Supplementary Appendix

Supplement to: Manganese primed immunochemotherapy in platinum-resistant/refractory ovarian cancer: a randomized, single-blind, placebo-controlled, phase 2 trial. Authors: Mei et al.

Contents

Supplementary Methods

Administration modes assessment

The safety and anti-tumor effects of various modalities and frequencies of administration were assessed using a murine tumor model. Six to eight-week-old female C57BL/6 mice were bred and kept under specific pathogen-free conditions in the Laboratory Animal Center of Chinese PLA General Hospital. All animal experiments were undertaken in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals, with the approval of the Scientific Investigation Board of Chinese PLA General Hospital. C57BL/6 mice were injected subcutaneously into the right back flank with 1×10^6 B16F10 cells in 100 μL Matrigel. Tumor-bearing mice were treated intranasally or intravenously with 5mg/kg $MnCl_2$ daily (continuous) or every 2 days (intermittent) for three times, starting from 5 days after transplant. Tumor growth and body condition of tumor-bearing mice were monitored daily and measured every 2 days. Tumor volume was determined as length (mm) × width^2 (mm^2) × 0.5. Body condition was scored via a 11-point scale covering appearance, behavior, provoked behavior, clinical signs, and body habitus (Figure S2)[1]. The mice were euthanized on day 15 and peripheral blood and tumors were obtained for the purpose of cytokine secretion and FACS analysis.

Supplemental Results

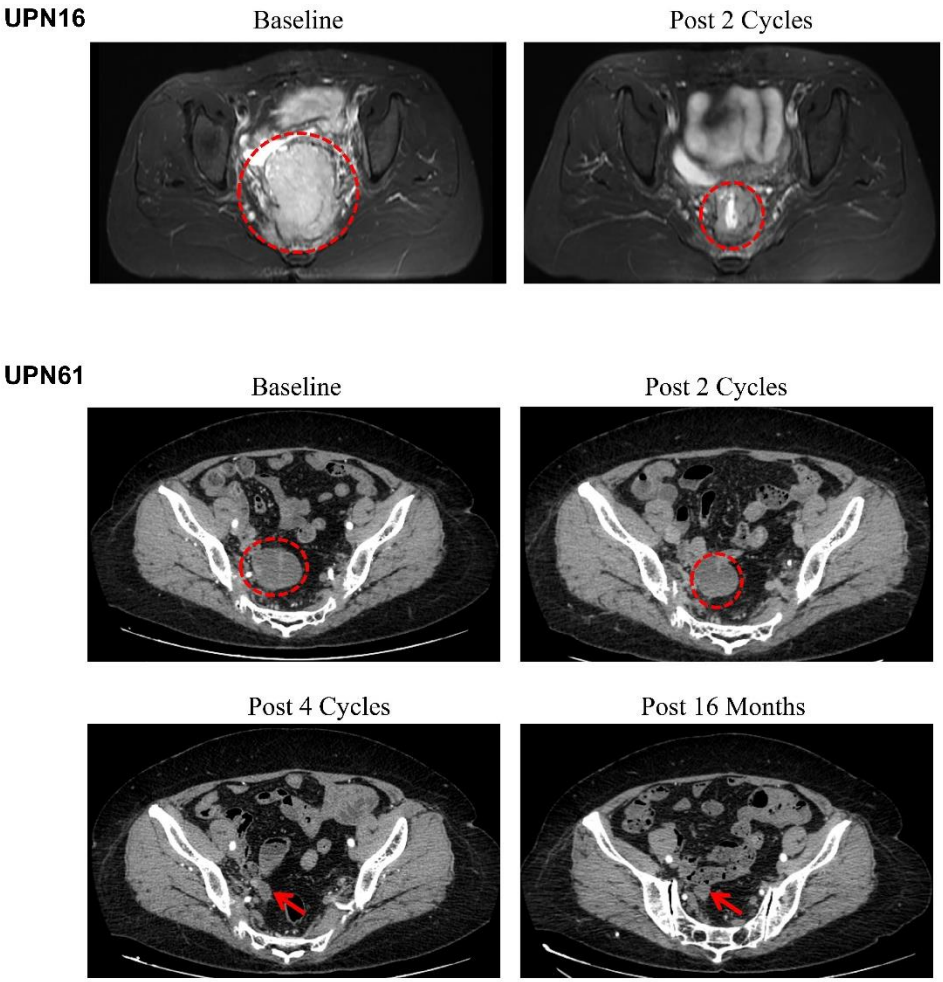
Administration modalities

In accordance with our published data[2], both intranasal and intravenous Mn^{2+} treatment resulted in a considerable reduction in the B16F10 tumor model mice (Figure S2). Both continuous and intermittent treatment through both routes demonstrated comparable antitumor efficacy. However, dissimilarities of safety profile deserve particular attention. Nevertheless, it is crucial to give special consideration to the variations in safety profiles. According to Figure S3, the mice that were subjected to daily continuous therapy exhibited a significant decrease in body weight and condition scores in comparison to those who received intermittent administration and were left untreated. The FACS analysis of tumors demonstrated a notable recruitment and stimulation of CD8+ T cells in all treatment groups, accompanied by the maturation and activation of monocytes. This suggests a preventive defense mechanism by enhancing the innate immune response, which was further confirmed in the peripheral blood (Figure S3). In addition, it was observed that the levels of different innate interferon response cytokines (IFNα/IFNβ/IL-6/CXCL10) and T cell response cytokines (IFNγ/TNFα) were significantly increased in the peripheral blood (Figure S3), which aligns with the previously reported role of Mn^{2+} as a cGAS-STING activator. It is worth mentioning that the administration of $MnCl_2$ either intranasally or intravenously on a daily basis resulted in a robust production of type-I TFN and IL-6 in the peripheral blood (Figure S3). This finding is consistent with previous results from our phase I study that showed cytokine-release syndrome-like clinical features and a

decrease in the body condition score of mice (Figure S3). The data presented collectively indicate that intermittent administration may be a more applicable and safer route of administration. Moreover, the rationale behind utilizing intranasal delivery in this clinical study can be attributed to its operational convenience, equivalent anti-tumor efficacy, and marginal superiority in body condition score when compared to intravenous therapy (Figure S3).

Supplementary Figures

Figure S1. Representative cases of patients with PR in the Mn^{2+} group

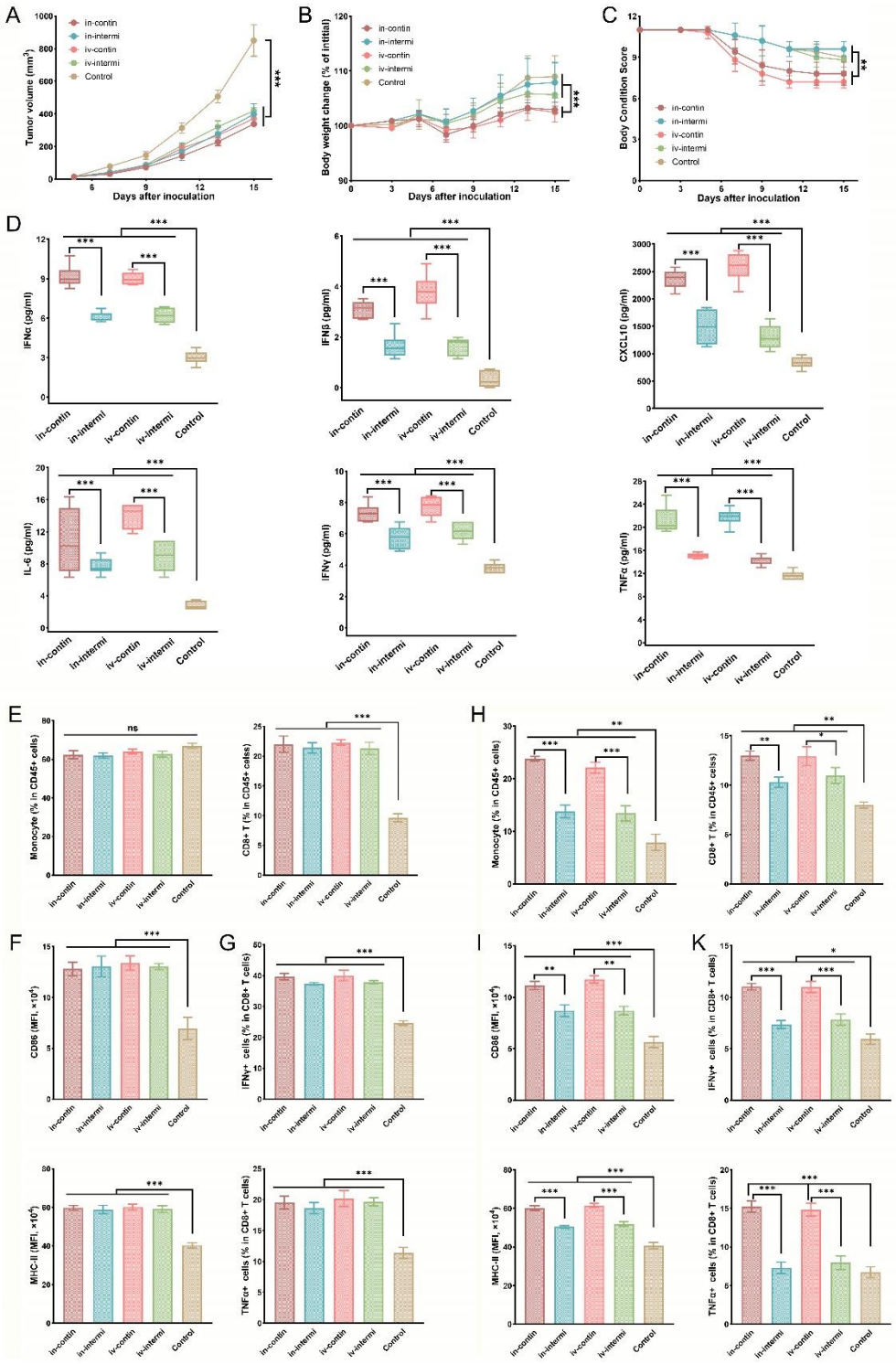


Patients with bulky lesions obtained rapid regression and achieved partial response, confirmed by MRI (UPN16) or CT (UPN61).

Figure S2. 11-point scale of body condition scoring

Category	Condition	Score
Appearance	Bright eyes, well-groomed shiny hair	2
	Dull fur, lack of grooming	1
	Piloerection, hunched back, very rough coat, abnormal posture	0
Behavior	Active, interactive, alert	2
	Isolated, pronounced decreased activity, decreased alertness	1
	Immobile, weak, vocalizations	0
Provoked behavior	Quickly moves away	3
	Slow to move	2
	Moves away after short period of time	1
	Does not move	0
Clinical signs	Normal body temperature, normal respiratory rate	2
	Decreased body temperature, slow respiratory rate	1
	Hypothermia, respiratory rate markedly reduced	0
Body score condition	Normal	2
	Thin	1
	Emaciated	0

Figure S3. Assessment of administration mode via murine tumor model



Tumor growth, body weight, condition score and cytokine levels were shown in panel A to D. The FACS analysis of tumor and peripheral blood were exhibited in panel E to G and H to K, respectively.

Abbreviations: in, administrated intranasally; iv, administrated intravenously; contin, continuous; intermi, intermittent.

Supplementary Tables

Table S1. subgroup analyses of ORR in the Mn^{2+} group

Subgroup	ORR (95% CI)		*P*-value
Age <57 *v.s.* ≥57	48.1 (29.6-67.2)	75.0 (55.1-88)	0.0540
ECOG 0 *v.s.* 1-2	61.1 (44.0-75.9)	63.2 (38.4-83.7)	0.8820
Previous treatment lines <4 *v.s.* ≥4	72.7 (49.6-87.8)	54.5 (37.1-71.0)	0.2580
Primary PFI <6M *v.s.* ≥6M	54.3 (37.3-70.3)	75.0 (50.4-89.8)	0.1580
Most recent PFI <1M *v.s.* ≥1M	56.7 (38.1-73.5)	68.0 (46.7-83.7)	0.4190
Prior Bevacizumab treatment Yes *v.s.* No	63.0 (42.8-79.4)	60.7 (41.1-77.4)	1.0000
Prior Nab-Paclitaxel treatment Yes *v.s.* No	58.1 (39.7-74.4)	66.7 (45.0-83.0)	0.5840
Prior anti-PD-1 treatment Yes *v.s.* No	40.0 (13.4-74.2)	66.7 (51.4-79.1)	0.1560
Prior PARPi treatment Yes *v.s.* No	63.2 (38.7-82.3)	61.1 (44.0-75.9)	1.0000
BRCA mutation pos. *v.s.* neg.	85.7 (53.5-96.9)	52.8 (36.2-68.8)	0.0504
HRD pos. *v.s.* neg.	68.8 (41.1-87.4)	58.8 (41.3-74.4)	0.5493

Pos., positive; neg. negative.

Reference

1. Rupert JE, Narasimhan A, Jengelley DHA, et al. Tumor-derived IL-6 and trans-signaling among tumor, fat, and muscle mediate pancreatic cancer cachexia. J Exp Med. 2021;218(6):e20190450.

2. Lv M, Chen M, Zhang R, et al. Manganese is critical for antitumor immune responses via cGAS-STING and improves the efficacy of clinical immunotherapy. Cell Res. 2020;30(11):966-979.