Succinate based biomaterials modify immune system and reduce tumour growth in aging immunocompetent mice

Sahil Inamdar[1], Joslyn L. Mangal[1], Abhirami Suresh[1], Nathan Ng[1], Alison Sundem[1], Xiaojin Shi[2], Marion Curtis[2], Haiwei Gu[3], Abhinav P. Acharya[1]

[1]Arizona State University, Tempe, AZ, [2]Mayo Clinic, Phoenix, AZ, [3]Florida International University

Introduction: Melanoma is most frequently diagnosed in the aging population (median age: 65) where the T cell population is severely reduced and has diminished ability to protect against cancer.[55, 56] Interestingly, although innate immune cells are also functionally defective in this system, their overall population is not severely diminished, and thus may be functionally modified to become effective in reducing tumour growth. In this study, aged mice (>18 months) were injected with YUMM1.1 ($BRAF^{V600E}$ mutated, mimics human melanoma mutation) murine melanoma cells along with PES MPs to determine the response of innate immune cells on tumour growth. To avoid trafficking of PES MPs by phagocytic cells away from the tumour site, >20 µm PES MPs were utilized.

Methods: Metabolite-based polymeric particles were generated by oil in water emulsions. These particles were characterized using dynamic light scattering (DLS), scanning electron microscopy (SEM), nuclear magnetic resonance (NMR) and release kinetics. Flow cytometry and enzyme-linked immunosorbent assay (ELISA) determined the modulation of DCs and adaptive immunity by microparticles *in vitro*. Female C57BL/6j mice, >18 months old and age-matched, were used for the aging mice study. For inoculation, YUMM1.1 cells were counted and resuspended in either (i) sterile PBS, (ii) sterile PBS containing 1 mg/100 µL (>20 µm) PLGA MPs or (iii) sterile PBS containing 1 mg/100 µL (>20 µm) PES MPs to obtain a solution of $7.5x10^6$ cells/mL. Finally, each mouse was s.c. injected on either side with either (i) and (iii) or (ii) and (iii). Mice were intraperitoneally injected with 20 mg/kg PLX4720 (unless otherwise mentioned).

Results: Succinate-based polymers were synthesized and subsequently formed into polymeric-microparticle (>20 µm). Notably, a significant decrease in the pro- to anti-inflammatory Mϕs phenotype ($CD80^+C86^+$ of F4/80 to $CD206^+CD163^+$ of F4/80) was observed in with PLX4720 treated Mϕs as compared to untreated Mϕs. Moreover, a significant increase in the pro- to anti-inflammatory ratio of Mϕs phenotype was observed when Mϕs were treated with PES MPs along with PLX4720. After administration of the formulations *in vivo,* significant differences in the tumours were observed from day 28 to 33 in tumours injected with PES MPs as compared to untreated and PLGA MPs treated tumours indicating that PES MPs by themselves are able to reduce tumour growth. Interestingly, tumours treated with PLX4720 along with PES MPs were significantly smaller as compared to PLX4720 only treated tumours further confirming that the presence of PES MPs in the tumour microenvironment (TME) has an effect on tumour growth

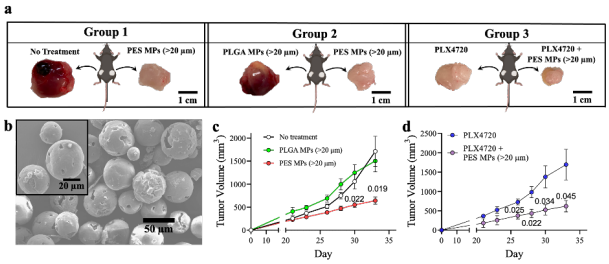

Figure 1: PES MPs (>20 µm) reduce tumour growth in aging immunocompetent mice. a, Schematic of the three treatment groups; Group I – left: No treatment, right: PES MPs (>20 µm); Group II – left: PLGA MPs (>20 µm), right: PES MPs (>20 µm); Group III – left: PLX4720, right: PLX4720 + PES MPs (>20 µm). **b,** SEM images indicate spherical PES MPs >20 µm in size. **c,** Tumours treated with PES MPs (>20 µm) grew significantly slower from than untreated or PLGA MPs (>20 µm) treated from day 30 onwards (n=3 for no treatment and PLGA MPs (>20 µm); n=6 for PES MPs (>20 µm)). **d,** Tumours treated with PES MPs (>20 µm) along with PLX4720 grew slower as compared to PLX4720 treated tumours. A significant reduction in tumours was seen from day 26 onwards in PLX4720 + PES MPs (>20 µm) treated tumours (n=4). Data depicted as average ± std error.

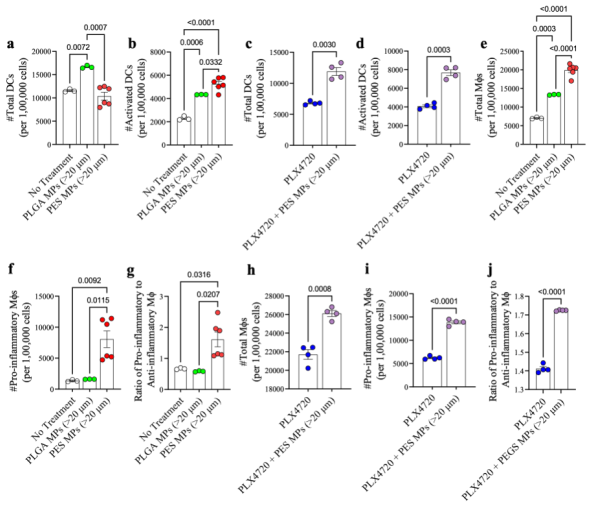

Figure 7: PES MPs (>20 µm) generate innate and adaptive pro-inflammatory responses in aging immunocompetent mice. a,b,c,d, Although significantly higher number of DCs were observed in PLGA MPs treated tumours as compared to PES MPs treated or untreated tumours **(a)**, significantly higher number of activated DCs **(b)** (n=3 for no treatment and PLGA MPs (>20 µm); n=6 for PES MPs (>20 µm). Similarly, significant increase in the number of DCs **(c)** and activated DCs **(d)** per 1,00,000 cells in the TME were observed in mice treated with PLX4720 + PES MPs (>20 µm) as compared to PLX4720 only treated tumours. **e,f,g,h,i,j,** A significant increase in the total Mϕs **(e)** and activated Mϕs **(f)** along with a significantly higher ratio of pro-inflammatory to anti-inflammatory Mϕs **(g)** was observed in tumours treated with PES MPs (>20 µm) as compared to other treatment groups. Similarly, significant increase in the number of Mϕs **(h)** and pro-inflammatory Mϕs **(i)** per 1,00,000 cells in the TME were observed in mice treated with PLX4720 + PES MPs (>20 µm) as compared to PLX4720 only treated tumour. Impressively, there was a significant (~1.3-fold) difference in the ratio of pro-inflammatory to anti-inflammatory Mϕs **(j)** in mice treated with PLX4720 + PES MPs (>20 µm) as compared to PLX4720 only treated tumour (n=4). Data depicted as average ± std error.

Conclusion: Succinate based biomaterials generate a robust anti-tumour response, necessary for melanoma treatment in aging immuno-defective mice.