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

Vela M[1], González-Navarro P[1], Brito A[1], Valentín J[1], Bueno D[2], Escudero A[3], Fernández L[4], Pérez-Martínez A[2].

[1]Translational Research in Pediatric Oncology, Hematological Transplantation & Cell Therapy (IdiPAZ). [2]Pediatric Hemato-Oncology (La Paz Universitary Hospital). [3]Pediatric Molecular Hemato-Oncology (INGEMM). [4]Hematological Malignancies Clinical Research Unit (CNIO).

maria.vela@idipaz.es

Background Metastatic sarcoma in children and adolescents has a 5-year survival rate of less than 20%. Current therapies, consistent in radical surgery and neo-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–stromal interactions, sensitize sarcoma cells to cytotoxic drugs, and reduce tumor growth and metastatic burden.

Activated and expanded NK cells (NKAE) have shown cytoxicity 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.

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

RH30 cell line showed the highest CXCR4 expression.

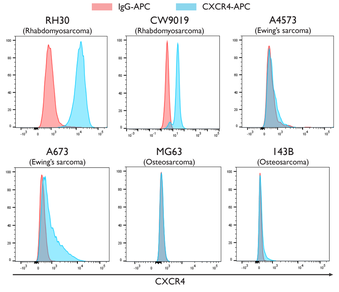

Fig 1. Expression of CXCR4 by different sarcoma cell lines (RH30, CW9019, A4573, A673, MG63 and 143B) was analyzed by flow cytometry.

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

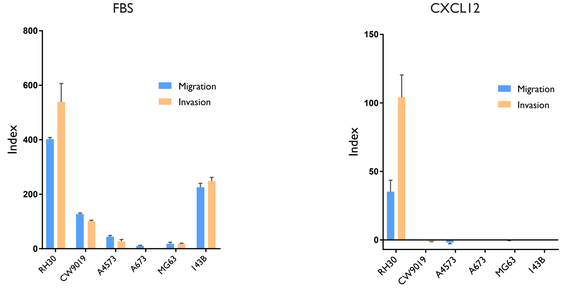

Fig 2. Migration capacity towards CXCL12 (100 ng/ml) or fetal bovine serum (FBS, 10%) was tested using 8 µm-pore membranes Transwell assays (48 hours). Invasion capacity was measured under the same conditions using Matrigel-coated Transwell membranes.

II. *In vitro* migration and invasion inhibition of alveolar rhabdomyosarcoma cells

Co-culturing Peripheral Blood Mononuclear Cells (PBMC) with K562mbIL15-41BBL allows to selectively expand NK cells.

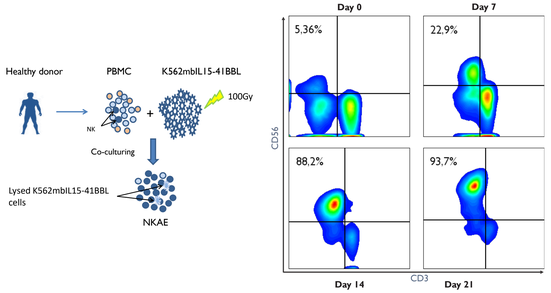

Fig 3. PBMC were obtained from a healthy donor, and co-cultured with 100 Gy irradiated K562mbIL15-41BBL cell line. At day 21 of the expansion, >90% of NAKE ($CD3^-$ $CD56^+$) population was achieved.

The combination of MDX1338 and NKAE cells completely abrrogated RH30 cells migration and invasion towards CXCL12.

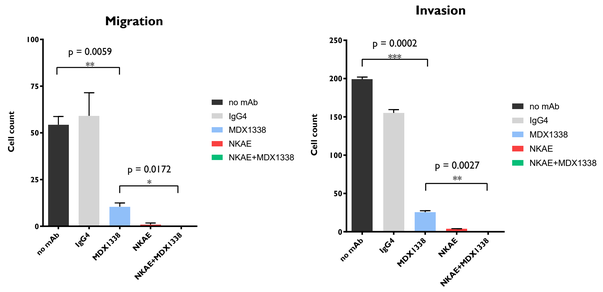

Fig 4. MDX1338 and NKAE cells mediated inhibition of RH30 cells migration and invasion towards a gradient of human recombinant CXCL12 chemokine was tested using Transwell plates.

III. *In vivo* tumor implant inhibition by MDX1338 and NKAE cells

The MDX1338 treatment alone moderately inhibited RH30 tumor implant, while NKAE treatment completely prevented it.

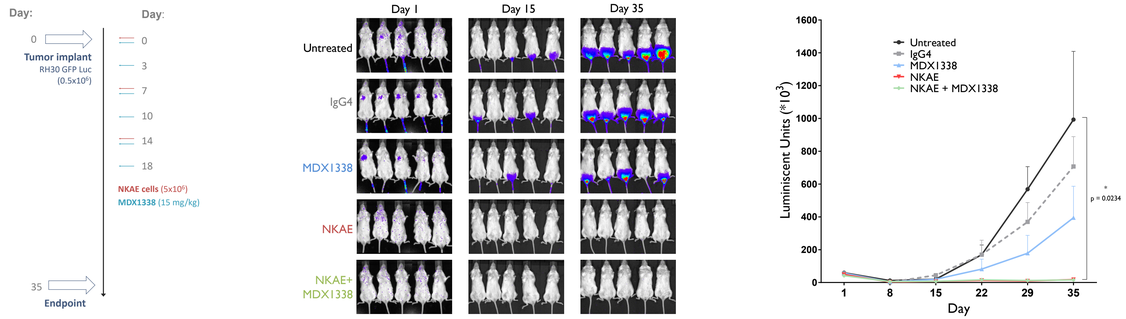

Fig 5. Lentiviral particles expressing GFP and luciferase were used to transduce RH30 cell line. GFP+ Luc+ rhabdomyosarcoma cells were inoculated intravenously in immunodeficient NSG mice (NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl} /SzJ) to generate an *in vivo* model of metastatic sarcoma. Five treatment arms were established: untreated; IgG4; MDX1338; NKAE; MDX1338+NKAE. Mice received six doses of mAb (15 mg/kg, twice a week), and three doses of NKAE (5 x 10^6 cells, once a week). Luminiscent tumors were monitored for 35 days.

IV. Sarcoma lung micrometastasis suppression

MDX1338 reduced RH30 lung micrometastasis incidence, while the combination of both MDX1338 and NKAE completely eliminated it.

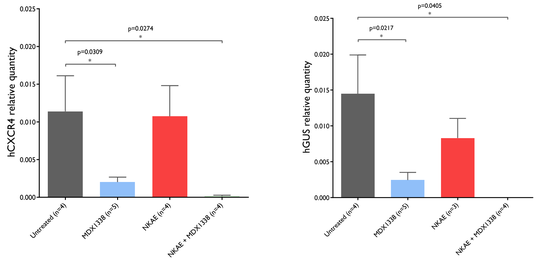

Fig 6. Lung micrometastasis were detected and quantified with qRT-PCR using a human CXCR4 and GUS specific TaqMAN probe. Indicated values are relative to hCXCR4 and hGUS expression by a 10^6 RH30 cells pellet.

Sarcoma micrometastases inhibition was further confirmed by histological methods.

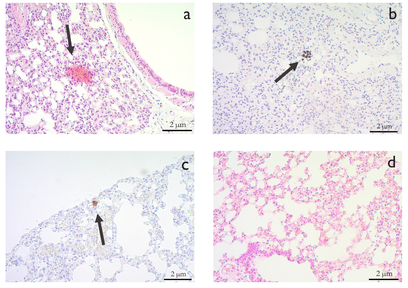

Fig 7. Sarcoma lung micrometastases were identified by hematoxylin & eosin staining (a), Alu sequences hybridization (b), and CXCR4-specific mAb stain (c). NKAE + MDX1338 treated mice showed no micrometastasis incidence (d).

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.