BiTE® Xenograft Protocol

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Abstract

This protocol describes methods for a mouse xenograft study designed to measure in vivo BiTE® efficacy against antigen-expressing human tumor cells. In a modified version, the protocol is used to measure BiTE®-mediated bystander killing. Mixtures of human EGFR-positive (unlabeled) and EGFR-negative (luciferase-labeled) cells are implanted in nude mice along with human T cells. Mice are treated daily with BiTE®, beginning one day after tumor implant for 20 days. Growth of EGFR-negative cells is measured by imaging (luminescence) and by calipers (tumor volume).

Citation: Sandra L. Ross, Marika Sherman, Patricia L. McElroy, Julie A. Lofgren, Gordon Moody, Patrick A. Baeuerle, Angela Coxon, Tara Arvedson BiTE® Xenograft Protocol. **protocols.io**

dx.doi.org/10.17504/protocols.io.h5eb83e

Published: 22 May 2017

Protocol

Overview

Step 1.

- Female athymic nude mice approximately 7 weeks old (Charles River Laboratories) are used for the study
- Human tumor cell lines are cultured in McCoy's medium with 10% heat-inactivated fetal bovine serum (FBS) (HCT116) or RPMI with 10% FBS (SW620-LUC) at 37º C with 5% CO_2.
- Human pan T cells (AllCells) are thawed according to supplier instructions and cultured in RPMI with 10% FBS at 37º with 5% CO_2. T cells are activated by culturing with beads coated with anti-CD2, -CD28 and CD3 antibodies following the manufacturer's instructions (Miltenyi Biotec T cell activation/expansion kit). Three days after establishment of the culture, huIL-2 (Miltenyi Biotec) is added to the medium at a concentration of 0.1 µg/mL.
- Tumor cells mixed with activated T cells at a 1:1 ratio are implanted subcutaneously on the left flank of the mice, in a 1:1 mix of serum-free RPMI and Matrigel basement membrane matrix (BD Biosciences).
- Control and treatment BiTEs® are diluted to the proper concentration in PBS and administered intraperitoneally.
- Imaging of bioluminescence from luciferase-labeled cells is conducted using the IVIS Spectrum in vivo imaging system (PerkinElmer); mice are administered 150 mg/kg D- Luciferin (Sigma, diluted in PBS) intraperitoneally 15 minutes before image capture.
- Tumor volumes are obtained using handheld digital calipers to measure tumor length, width and height to calculate cubic millimeters; mice are weighed using a digital balance.
- Tumors are collected at the end of the study and placed in neutral buffered formalin (NBF).

Prepare T cells

Step 2.

1. Prepare activating beads according to manufacturer's instructions; briefly, prepare mixture of activating antibodies, add unlabeled beads, mix gently at 4º for 2 hours, store at 4º.
2. Thaw T cells according to supplier instructions and place in tissue culture (TC) flask, at a concentration of approximately 2 x 10^6 cells/mL.
3. Add activating beads at a bead-to-cell ratio of approximately 1:4. Wash beads one time to remove excess antibody before adding to T cells.
4. After 3 days, add medium with IL-2. Keep cell density at approximately 2 x 10^6 cells/mL, adding fresh media with IL-2 and expanding into additional flasks every 2 to 3 days. Viability should remain ≥ 90%. Expand to approximately 50% more than number needed for implantation; extra is needed due to loss during de-beading prior to implantation.

Prepare tumor (target) cells

Step 3.

1. Thaw tumor cells and place in proper medium in TC flasks and incubate.
2. Expand to cell numbers necessary for implant (plan for approximately 30% extra cells for each cell line).

Implant admixed tumor (target) cells and T cells

Step 4.

Day 0:

1. Thaw a vial of Matrigel; keep cold.
2. Count T cells to determine viability and cells/mL. Viability should be ≥ 90%. Determine volume of T cells needed (with extra) based on cell count and remove beads by using MACSiMAG Separator magnetic column (Miltenyi Biotec) or similar. Count de-beaded T cells and keep on ice.
3. Harvest appropriate number of tumor cells using 0.05% trypsin with EDTA (1X) (Gibco), adding medium with FBS after cells have lifted off the flask; pool cells, determine cell count and viability (should be ≥ 90%); keep on ice.
4. Mix T cells and tumor cells at a 1:1 ratio. (Example: add 250,000 T cells to 250,000 tumor cells.) For groups containing two tumor cell lines, combine equal numbers of each tumor cell line (e.g. 250,000 HCT116 and 250,000 SW620-LUC) and add T cells equal to total number of tumor cells (500,000).
5. Wash T cell and tumor cell mixture in serum-free RPMI (centrifuge at 300 G, 5 minutes, 4º). Resuspend pellet in a 1:1 mixture of serum-free RPMI and Matrigel, in a volume that will give each mouse the desired number of cells in 0.1 mL. Keep cell solution on ice.
6. Inject 0.1 mL of T cell/tumor cell mixture subcutaneously on the flank of each mouse, using a 0.5 mL syringe with a 28 gauge needle. Fill only one syringe at a time and keep remaining cell solution on ice so that the Matrigel does not thicken.

Baseline bioluminescence and initiation of BiTE® treatment

Step 5.

Day 1:

1. Image all mice with IVIS bioluminescence imaging system to obtain baseline image. Treat mice with 150 mg/kg IP of D-Luciferin 15 minutes prior to imaging. Image tumors with mice in lateral

recumbency with tumor side up.

2. Dilute BiTEs® with PBS to proper concentration.
3. Treat mice with control or active BiTE®: administer IP.

Ongoing BiTE® dosing and tumor measurement

Step 6.

Day 2 and continuing until end of study:

- Treat mice daily with BiTEs®.
- Image 2 times per week, and measure tumors with calipers.
- Weigh mice 2 times per week once tumors are palpable.
- At end of study, euthanize mice according to IACUC guidelines and harvest tumors; place in NBF.