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CT-mediated intratumoral delivery of PD-L1 antibody mitigates immunotherapy-related colitis without compromising its antitumor function in bladder cancer-bearing mice

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Abstract Immune checkpoint inhibitors (ICIs) have been the mainstay of immunotherapy against cancer. However, the systemic administration of ICIs is accompanied by considerable immunotherapy-related toxicities. To explore whether the local delivery of PD-L1 antibody via a sustained-release gel-forming carrier mediates effective anticancer function but causes less side effects on colitis, CT, a previously reported depot system was employed to locally deliver PD-L1 antibody together with curcumin to the tumor bed of bladder cancer-bearing ulcerative colitis model mice. Our results demonstrated that the systemic administration of PD-L1 antibody by IP injection synergized with the intra-tumoral injected curcumin via CT in inhibiting tumor growth, but exacerbated colitis of UC model mice. Notably, CT-mediated intra-tumoral injection of PD-L1 antibody and curcumin enabled sustained-release of the loaded PD-L1 antibody and curcumin, which exhibited effective anticancer function but caused negligible side effects on colon of UC model mice. These suggested CT a promising agent for local delivery of anticancer drugs, which maintains effective anticancer function while sharply reduced the adverse side effects associated with the drugs.

Introduction

Immune checkpoint inhibitors (ICIs) have revolutionized cancer therapy and are currently the mainstay of cancer immunotherapies(1) . PD-1/PD-L1 monoclonal antibodies have been

very successful in the treatment of a variety of malignancies refractory to other therapies and were approved for the management of many malignant tumors (2, 3). Although ICIs are associated with less frequent AEs as compared to chemotherapy, immunotherapy-related toxicities, well known as immune-related adverse events (irAEs), could arise from ICIs. Common irAEs appear to involve generalized epithelial inflammation, which results in the manifestations of rash, colitis, pneumonitis and so on (4, 5).

Ulcerative colitis (UC) is a chronic, nonspecific inflammatory and autoimmune disorder. It mainly involves the colon and rectum. It is believed that in genetically susceptible patients, the interaction between multiple environmental factors and intestinal microbiota causes a dysregulation of the immune system, ultimately leading to chronic intestinal inflammation. PD-1/PD-L1 inhibitors were reported to induce UC flares in CPUC of whom those in active phase of UC are more inclined to experience symptom worsening (6-10). Therefore, local drug delivery systems that could be applied at the targeted tumor sites to enable intratumorally controlled and sustained drug release are a promising strategy to obtain greater efficacy and lighter adverse effects. CT is a biocompatible, gel-forming and highly tunable polymer whose encapsulation efficiency and release sustainability of the gel formed could be tuned by changing the molecular weight of the polymer (11). Our in vivo studies have shown that CT efficiently delivered chemotherapeutic agents and served as a prototype for further development of antidrug carrier for local delivery (11).

Previously, we have generated a self-indicating, gel-forming and highly biocompatible cellulose derivative, being named as CT (12). The polymer can undergo molecular entanglement in an aqueous environment enables to form a depot of bioactive agents *in situ* (13). It shows high biocompatibility, release sustainability and intrinsic self-indicating capacity (13). In a preclinical trial on a mouse xenograft model established by using BGC-823 cells, the derivative has enhanced the efficiency of chemotherapy mediated by paclitaxel while reducing the systemic toxicity caused by the delivered drug (13). In this study, by using CT as a carrier to loaded curcumin and PD-L1 antibody, our objective is to develop an injectable formulation for treatment of bladder cancer and to evaluate the *in vivo* therapeutic efficacy of the developed formulation. Curcumin is an active anticancer agent proved by in vitro studies and in vivo studies when locally injected (14-16). However, the systemic delivery of curcumin either by oral administration or by intravenous injection has not been successfully established(17, 18). Local delivery of curcumin counteracts and reverts immunosuppressive tumor microenvironment (18, 19). Curcumin was reported to synergize with PD-1/PD-L1 blockade in anticancer studies(20-22). Considering these facts, to achieve the effective concentration of PD-L1 antibody in TME, the dose locally administered via CT could be sharply reduced as compared that via systemic administration. As a result, CT-mediated local delivery of PD-L1 antibody is expected to witness significant lower systemic level of PD-L1 antibody, which hopefully results in less toxicity to the organs beyond that locally injected.

Materials and methods

Reagents

2-methylpropenoic acid, 1-chloro-2,3-epoxypropane, 4-dimethylaminopyridine (DMAP), and various other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). DSS (MW 36,000-50,000) was obtained from Yeasen Biotechnology (Shanghai, China). Anti-mouse PD-

L1 antibody (BE0101) was purchased from Bio X Cell. PD-L1 Protein was purchased from Sino Biological (50010-M08H). Curcumin was purchased from Sigma-Aldrich (Saint Louis, MO). Anti-CD8 alpha antibody (EPR21769) was bought from Abcam (Cambridge, UK). HRP-labeled Goat Anti-Rabbit IgG and Alexa Fluor 488 Goat Anti-Rabbit IgG were obtained from Beyotime.

CT synthesis and gel fabrication

CT was synthesized as previously described from modified cellulose (degree of hydroxypropylation = 7–12%; degree of methylation = 28–30%) whose 2% (w/v) aqueous solution exhibited the viscosity of 6 mPa·s at ambient conditions (13). To induce gelation, CT was first dispersed in anhydrous DMSO at a concentration of 1% (w/v). The solution was then added to 50 mL of distilled water. A gel was formed after incubation of the solution at ambient conditions for 10 min.

Determination of the encapsulation efficiency (EE)

Curcumin [4% (w/v)] and/or PD-L1 [0.4% (w/v)] were added to 25 mL of a 5% (w/v) DMSO solution of CT, followed by magnetic stirring at ambient conditions for 30 min. After that, the solution was added to distilled water. The system was incubated at ambient conditions for 10 min to induce gelation. The generated drug-loaded gel was obtained by centrifugation at a relative centrifugal force of 10,000 x g for 30 min. The supernatant was removed.

The concentration of unloaded PD-L1 was determined by using the Bradford reagent (Sigma-Aldrich, Missouri, USA). The concentration of unloaded curcumin was found by using reverse-phase ultra-high performance liquid chromatography (UPLC), coupled with a triple quadrupole mass spectrometer (UPLC-MS/MS). The EE was determined using the following equation:

$$EE = \frac{m_l}{m_t} \times 100\%$$

where m_l is the mass of the agent successfully loaded into the gel, and m_t is the total mass of the agent added during the agent encapsulation process.

Determination of the agent release profile

1 g of the lyophilized drug-loaded gel was added to 10 mL of PBS (pH 7.4). The system was then incubated at 37 °C in a 5% CO_2 atmosphere. 1 mL of the release medium was withdrawn at regular time intervals. The volume of the medium withdrawn from the system was replaced with the same volume of PBS. The quantity of the loaded PD-L1 released from the drug-loaded gel was determined by using the Bradford reagent (Sigma-Aldrich, Missouri, USA). The amount of the loaded curcumin released from the gel was found by using UPLC-MS/MS. The percentage of cumulative release of the loaded curcumin and PD-L1 was calculated using the following equation:

$$Cumulative\ release\ (\%) = \frac{\sum_{t=0}^{t} m_t}{m_\infty} \times 100\%$$

where m_t is the mass of the agent released from the gel at time t, and m_∞ is the total mass of the agent loaded into the gel.

Morphological examination

Lyophilized CT was sputter-coated with gold. The morphological features were observed by using a scanning electron microscope (JSM-6380; JEOL, Tokyo, Japan) operated at an accelerating voltage of 10 kV.

Rheological measurements

The viscosity of a DMSO solution of CT before and after gelation in distilled water was determined by using a Brookfield DV-III Ultra programmable rheometer (Brookfield Engineering Laboratories Inc., Middleboro, MA, USA) with spindles (CP-40). Viscosity parameters were measured at ambient conditions at different shear rates.

Determination of the water content (WC) and swelling ratio

A lyophilized gel was weighed and immersed in 100 mL of PBS (pH 7.4). The gel was retrieved by centrifugation at a relative centrifugal force of 4000 × g for 5 min at regular time intervals. Excess PBS was removed from the gel by blotting. The water content of the gel was determined using the following equation:

$$Water\ content\ (\%) = \frac{m_s - m_d}{m_s} \times 100\%$$

where m_s is the mass of the swollen gel, and m_d is the mass of the dried gel.

Evaluation of the erosion behavior

The erosion behavior of the gel was determined as previously described (12). In brief, a known amount of a lyophilized gel was immersed in PBS (pH 7.4). The system was incubated at 37 °C. The gel was retrieved by centrifugation at a relative centrifugal force of 4000 × g for 5 min after a specified time interval. It was then dried in an oven at 65 °C. The final dry mass of the gel was recorded.

Cell culture

Murine bladder cancer cell line MB49 was obtained from EK-Bioscience (Shanghai, China). The cells were cultured in RPMI-1640 medium with 10% FBS and 1% penicillin–streptomycin at a humidified 5% CO_2 incubator at 37 °C.

Mice

Eight-week-old C57BL/6 female mice weighing 18-20g were purchased from Shanghai Slack Laboratory Animal Co, Ltd. (Shanghai, China) and maintained in a standard 12-h light/12-h dark cycle at a room temperature of 23±2 °C and a relative humidity between 50% and 60%.

All mice were housed in accordance with the National Institutes of Health guidelines for laboratory animals. The study was approved by the Animal Ethics Committee of Zhejiang Provincial People's Hospital.

Induction of DSS colitis in mice and drug treatment

After 7 days of acclimatization to the experimental environment, the experimental colitis mice model was established by administering 3% (w/v) DSS, MW, 36,000-50,000 kDa (MP Biochemicals, Aurora, OH, USA) dissolved in standard drinking water (days 0-7). The normal group received drinking water and the DSS group received drinking water containing 3% DSS. Body weight and stool condition were measured daily from the initiation of DSS feeding.

Culture MB49 bladder cancer cells, take 5×10^5 cells in logarithmic growth phase mice, and implant 50μl into the subcutaneous of the right back of the mice, which suggested the subcutaneous tumor model of mouse bladder cancer. A tumor of about 150-250 mm^3 in size could be palpated on the back of the mice on the seventh day, which was considered successful for modeling. The mice were randomly divided into three groups: blank group, CT-mediated local delivery of curcumin, CT-mediated local delivery of curcumin and PD-L1 antibody (2mg/kg), CT-mediated local delivery of curcumin and IP injection of PD-L1 antibody (10mg/kg).. Use digital calipers for vertical tumor diameters.

The tumor volume was calculated by this formula: Tumor volume (mm3) = length × $width^2/2$.

Modified disease activity index (mDAI)

Body weight, stool consistency and rectal bleeding were monitored every day. The daily body weight changes were calculated as the percentage of the initial weight on Day 1 of DSS feeding. Occult blood was assessed using a fecal occult blood test kit.

The **modified disease activity index** (mDAI) was calculated based on the scoring system shown in supplemental Table 1(23, 24).The mDAI was calculated by grading on a scale of 0 to 5 : mDAI = (Weight loss + stool condition + gross bleeding)/3. Mice that died as a result of severe inflammation were scored a top score of 5.

Histological processing and analysis

Mice were sacrificed at the end of the experiment, and the entire colon was dissected, fixed in 10% formalin, paraffin-embedded and sectioned, and then stained with hematoxylin and eosin (H&E). The histological damage score was evaluated based on three independent parameters: the extent of inflammation (0~3: none, mucosal, submucosal, and transmural), the extent of inflammatory cell infiltration (0~3: none, mild, moderate, and severe), and crypt architecture damage (0~2: none, regeneration, and destruction). The scores derived from the above parameters were combined to generate the final score, which ranged from 0 to 8.

Elisa

The PD-L1 protein was encapsulated on a 96-well enzyme labeled plate overnight at 4°C.For the standard curve, antibodies were serially diluted at different concentrations. Plate was washed with washing buffer (0.01 M phosphate-buffered saline with 0.05% tween-20) prior to blocking with blocking buffer (0.01 M phosphate-buffered saline with 5% fetal bovine serum) and incubated at 37°C for 2 h. Plate was washed prior to incubation with detecting serums diluted in blocking buffer for 1 h at 37°C. Plate was washed and incubated with HRP-labeled Goat Anti-Rabbit IgG diluted in blocking buffer at 1:250 and incubated at 37°C for 1 h. Plate was washed and incubated TMB substrate solution (P0209, Beyotime) at 37°C for 30 min. The reaction was stopped by Stop solution for TMB substrate. Plate was read using plate reader at 450 nm.

Immunohistochemistry

Tumors were paraffin embedded for further histologic analysis. Paraffin blocks were sectioned

at 5μm and dried for 1 h at 65 °C before the procedures of deparaffinization, rehydration and epitope retrieval in the pretreatment module at 95 °C for 20 min in 50× Tris/EDTA buffer. Before staining of sections, endogenous peroxidase was blocked. Sections were incubated with specific antibodies targeting CD8 (1:2000, abcam). Staining was by goat anti-rabbit horseradish peroxidase, visualized with 3,3′-diaminobenzidine (DAB) as a chromogenic substrate and counterstained with haematoxylin. Histological quantifications were performed with ImageJ software.

Immunofluorescence analysis

Tumor tissues were embedded in paraffin and sectioned. After deparaffinized, rehydrated, antigenically repaired, blocked from endogenous peroxidases, sections were blocked with 5% BSA overnight and then incubated with Alexa Fluor 488 Goat Anti-Rabbit IgG (1:1000, Beyotime, A0423) for 10 min. The sections were counterstained with 4',6-diamidino-2-phenylindole (DAPI). Immunofluorescence images were captured using EVOS™ M7000 imaging system and analyzed using ImageJ software.

Statistical analysis

Data were presented as the mean ± SEM and statistically evaluated by one-way ANOVA. Statistical analyses were conducted using GraphPad Prism 9.4.1 software. In all statistical comparisons, a P value less than 0.05 was used to indicate a significant difference.

Results and discussions

1. Preparation and gelation of CT for drug delivery

Generation of CT started from methylation and hydroxypropylation of cellulose, followed by transesterification in the presence of a polar aprotic solvent as the reaction medium. The process of gelation induced by injection of the CT solution into an aqueous environment is evidenced by changes in the apparent viscosity of the CT solution. As shown in the **Figure 1**, the apparent viscosity of the CT solution decreased at the shear rate increased. This phenomenon occurred in the sol form, as well as the gel form, of the CT solution. This suggests that the CT solution and the generated gel display pseudoplastic behavior. In addition, compared to the sol form of the CT solution, the gel form showed much higher apparent viscosity. This is explained by the occurrence of gelation upon injection of the CT solution into an aqueous medium, leading to a decline in the solubility of CT and subsequent molecular entanglement. The network formed *in situ* enables CT to serve as a depot system for entrapment and sustained release of drug molecules.

The polymeric network formed by CT, upon injection of the CT solution into an aqueous medium, was imaged by using SEM. As shown in **Figure 2A**, a highly porous microstructure of the CT gel was observed. Such porous microstructure will be exploited in this study for entrapment and sustained release of curcumin and PD-L1, and for development of an injectable formulation for treatment of bladder cancer in the subsequent part of this study. The swelling capacity of the gel as depicted by changes in water content was revealed in **Figure 2B**. Such capacity, along with the gradual erosion of the gel as shown in **Figure 2C**, enables the CT gel to be swollen with an aqueous medium, which can then serve as the medium of diffusion for the loaded agent, and allows the loaded agents to be released from the gel in a sustained and steady manner.

2. Performance of CT in drug encapsulation and release

The EE of CT for curcumin and PD-L1 was found to be 82.3% and 42.2%, respectively (**Figure 3A**). Compared to that for curcumin, the EE for PD-L1 was lower. This is attributed to the higher aqueous solubility of PD-L1, leading to a higher loss of the encapsulated agent during the drug loading process. After both curcumin and PD-L1 were co-loaded into CT, the EE for curcumin and PD-L1 was 84.3% and 43.3%. Changes in the EE for both curcumin and PD-L1 were found not to be statistically significant after the co-loading process. This suggests that the process of co-loading has negligible influence on the EE of individual agents loaded into CT, and that physical interactions between curcumin and PD-L1 are minimal.

The release profiles of curcumin and PD-L1 from the CT gel were shown in **Figure 3B**. Compared with that of PD-L1, the release rate of curcumin was much lower. This can be explained by the lower aqueous solubility of curcumin, leading to a lower rate of Fickian diffusion from the polymeric network. The profiles of release of curcumin and PD-L1 from CT-Cur and CT-PD-L1 were found not to be influenced even after both curcumin and PD-L1 were co-loaded into the gel. This is favorable because this implies that when a curcumin/PD-L1 co-formulation is generated by using CT as a carrier, the EE and release profiles of each of the delivered agents can be easily predicted and individually tuned to meet practical needs, without having the optimization process being complicated by possible agent interactions and mutual influence.

2. PD-L1 antibody exacerbated symptoms and histopathological changes of colitis in DSS-induced UC mice.

PD-1/PD-L1 inhibitors are most widely used ICIs. To evaluate the safety of these ICIs for UC, a UC mouse model was generated by feeding C57BL/6 mice DSS-containing water (Supplemental Figure 1). The UC model mice were characterized by body weight loss, diarrhea, and bloody feces. Consistent with previous reports, we showed that UC was successfully induced in mice fed DSS-containing water for 7 consecutive days, and significant body weight loss was observed for these UC model mice compared with those fed non-DSS water (Fig. 1A, B) (Panel A, p=0.0164; Panel B, p=0.0225). Compared with the UC model mice injected ip with PBS or PD-1 antibody, those injected ip with PD-L1 antibody had even lower body weight (Fig. 1A, B) (Panel A, p=0.0475; Panel B, p=0.0371). In line with the change in body weight, the mDAI was significantly increased by PD-L1 antibody compared with PBS (p=0.0256) and PD-1 antibody (p=0.0498) (Fig. 1C). A shortening of the colon has been regarded as another indicator of colon inflammation severity. On Day 28, DSS administration had induced significant shortening of colon length compared with control water administration (Fig. 1D) (p=0.0256). In line with the above endpoints evaluating UC severity, PD-L1 antibody markedly shortened the colon length compared with PBS (p=0.0066) or PD-1 antibody (p=0.0112) (Fig. 1D, E). Human PD-1 antibodies were supposed to have a trend towards inducing more severe and frequent AEs than PD-L1 antibodies in patients with cancer (25). However, our results suggested a more detrimental effect of PD-L1 antibody than that of PD-1 antibody for UC in C56BL/6 mice, which might ascribe to a possible higher titer of the mouse PD-L1 antibody than that of the mouse PD-1 antibody used in the current study.

To corroborate the findings in DSS-induced UC mice, mouse colons dissected from sacrificed

mice were analyzed to evaluate the histopathological changes. In agreement with the above observations, mice fed DSS-water showed a distortion of crypts, a loss of goblet cells, severe epithelial injury and massive inflammatory cell infiltration in the mucosa and submucosa (Fig. 1F, G) (p=0.0003). Compared with the colons from UC model mice injected ip with PBS, those injected ip with PD-1 or PD-L1 antibody exhibited more extensive infiltration of inflammatory cells and a higher histological damage score, which was statistically significant for the PD-L1 antibody (p=0.0354) (Fig. 1G). These results histopathologically indicated that PD-L1 antibody worsened colitis of UC model mice.

3. CT-mediated local delivery of curcumin and PD-L1 antibody inhibited tumor growth and promoted CD8$^+$ T cell infiltration

Curcumin was previously proven as an effective anticancer agent in preclinical studies. Consistently, we showed that curcumin significantly inhibited tumor growth of subcutaneous bladder cancer xenograft in C57BL/6 mice and there was a trend towards improved anticancer effects with CT-mediated local delivery of curcumin (Fig. 2A, B). To investigate whether curcumin is effective for bladder cancer-bearing UC model mice and if PD-L1 antibody has the synergistic anticancer effect with curcumin, C57BL/6 mice were subcutaneously inoculated with bladder cancer cells followed by feeding with DSS for 7 days (Supplemental Fig. 2). Curcumin alone or in combination with PD-L1 antibody was injected intratumorally via CT. CT-mediated local delivery of the combination of curcumin and PD-L1 antibody significantly inhibited tumor growth in UC model mice ($p < 0.05$) (Fig. 2C, D). PD-L1 antibody administered by intraperitoneal injection was nearly as effective as that locally delivered with CT, however, intraperitoneal injection of PD-L1 antibody was associated with more death related to colitis as compared to local injection (Fig. 2C). The infiltration of CD8$^+$ T cells was associated with the anti-cancer efficacy of PD-1/PD-L1 inhibitors (26-28). To explore whether the local co-administration of curcumin and PD-L1 antibody via CT increased CD8$^+$ T cell infiltration, hematoxylin and eosin (H&E) as well as CD8 staining were performed (Fig. 2E, F). Compared with the control, local injection of CT loaded curcumin alone or in combination with PD-L1 antibody injected ip significantly increased the infiltration of CD8$^+$ T cells, and the local injection of CT loaded curcumin and PD-L1 antibody caused the most significant CD8$^+$ T cell infiltration (Fig. 2F, G) ($P < 0.01$), indicating a key role of CD8$^+$ T cells in tumor growth inhibition associated with CT-mediated local delivery of the combination of curcumin and PD-L1 antibody. In addition to the TME, David M et al observed targeted delivery of ICIs into lymphoid tissues where these stem-like CD8 T cells reside at high frequencies. Administration of ICIs can augment ICB therapy by improving immunomodulation within tumor-draining lymph nodes (TdLNs).

4. Intra-tumoral delivery of PD-L1 antibody via CT was less toxic on colon by way of colitis

It was reported that in CPUC, ICIs caused UC exacerbations, and steroid hormones or mesalazine dosing were required to curb UC symptoms, with ICI discontinuation required in some patients (29, 30). Therefore, we next asked whether local injection of PD-L1 antibody via CT was less toxic to mouse colon in terms of colitis than ip administration of PD-L1 antibody. By measuring body weight, we showed that the body weight of mice who were intratumorally injected with CT-loaded curcumin and PD-L1 antibody was comparable to that of mice who were intratumorally injected with CT-loaded curcumin only, but significantly greater than that of

mice who were intratumorally injected with CT-loaded curcumin and ip injected with PD-L1 antibody (Fig. 3A). As expected, DSS significantly caused colon shortening and ip injection of PD-L1 antibody further significantly shortened colons of mice who were intratumorally injected with CT-loaded curcumin; however, compared with intratumoral administration of curcumin, local injection of PD-L1 antibody together with curcumin via CT did not cause colon shortening (Fig. 3B). Determined by mDAI socres, ip injection of PD-L1 antibody induced significantly higher mDAI scores as compared with local injection of PD-L1 antibody via CT which induced similar mDAI scores to local injection of curcumin via CT (Figure 3C). In line with the above changes of body weight and mDAI scores, H&E staining of colon sections revealed a less severe colonic architecture damage and less infiltration of inflammatory cells with no apparent ulceration in mice treated with local injection of curcumin and PD-L1 antibody via CT than in mice treated with ip injection of PD-L1 antibody combined with local injection of curcumin via CT (Fig. 3D, E), indicating a safer adverse profile associated with local delivery of PD-L1 antibody by CT as compared with ip injection of PD-L1 antibody.

5. CT-mediated intratumoral delivery of PD-L1 antibody decreased its systemic distribution

Since 6-fold lower dose of PD-L1 antibody was injected intratumorally via CT than that injected ip, we next asked whether the intratumoral distribution of PD-L1 antibody in the mice locally injected with CT loaded PD-L1 antibody was more scarce than that in the mice ip injected with PD-L1 antibody. Surprisingly, despite of less PD-L1 antibody administered intratumorally, comparable intratumoral distribution of PD-L1 antibody was seen between the mice intratumorally injected with CT-loaded PD-L1 antibody and those ip injected with PD-L1 antibody (Figure 4A, B). To investigate whether the mice intratumorally injected with CT-loaded PD-L1 antibody have less systemic PD-L1 antibody, the concentration of PD-L1 antibody in the peripheral serum was measured by ELISA. The results demonstrated that serum PD-L1 antibody concentration was significantly lower in the mice intratumorally injected with CT-loaded PD-L1 antibody than that in the mice ip injected with PD-L1 antibody (Figure 4C). Accordingly, we further determined the distribution of PD-L1 antibody in mouse colons. In line with the serum distribution of PD-L1 antibody, there was significantly less PD-L1 antibody in colons of mice intratumorally injected with CT-loaded PD-L1 antibody than in mice ip injected with PD-L1 antibody (Fig. 4D, E). These results suggest that the less severe colitis in the mice intratumorally injected with CT loaded PD-L1 antibody likely ascribes to less systemic PD-L1 antibody. Interestingly, 5 days post injection of PD-L1 antibody, compared with the mice ip injected with PD-L1 antibody, those intratumorally injected with CT loaded PD-L1 antibody exhibited significantly stronger staining of PD-L1 antibody in tumor tissues and lower concentration of PD-L1 antibody in peripheral serum (Figure 4B, C). These implied the controlled releasing of PD-L1 antibody mediated by CT.

Conclusions

In conclusion, we have successfully delivered PD-L1 antibody intratumorally using CT, which effectively synergized with local delivered curcumin to inhibit tumor growth. PD-L1 antibody accumulated at the TME when injected intratumorally via CT. CT-mediated local delivery of PD-L1 antibody resulted in controlled release of PD-L1 antibody and significantly reduced its systemic distribution. Compared with ip injection of PD-L1 antibody, CT-mediated local delivery

of PD-L1 antibody showed significant less colon distribution of PD-L1 antibody and was safer for UC model mice in terms of colitis. Hence, CT-mediated local delivery of ICIs is potentially an effective and safe strategy for cancer patients with UC, which warrants further investigation.

References

1. Bagchi S, Yuan R, Engleman EG. Immune Checkpoint Inhibitors for the Treatment of Cancer: Clinical Impact and Mechanisms of Response and Resistance. Annual review of pathology. 2021;16:223-49.
2. Pennock GK, Chow LQ. The Evolving Role of Immune Checkpoint Inhibitors in Cancer Treatment. The oncologist. 2015;20(7):812-22.
3. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nature reviews Cancer. 2012;12(4):252-64.
4. Marabelle A, Tselikas L, de Baere T, Houot R. Intratumoral immunotherapy: using the tumor as the remedy. Annals of Oncology. 2017;28:xii33-xii43.
5. Thompson JA, Schneider BJ, Brahmer J, Achufusi A, Armand P, Berkenstock MK, et al. Management of Immunotherapy-Related Toxicities, Version 1.2022, NCCN Clinical Practice Guidelines in Oncology. Journal of the National Comprehensive Cancer Network : JNCCN. 2022;20(4):387-405.
6. Grover S, Ruan AB, Srivoleti P, Giobbie-Hurder A, Braschi-Amirfarzan M, Srivastava A, et al. Safety of Immune Checkpoint Inhibitors in Patients With Pre-Existing Inflammatory Bowel Disease and Microscopic Colitis. JCO oncology practice. 2020;16(9):e933-e42.
7. Bergqvist V, Hertervig E, Gedeon P, Kopljar M, Griph H, Kinhult S, et al. Vedolizumab treatment for immune checkpoint inhibitor-induced enterocolitis. Cancer immunology, immunotherapy : CII. 2017;66(5):581-92.
8. Gutzmer R, Koop A, Meier F, Hassel JC, Terheyden P, Zimmer L, et al. Programmed cell death protein-1 (PD-1) inhibitor therapy in patients with advanced melanoma and preexisting autoimmunity or ipilimumab-triggered autoimmunity. European journal of cancer (Oxford, England : 1990). 2017;75:24-32.
9. Kähler KC, Eigentler TK, Gesierich A, Heinzerling L, Loquai C, Meier F, et al. Ipilimumab in metastatic melanoma patients with pre-existing autoimmune disorders. Cancer immunology, immunotherapy : CII. 2018;67(5):825-34.
10. Iwamoto M, Kato K, Moriyama M, Yamaguchi K, Takahashi S. Remission of ulcerative colitis flare-up induced by nivolumab. International journal of colorectal disease. 2020;35(9):1791-5.
11. Lai W-F, Huang EM, Wong W-T. A gel-forming clusteroluminogenic polymer with tunable emission behavior as a sustained-release carrier enabling real-time tracking during bioactive agent delivery. Applied Materials Today. 2020;21.
12. Wing-Fu L, Dayong G, Mangin W, Aaron D, Andrey LR, Tingcao H, Wing-Tak W. A self-indicating cellulose-based gel with tunable performance for bioactive agent delivery. Journal of Drug Delivery Science and Technology. 2021;63:102428.
13. Wing-Fu L, Eric MH, Wing-Tak W. A gel-forming clusteroluminogenic polymer with tunable emission behavior as a sustained-release carrier enabling real-time tracking during bioactive agent delivery. Applied Materials Today. 2020;21:100876.
14. Chang M, Wu M, Li H. Antitumor Effects of Curcumin and Glycyrrhetinic Acid-Modified

Curcumin-Loaded Cationic Liposome by Intratumoral Administration. Evidence-based complementary and alternative medicine : eCAM. 2020;2020:4504936.
15. de Oliveira TV, Stein R, de Andrade DF, Beck RCR. Preclinical studies of the antitumor effect of curcumin-loaded polymeric nanocapsules: A systematic review and meta-analysis. Phytotherapy research : PTR. 2022;36(8):3202-14.
16. Hao M, Chu Y, Lei J, Yao Z, Wang P, Chen Z, et al. Pharmacological Mechanisms and Clinical Applications of Curcumin: Update. Aging and disease. 2023;14(3):716-49.
17. Dhillon N, Aggarwal BB, Newman RA, Wolff RA, Kunnumakkara AB, Abbruzzese JL, et al. Phase II trial of curcumin in patients with advanced pancreatic cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 2008;14(14):4491-9.
18. Shureiqi I, Baron JA. Curcumin chemoprevention: the long road to clinical translation. Cancer prevention research (Philadelphia, Pa). 2011;4(3):296-8.
19. Prasad S, Saha P, Chatterjee B, Chaudhary AA, Lall R, Srivastava AK. Complexity of Tumor Microenvironment: Therapeutic Role of Curcumin and Its Metabolites. Nutrition and cancer. 2023;75(1):1-13.
20. Gong F, Ma JC, Jia J, Li FZ, Wu JL, Wang S, et al. Synergistic effect of the anti-PD-1 antibody with blood stable and reduction sensitive curcumin micelles on colon cancer. Drug delivery. 2021;28(1):930-42.
21. Guo L, Li H, Fan T, Ma Y, Wang L. Synergistic efficacy of curcumin and anti-programmed cell death-1 in hepatocellular carcinoma. Life sciences. 2021;279:119359.
22. Hayakawa T, Yaguchi T, Kawakami Y. Enhanced anti-tumor effects of the PD-1 blockade combined with a highly absorptive form of curcumin targeting STAT3. Cancer science. 2020;111(12):4326-35.
23. Yan YX, Shao MJ, Qi Q, Xu YS, Yang XQ, Zhu FH, et al. Artemisinin analogue SM934 ameliorates DSS-induced mouse ulcerative colitis via suppressing neutrophils and macrophages. Acta pharmacologica Sinica. 2018;39(10):1633-44.
24. Jeon YD, Lee JH, Lee YM, Kim DK. Puerarin inhibits inflammation and oxidative stress in dextran sulfate sodium-induced colitis mice model. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2020;124:109847.
25. Ou SL, Luo J, Wei H, Qin XL, Du SY, Wang S, Jiang Q. Safety and Efficacy of Programmed Cell Death 1 and Programmed Death Ligand-1 Inhibitors in the Treatment of Cancer: An Overview of Systematic Reviews. Frontiers in immunology. 2022;13:953761.
26. Acúrcio RC, Pozzi S, Carreira B, Pojo M, Gómez-Cebrián N, Casimiro S, et al. Therapeutic targeting of PD-1/PD-L1 blockade by novel small-molecule inhibitors recruits cytotoxic T cells into solid tumor microenvironment. Journal for immunotherapy of cancer. 2022;10(7).
27. Huang Q, Wu X, Wang Z, Chen X, Wang L, Lu Y, et al. The primordial differentiation of tumor-specific memory CD8(+) T cells as bona fide responders to PD-1/PD-L1 blockade in draining lymph nodes. Cell. 2022;185(22):4049-66.e25.
28. Chen D, Barsoumian HB, Yang L, Younes AI, Verma V, Hu Y, et al. SHP-2 and PD-L1 Inhibition Combined with Radiotherapy Enhances Systemic Antitumor Effects in an Anti-PD-1-Resistant Model of Non-Small Cell Lung Cancer. Cancer immunology research. 2020;8(7):883-94.
29. Portenkirchner C, Kienle P, Horisberger K. Checkpoint Inhibitor-Induced Colitis-A Clinical Overview of Incidence, Prognostic Implications and Extension of Current Treatment Options.

Pharmaceuticals (Basel, Switzerland). 2021;14(4).
30. Zhang J, Guo Y, Fang H, Guo X, Zhao L. Oncolytic virus oHSV2 combined with PD-1/PD-L1 inhibitors exert antitumor activity by mediating CD4 + T and CD8 + T cell infiltration in the lymphoma tumor microenvironment. Autoimmunity. 2023;56(1):2259126.