Reviewer's report

Title: In vitro and in vivo anticancer properties of a Calcarea carbonica derivative complex (M8) treatment in a murine melanoma model

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Reviewer: Haili Qian

Reviewer's report:

This manuscript contains work on the Calcarea carbonica in inhibiting invasion and inducing immunosuppression of B16F10 melanoma model. The authors examined the effects of Calcarea carbonica in vitro and in vivo. Calcarea carbonica is a new immune system stimulator they used to suppress lung metastasis of melanoma cells. It did not change the expression of some key tumoral process proteins but showed ability to suppress cell adhesion in Matrigel model. Also, by lung inhalation, it stimulates the immune system to fight lung metastasis of B16F10 melanoma cells. The authors showed good data to support their conclusion. There are minor questions to this manuscript: 1. The author used lung inhalation as a way to stimulate the immune system to suppress the lung metastasis of melanoma cells. To exclude the direct suppression of the M8 complex on the tumor growth, it is better to administer the M8 complex by other ways but not lung inhalation to confirm the immune effects of M8 on metastasis. Or try the effect of lung inhalation on the subcutaneous tumor model. 2. Please include the methods to count the number of lung metastatic lesions. 3. Please include the statistics the authors used in the Methods section.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no competing interests.