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Natural killer cell depletion in vivo (mouse)

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ABSTRACT

This protocol describes a validated procedure for antibody depletion of natural killer cells in mice (C57Bl/6J and 129SvEv strains), combined with the injection of cancer cells i.v. It is based on combining a few references and testing the optimal antibody concentration and frequency of injection to achieve good level of depletion, minimise animal injection frequency, and reagent consumption.

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BEFORE STARTING

Before starting the experiment, validate the level od depletion of your batch of antibody by FACS (use spleen or blood sample to check the number of NK cells 4 or 7 days after antibody administration). Make sure to use a staining antibody for a marker other than the one targeted with a depletion antibody. I use Nkp46.

My protocol is based on depleting the NK cells for 4 days (two doses) before injecting tumour cells, followed by a weekly administration of the depleting antibody afterwards.

This schedule should be checked and adapted for any other setting/experiment.

For NK cell depletion in **C57Bl/6** mice, use the following antibody and corresponding isotype control:

Isotype control is Mouse IgG2a, κ (InVivoMAb, clone C1.18.4, catalog number BE0085)
NK cell neutralising antibody is Nk1.1 (Clone PK136, generated in-house from a hybridoma line, or purchased commercially)

Most references use 200-300 ug of NK1.1 antibody per mouse, with various frequencies, from 1x week to 3x week

Smyth et al. 1998 (200 ug)
Nishikado et al. 2011 (300 ug)
Victorino et al. 2015 (200 ug)

For NK cell depletion in **129SvEv mice**, use the following antibody, because 129S strain NK cells do not express Nk1.1:

Polyclonal rabbit IgG (InVivoMAb, BE0095, Isotype Control)
Asialo GM1 Polyclonal Antibody, Functional Grade, eBioscience™ (ThermoFisher, Catalog # 16-6507-39)

Dose:
10 uL (anti-asialo GM1), diluted to 100 uL with PBS prior to the injection

N.B. Anti-asialo GM1 antibody also depletes basophils.

1 Mice will receive an i.p. *injection* of the antibody in 200 µl PBS (Day 1) e.g. Monday

2 Mice will receive an i.p. injection of the antibody in 200 µl PBS (Day 4) e.g. Thursday

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3 Mice will receive tail vein injection of the cell suspension in 100 µl PBS (Day 5) e.g. Friday

4 Mice will receive an i.p. injection of the antibody in 200 µl PBS (Day 11, 18, 25) i.e. once per week

5 Mice will be culled after 28 days from the day of tumour cell injection or at an appropriate time-point for any particular experimental setting.

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