Changes in tumor infiltrating leukocytes in neuroblastoma treated with oncolytic virotherapy: insights from preclinical models and patients

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INTRODUCTION
The prognosis of metastatic or relapsed NB is very poor, practically incurable. New therapeutic strategies for increasing cure rates are clearly needed. Our group has been developing a strategy for the treatment of refractory and metastatic childhood solid tumors, based on the administration of Celyvir: autologous mesenchymal cells that carry inside the oncolytic adenovirus ICOVIR-5 (EurAd2002003646-16; ClinicalTrials Identifier: NCT01844661). The possibility that oncolytic virotherapy stimulates antitumor immune responses opens new possibilities in the field of cancer immunotherapy. Preexisting lymphocytic infiltration in tumors (TILs) is associated with better prognosis in a variety of cancers. Recent studies also indicate that lymphocyte responses can identify those patients most likely to benefit from immune targeted therapies, suggesting that the effectiveness of immunotherapy can be improved through strategies that induce inflammation of the tumor. In our clinical experience we have found that the local and systemic administration of Celyvir causes changes within TILs, associated with a beneficial clinical response.

The main objective of this project is to characterize Celyvir-induced changes in TILs and tumor stroma using a murine NB model (which recapitulates the main genetic and clinical aspects of NB with amplified MYCN) and a murine Celyvir therapy similar to human.

METHODOLOGY

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**Murine Celyvir**

Mouse adenovirus type 1 (MAV-1) E1A protein contains three conserved regions (CR1, CR2 and CR3) that are comparable to the conserved regions found in AAV E1A, which interacts with members of the pRB family. MAV-1 Δ3202 contains a CR2 deletion which confers oncolytic properties, similar to the J24 oncolytic AAV vector (Smith et al. 2012). Interaction of Mouse Adenovirus Type 1 Early Region 1A Proteins with Cellular Proteins pRB and p107. Virology 1996;224:184-197. MAV-1 Δ102 virus was known to replicate and to have a potent anti-tumor activity in a panel of murine tumor cell lines.

**Human Celyvir**

Bone marrow mesenchymal stem cells (MSCs) were obtained from the iliac crest of patients. MSCs production complied with the principles of Good Manufacturing Practice. MSCs received 48 h irradiation, were then infected with ICOVIR-5, washed and resuspended in culture supplemented with human albumin, and infused through a central line.

**RESULTS**

Changes in the phenotype of tumor infiltrating T lymphocytes of a patient after therapy. Flow cytometry of tumor biopsies obtained at different moments during CELYVIR therapy (stable disease and end-stage disease) showed notable changes in the CD4/CD8 ratio and T-helper/memory effector immunophenotypes of TILs.

**CONCLUSIONS**

1. Systemic administrations of Celyvir induced changes at local tumor sites. These changes affected both the infiltration by immune competent cells (lymphoid and myeloid cells, effectors and regulators) and the tumor-tolerant microenvironment. The cellular component of our strategy functions not only as carrier for the oncolytic virotherapy but has a role in the immune responses taking place after CELYVIR infusions. Works are in progress in order to functionally characterize the changes in the tumor infiltrating immune cells and in the tumor-tolerant environment.

2. The immunocompetent animal model and the conditionally-replicative MAV-1 Δ3202 should allow us to dissect crucial aspects of the mechanism of action of Celyvir, helping in optimizing this strategy in the clinic setting.

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**NB neurospheres derived from TH-MYCN (22G17) mice were cultured in DMEM:F12 (50%), 1xB27 (w/o VitA), 40 µg/mL of mFGF and 20µg/mL of rmIGF1. At day 0, 106 tumor cells were orthotopically implanted on the adrenal gland of WT 129/Si6 mice. At week 2 and 3, mice were either treated with murine Celyvir (ICOVIR-5 infected with MAV-1 Δ3202) or untreated. Similarly to human Celyvir, we infected adipoocytes derived from mice with MAV-1 Δ3202 oncolytic vector at a MOI of 200 during 90 min. After infection, cells were washed and prepared to treat mice, and mICOVIR was i.v. injected. At week 4, mice were sacrificed and tumors were analyzed by flow cytometry (leukocyte infiltration) or qRT-PCR (genes of tumor microenvironment).

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