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A synergistic antitumor effect of PLAG with aPD-1 by modulating the adenosine signaling pathway in ICI low-sensitivity CRC model

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ABSTRACT

Background: Immune checkpoint inhibitors (ICIs) have been proven to be very effective and have fewer side effects than conventional anticancer drugs by responding specifically to tumors. However, tumors developed resistance through various tumor-friendly factors, gradually weakening the effects of ICI. These factors suppress the activation of cytotoxic T-lymphocytes (CTLs), thereby inhibiting the ICI efficacy on tumors. Therefore, it is very important to develop a combination therapy that induces the anticancer efficacy of ICI by suppressing resistance factors produced by tumors.

Our previous results demonstrated that 1-palmitoyl-2-linoleoyl-3-acetyl-rac-glycerol (PLAG) effectively controls the tumor-friendly factors (neutrophils infiltration, adenosine, etc.) while increasing CTL infiltration to suppress tumor progression. Therefore, we propose that PLAG is one of the most effective alternatives to increase the antitumor efficacy of ICI.

Method: The effects of anti-programmed cell death-1 (aPD-1) and PLAG were investigated in syngeneic mice. CT26 colorectal carcinoma (CRC) cell was inoculated into the Balb/c mice S.C. and maintained for 4 days. After that, PLAG (25/50/100 mpk) was daily administered orally for 3 weeks w/wo 5 mpk aPD-1 (RMP1-14) via IP once/week. AZD4635 was daily administered orally at 30 mpk, and anti-CD73 by IP at 10 mpk twice/week. Tumor growth was measured in 3-day intervals.

Result: In the CT26 ICI low-sensitivity CRC model, aPD-1 inhibited tumor growth by 39%, whereas the aPD-1/PLAG treatment reduced 85% of tumor growth (~75% suppression compared to the aPD-1 single treatment). Tumor weight was reduced by 17.5% in aPD-1, whereas aPD-1/PLAG treatment decreased by 68% (~60% compared with the aPD-1 single treatment). PLAG treatments (PLAG only group: 4 out of 6, PLAG/aPD-1 combination group: 5 out of 6) significantly improved the survival rate of tumor-bearing mice compared to control or aPD-1 only group (2 out of 6) ($p<0.0019$). PLAG significantly increased tumor infiltration of CTLs while effectively controlling infiltration of tumor-friendly active neutrophils, as well as induced M1-type macrophage polarization ($p<0.05$). Importantly, PLAG suppressed the production of adenosine and ATP, which is involved in tumor progression. PLAG showed superior antitumor efficacy compared to current ICI combination therapies targeting the adenosine signaling pathway (AZD4635: A2AR antagonist, anti-CD73: inhibition of extracellular adenosine production). PLAG improved survival rate (AZD4635: 3 out of 6, PLAG: 4 out of 6; $p<0.0394$) and reduced the tumor size by 14% compared with AZD4635/aPD-1.

Conclusion: Collectively, our findings show that PLAG inhibits tumor growth by suppressing massive adenosine production, which may increase the antitumor efficacy of aPD-1 through improved CTLs infiltration. Therefore, we propose that PLAG could be a novel therapeutic strategy for patients with ICI-resistant tumors.

EXPERIMENTAL DESIGN

Drug	Dose (mpk)	Excipient	Delivery Method
PLAG	25, 50, 100	Sterile PBS	Oral Administrate (OA), Daily
aPD-1	5		IP, once/week

Drug delivery time		
PLAG	Daily / 17:00~17:30	
aPD-1	Tue. / 17:30	

Drug	Dose (mpk)	Excipient	Delivery Method
PLAG	50	Sterile PBS	Oral Administrate (OA), Daily
AZD4635	30	Corn oil	Oral Administrate (OA), Daily
aCD73	10	Sterile PBS	Intraperitoneal (IP), Once/week
aPD-1	5	Sterile PBS	Intraperitoneal (IP), Once/week

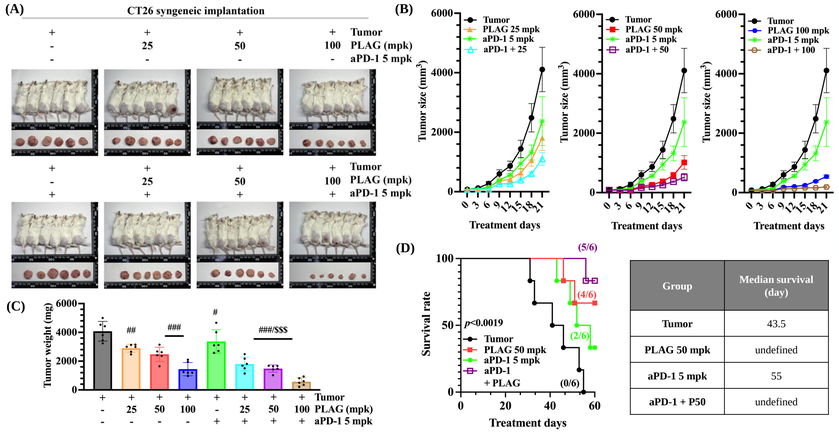
Drug delivery time		
PLAG, AZD4635	17:00~17:30	
aCD73	Mon. Fri. 17:30	
aPD-1	Tue. 17:30	

1. Compound concentration
- PLAG : 25 / 50 / 100 mpk
- aPD-1 : 5 mpk
- AZD4635 : 30 mpk
- aCD73 : 10 mpk

2. Compound delivery
- O.A : PLAG, AZD4635 (QD)
- I.P : aPD-1 (QW)
  aCD73 (Twice a week)

RESULT

1. PLAG synergistically increases the anti-tumor efficacy of aPD-1



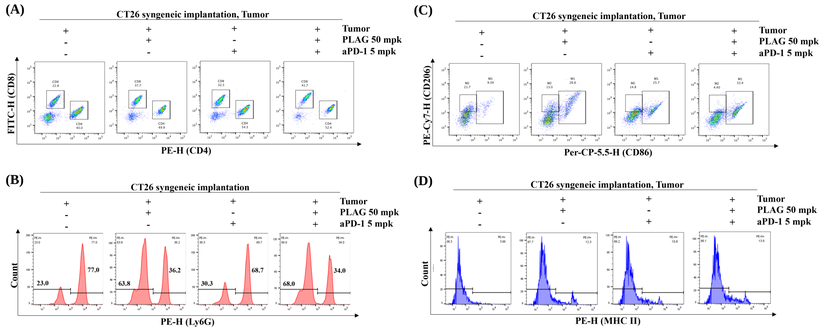
Group	Median survival (day)
Tumor	43.5
PLAG 50 mpk	undefined
aPD-1 5 mpk	55
aPD-1 + P50	undefined

Cotreatment with PLAG and aPD-1 inhibits ICI low-sensitivity CRC growth in a CT26 model

(A) Tumor burden and tumor size were measured on the day of sacrifice in untreated control mice and treated with PLAG and aPD-1. **(B)** Increase in size of implanted tumors from mice in each treatment group, measured at 3-day intervals. **(C)** Tumor weight were measured on the day of sacrificed. **(D)** 60-days survival rate study according to aPD-1 and concurrent treatment with PLAG.

Compared with the negative control: *P<0.033, **P<0.002, ***P<0.001; Compared with the tumor only: #P<0.033, ##P<0.002, ###P<0.001; Compared with the aPD-1 only: $P<0.033, $$P<0.002, $$$P<0.001 (each experiment n=6). N.S, Not significant. Mean ± SD.

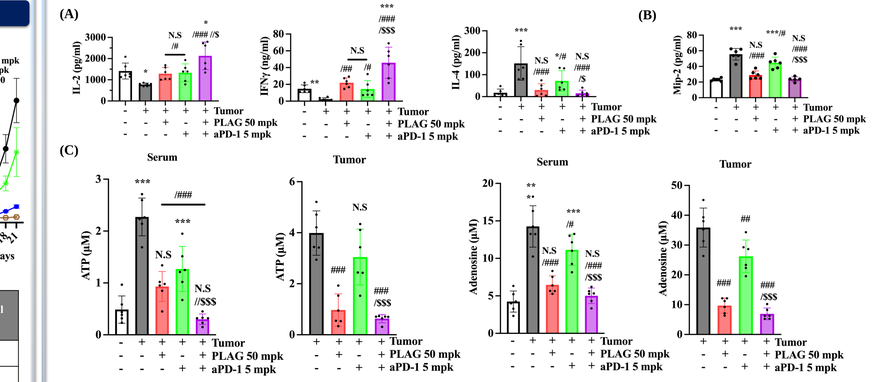
2. PLAG modulate tumor infiltrated immune cell population



Analysis of tumor-infiltrating immune cell population in tumors treated with PLAG and aPD-1

(A) Among CD3+ cells (lymphocyte), CD4+ (help T lymphocyte) and CD8+ cells (cytotoxic T lymphocyte) in tumor were measured in mice from each treatment group by FACS. **(B)** Among CD45+ cells (leukocytes), Ly6G+ and CD11b+ cells (neutrophils) in tumor were measured in mice from each treatment group by FACS. **(C)** Among CD45+ (leukocytes) and CD11b+ (Myeloid cell), CD206 (M2 type macrophage) and CD86+ (M1 macrophage) in tumor were measured in mice from each treatment group by FACS. **(D)** Among CD45+ (leukocytes) and CD11b+ (Myeloid cell), MHC Class II cells in tumor were measured in mice from each treatment group by FACS.

3. PLAG properly modulates immune cell activity and migration related factors

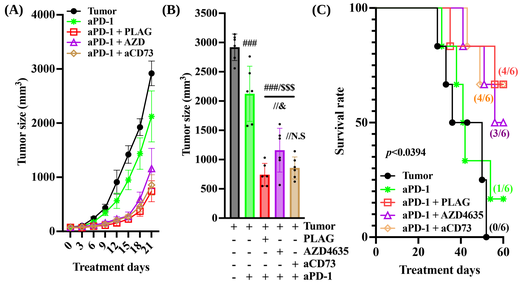


Verification of immune cell modulation related factor release and restored abnormally increased DAMPs by tumor to normal following PLAG treatment.

(A) Validation of changes in release of factors involved in T lymphocyte population regulation. **(B)** Quantitative verification of release changes of neutrophil infiltration-related factors. **(C)** Quantitative analysis of changes in excessively increased ATP and adenosine concentration.

Compared with the negative control: *P<0.033, **P<0.002, ***P<0.001; Compared with the tumor only: #P<0.033, ##P<0.002, ###P<0.001; Compared with the aPD-1 only: $P<0.033, $$P<0.002, $$$P<0.001 (each experiment n=6). N.S, Not significant. Mean ± SD.

4. PLAG has comparatively equivalent effects compared to adenosine target comparator drugs



Cotreatment aPD-1 with PLAG and adenosine target comparator drugs (AZD4635, aCD73) inhibits ICI low-sensitivity CRC growth in a CT26 model

(A) Increase in size of implanted tumors from mice in each treatment group, measured at 3-day intervals. **(B)** Tumor weight were measured on the day of sacrificed. **(C)** 60-days survival rate study according to aPD-1 and concurrent treatment with PLAG and target compounds.

Compared with the negative control: *P<0.033, **P<0.002, ***P<0.001; Compared with the tumor only: #P<0.033, ##P<0.002, ###P<0.001; Compared with the aPD-1 only: $P<0.033, $$P<0.002, $$$P<0.001 (each experiment n=6). N.S, Not significant. Mean ± SD.

CONCLUSION

- **Compared to the positive control group, the aPD-1 treatment alone reduced 39%, whereas the PLAG concurrent treatment group synergistically reduced tumor size by 85% (25 mpk; 72.5% / 50 mpk; 86.9% / 100 mpk; 95.3%)**
- **PLAG changes the tumor microenvironment into an anti-tumor condition by appropriately regulating immune cell infiltration.**
- **In particular, PLAG regulates excessive release of adenosine, and has an anticancer effect comparable to that of adenosine target drug (AZD4635 and aCD73).**
- **These results suggest that PLAG therapy may be leveraged to effectively inhibit CRC patients to help promote complete recovery.**

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