Anti CXCR4 antibody combined with activated and expanded killer cells for sarcoma immunotherapy

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1. Analysis of CXCR4 expression, migration and invasion capacity of different sarcoma cell lines

RH30 cell line showed the highest CXCR4 expression.

RH30 cells are able to migrate and invade towards a gradient of CXCL12 chemokine, CXCR4 specific ligand.

Fig 1. Expression of CXCR4 by different sarcoma cell lines (RH30, CR809, A549, A57, MG63 and 143B) was analysed by flow cytometry.

Fig 2. Migration capacity towards CXCL12 (100 ng/ml) or fetal bovine serum (FBS, 10%) was tested using 8 µm-pore membranes Transwell assays (48 hours). Invasion capacity was measured under the same conditions using Matrigel-coated Transwell membranes.

The combination of MDX1338 and NKAE cells completely abrogated RH30 lung micrometastasis.

Fig 3. P53MC were obtained from a healthy donor, and co-cultured with 100 Gy irradiated K562/imm-pLuc+IL2-41BBL cells. At day 21 of the expansion, >98% of NKAE (CD56+CD3−) population was achieved.

Fig 4. MDX1338 and NKAE cells mediated inhibition of RH30 cells migration and invasion towards CXCL12.

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Fig 5. Luminiscent particles expressing GFP and hUranus were used to transduce BDF1 cells. GFP+ Luc+ thalidomidosarcoma cells were inoculated intravenously in immunodeficient NSG mice. Luminiscent units (Luminiscent Units (LU) (107 cells, once a week)). Luminiscent tumors were monitored for 25 days.

Fig 6. Lung micrometastasis were detected and quantified with qRT-PCR using a human CXCR4 and GUS specific TaqMAN probe. Indicated values are relative to hCXCR4 and hGUS expression by a 1µg RH30 cells pellet.

Sarcoma micrometastases inhibition was further confirmed by histological methods.

Conclusions

Our in vitro and in vivo studies show a synergistic role of anti-CXCR4 antibody MDX1338 and NKAE cell therapy to prevent rhabdomyosarcoma cell invasion, tumor implant and metastasis formation. These preclinical results constitute the first evidence of the efficacy of this combined immunotherapy treatment to prevent sarcoma disease dissemination.

Background

Sarcoma lung micrometastases were identified by hematoxylin & eosin and Day 7 and Day 21

2 µm

p=0.0309

by flow cytometry.

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Background

Sarcoma metastasis in children and adolescents has a 5-year survival rate of less than 20%. Current therapies, consistent in radical surgery and non-adjuvant chemotherapy, remain ineffective. Consequently, new therapeutic venues are required.

CXCR4 is upregulated in 33.3 - 73.3% of sarcomas and its overexpression contributes to tumor growth, invasion, angiogenesis, metastasis, relapse, and therapeutic resistance. CXCR4 signaling blockade by MDX1338 (Bristol-Myers Squibb) may disrupt tumor-endothelial interactions, sensitize sarcoma cells to cytotoxic drugs, and reduce tumor growth and metastatic burden.

Activated and expanded NK cells (NKAE) have shown cytotoxicity against osteosarcoma and Ewing’s sarcoma in vitro and in vivo. We have tested the synergistic effect of NK cell therapy in combination with anti CXCR4 antibody immunotherapy for the inhibition of sarcoma metastasis.

Co-culturing Peripheral Blood Mononuclear Cells (PBMC) with K562/imm-pLuc+IL2-41BBL allows to selectively expand NK cells.

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