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Suppressive effect of PLAG on tumor progression and its synergistic therapeutic effect with ICI therapy through adenosine clearance

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ABSTRACT

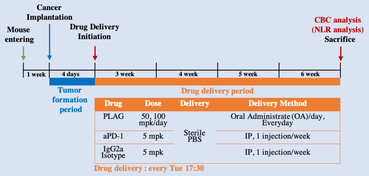
Background: There are limited side effects with ICI therapy such as not responding or reduced therapeutic effect caused by its tolerance. In particular, adenosine-induced tumor progression and drug adaptability are the obstacles in use of ICI therapy. Removing adenosine around the tumor is a prerequisite factor of improving the effect of ICI therapy along with suppression of tumor progression.

Methods: To investigate the anti-tumor effect of PLAG with the PD-1 antibody (aPD-1), the syngeneic model was used (n=6/group). LLc-1 lung carcinoma cells were implanted into the C57BL/6J mice via subcutaneous. PLAG was daily administrated for 4 weeks with or without 5 mpk of aPD-1 (RMP1-14). it was delivered via IP injection biweekly. The adenosine levels and infiltrated immune cell populations in the tumor and blood were analyzed weekly until the sacrifice day.

Results: The tumor size decreased by 65%/81% in a dose-dependently compared to the positive control. Especially, the tumor completely disappeared in 1 animal in treated with aPD-1 and 50 mpk of PLAG, and the 2 animals in treated with 100 mpk. The adenosine levels of blood and tumor burden in the PLAG-treated group decreased by more than 50% compared to the positive control group. It was also confirmed that the adenosine level was about 25% lower in the PLAG-treated group compared to the aPD-1 alone. We found that the expression of A2B receptor in the tumor was significantly reduced in PLAG-treated group. Interestingly, the tumor growth inhibitory effect of PLAG was about 23% better than MRS1754, an A2B receptor antagonist. MRS1754-treated group showed significantly high adenosine level in the blood and tumor, whereas, in the PLAG-treated group, similar adenosine level in blood was continuously maintained as the negative control and its level in the tumor was decreased compared to the positive control. Increasing cancer cell growth and adenosine secretion were shown at 8 h post-treatment of adenosine. PLAG co-treatment decreased the adenosine level by half rapidly and then reduced continuously. In particular, degradation of the A2B receptor was observed after 4 h in PLAG treated cells. Also lysosomal activity during the trafficking of the receptor was elevated within a short period by PLAG and then rapidly decreased at the same time as the signal protein change. PLAG may induce lysosomal protein degradation regulation to A2B receptor degradation through overexpression of αARR. The effect of PLAG disappeared when αARR was knock-down.

Conclusion: Extracellular adenosine level of tumor in ICI therapy could be the critical factor to be a successful anticancer treatment. To attenuate adenosine levels, a number of targeted therapies are being developed. Unlike these targeted therapeutics, PLAG fundamentally blocks cancer growth by adenosine clearance. Therefore, PLAG has its own anticancer effect and can maximize the effect of ICI therapy.

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

1. Compound concentration

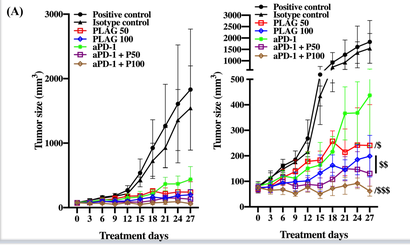
- PLAG : 50, 100 mpk
- PD-1 immune-checkpoint inhibition antibody (aPD-1) : 5 mpk (BioXcell, RMP1-14 clone)
- IgG2 isotype antibody : 5 mpk (BioXcell)

2. Compound delivery

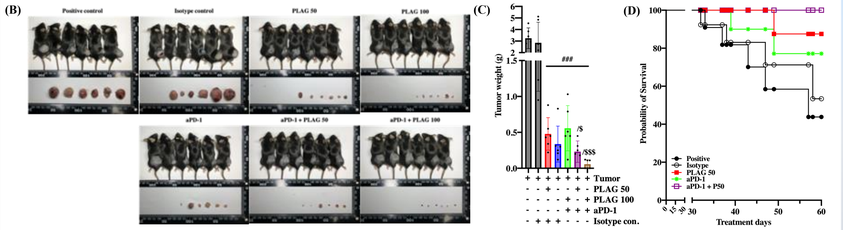
- O.A : PLAG (Daily)
- I.P : aPD-1, Isotype control (1 injection/week)

RESULT

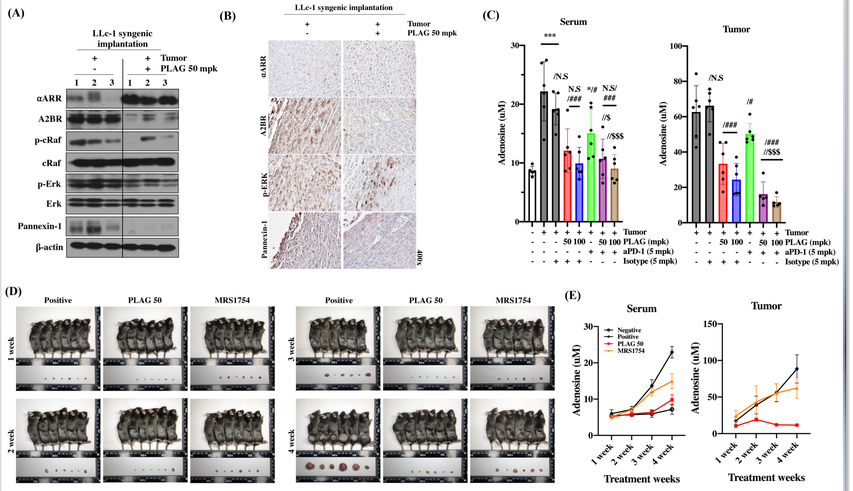
1. Increased inhibitory effect of aPD-1 on tumor progression by PLAG treatment



(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-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. **(D)** Analysis of survival rate in PLAG or aPD-1 co-treatment.

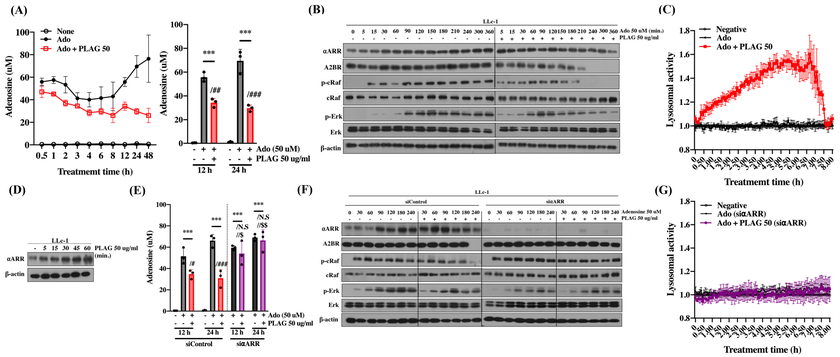


2. Clearance of extracellular adenosine by PLAG treatment



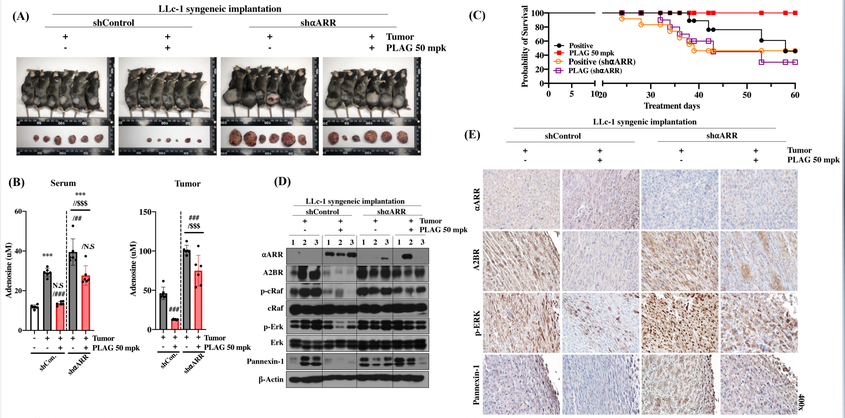
(A) Confirmation of protein signaling pathways in tumor by PLAG treatment. **(B)** Analysis of protein signaling modulation effect by PLAG treatment in tumor tissue through IHC staining. **(C)** Analysis of adenosine concentration on sacrifice day according to PLAG and aPD-1 treatment. Compared with the negative control: *P<0.05, ***P<0.001; Compared with the positive control: #P<0.05, ###P<0.001; Compared with the aPD-1 only treat group: $P<0.05, $$$P<0.001 (each experiment n=6). N.S, Not significant. Mean ± SD. **(D)** Verification of changes in cancer tissue size by week following treatment with PLAG and MRS1754, a target antagonist of A2BR. **(E)** Confirmation of adenosine concentration by week according to PLAG and MRS1754 treatment.

3. PLAG regulation of the A2BR signaling pathway via αARR-dependent



(A) Confirmation of changes in adenosine concentration over time according to PLAG treatment. **(B)** Confirmation of A2BR degradation and the following signal path changes according to PLAG treatment. **(C)** Measurement of changes in intracellular lysosomal activity over time according to PLAG treatment. **(D)** PLAG induced αARR expression. **(E)** Verification of changes in PLAG effect according to αARR Knock-down (KD). At 12/24 hours, the effect of PLAG inducing adenosine clearance with or without αARR was confirmed. Compared with the negative control: ***P<0.001; Compared with the positive control: #P<0.05, ###P<0.001; Compared with the shControl group: $P<0.01 $$$P<0.001 (each experiment n=3). N.S, Not significant. Mean ± SD. **(F)** Confirmation of the inducing the A2BR degradation and the following signal path change via PLAG treatment according to the presence or absence of αARR. **(G)** Confirmation of changes in lysosomal activity induction of PLAG according to αARR-KD.

4. PLAG reduced the tumor progression in a αARR-dependent manner



(A) Confirmation of changes in morphology and tumor size of mice with or without αARR on the day of sacrifice. **(B)** Analysis of adenosine concentration on sacrifice day. Compared with the negative control: *P<0.05, ***P<0.001; Compared with the positive control: #P<0.05, ###P<0.001; Compared with the shControl group: $$$P<0.001 (each experiment n=6). N.S, Not significant. Mean ± SD. **(C)** Analysis of survival rate in PLAG with or without αARR. **(D)** Confirmation of protein signaling pathways in tumor by PLAG treatment. **(E)** Analysis of protein signaling modulation effect by PLAG treatment in tumor tissue through IHC staining.

CONCLUSION

- **PLAG not only increases the anti-tumor effect of aPD-1 more effectively, but it can suppress tumor progression on its own.**
- **In particular, PLAG treatment effectively removes high concentrations of adenosine around the tumor and blood caused by the tumor.**
- **PLAG's adenosine removal effect continues to appear from the initial tumor growth period, and through this effect, PLAG effectively suppresses the tumor growth.**
- **PLAG degrades intracellular A2B receptors through induction of lysosomal activity, thereby effectively blocking adenosine-related signaling pathways. PLAG exhibits this effect through induction of the expression of αARR.**
- **The anti-tumor effect of PLAG is dependent on the αARR expression, and this effect is due to degradation of A2BR and removal of extracellular adenosine.**