# Evaluation of Vaccine Induced Antitumor Response with Cationic Nanoparticles in Colorectal Cancer

**Md Meraj Anjum, L.S.L Janardhanam, Ramkrishna Sen, Mohammad Al-Natour, Sean M. Geary, and Aliasger K. Salem***

*Department of Pharmaceutical Sciences and Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa 52242, USA*
*Contact information: Aliasger-salem@uiowa.edu, mdmeraj-anjum@uiowa.edu*

Control ID#**2028598**

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## Introduction

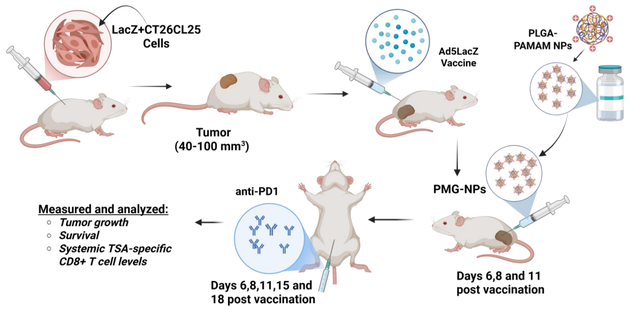
Colorectal cancer (CRC) is a significant global health issue, being the third most common malignancy and the second biggest cause of cancer-related deaths globally. In 2020, CRC accounted for more than 1.9 million new diagnoses and over 935,000 fatalities worldwide[1].

Conventional therapeutic approaches to metastatic CRC, such as chemotherapy, and radiotherapy, have can marginally improve survival rates; however, they are also linked to considerable adverse effects and inconsistent efficacy.

Tumor antigens (TAs) are proteins that are either exclusively expressed in tumor cells or are expressed at significantly greater levels than in normal cells and classified as tumor-specific antigens (TSAs) and tumor-associated antigens (TAAs), respectively. TAs can elicit an immunological response to suppress TA-expressing tumors.

Nanoparticles (NPs) can be made from combining polyamidoamine (PAMAM) dendrimers and poly(lactide-coglycolide) (PLGA) resulting in NPs with highly controllable sizes and surface chemistry, that are promising candidates for many biomedical applications, including drug and gene delivery. Here, we prepared novel NPs made from PLGA and PAMAM generation 5, 6 or 7 (resulting in NPs PMG5, 6 or 7) which appear to have a significant antitumor effect when delivered intratumorally subsequent to a model TA vaccine (Ad5LacZ: *LacZ* encoding for model TSA, b-galactosidase) administration. We previously showed this therapy worked well in a melanoma model; now we wish to test the effectiveness of the treatment in a CRC model[2].

## Rationale




## Objectives

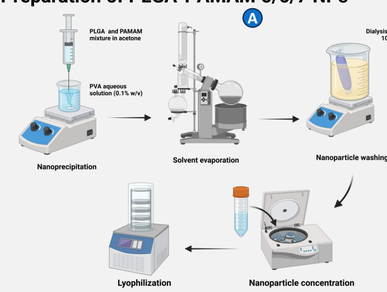
- The study was aimed at testing NPs PMG5, 6 or 7, made from PAMAM generation 5, 6 or 7 and PLGA, as adjuvants to a model TA vaccine by delivering the NPs intratumorally resulting in therapeutic synergy between the vaccine and the NPs.
- The study was also intended to check if addition of immune checkpoint blockade (anti-PD1) in the treatment further improved the antitumor activity and survival probability

## Conclusion

- We successfully synthesized and characterized cationic NPs made from PLGA and PAMAM-5/6/7. We have also demonstrated that by combining a single vaccination of Ad5-LacZ (delivered contralaterally to the tumor) with subsequent intra-tumoral administrations of PMG NPs, we can significantly increase the anti-tumor efficacy of the Ad5-LacZ cancer vaccine in a murine CRC model.
- We further, showed that inclusion of anti-PD1 (intraperitoneal) in the treatment resulted in enhancement in the survival/tumor free survival.

## Methods and Results

### Preparation of PLGA-PAMAM 5/6/7 NPs




### Nanoparticle Characterization

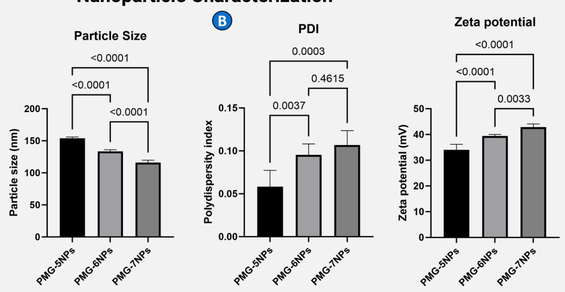



**Figure 1**: Diagram summarizing the major stages involved in the synthesis of PMG-5/6/7 NPs (**A**). The hydrodynamic diameter (particle size), size distribution (PDI) and net surface charge (zeta potential) of the PMG-5/6/7 NPs. Data indicated as mean ± SD., n = 6 (**B**).

### Animal study

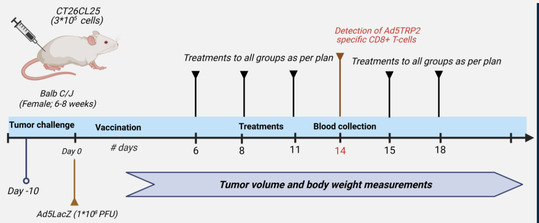



**Table 1:** Details of experimental groups and respective treatments; groups where PMG-5/6/7 is indicated means 3 groups were generated, each treated with PMG-5, PMG-6 or PMG-7; PV = post vaccination; IT = intratumoral; IP = intraperitoneal

| Groups | Treatments | Groups | Treatments |
|---|---|---|---|
| Normal Control | No treatment | PMG-5/6/7 NPs | 1.6 mg/50 µL PMG5/6/7NPs IT (Days 6, 8 and 11 PV) |
| Ad5LacZ+ PBS/sucrose | 50 µL PBS/Sucrose IT (Days 6, 8 and 11 post vaccination) | PMG-5/6/7+ anti-PD1 | 1.6 mg/50 µL PMG5/6/7NPs IT (Days 6, 8 and 11 PV) & 10mg/kg anti-PD1 IP (Days 6, 8, 11, 15 and 18 PV) |
| Ad5LacZ+ PMG-5/6/7 | 1.6 mg/50 µL PMG5/6/7NPs IT (Days 6, 8 and 11 PV) | Ad5LacZ + anti-PD1 | 10mg/kg anti-PD1 IP (Days 6, 8, 11, 15 and 18 PV) |
| Ad5LacZ + PMG-5/6/7+ anti-PD1 | 1.6 mg/50 µL PMG5/6/7NPs IT (Days 6, 8 and 11 PV) & 10mg/kg anti-PD1 IP (Days 6, 8, 11, 15 and 18 PV) | anti-PD1 | 10mg/kg anti-PD1 IP (Days 6, 8, 11, 15 and 18 PV) |

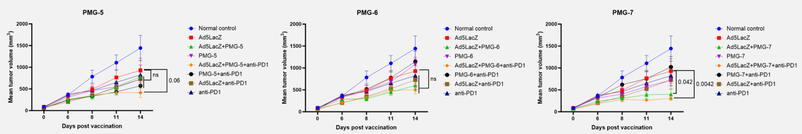



**Figure 2:** Comparative tumor regression plots showing antitumor effect of various treatment groups involving PMG-5/6 and 7- NPs. Data presented as mean ±SEM (n=6-8). Two-way ANOVA followed by Tukey's test.

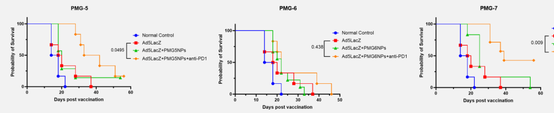



**Figure 3:** Survival curve of mice treated with different treatments after being challenged with tumors. The probability was determined by the log-rank test with all groups compared to the Ad5LacZ group, adjusted for multiple comparisons.

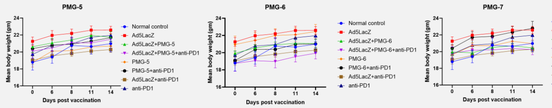



**Figure 4:** Graph showing the average weight of mice over time. Data are plotted as mean ± SEM

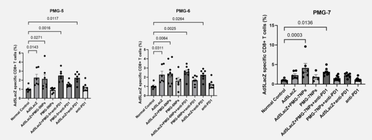



**Figure 5:** levels of Ad5LacZ-specific CD8+ T lymphocytes (on day 14 after vaccination) using direct immunostaining and flow cytometry (expressed as a percentage of total CD3 + CD8 +T lymphocytes). Error bars represent SEM. P values were determined by one-way ANOVA with Tukey posttest

## Results

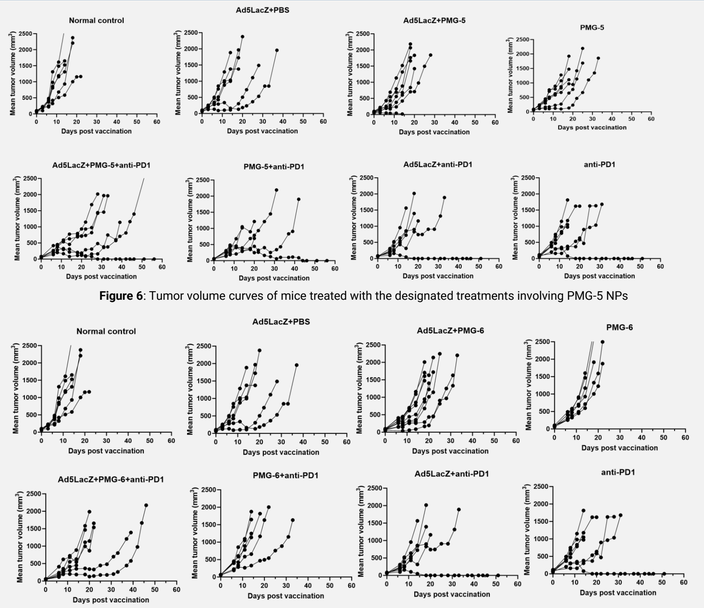



**Figure 6**: Tumor volume curves of mice treated with the designated treatments involving PMG-5 NPs

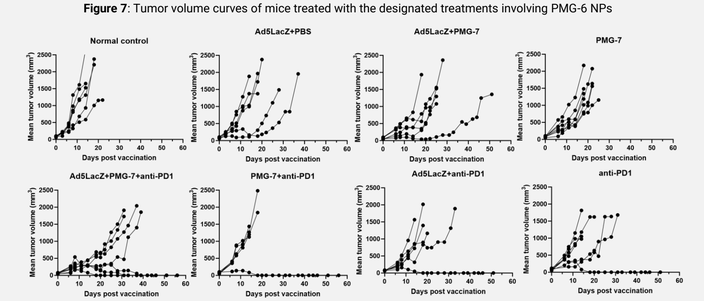



**Figure 7**: Tumor volume curves of mice treated with the designated treatments involving PMG-6 NPs


**Figure 8**: Tumor volume curves of mice treated with the designated treatments involving PMG-7 NPs

## References & Acknowledgement*

1. Y. Tang et al., Cancer Letters 588 (2024) 216798
2. Smith et al., Sci. Adv. 8, eabk3150 (2022)

*The authors are thankful to Gastro-Intestinal Research Foundation (GIRF) for financially funding this project

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