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CASE REPORT

NK-92 cellular therapy for pediatric relapsed/refractory Ewing sarcoma

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Abstract

Relapsed/refractory Ewing sarcoma prognosis is dreadful, especially for recurrences within the first 2 years after initial diagnosis. It is obvious that there is an urgent need for novel treatment strategies for this dismal situation. NK-92 is an activated NK cell line with high cytotoxicity against malignant cells. Here, we present a relapsed/refractory Ewing sarcoma case who had no response to conventional strategies and recieved intratumoral NK-92 cell injections. We observe that intratumoral injection of NK-92 is safe, has no toxicity and shows preliminary evidence of tumor response in relapsed/refractory Ewing Sarcoma.

Keywords Ewing's sarcoma · Immunotherapy · NK-92

Abbreviations

CAR	Chimeric antigen receptor
CD	Cluster of differentiation
EWS	Ewing sacoma
GMP	Good manufacturing practice
MHC	Major histocompatibility complex
MRI	Magnetic resonance ımaging
NK	Natural killer
OS	Overall survival
PET/CT	Positron emission tomography and computed tomography
VTC	Vincristine–topotecan–cyclophosphamide

Introduction

Ewing sarcoma (EWS) is the second most common malignant bone tumor in children. Although multimodal treatment including multiagent chemotherapy, surgery, and radiotherapy improved the overall survival up to 75% for localized disease, the outcome for initially metastatic and reccurent disease remains poor. Unfortunately, post-recurrence survival within the first 2 years after primary diagnosis is very dismal (5-year OS 7%) [1, 2].

Besides the success rate of conventional multimodal treatment in EWS, novel strategies are required especially for reccurent disease. Recently, there have been imperessive developments in cellular therapies for solid tumor treatments. Cellular therapies are widely investigated as single agent or as a part of combination with conventional therapies.

Strategies based on NK cell targeting in EWS could be favored by reduced expression of MHC class I and high expression of ligands for activating NK cell receptors in EWS [3]. NK-92 cell line-based therapies are outstanding examples of NK cell targeting. NK-92 was derived from a patient with a NK-cell non-Hodgkin lymphoma with a CD56+/CD3−/CD16− immunophenotype and retains cytotoxic antitumor activity [4]. NK-92 is an activated NK cell line with high cytotoxicity against malignant cells [5, 6].

Here, we present our experience in a relapsed/refractory EWS case, who had no response to conventional strategies and was treated with intratumoral NK-92 injections. We

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claim that it is worthy to try immunotherapy in such a dismal situations.

Case

A 9-year-old boy presented with a right iliac mass. Biopsy was performed and pathological evaluation was compatible with Ewing sarcoma. It was a localized disease without metastasis. Euro Ewing 99 protocol was applied and he was followed up in remission. After 16 months from the initial diagnosis, he presented again with a mandibular mass and was referred to our center for further treatment.

At admission, he had a 10 × 16 cm bulky mass on the left side of his face (Supplemental Figure). In PET-CT and MRI imagings, two other masses were detected. One of them measured 7 × 6 cm at the right iliac region reaching out to the posterior bladder. The second one measured 4 × 3 cm at the right upper femur. Biopsy was performed from the facial bulky mass, and this was compatible with Ewing sarcoma metastasis.

The patient was diagnosed with relapsed metastatic Ewing sarcoma. Salvage multi-drug chemotherapy and additional radiotherapy did not work. After unsuccesful conventional salvage therapies, we decided to apply experimental intratumoral immunotherapy combined with chemoterapy. The NK-92 cell line was selected for experimental intratumoral immunotherapy. NK-92 cells were expanded from a master cell bank of NK-92 cells in GMP conditions of Acıbadem Labcell Laboratories.

The NK-92 cell line was selected for experimental intratumoral immunotherapy. The NK-92 cell line was provided from ATCC company. NK-92 cells were expanded from a master cell bank of NK-92 cells in GMP conditions with a modified method as mentioned below. The cells were sterile and free of mycoplasma, fungus and human or animal pathogen viruses. For the generation of individual cell dosages, a vial of the master cell bank of NK-92 cells was thawed and expanded in the presence of 500 IU human recombinant IL-2 (Proleukin, Novartis, Basel, Switzerland) until cell growth was established. The G-Rex culture system was prefilled with 3 L of X-Vivo 10 medium with 500 IU IL-2 and 5% human serum. NK-92 cells were added at a concentration of 1×10^7 cells/mL. The day before harvest, an additional 500 IU/mL of Proleukin was added to the culture bags.

Finally, the NK-92 cells were spun down again and resuspended in 100 mL 1% human albumin. The cell numbers were assessed with the use of a Sysmex XT 1800 automated cell analyzer. Before release, cell viability was determined by means of 7AAD exclusion. Additionally, the potency was assessed in a flow cytometric cytotoxicity assay against K562. NK-92 cell preparations were released if they fulfilled the following criteria: negative sterility at the time of batch culture initiation, viability > 80% and cytotoxicity > 50% at effector-to-target ratio of 10:1 against K562. Cells were irradiated with 10 Gy before injections.

This experimental immunotherapy was performed only after the consent of patient and additionally the confirmation document of Ministry of Health with respect to ethical and medical aspects (27.9.18–56733164/203). We decided to apply intratumoral NK-92 injections only into the facial mass, because of its bulky and superficial characteristic that made it easy to apply and evaluate the response. Intratumoral injections were performed by the guidance of interventional radiology. We made NK-92 cellular application totaly three times every other day with ultrasound assistance. In every application, we injected 5×10^6 cells/cm^2; our cellular product was totally 20 ml consisting of 2×10^8 NK-92 cells in every vial for one application. In every application day, we applied NK-92 cells into different points of the bulky mass. We tried to apply as many points as we could and to apply at least 3 cm apart. With the help of ultrasound assistance, we avoided any harm to the non-tumoral tissues.

All injections were well tolerated by the patient. No complications were observed associated with intratumoral NK-92 injections except local swelling just after the procedure, which resolved spontaneously within hours.

At the begining of the injections, the tumor size was measured in three dimensions (18 × 16 × 13 cm). 1 week after the last injection, the mass progressed approximately 2 cm in every dimension (20 × 18 × 15 cm). This was accepted as pseudoprogression due to immunotherapy [7]. 2 weeks later, still there was no response, but unfortunately new metastatic lesions were detected at the liver. Vincristine, topotecan, and cyclophosphamide (VTC) chemotherapy was added to therapy as planned before. After two cycles of VTC and 8 weeks apart, the intratumoral NK-92 injections facial mass began to regress, but new metastatic lesions were detected in the brain and lungs. At the 12th week of intratumoral NK-92 cell injection, the facial mass regressed approximately 4 cm in every dimension (Fig. 1), but the pelvic mass stayed stable in size (initial 65 × 82 × 72 mm, after therapy 63 × 7 × 73 mm) (Fig. 2). The patient was lost because of multiorgan failure at the 14th week of immunotherapy with a definite response only at the facial mass.

Discussion

Relapsed/refractory EWS prognosis is dreadful, especially for recurrences within the first 2 years after initial diagnosis (5 year OS is 7%) [1, 2]. It is obvious that there is an urgent need for novel treatment strategies for this dismal situation.

NK-92 cells were clinically tested for various tumor types before. Although the results were not so promising, there were some reponses in very refractory type of tumors [8, 9].

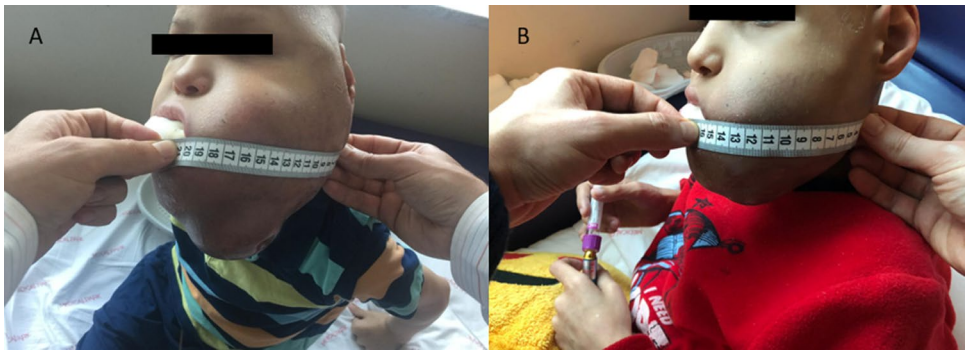

Fig. 1 Regression of bulky facial mass, before NK-92 (**a**) and after NK-92 (**b**)

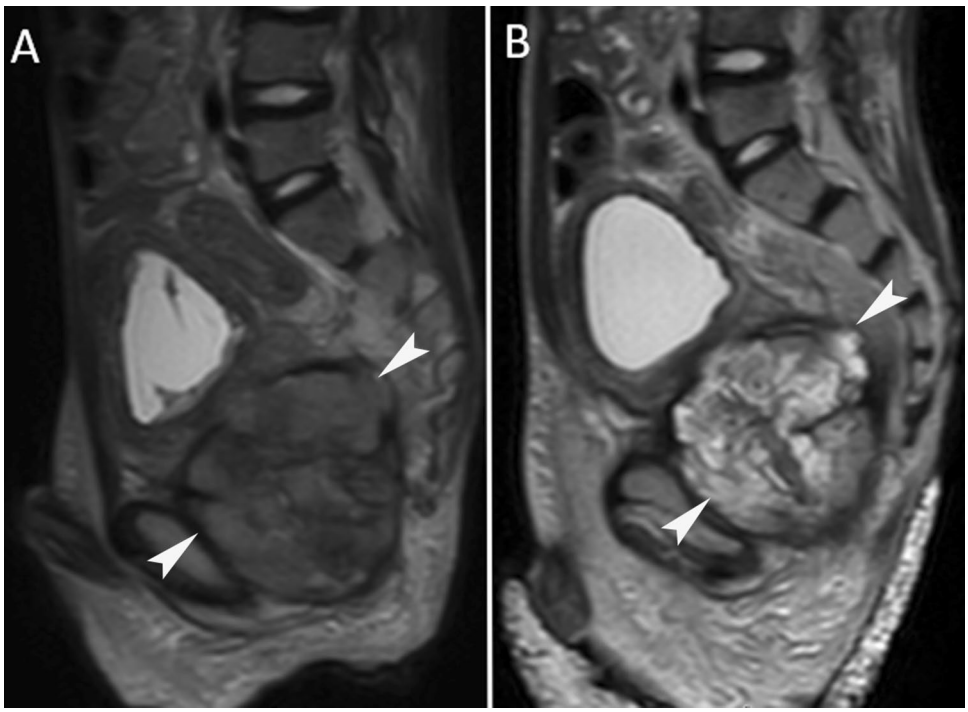

Fig. 2 Sagittal lower abdomen T-2 weighted images show a presacral, lobulated and heterogeneous mass and stable disease despite chemo/radiotherapy (initial **a**, after therapy **b**)

Besides solid tumors, NK-92 adminstration was tested on hematologic malignancies relapsing after autologous hematopoietic stem cell transplantation [10]. Nowadays, there are studies on using NK-92 cells with CAR setting in cancer treatment [9].

This report is unique in NK-92 cell treatments with route of adminstration and type of tumor. This is the first case using NK-92 cells in EWS and cells are administered intratumorally. Although we had response to local adminstration, we had no chance to use systemic NK-92 intravenously before disease disseminated, resulting in multiorgan failure.

Although dissemination of disease could not be prevented in our case, this entity is also our proof of eficacy. After multi-line salvage therapies, the primary mass had no response and new metastasis occured, but only the facial mass that was treated with local NK-92 regressed definitely.

Initially, our aim to prefer combined systemic chemotherapy is not to leave active disease untreated while applying local intratumoral therapy. Then we argued that it is possible to have a synergic effect with combined immuno-chemotherapy. Although there is no significant regression on facial mass only with immunotherapy, a definite response was detected after the end of the second VTC cycle. Progressive and metastatic lesions revealed that salvage VTC therapy could not work on systemic disease, but it could help NK-92 in eliminating facial tumor load.

It is very interesting to observe this synergic effect of immuno-chemotherapy. The mechanism of this effect is mostly unknown and still being explored. There are systemic and local tumoral microenviromental factors possibly involved in this mechanism. It seems that the clinical success of combination therapies depends on the type of drugs, different cellular modalities and timing of combination [11–13]. In this case, we explain the mechanism of combination therapy as follows: at first, immunotherapy killed tumor cells and also built an inflammatory microenviroment that

we called pseudoprogression; then chemotherapy completed the work by destroying this inflammatory tissue. After all, we could observe the real regression which had been done by immunotherapy.

This study involves one cycle of intratumoral injections of NK-92 cells with three injections on every other day at a total dose of 6×10^8 cells. We observe that intratumoral injection of NK-92 is safe, has no toxicity and shows preliminary evidence of tumor response in relapsed/refractory EWS. Although the non-target lesions progressed and the patient was lost due to disseminated metastasis, this local adminstration could be a reference point for the proof of NK-92 efficacy on EWS. It could be claimed that systemic adminstration also combined with chemotherapy would be more efficent. Further studies are needed to test this entity.

Compliance with ethical standards

Conflict of interest There is no conflict of interest.

Informed consent Informed consent has been properly documented for all the figures.

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