

EXPERIMENTAL PROTOCOL: Immune checkpoint blockade *in vivo*

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Introduction

This protocol is designed to illustrate tumor growth kinetics and response to immune checkpoint blockade (ICB) with anti-PD-1 and/or anti-CTLA-4 in the most commonly used mouse models. Results may vary significantly between animal facilities, cellular derivation, etc...

All *in vivo* procedures must comply with local regulations and included in an experimental protocol.

Reagents

- PBS
- anti-PD-1 (data obtained with clone 29F.1A12, BioXcell)
- anti-CTLA-4 (data obtained with clone 9D9, BioXcell)
- 23G/25G needle and syringes

Before you begin

- Ensure hood is clean (DO NOT do cell culture in a hood at the same time) before you start
- It is recommended to aliquot antibodies for each treatment day to avoid contamination
- The combination of anti-PD-1 + anti-CTLA-4 can be toxic in some models and cause animal death. The highest recommended dose is 200ug e.a. at days 6, 9 and 12 after tumor inoculation (a fourth dose could be fatal).

PROCEDURE

CALCULATE DILUTIONS FOR ICB

1. Determine what volume of IO you'll need:
 - a. Make each dose 100 uL

- b. Calculate one dose per treated mouse, per treated day
- c. Include extra for each day of dosing
- d. EXAMPLE:
 - i. 20 mice being dosed, d6/d9/d12
 - ii. $(100 \text{ uL/dose} * 20 \text{ mice}) = 2 \text{ mL}$ each day... budget 2.3 mL each day
 - iii. $(2.3 \text{ mL/day} * 3 \text{ treatment days}) = 6.9 \text{ mL total}$

Calculate dilutions based on desired final concentration of antibody

- a. Determine concentration of drug required to deliver desired amount in 100 uL
 - i. 100 ug dose in 100 uL = 1 mg/mL
 - ii. 200 ug dose in 100 uL = 2 mg/mL
- b. Dilution calculation from the stock concentration of antibodies in batch you use
 - i. $(\text{Final conc}) * (\text{Final volume}) = (\text{Stock conc}) * (\text{_____ stock volume needed})$

EXAMPLE: $(1 \text{ mg/mL}) * (6.9 \text{ mL total}) = (\text{Stock at } 8.13 \text{ mg/mL}) * (\text{_____})$

Divide to get 0.849 mL of stock antibody

- ii. Remaining volume (up to total final volume) from sterile 1x PBS
EXAMPLE: $6.9 \text{ mL final} - 0.849 \text{ mL antibody} = 5.851 \text{ mL } 1x \text{ PBS}$

TREATMENT RESPONSE

Representative graphs showing tumor growth with indicated tumor models and treatments. Tumors were induced by subcutaneous injection of 10^6 cells/mouse. Data is represented as mean \pm SEM

