Investigating the role of platelets in cancer patients undergoing treatment with PD-1/PD-L 1 inhibitors

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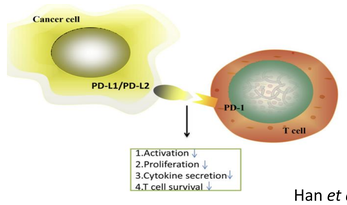
NDSU PHARMACEUTICAL SCIENCES

Introduction

- Programmed cell death protein 1 (PD-1) is a cell surface receptor present on T lymphocytes that once bound to its ligands (PD-L1) negatively regulates immune cell functions.
- Cancer cells that acquire PD-L1 expression become resistant to cancer immune surveillance and clearance.
- PD-1/L1 inhibitors block this interaction between T lymphocytes and the tumor and tumor microenvironment with significant anti-tumor efficacy.



Han *et al.*, 2020

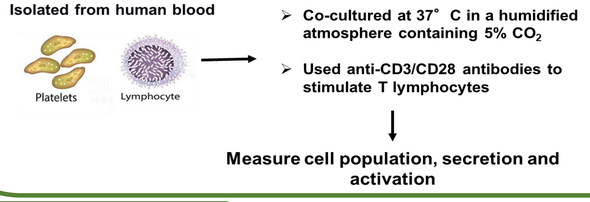
- Platelets are also a significant source of PD-L1 during cancer. However, the role of platelets in cancer growth through the PD-1/PD-L1 signaling pathway is still largely unknown.

Aim

The aim of the project is to investigate whether targeting the PD-1/PD-L1 pathway prevents cancer growth by altering platelets functions and interaction with the immune system

Method

1. **Whole Blood samples:** were collected in 3.2% sodium citrate from cancer patients before (pre-therapy) and after 3 weeks of immuno-therapy (post-therapy).
2. **Whole blood/Optical Lumi-Aggregometer:** to measure platelet aggregation and secretion in blood samples
3. **Flow cytometry:** to measure the Percentage of specific T cell populations and p-selectin surface expression on platelets
4. **Hemavet® Multispecies Hematology System:** to count blood cell populations
5. **Platelet and PBMC co-culture**



Summary

So far, our data show that 1) platelets are highly responsive in cancer patients and 2) the 3-week treatment with PD-1/L1 inhibitor has shown a trend of decrease in platelet aggregation and interaction with T cells.

Results

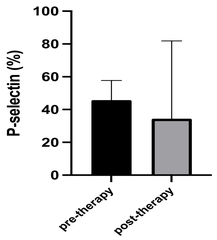


Figure 1. P-selectin expression on platelets in cancer patients before and after immunotherapy. Blood samples were collected from cancer patients before (pre-therapy, n=3) and after 3 weeks of immuno-therapy (post-therapy, n=2). P-selectin surface expression was measured on the platelet surface in the whole blood samples using flow cytometry. P-selectin expression was not observed in the healthy control.

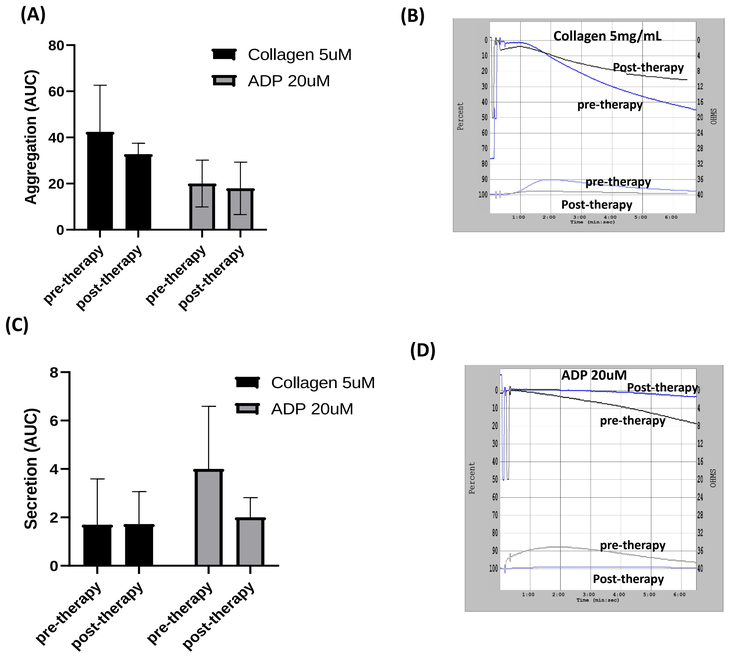


Figure 2. Aggregation and secretion in the whole blood of cancer patients before and after immune therapy. Aggregation (**A**) and secretion (**C**) were measured using a Whole blood/Optical Lumi-aggregometer in blood samples of cancer patients. Aggregation and secretion were induced using Collagen (5μg/mL), **B**) or ADP (20μM, **D**). Samples were collected before (pre-therapy) and after the 3-week treatment (post-therapy) (n=3)

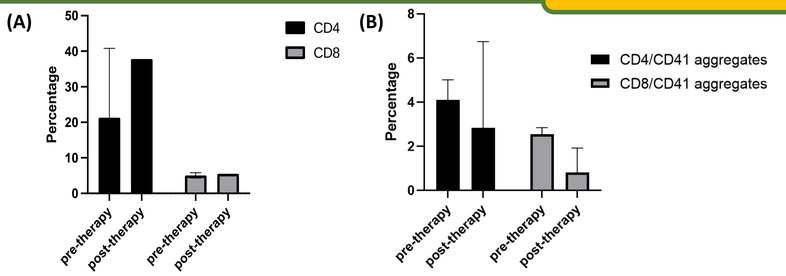


Figure 3. CD4⁺ and CD8⁺ T cell populations and circulating platelet/T cell aggregates in blood samples of cancer patients before and after immunotherapy. Whole blood samples were collected from cancer patients before (pre-therapy) and after 3 weeks of immuno-therapy (post-therapy). CD4⁺ and CD8⁺ T cell populations and platelet/T cell aggregates were analyzed through flow cytometry (n=2).

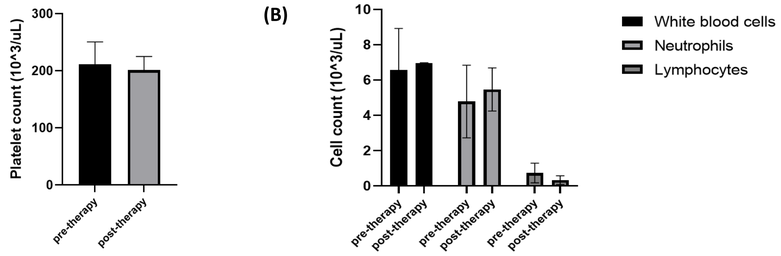


Figure 4. Platelet and white blood cell counts in samples of cancer patients before and after immunotherapy. Cell counts were measured using Hemavet Multispecies Hematology System. Graphs show counts of platelets (A), white blood cells (B), lymphocytes (B) and neutrophil (B) in blood samples from cancer patients before (pre-therapy) and after 3 weeks of immuno-therapy (post-therapy). Values are expressed as 1x10³cells/μL, mean ± S.E.M. n=2.

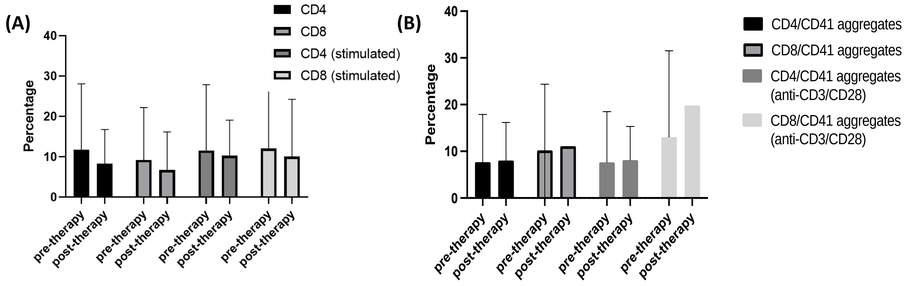


Figure 5. CD4⁺ and CD8⁺ T cell populations and platelet/T cell aggregates in patient platelets and PBMCs co-culture. Platelets and PBMC were isolated from cancer patients and co-cultured for 48 hours. T cells were stimulated using anti-CD3 and anti-CD28 antibodies. CD4⁺ and CD8⁺ T cell populations and platelet/T cell aggregates were analyzed through flow cytometry (n=2).