

Figure S1. Triptolide can Enhance the Anti-tumour Effect of VNP20009 and Improve its Colonization.

A) The treatment schedule of triptolide and VNP20009 (VNP) for mice bearing B16F10 melanoma xenografts, and the volume of tumours in different treatment groups. For the intraperitoneal treatment of triptolide, the dose was 20 $\mu\text{g}/\text{kg}$ in 200 μL once every day from d 4 to d 14 post the inoculation of melanoma cells. For the intratumoral treatment of VNP20009 (VNP), the dose was 5×10^4 CFU/mouse in 50 μL on d 7 post the inoculation of melanoma cells. Mean \pm S.D., $n=7$. B) The body weight of mice receiving VNP20009 and triptolide intraperitoneally, relevant to Fig. 1A. Mean \pm S.D., $n=7$.

Figure S2. The Effects of Gr-1-neutralizing Antibody Treatment.

A) Gr-1⁺ cells in the peripheral blood were analyzed by the flow-cytometry after the depletion treatment on d 3 post the depletion. Mean \pm S.D., $n=3$. B) The body weight of mice receiving VNP20009, triptolide and Gr-1 neutralizing antibody, relevant to Fig. 2B. Mean \pm S.D., $n=5$.

Figure S3. Effects of intratumoural VEGF neutralization combined with VNP20009 treatment on the growth of tumours. The body weight of mice receiving VNP20009 and VEGF-neutralizing antibody, relevant to Fig. 4E. Mean \pm S.D., $n=5$.

Figure S4. The Overall Schematic Diagram of the synergistic effects of VNP20009 and triptolide. Conventional monotherapy of VNP20009 causes necrosis of tumour cells and suppresses tumour-angiogenesis, whose effects are diminished by the tumour-infiltrating neutrophils through confining VNP20009 in the necrotic centre. However, the combination therapy of triptolide and VNP20009 suppresses the activity of neutrophils to improve the tumour-colonization of VNP20009. At the same time, triptolide and VNP20009 also reduce the tumour-

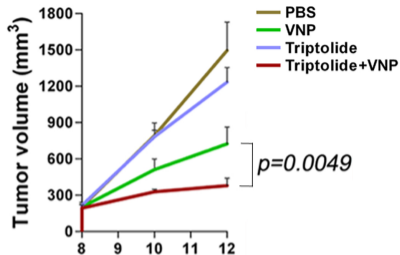
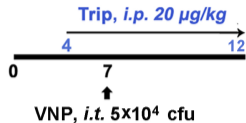
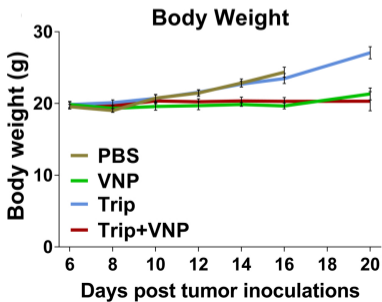
angiogenesis in a synergistic manner.

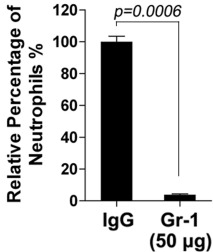
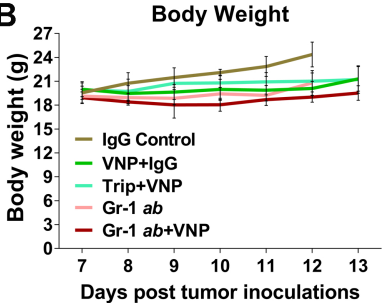
Figure S5. Triptolide Can Enhance the VNP20009-induced Tumour Infiltration of CD8⁺ T Cells.

A) Immunohistochemistry of CD8⁺ T cells infiltrating melanoma tissues. The red arrows indicate the positive cells. B) The flow-cytometry of CD8⁺ T cells in tumours. Anti-CD8-FITC antibody and normal IgG-FITC antibody were used for the analysis. Totally, 20,000 cells were collected for each sample. Mean \pm S.D., $n=3$.

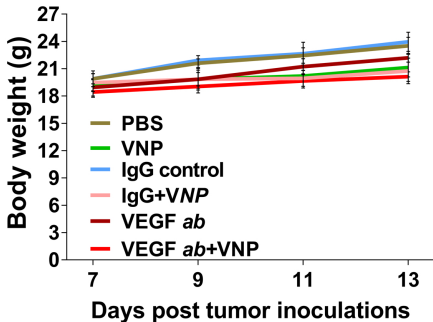
Figure S6. A High Dose of Triptolide Results in Systematic Toxicity and Loss of Anti-tumor Effects in the Combination Therapy of VNP20009 and Triptolide.

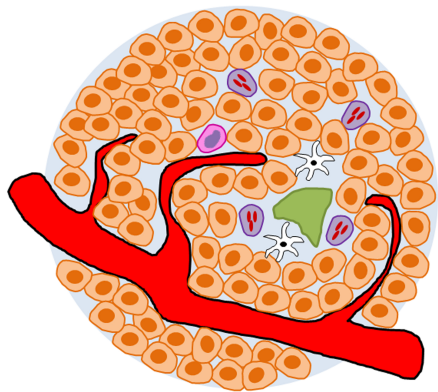
A) The treatment schedule of triptolide and VNP20009 (VNP) for mice bearing B16F10 melanoma xenografts. Triptolide was given intraperitoneally once every day from d 4 to d 10 at 20 or 200 $\mu\text{g}/\text{kg}$ in 200 μL PBS. B) The body weight of mice receiving VNP20009 and triptolide on d 12 post the inoculation of melanoma cells. Mean \pm S.D., $n=3$. C) The spleen weight of mice receiving VNP20009 and triptolide on d 12 post the inoculation of melanoma cells. Mean \pm S.D., $n=3$. D) The liver weight of mice receiving VNP20009 and triptolide on d 12 post the inoculation of melanoma cells. Mean \pm S.D., $n=3$. E) The tumour weight of mice receiving VNP20009 and triptolide on d 12 post the inoculation of melanoma cells. Mean \pm S.D., $n=3$.

A**B**

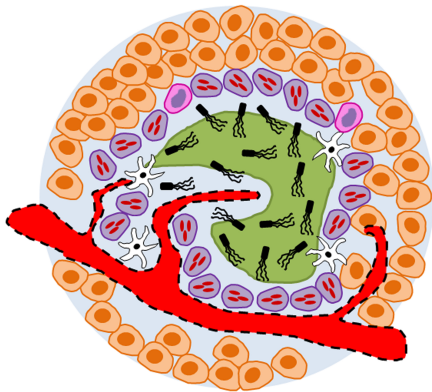
A**B**

Body Weight

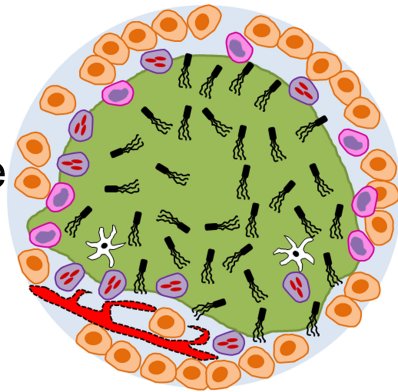











VNP →



VNP + Triptolide →

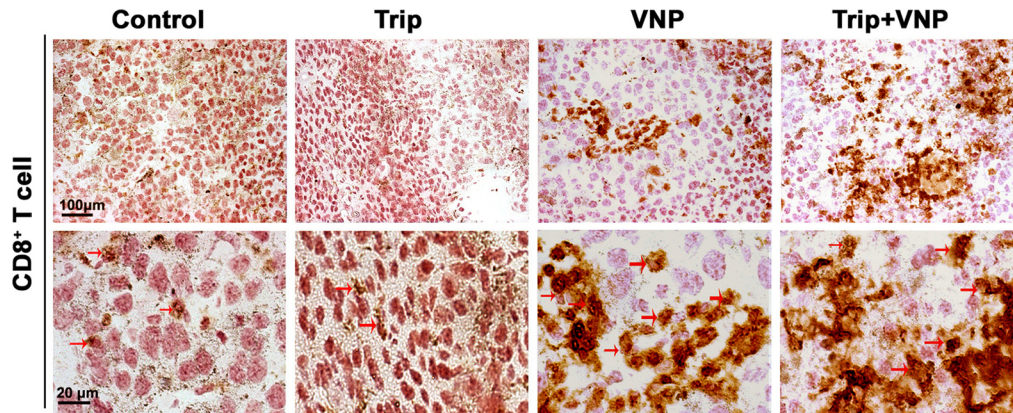


-  tumor cell
-  blood vessel
-  salmonella
-  necrotic area

-  neutrophil
-  macrophage
-  CD8+ T cell

angiogenesis ↓
 neutrophils ↑↑
 necrosis ↑

angiogenesis ↓↓
 neutrophils ↑
 CD8+ T cells ↑
 necrosis ↑↑

A**B**