of Biological Chemistry. 2001;276(5):3666-73.
5. Tran CW, Saibil SD, Le Bihan T, Hamilton SR, Lang KS, You H, et al. Glycogen Synthase Kinase-3 Modulates Cbl-b and Constrains T Cell Activation. J Immunol. 2017;199(12):4056-65.
6. Rudd CE, Chanthong K, Taylor A. Small Molecule Inhibition of GSK-3 Specifically Inhibits the Transcription of Inhibitory Co-receptor LAG-3 for Enhanced Anti-tumor Immunity. Cell Rep. 2020;30(7):2075-82.e4.