Intrinsic cell-related differences in carrier cells associated to clinical outcome in children with neuroblastoma treated with oncolytic virotherapy

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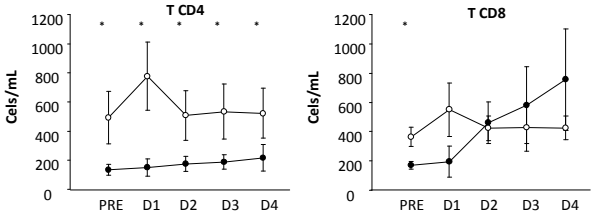
INTRODUCTION

We report here our clinical experience of a program of compassionate use of CELYVIR: autologous marrow-derived mesenchymal stem cells (MSCs) carrying ICOVIR-5, an oncolytic adenovirus, for treating children with advanced metastatic neuroblastoma. Patients could be distinguished based on their response to therapy: those who had a clinical response (either complete, partial or stabilization), and those who did not respond. We found differences between patients who responded versus those who did not when analyzing their respective MSCs, at the expression levels of adhesion molecules (CCR1, CXCR1 and CXCR4) and in migration capacities in transwell assays, and in immune-related molecules (IFNγ, HLA-DR). These results suggest interpatient differences in the homing and immune modulation capacities of the therapy administered. In addition, the pretherapy immune T cell status and the T effector response were markedly different between responders and non-responders.

RESULTS

Changes in circulating lymphocytes during treatment with CELYVIR



*p<0.05

Figure 1. We followed the absolute numbers of circulating leukocytes before and after CELYVIR therapy and found changes in all patients. Numbers of leukocytes of the innate immunity such as neutrophils (NT), monocytes (MO), natural killer (NK) cells, and of the adaptive response like B-lymphocytes were not significantly different when comparing responder and non responder patients (not shown). Patients who responded had significantly higher counts of T lymphocytes pre-therapy (p=0.0157, Wilcoxon test). Absolute numbers of T lymphocytes remained higher, mainly in CD4 (not significantly for CD8), along therapy among the patients that showed a clinical response.

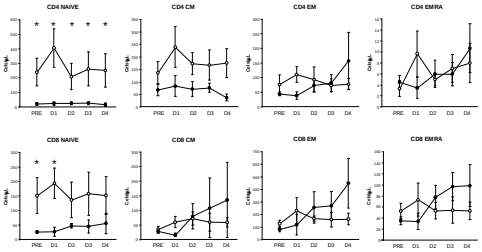


Figure 2. Naïve (CD45RA+CCR7+) CD4 and CD8 T lymphocyte numbers in children who responded were always above those of children with no response. Major changes in the numbers of these T cell subpopulations affected to the effector memory (CD45RA-CCR7-) and effector memory CD45RA (CD45RA+CCR7-) subsets of children who did not respond to the therapy, both in CD4 and CD8 T lymphocytes, and also in CD8 central memory (CD45RA-CCR7+). Compared to responder patients who presented an almost flat kinetic, non-responders showed a trend towards increasingly higher numbers of these subpopulations during therapy. These differences were not statistically significant. Finally, we also analyzed the subpopulation of circulating CD4+CD25+CD127low/negative containing T regulatory (Treg) lymphocytes, and did not find statistically significant differences between both groups of patients (not shown). * p<0.05.

Adhesion molecule profile of MSCs and clinical outcome

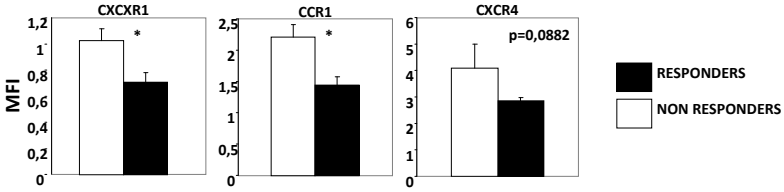


Figure 3. Adhesion molecule profile of MSCs and clinical outcome.
MSCs exhibit tropism for damaged tissues as well as the tumor microenvironment and many different receptors have been implicated in the homing of MSCs. We studied expression levels of several adhesion molecules (integrins, selectins, chemokine receptors) on the surface of the CELYVIR product and found that irradiated and infected MSCs of patients that had a positive clinical response expressed significantly (*p<0.05) higher levels of CXCR1 and CCR1 than MSCs of patients with no response. In addition, the levels of CXCR4 tended to be higher, almost statistically significant (p<0.1), in the group of patients that responded after receiving CELYVIR.

MSCs migrated towards chemotactic stimuli and neuroblastoma cells

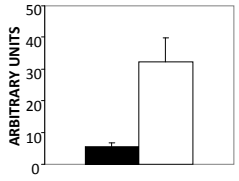
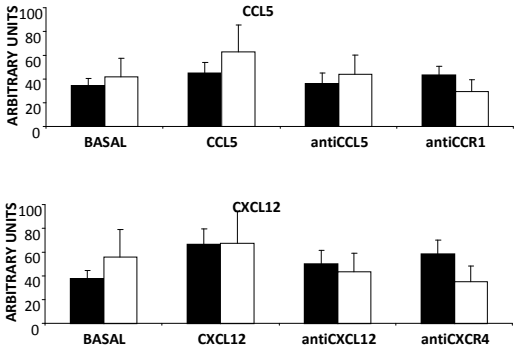


Figure 4. Since chemokine receptors expressed by MSCs were associated to clinical outcome, we studied whether the results on expression levels translated into functional differences.

Neuroblastoma cells were placed in the lower chamber and used as chemotactic stimuli. The MSCs used in the production of CELYVIR responded by significantly increasing their migration toward tumor cells.



We also compared chemotactic responses of CELYVIR products of patients that had a positive clinical response versus those of patients with no response, in the above mentioned transwell assays. Although the migration values were higher in MSCs from patients who had clinical responses, the differences were not statistically significant.

Immune molecule profile of MSCs and clinical outcome

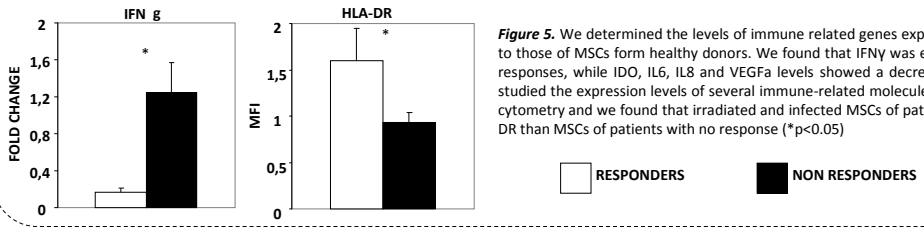


Figure 5. We determined the levels of immune related genes expressed by the infused MSCs, using RTqPCR assays. Expression levels were normalised to those of MSCs form healthy donors. We found that IFNγ was expressed at significantly lower levels by the CELYVIR product of children with clinical responses, while IDO, IL6, IL8 and VEGFa levels showed a decrease in these patients which almost reached statistical significance (p<0.1). We also studied the expression levels of several immune-related molecules (B7H family, HLA) on the surface of MSCs of patients treated with CELYVIR by flow cytometry and we found that irradiated and infected MSCs of patients that had a positive clinical response expressed significantly higher levels of HLA-DR than MSCs of patients with no response (*p<0.05)

CONCLUSIONS

Our results confirm that the use of mesenchymal progenitor cells carrying an oncolytic adenovirus is a safe procedure in treating children with advanced NB, which can be administered in a multidose protocol with very high quantities of virus and an excellent tolerance. The presence of MSCs in the medicine product may have an impact in the response to the therapy beyond their role as cell carriers. Several characteristics of the MSCs, related to their migratory capacities and response to the viral infection, may help us in designing an optimum version of this new antitumor strategy

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