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The effect of PLAG on the PD-L1 immune-checkpoint drug therapy in the MB49 bladder cancer syngeneic model

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AACR American Association for Cancer Research

ABSTRACT

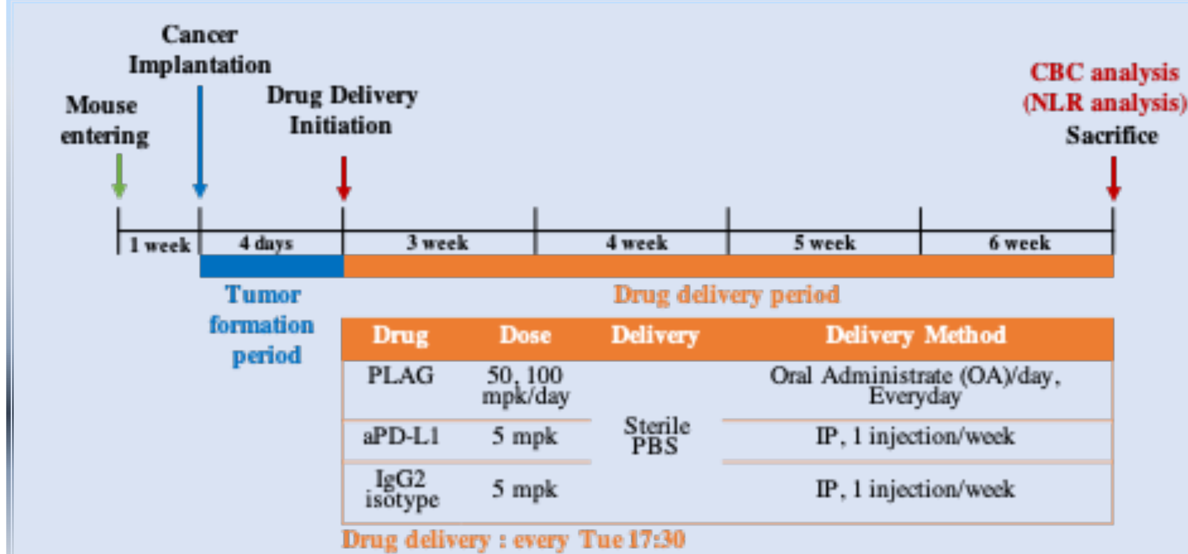
Background: The development of immune-checkpoint drugs, a fourth-generation anticancer drug, is a significant breakthrough in anticancer research. Cancer therapy using immune-checkpoint drugs are still enlarged. To improve the efficacy of immune-checkpoint drugs, modulation of the tumor microenvironment is essentially required. In this experiment, we examined the elevated anti-cancer efficacy of 1-Palmitoyl-2-Linoleoyl-3-Acetyl-rac-Glycerol (PLAG), which has been demonstrated to attenuate tumor infiltrating neutrophils (TINs) in the tumor, along with the PD-L1 immune checkpoint inhibitor treatment.

Methods: The syngeneic model was used (n=6) to investigate the enhanced anti-tumor effect of PD-L1 antibody with the addition of PLAG. MB49 murine bladder cancer cells were implanted into the C57BL/6 mice subcutaneously and bred for 5 weeks. After a week from tumor implantation, PLAG at different dosages (50/100 mpk) were daily administered orally for another 4 weeks with or without 5 mpk PD-L1 antibody (10F.9G2). PD-L1 antibody was delivered via IP injection once a week.

Results: The PLAG treatment groups demonstrated that the tumor burden decreased in a concentration-dependent manner. In 50 and 100 mpk of PLAG treated mice, the tumor burden was decreased to a significant value compared to a positive control ($p < 0.05$). In the group treated with the PD-L1 antibody alone, the growth rate of the tumor decreased until about 2 weeks. In the group treated simultaneously with PLAG and PD-L1 antibody, the growth of the tumor was significantly reduced compared to the group treated with PD-L1 antibody alone. As a result of calculating neutrophils and lymphocytes every two weeks, the neutrophil-to-lymphocyte ratio (NLR) level in the group treated with PLAG and PD-L1 was significantly decreased compared to that treated with PD-L1 antibody alone. Besides, the number of TINs were effectively reduced by PLAG treatment alone. The interruption of tumor growth and reduced chemokine secretion were also observed in the PLAG treated groups. In summary, our data suggest that PLAG provides an enhanced PD-L1 antibody effect on the regression of tumor burden in the syngeneic mice model via reducing the number of TINs.

Conclusion: PLAG may be utilized for improving the efficacy of the PD-L1 antibody on reducing the tumor burden at the devastating tumor microenvironment.

EXPERIMENTAL DESIGN



Drug	Dose	Delivery	Delivery Method
PLAG	50, 100 mpk/day	Sterile PBS	Oral Administrate (OA)/day, Everyday
aPD-L1	5 mpk		IP, 1 injection/week
IgG2 isotype	5 mpk		IP, 1 injection/week

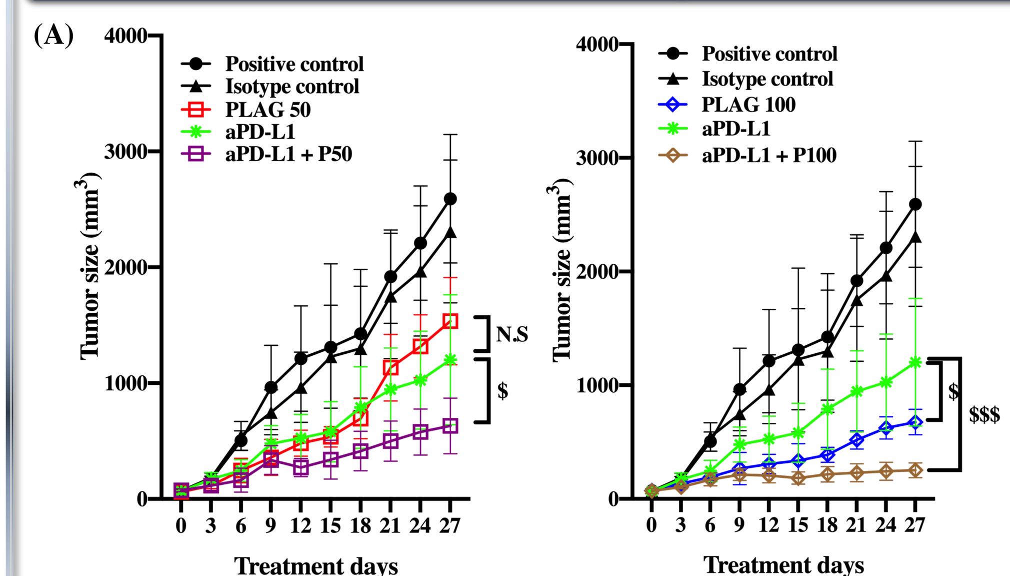
Drug delivery : every Tue 17:30

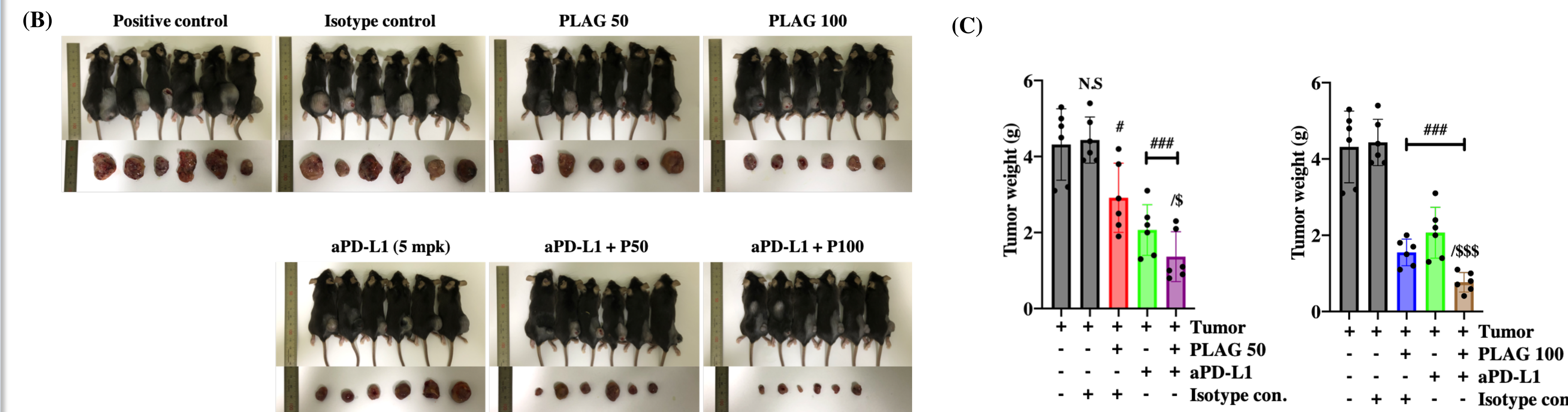
1. Compound concentration
   - PLAG : 50, 100 mpk
   - PD-L1 immune-checkpoint inhibition antibody (aPD-L1) : 5 mpk (BioXcell, 10F.9G2 clone)
   - IgG2 isotype antibody : 5 mpk (BioXcell)

2. Compound delivery
   - O.A : PLAG (Daily)
   - I.P : aPD-L1 (5 mpk, 1 injection/week)
   - I.P : Isotype (5 mpk, 1 injection/week)

RESULT

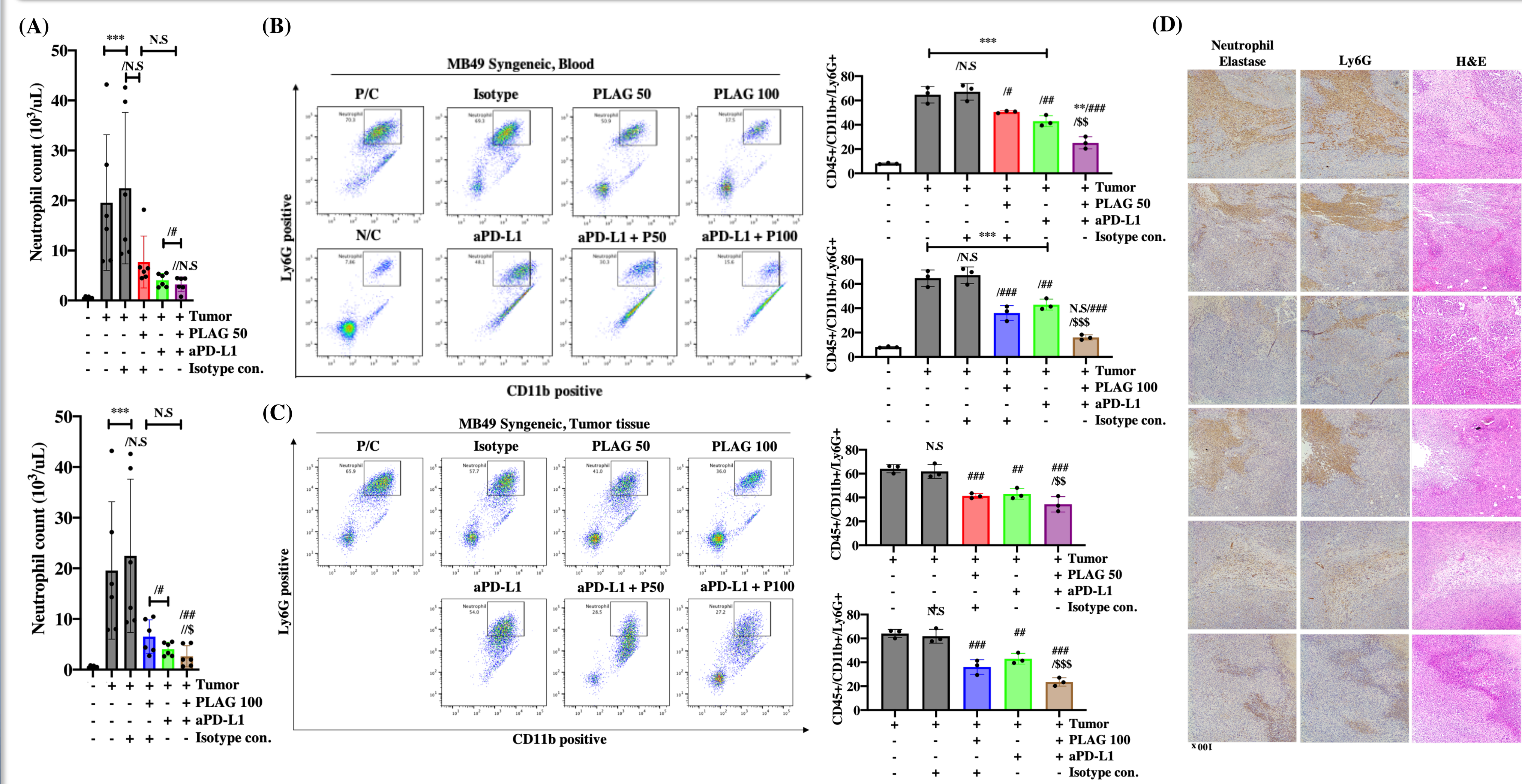
1. Increased inhibitory effect of aPD-L1 on tumor progression by PLAG treatment





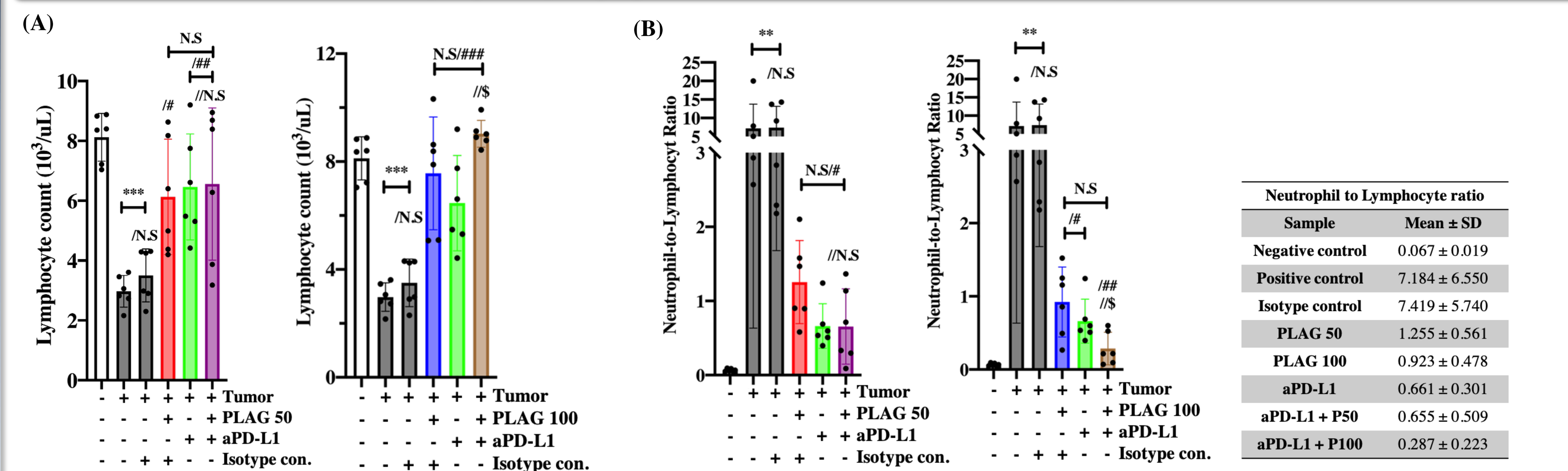
(A) Analysis of tumor size change in each group estimate 3 days interval. (B) Confirmation of changes in morphology and tumor size of mice on the day of sacrifice. (C) Tumor weight analysis in PLAG or aPD-L1 co-treat mice evaluated at the sacrificed day. Compared to the positive control: #P<0.05, ###<0.001; Compared with the aPD-L1 only treat group: $P<0.05, $$$P<0.001 (each experiment n=6). N.S, Not significant. Mean ± SD

2. Control of neutrophil population and tumor infiltration by PLAG treatment

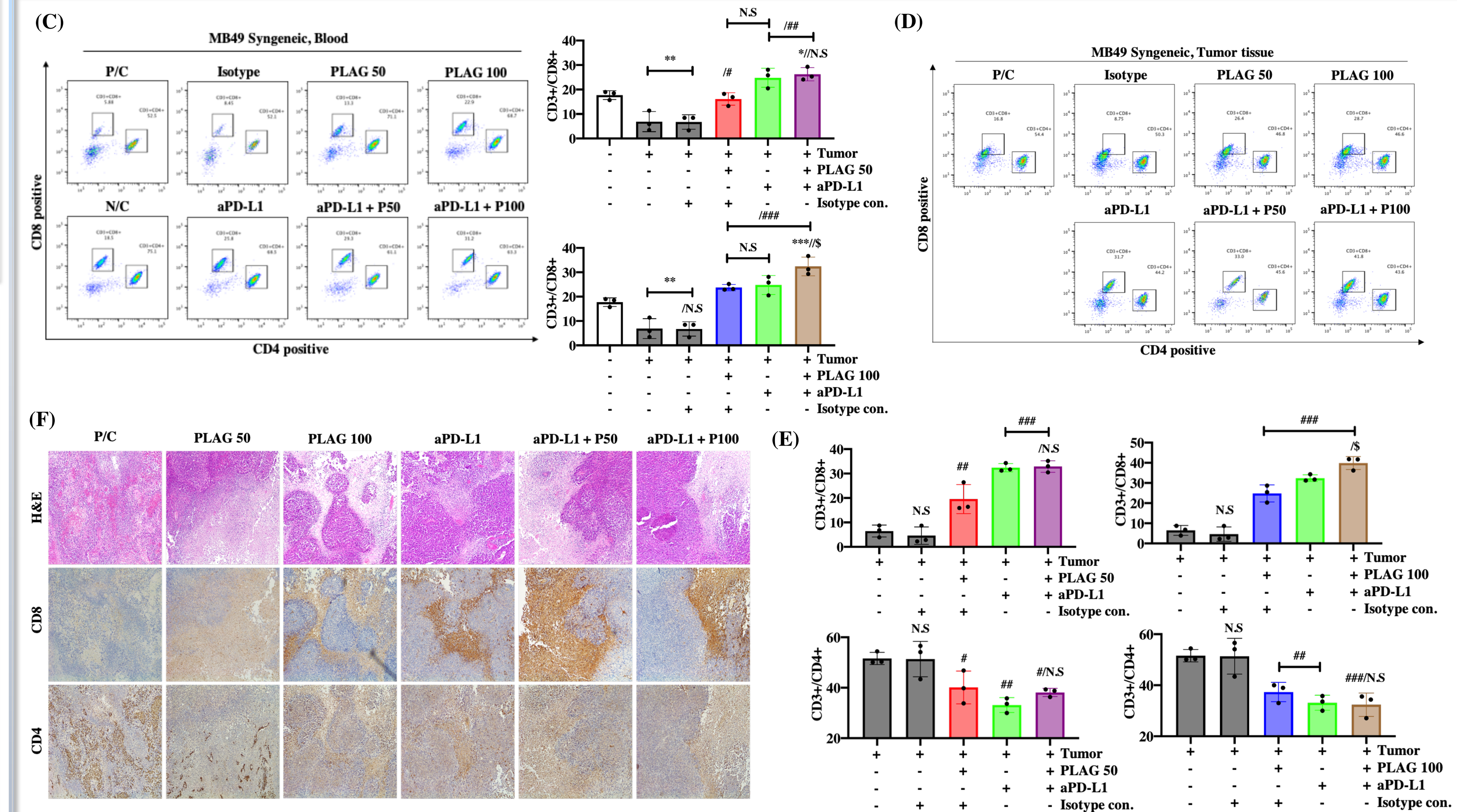


(A) Validation of PLAG modulating neutrophil count via complete blood count (CBC) analysis. (B) Analysis of blood Ly6G and CD11b positive cell sorting results according to PLAG and aPD-L1 treatment. (C) Analysis of tissue infiltrated Ly6G and CD11b positive cell sorting results according to PLAG and aPD-L1 treatment. (D) Analysis of neutrophil infiltration control effect by PLAG treatment in tumor tissue through IHC staining. Ly6G: neutrophil population; Neutrophil Elastase: active neutrophil. 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-L1 only treat group: $$P<0.01, $$$P<0.001 (each experiment n=3). N.S, Not significant. Mean ± SD.

3. Control of lymphocyte population and tumor infiltration by PLAG treatment

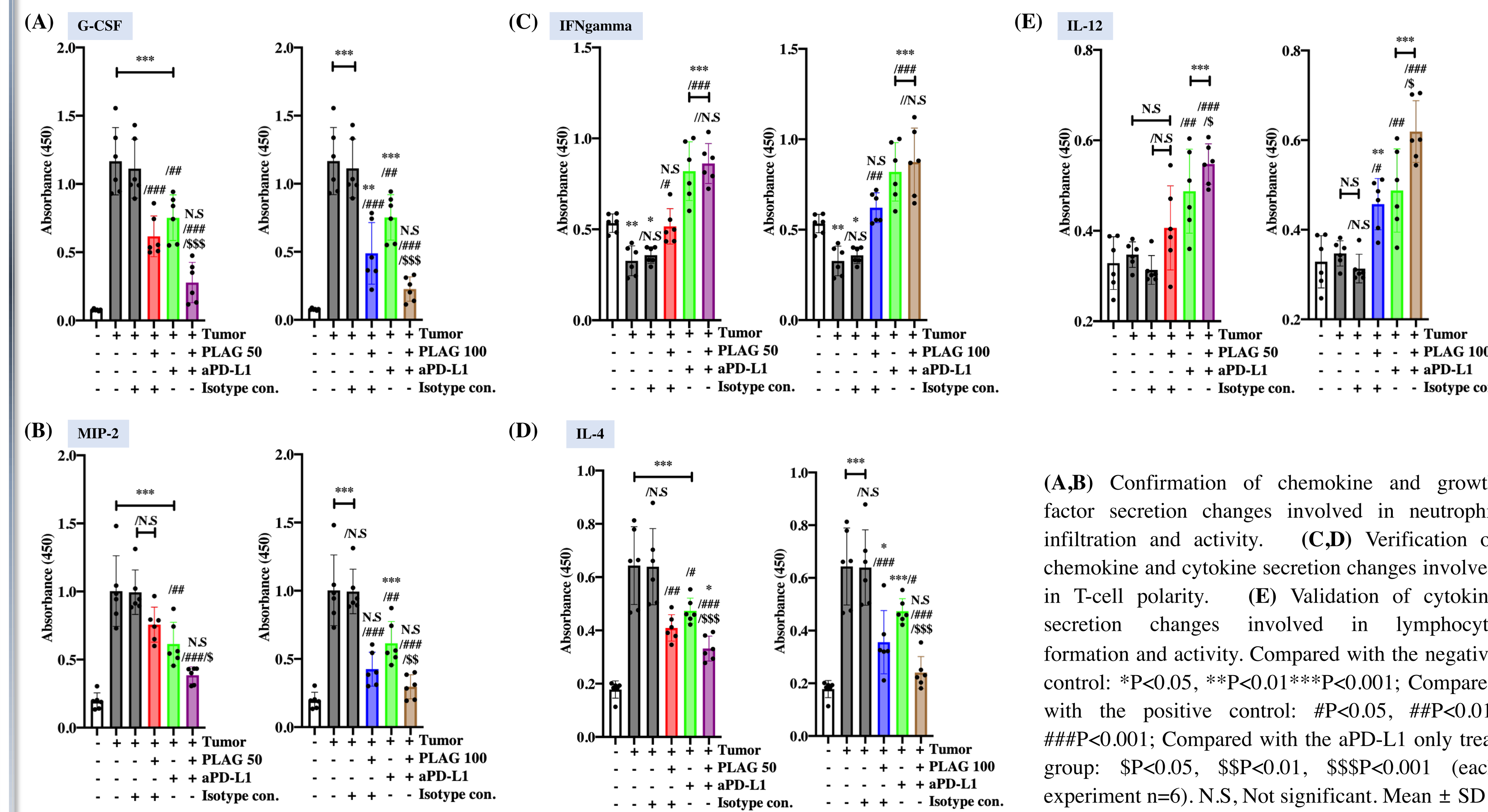


Neutrophil to Lymphocyte ratio	
Sample	Mean ± SD
Negative control	0.067 ± 0.019
Positive control	7.184 ± 6.550
Isotype control	7.419 ± 5.740
PLAG 50	1.255 ± 0.561
PLAG 100	0.923 ± 0.478
aPD-L1	0.661 ± 0.301
aPD-L1 + P50	0.655 ± 0.509
aPD-L1 + P100	0.287 ± 0.223



(A) Validation of PLAG modulating lymphocyte count via complete blood count (CBC) analysis. (B) Quantitative analysis of NLR levels in blood according to PLAG treatment. (C) Analysis of blood CD4 and CD8 positive cell sorting results according to PLAG and aPD-L1 treatment. (D,E) Analysis of tissue infiltrated CD4 and CD8 positive cell sorting results according to PLAG and aPD-L1 treatment. (F) Analysis of lymphocyte infiltration control effect by PLAG treatment in tumor tissue through IHC staining. 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-L1 only treat group: $$P<0.01, $$$P<0.001 (each experiment n=3). N.S, Not significant. Mean ± SD.

4. Validation of cytokine and chemokine secretion involved in immune cell population by PLAG treatment



(A,B) Confirmation of chemokine and growth factor secretion changes involved in neutrophil infiltration and activity. (C,D) Verification of chemokine and cytokine secretion changes involved in T-cell polarity. (E) Validation of cytokine secretion changes involved in lymphocyte formation and activity. Compared with the negative control: *P<0.05, **P<0.01***P<0.001; Compared with the positive control: #P<0.05, ##P<0.01, ###P<0.001; Compared with the aPD-L1 only treat group: $P<0.05, $$P<0.01, $$$P<0.001 (each experiment n=6). N.S, Not significant. Mean ± SD

CONCLUSION

- PLAG not only increases the anti-tumor effect of aPD-L1 more effectively, but it can suppress tumor progression on its own
- In particular, tumor infiltrating neutrophils (TINs), which increases tumor progression, is effectively reduced by PLAG
- Through effectively reducing the number of TINs by PLAG, the anti-tumoral effect by cytotoxic T-lymphocytes (CTLs) is further increased