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The synergistic anticancer effect of PLAG on the PD-1 immune-checkpoint inhibitor treatment in the LLC-1 syngeneic model

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ABSTRACT

Background: Although immune checkpoint inhibitor (ICI) therapy usage has been increasing for various indications, some patients of various types of cancer were shown to not respond to ICI. To improve ICI response rate, a combination therapy targeting additional mechanisms to prevent tumor immune evasion by modulating the tumor microenvironment may be needed

Methods: To investigate the enhanced anti-tumor effect of the anti-PD-1 antibody (aPD-1) with the addition of 1-palmitoyl-2-linoleoyk-3-acetyl-rac-glycerol (PLAG), the syngeneic model was used (n=6/group), LLC-1 lung carcinoma was implanted into C57BL/6 mice subcutaneously. PLAG was daily administrated for 4 weeks with or without aPD-1 (RMP1-14). aPD-1 was delivered via IP injection once a week. The degree of infiltrated lymphocyte population and neutrophils in the tumor and blood on the sacrificed day were analyzed.

Results: In PLAG treated (50 and 100 mpk) mice group, the tumor burden was significantly reduced compared to a positive control ($p < 0.05$). In the group treated with aPD-1 alone, the tumor growth decreased by about 65% compared to the positive control. However, in mice co-treated with PLAG, the tumor was significantly reduced (18%) compared to the aPD-1 alone. The neutrophil-to-lymphocyte ratio levels in the group co-treated with PLAG were decreased remarkably compared to the aPD-1 alone. In particular, the degree of neutrophil infiltration in the tumor was effectively reduced upon PLAG treatment. Besides, the activity and infiltration of cytotoxic T-Lymphocyte (CTLs) in the tumor were effectively increased in the group co-treated with PLAG compared to the aPD-1 alone. Such improvement was caused by a significant reduction of the population of Th17 which induced massive neutrophil infiltration in the tumor, compared to the positive control.

Conclusion: PLAG enhanced the anti-cancer effect of aPD-1 synergistically on the regression of tumor burden via decreasing the tumor-infiltrating neutrophils and Th17 population while increasing the CTLs. Therefore, combining aPD-1 with PLAG, which has excellent safety profiles, may contribute to enhancing the antitumor response of aPD-1 while lowering immune-related toxicities by reducing the dose of ICI.

EXPERIMENTAL DESIGN

Drug	Dose	Delivery	Delivery Method
PLAG	50, 100 mpk/day	Sterile PBS	Oral Administrate (OA)/day, Everyday
aPD-1	5 mpk		IP, 1 injection/week
IgG2a Isotype	5 mpk		IP, 1 injection/week

Drug delivery : every Tue 17:30

1. Compound concentration

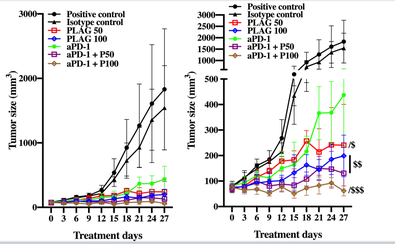
- PLAG : 50, 100 mpk
- PD-1 immune-checkpoint inhibition antibody (aPD-L1) : 5 mpk (BioXcell, RMP1-14 clone)
- IgG2 isotype antibody : 5 mpk (BioXcell)

2. Compound delivery

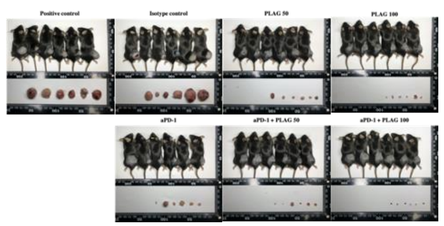
- O.A : PLAG (Daily)
- I.P : aPD-1 (5 mpk, 1 injection/week)
- I.P : Isotype (5 mpk, 1 injection/week)

RESULT

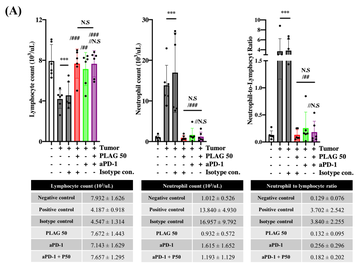
1. Synergistic anti-tumor effect of PLAG with anti-PD-1 antibody(aPD-1)



(A) Analysis of tumor size change in each group estimate 3 days interval. **(B)** Confirmation of changes in morphology and tumor size of mice on the day of sacrifice. **(C)** Tumor weight analysis in PLAG or aPD-1 co-treat mice evaluated at the sacrificed day. Compared to the positive control: ###<0.001; Compared with the aPD-1 only treat group: $P<0.05, $$P<0.05, $$$P<0.001 (each experiment n=6). N.S, Not significant. Mean ± SD



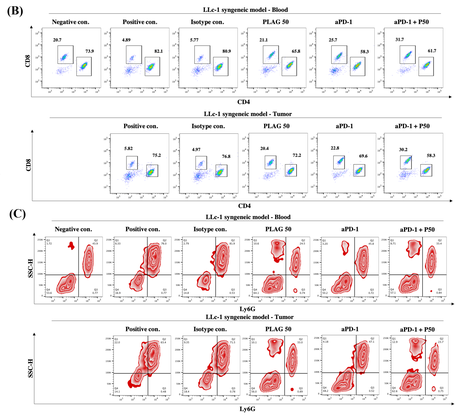
2. Effects on the immune cell population and tumor infiltration by PLAG and aPD-1 treatment

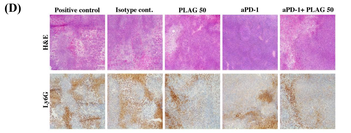


Lymphocyte count (10³/uL)	
Negative control	7.932 ± 1.626
Positive control	4.187 ± 0.918
Isotype control	4.547 ± 1.314
PLAG 50	7.672 ± 1.443
aPD-1	7.143 ± 1.629
aPD-1 + P50	7.657 ± 1.295

Neutrophil count (10³/uL)	
Negative control	1.012 ± 0.526
Positive control	13.840 ± 4.930
Isotype control	16.957 ± 9.792
PLAG 50	0.932 ± 0.572
aPD-1	1.615 ± 1.652
aPD-1 + P50	1.193 ± 1.129

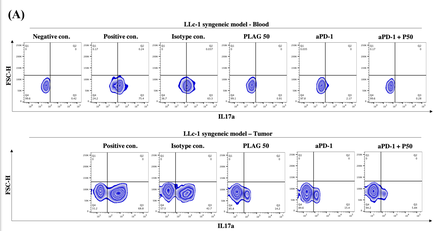
Neutrophil to lymphocyte ratio	
Negative control	0.129 ± 0.076
Positive control	3.702 ± 2.542
Isotype control	3.840 ± 2.255
PLAG 50	0.132 ± 0.095
aPD-1	0.256 ± 0.296
aPD-1 + P50	0.182 ± 0.202

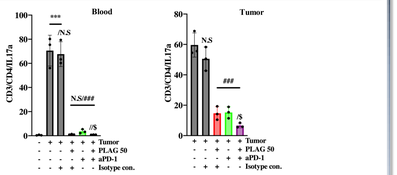




(A) Validation of PLAG modulating immune-cell count via complete blood count (CBC) analysis. **(B)** Analysis of blood/tumor CD4 or CD8 positive cell sorting results according to PLAG and aPD-1 treatment. **(C)** Analysis of tissue infiltrated Ly6G positive cell sorting results according to PLAG and aPD-1 treatment. **(D)** Analysis of neutrophil infiltration control effect by PLAG treatment in tumor tissue through IHC staining. Ly6G: neutrophil population. Compared with the negative control: ***P<0.001; Compared with the positive control: #P<0.05, ##P<0.01, ###P<0.001; Compared with the aPD-1 only treat group: $$P<0.01, $$$P<0.001 (each experiment n=6). N.S, Not significant. Mean ± SD.

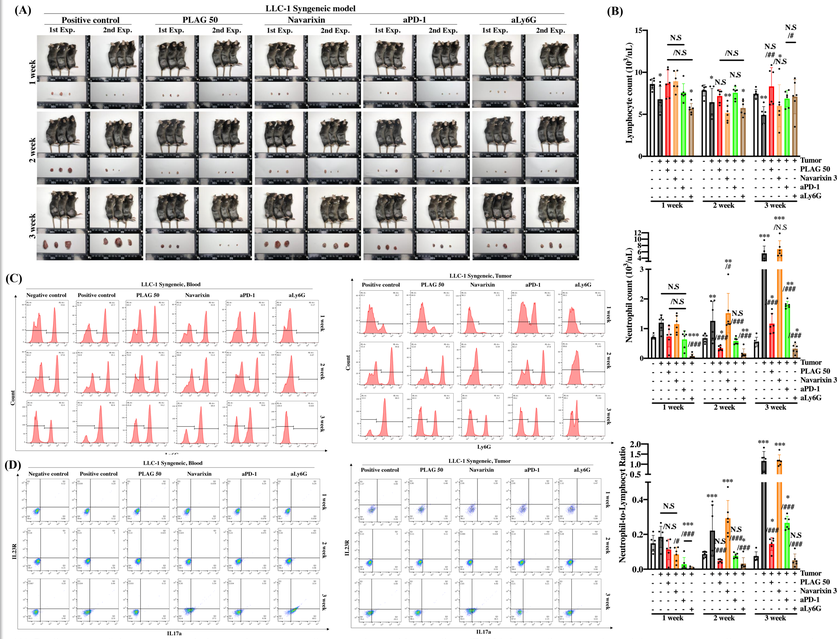
3. Effects on the modulation of Th17 population and tumor infiltration by PLAG and aPD-1 treatment





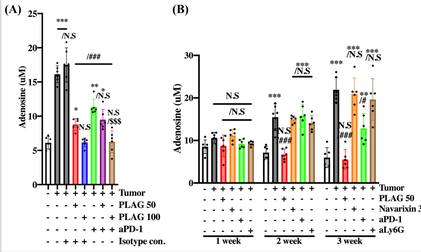
(A) Analysis of blood/tumor Th17 cell population sorting results according to PLAG and aPD-1 treatment. Compared with the negative control: ***P<0.001; Compared with the positive control: ###P<0.001; Compared with the aPD-1 only treat group: $P<0.01 (each experiment n=3). N.S, Not significant. Mean ± SD.

4. PLAG acts as a modulator, not an inhibitor of neutrophil infiltration and migration



(A) Confirmation of changes in morphology and tumor size of mice on the weekly sacrifice. **(B)** Validation of compounds modulating immune-cell count via complete blood count (CBC) analysis. **(C)** Analysis of tissue infiltrated Ly6G positive cell sorting results according to compounds treatment. **(D)** Analysis of blood/tumor Th17 cell population sorting results according to PLAG and aPD-1 treatment. (each experiment n=6). Navarixin: CXCR2 antagonist; aLy6G: anti-Ly6G antibody treatment. Compared with the negative control: ***P<0.001; Compared with the positive control: #P<0.05, ##P<0.01, ###P<0.001; Compared with the aPD-1 only treat group: $$P<0.01, $$$P<0.001 (each experiment n=6). N.S, Not significant. Mean ± SD.

5. PLAG prevents the increase of DAMP by tumor progression through the rapid removal of DAMP



(A) Analysis of adenosine concentration in plasma on sacrifice day according to PLAG and aPD-1 treatment. **(B)** Analysis of adenosine concentration in plasma on weekly sacrificed according to compounds treatment. Navarixin: CXCR2 antagonist; aLy6G: anti-Ly6G antibody treatment. Compared with the negative control: ***P<0.001; Compared with the positive control: #P<0.05, ##P<0.01, ###P<0.001; Compared with the aPD-1 only treat group: $$P<0.01, $$$P<0.001 (each experiment n=6). N.S, Not significant. Mean ± SD.

CONCLUSION

- **PLAG has not only a synergistic anti-tumor effects on the tumor progression with aPD-1, but it suppress tumor progression on its own.**
- **PLAG reduced tumor infiltrating neutrophils (TINs) via an rapid removal of DAMP (adenosine) originated from tumor.**
- **By removal of the initial DAMP(adenosine) by PLAG, the massive infiltration of neutrophils to the tumor region is not occurred.**
- **PLAG reduced the Th17 population and tumor-infiltrating Th17 cells involved in excessive neutrophil infiltration into tumor site.**
- **In conclusion, combination of aPD-1 and PLAG may improve treatment outcomes of aPD-1, compared to aPD-1 alone, contributing to enhancing anti-tumor immune responses via treating the suppressive TME. Presumably, PLAG treatment may transform the immunosuppressive TME into an immune-enhanced TME via inhibition of neutrophil recruitment into the TME and enhancement of anti-tumor immunity of T cells.**