A Novel Murine Tumor Model for Assessing the Efficacy of Neoadjuvant Dosing Regimens for Immunotherapies

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INTRODUCTION

Immunotherapy extends survival for patients with several cancer types, especially in combination with surgery. Recent evidence demonstrated improved efficacy with neoadjuvant/adjuvant pembrolizumab in combination with debulking surgery in patients with melanoma[1]. However, evaluating these regimens preclinically is challenging due to the rarity of appropriate models. To address this question, we have established a novel murine tumor model involving partial tumor-debulking surgery, which enables monitoring of the temporal response to immunotherapy in neoadjuvant/adjuvant, adjuvant and neoadjuvant settings.

METHODS

MC38 Colon Carcinoma Tumor Model
Female C57BL/6 mice were subcutaneously inoculated with 0.5 X 10^6 colon carcinoma MC38 cells into the right flank.

Immunotherapy
mNemvaleukin (mNemva) is a cytokine-based immunotherapeutic that selectively expands cytotoxic NK cells and antigen experienced $CD8^+$ T cells. When tumors reached ~100 mm^3, mice were subcutaneously administered PBS or mNemva at 6 mg/kg.

Partial tumor-debulking
Surgery was performed under general anesthesia, induced and maintained by isoflurane. Pre-emptive analgesia was provided by buprenorphine. Surgical area was disinfected prior to making a 10 mm incision into the skin above the tumor with a scalpel. Then, 2-3 mm of the skin on both sides of the incision were detached from the underlying tumor with forceps. Tumor tissue was manually expressed through an incision. The wound was closed by simple interrupted closing pattern using a cutting surgical needle attached to a non-absorbable monofilament suture material.

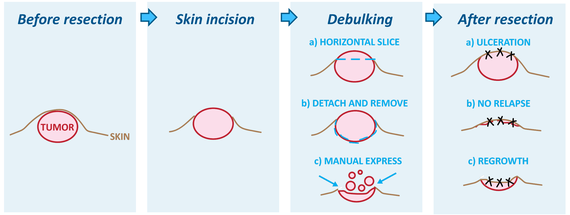

Figure 1. Tumor debulking techniques. To achieve 100% regrowth of tumors in the control group, several approaches were tested: a) horizontal slice that results in tumor ulceration, which requires euthanasia of an animal, b) detach and remove that often lacks relapse, and c) manual express, which outcomes in 100% regrowth of tumors in the control group.

Efficacy assessment
Effects of treatments were evaluated by the number of complete responders, percentage of tumor growth inhibition (TGI) and median survival. TGI was calculated on the last day when all animals were still enrolled in the study.

Re-challenge
Several weeks after the initial studies were finalized, complete responders were subjected to a re-challenge experiment, in which tumor growth was assessed again after subcutaneous inoculation of MC38 cells into the left flank.

References
1. Patel S et al. Neoadjuvant versus adjuvant pembrolizumab for resected stage III-IV melanoma [ESMO abstract LBA6]. *Annals of Oncology* 2022;33(suppl_7):S808-S869.

RESULTS

Neoadjuvant/adjuvant setting

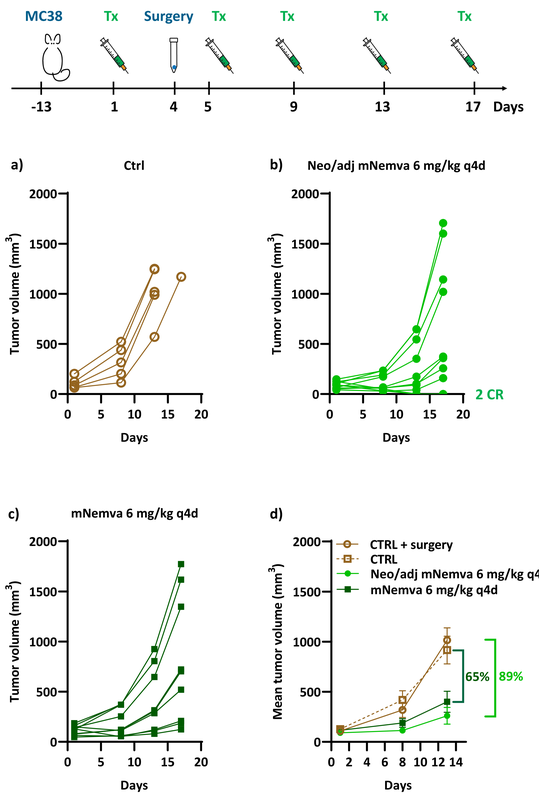

Figure 2. Representative tumor growth curves for a) control group and b) mNemva group in the neoadjuvant/adjuvant setting. (a) All tumors in the control group of animals reached predetermined tumor volume endpoint. (b) 2/10 tumors reached complete remission (CR) in mNemva group of neoadjuvant/adjuvant setting. (c) Five doses of mNemva without partial tumor-debulking surgery result in no CR, however, the treatment inhibited tumor growth for 65%. (d) Neoadjuvant/adjuvant mNemva results in superior tumor growth inhibition of 89% that is 24% higher in comparison to mNemva only.

Adjuvant setting

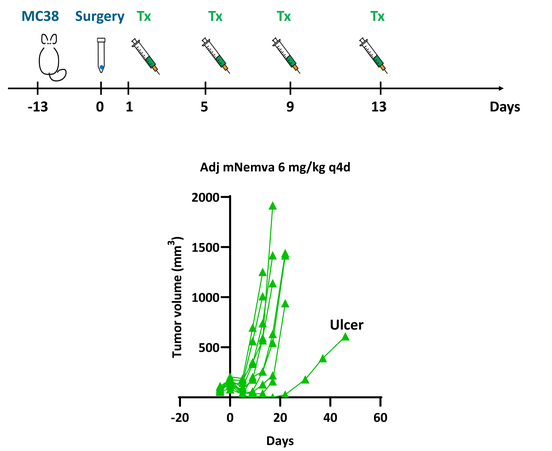

Figure 3. Tumor growth curves for the adjuvant setting with no animals in complete remission. No tumor growth inhibition was calculated because no control animals were enrolled in this experiment. Median survival was 17 days, which compares to median survival of the control group in neoadjuvant/adjuvant setting.

Re-challenge of mice enrolled in neoadjuvant/adjuvant setting

Mouse	Weeks between MC38 inoculations	Tumor growth in a re-challenge
1	11	No
2	12	No
3	12	No
4	22	No
5	22	No
6	30	No
7	30	No
8	30	No
9	30	Yes
10	30	Yes

Table 1. Re-challenge experiments with mice that were enrolled in the neoadjuvant/adjuvant setting. Following tumor re-challenge, 80% of prior complete responders are protected.

Neoadjuvant setting

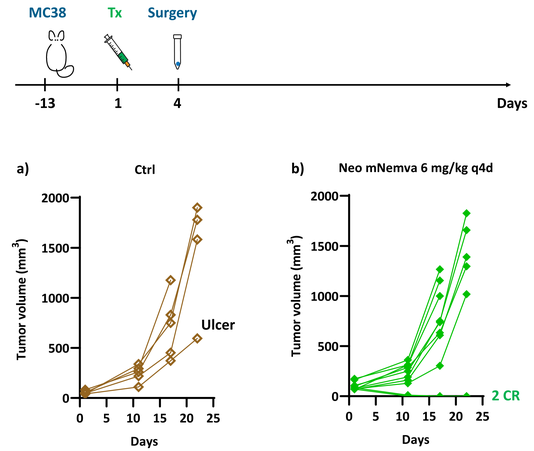

Figure 4. Tumor growth curves for the neoadjuvant setting. (a) All tumors in the control group of animals grew to a predetermined tumor volume endpoint. (b) 2/10 tumors reached complete remission (CR) in neoadjuvant setting. However, tumor growth inhibition was only 17% as calculated on day 17. Median survival was not improved in mNemva groups as it was 22 days for both the control group and mNemva-treated mice.

CONCLUSIONS

- **Subcutaneous MC38 colon carcinoma exhibits 100% regrowth if manual expression is employed as a debulking technique.**
- **Neoadjuvant/adjuvant treatment regimen with mNemva is superior to adjuvant and neoadjuvant in all aspects.**
- **While neoadjuvant/adjuvant and neoadjuvant settings yield 28% and 20% complete responders, respectively, no complete remission is observed in the adjuvant setting.**
- **Following tumor re-challenge, 80% of prior responders are protected, suggesting that mNemva induces an immunological memory in most mice.**

DISCLOSURES

- All authors are current or former employees of, and may hold stock in, Alkermes.

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