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Combination of vemurafenib and *in vivo* stimulation of gd T lymphocytes with zoledronate and interleukin 2: preclinical study on a BRAF mutated murine model.

BACKGROUND

Gd T cells are the most represented subset of melanoma-infiltrating lymphocytes. Combining their i*n vivo* stimulation with aminobisphosphonates with a BRAF-targeted could prolong therapeutic response and improve prognosis in metastatic melanoma.

SPECIFIC AIMS

Aim 1: To evaluate the antitumoral effects of *in vivo* zoledronate and IL-2 activated gd T cells using human BRAF-mutated melanoma xenografts in NOD scid g chain-knocked out (NSG) mice.

Aim 2: To compare the antitumoral effects of vemurafenib alone or in combination with *in vivo* zoledronate and Il-2 activated gd T cells using human BRAF-mutated melanoma xenografts in NOD scid g chain-knocked out (NSG) mice.

MATERIALS AND METHODS

90 NSG mice (10/group) will receive intraperitoneal (ip) human melanoma stem cells (MSCs) stably expressing luciferase and GFP. 80 mice will receive in addition, hr IL-2 and/or zoledronate (Zol) and highly purified human gd T cells (2×10^7/mouse).

Aim 1:

- Group 1: hMSC only (control)
- Group 2: hMSC + lymphocytes T gd
- Group 3: hMSC + lymphocytes T gd + zoledronate
- Group 4: hMSC + lymphocytes T gd + IL-2
- Group 5: hMSC + lymphocytes T gd + zoledronate + IL-2

Aim 2:

- Group 1: hMSC + lymphocytes T gd + vemurafenib
- Group 2: hMSC + lymphocytes T gd + vemurafenib+ zoledronate
- Group 3: hMSC + lymphocytes T gd + vemurafenib +IL-2
- Group 4: hMSC + lymphocytes T gd + vemurafenib +zoledronate + IL-2

Animals will be assessed for 6 months by the following parameters:

- Tumor progression, by measurement of tumoral size and *in vivo* luminometer imager, which allows localization of gd T cells;
- Number, phenotype and effector functions of circulating gd T cells at 1,3 and 6 months;
- Overall survival.

SIGNIFICANCE

The results of this study could build the preclinical bases for the development of combined therapies – targeted therapy/immunotherapy- and improve the therapeutical approach to metastatic melanoma, whose prognosis is still poor.

The combination of a BRAF-targeted therapy with *in vivo* stimulation of gd T lymphocytes with zoledronate and IL-2 could prolong clinical response in patients with metastatic BRAF-mutated melanoma.