Anti CXCR4 antibody combined with activated and expanded natural killer cells infusions efficiently inhibits sarcoma metastasis

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[1,2,3].

[1] Traslational Research in Pediatric Oncology, Hematopoietic Trasplantation & Cell Therapy, IdiPAZ, Madrid (Spain); [2] Pediatric Hemato-Oncology, La Paz Universitary Hospital, Madrid (Spain); [3] Pediatric Molecular Hemato-Oncology, INGEMM, Madrid (Spain); [4] Haematological Malignancies Clinical Research Unit, CNIO, Madrid (Spain).

***maria.vela@idipaz.es**

BACKGROUND

- Metastasis occurs in 20-55% of sarcoma patients and remains the main cause of death. We propose a novel immunotherapeutic approach based in anti CXCR4 antibody MDX1338 (Bristol Myers Squibb) in combination with Activated and Expanded Natural Killer (NKAE) cells therapy.
- CXCR4 is upregulated in 33.3-73.3% sarcomas. Its signaling blockade by MDX1338 may disrupt tumor-stromal interactions, sensitize sarcoma cells to cytotoxic drugs, and reduce tumor growth and metastatic burden.
- NKAE cells can eliminate malignant sarcoma cells as reported in assays showing NK cell cytotoxicity against osteosarcoma and Ewing's sarcoma *in vitro* and *in vivo*. Additionally, clinical data suggests that haploidentical donor NK cells may exert antitumor activity in children with solid tumors undergoing allogeneic hematopoietic stem cell transplantation.

OBJECTIVE

To test in *in vitro* and *in vivo* assays the synergistic effect of NK cell therapy in combination with anti CXCR4 antibody immunotherapy to prevent sarcoma metastasis.

RESULTS

I) Analysis of CXCR4 expression by different sarcoma cell lines

Alveolar rhabdomyosarcoma RH30 cell line showed the highest CXCR4 expression

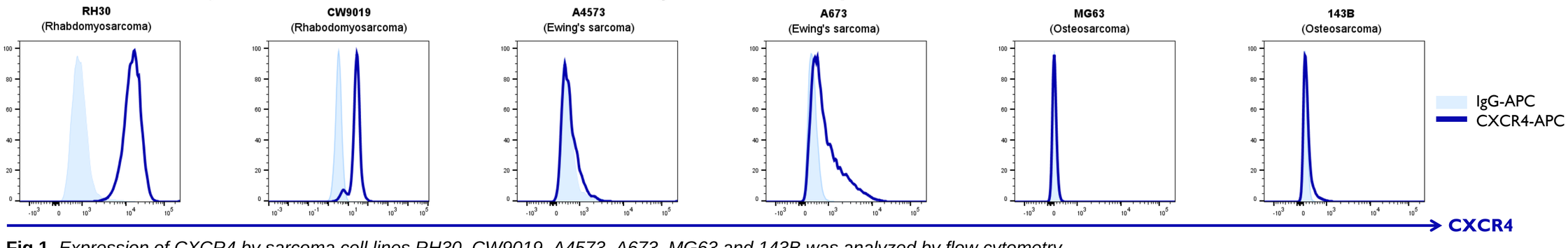

Fig 1. *Expression of CXCR4 by sarcoma cell lines RH30, CW9019, A4573, A673, MG63 and 143B was analyzed by flow cytometry.*

II) Migration and invasion capacity of different sarcoma cell lines

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

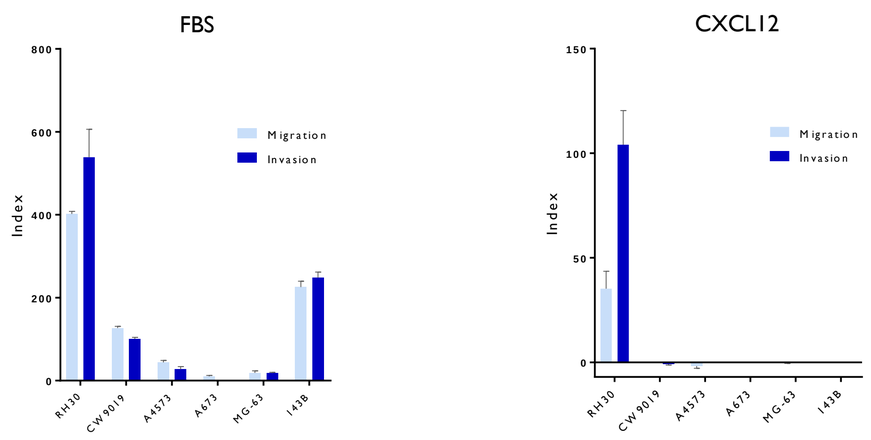

Fig 2. *Migration capacity towards fetal bovine serum (FBS, 10%) or human recombinant CXCL12 (100 ng/ml) was tested using 8 µm-pore membranes Transwell assays (48 hours). Invasion capacity was measured under the same conditions using Matrigel-coated membranes.*

III) *In vitro* migration and invasion inhibition of sarcoma cells

The combination of MDX1338 and NKAE cells completely abrogated RH30 cells migration towards CXCL12

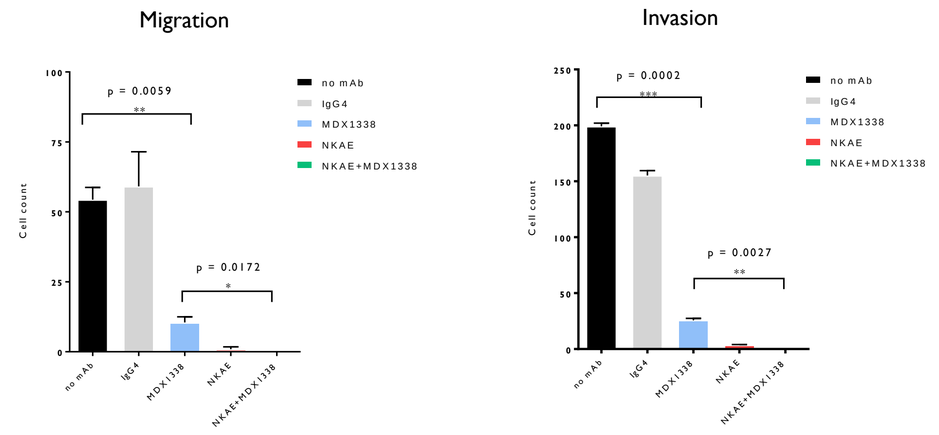

Fig 3. *MDX1338 and NKAE cells mediated inhibition of RH30 cells migration and invasion towards a gradient of CXCL12 chemokine was tested using Transwell plates. Antibody concentration was 300 µg/ml and NKAE:RH30 effector:target ratio was 5:1.*

IV) *In vivo* tumor implant inhibition by y MDX1338 and NKAE cells

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

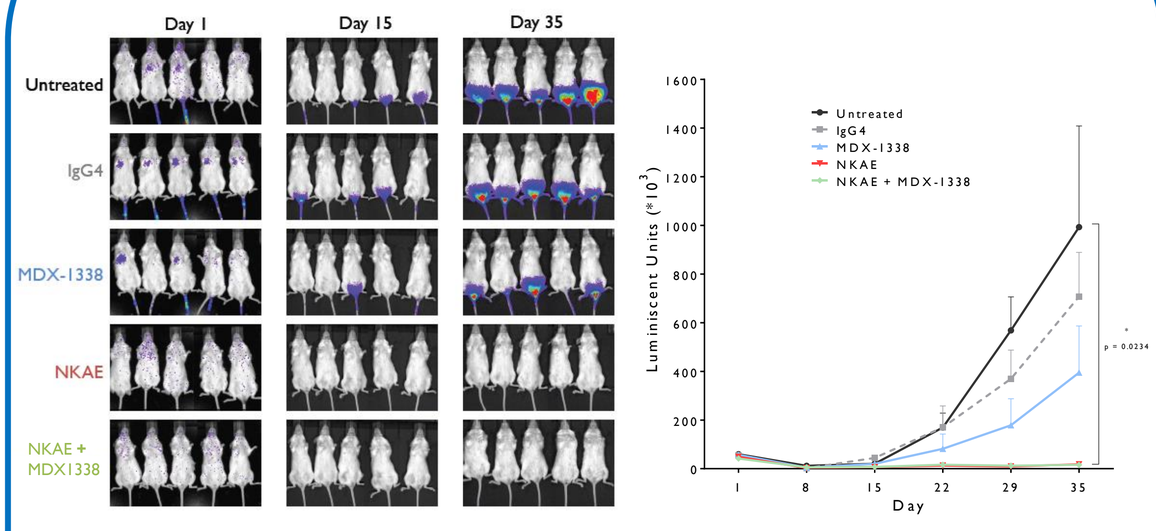

Fig 4. *Lentiviral particles expressing GFP and luciferase were used to transduce RH30 cell line. GFP⁺ Luc⁺ rhabdomyosarcoma cells were inoculated intravenously in immunodeficient NSG mice (NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ) to generate an in vivo model of metastatic sarcoma. Five treatment arms 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.*

V) Sarcoma lung micrometastasis suppression

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

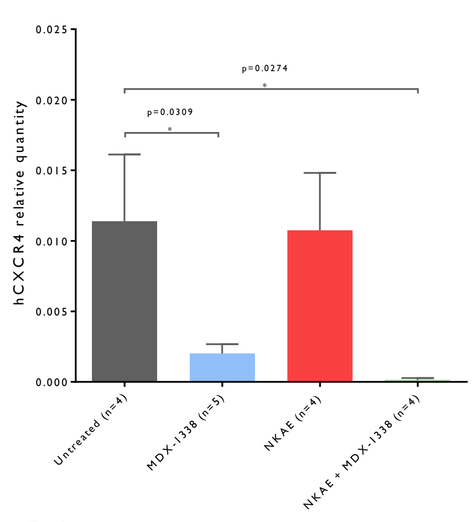

Fig 5. *Lung micrometastasis were detected and quantified with qRT-PCR using a human CXCR4 specific TaqMAN probe. Indicated values are relative to hCXCR4 expression by a 10^6 RH30 cells pellet.*

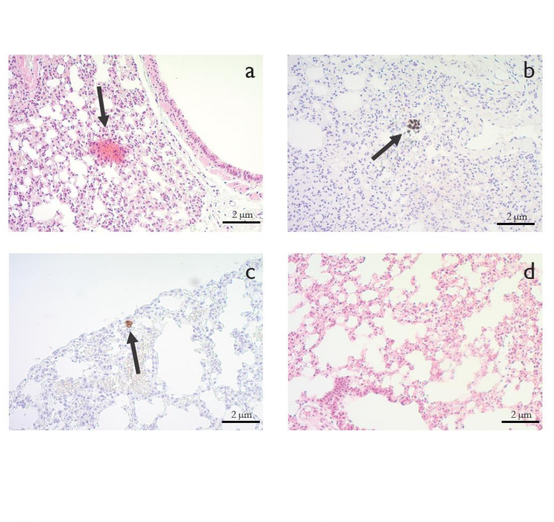

Fig 6. *Sarcoma lung micrometastases were identified by hematoxylin & eosin staining (a), Alu sequences hybridization (b), and CXCR4-specific mAb stain (c). NKAE + MDX1338 treated mice showed no micrometastasis incidence (d).*

CONCLUSION

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