Abstract

Background: The success of checkpoint inhibitors (CPIs) in treating LM is limited due to a liver’s inherent immunosuppressive nature, critically contributed by Myeloid-suppressive cells (MDCs). SD-101, a TLR9 receptor agonist delivered by PEDD device promotes an immune-activating anti-tumor microenvironment. Use of CPI in combination with the regionally delivered SD-101 enhances anti-tumor efficacy without a complete mechanistic understanding. Currently, CPIs are delivered intravenously which is inconvenient, costly and time consuming. Thus, there is a growing interest in developing alternate routes of administration. In this study we investigated whether anti-PD-1 delivered via subcutaneous (SQ) route is as effective as the systemic delivery and provide equivalent survival benefit to LM bearing mice.

Methods: LM model was developed by inoculating MC38-Luc cells via the spleen of 8-12 weeks old male C57/BL6 mice followed by splenectomy. After a week, subcutaneously labelled SD-101 (10µg/mouse) was delivered by using PEDD device, followed by anti-PD-1 delivered either via SQ or intraperitoneally (Sys). Anti-PD-1 was delivered, and tumor burden was monitored by in vivo imaging. Circulatory levels of pro-inflammatory cytokines were analyzed by using Luminex. Tissues were harvested on D3 or D10 to isolate CD45+ cells. For Nanostring analysis, the innate immune panels and for FC, MDSCs (CD11b+Gr1+), B cells isolated from D10 were used for flow cytometry (FC). Data is presented as mean ± SEM; n>4.

Results: SD-101 delivered via PEDD in combination with anti-PD-1 antibody delivered via SQ or Sys significantly reduced LM progression (Figure 1). Moreover, reduction of MDCs with increase in B, T, and M1 macrophages within the LM were observed, irrespective of the routes of delivery. The pro-inflammatory cytokines such as IFNγ and IFNγ significantly increased in the circulation of mice that received SD-101 as compared to the vehicle control. Nanostring analysis revealed that mono- and combination therapies inhibited myeloid cell differentiation and maintenance, angiogenesis, and increased cytokine, lymphocyte activation and TLR signaling pathways. Interestingly, combination of SD-101 and anti-PD-1 irrespective of the routes of delivery enhanced the survival of mice as compared to monotherapy and Veh control.

Conclusion: SD-101 administered regionally via PEDD as monotherapy reversed the tumor progression in mice with LM which was potentiated by combining anti-PD-1 administered via SQ or Sys and enhanced the survival of LM bearing mice, irrespective of the route of delivery.

Introduction and Methods

- SD-101, a class C TLR9 receptor agonist administered by PEDD promotes MDC depletion and immunostimulatory inflammation, in association with enhanced clinical outcomes in combination therapy.

- Currently, CPIs are delivered intravenously and there is a growing interest in subcutaneous (SQ) administration.

- We compared PEDD-SD-101 in combination with Sys or SQ CPI in a murine LM model.

Figure 1: PEDD-SD-101 effect on LM growth was enhanced by CPI irrespective of the delivery route

A. Schema

B. Tumor Burden

C. Bioluminescence image

Figure 2: Modulation of liver myeloid and lymphoid compartments by PEDD-SD-101 was preserved in combination with Sys or SQ CPI

A. Gating Strategy

B. MDSC

C. M-G/MDC Ratio

D. B-Cells

E. CD11c

F. T-Cells

G. M1-Macrophages

Figure 3: Peripheral immunostimulatory effects of PEDD-SD-101 in combination with Sys or SQ-pd-1

A. IFNγ

B. IP-10

Figure 4: PEDD-SD-101 in combination with Sys or SQ-pd-1 promoted transcriptomic changes consistent with enhancement of anti-tumor immunity in the liver TME

A. Venn Diagram of genes that were significantly up-/down-regulated by PEDD-SD-101/SQ/pd-1 compared to Veh. B. Heat map of genes that were significantly up-/down-regulated followed by ip-10 Ctrl, SD-101, Sys and QC treatment compared to Veh control

Figure 5: PEDD-SD-101 in combination with Sys or SQ α-pd-1 promoted transcriptomic changes consistent with enhancement of anti-tumor immunity in the liver TME

A. Pathway scoring

B. Expression of Ifnγ (i) and Granzyme (ii)

C. i-Lymphocyte activation and Checkpoint Signaling

D. T-Cell Activation and Checkpoint Signaling

E. Differentiation and Maintenance of Myeloid Cells

F. Cytokine Signaling

G. Cell Migration and Adhesion

H. Arginase

Figure 6: PEDD-SD-101 in combination with α-pd-1 irrespective of the route of administration improved the overall survival

Summary

- Tumor controlling effect of SD-101 delivered via PEDD was enhanced by α-pd-1 irrespective of the route of administration.

- Both the SQ and Sys resulted in superior outcomes in reducing frequencies of MDCs, predominantly immunosuppressive M-MDCS subpopulation and enhanced B, T, and dendritic cells within the liver tumor microenvironment (TME).

- SD-101 with/without α-pd-1 increased circulatory IFNγ and IP-10 leading to probable anti-tumor immunity.

- In the liver TME, SD-101 monotherapy and in combination with α-pd-1 either via SQ or Sys, enhanced genes that inhibited extracellular matrix remodeling and promoted pro-inflammatory, anti-tumorigenic, anti-angiogenic effects thereby driving pathways that promote anti-tumor immunity.

- SD-101 as a monotherapy and in combination with α-pd-1 irrespective of the route of administration improved the survival of mice with aggressive LM

Reference

Chandra C. Ghosh1, Lauren Counoyer2, Jennie Yujia Liu1, Alizee Ballarin1, Pragna Guha1, Bryan F. Cox1, Steven C. Katz1,2. (2022). PEDD deliver SD-101 and CPIs to Liver Metastasis (LM) to provide equivalent survival benefit to LM bearing mice with aggressive LM. Cancer Gene Ther.