

EXPRESSION OF GALECTIN-3 INHIBITORS FROM A SELF-REPLICATING RNA VECTOR AS TREATMENT FOR PEDIATRIC OSTEOSARCOMA

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Background Osteosarcoma is an aggressive bone tumor, primarily arising in the pediatric age. Despite years of intensive research, outcome for metastatic and non-responder patients is very poor and has not improved in the last 30 years. These tumors harbor a highly immunosuppressive environment, making the existing immunotherapies ineffective. Inhibition of galectin-3 (Gal3), a protein involved in immunosuppression,¹⁻³ adhesion of tumor cells,⁴⁻⁵ and metastases,⁶⁻⁹ has been demonstrated to reduce tumor progression in different tumor models, including osteosarcoma.¹⁰⁻¹² On the other hand, virotherapy based on recombinant Semliki Forest Virus (SFV), a self-replicating RNA virus, has shown therapeutic effect in orthotopic osteosarcoma mouse models.¹³

Methods We generated SFV vectors expressing truncated forms of Gal3, including its carboxy-terminal domain (SFV-Gal3-C) and its amino-terminal domain alone (SFV-Gal3-N) or fused to the Gal3 inhibitor peptide C12 (SFV-Gal3-N-C12). An additional construct expressed the C12 peptide¹⁴⁻¹⁵ (SFV-C12). We analyzed Gal3 expression in different murine and human osteosarcoma cell lines. Orthotopic osteosarcoma tumors, induced by intratibial injection of K7M2 murine cells, which showed high expression of Gal3, were treated with SFV vectors expressing Gal3 inhibitors or luciferase or with PBS (control). Animals were maintained under standard conditions, and all procedures were approved by the Institutional Ethical Committee (CEEA) in accordance with the guidelines of the University of Navarra, approval number 044-21.

Results Treatment with the SFV-Gal3-N-C12 vector showed the highest antitumor activity, significantly reducing tumor growth compared to control mice that received PBS. In fact, this vector prolonged animal survival, leading to 47% of complete regressions. Among the other vectors, SFV-Gal3-N and SFV-C12 were also able to transiently decrease tumor growth, although they had no impact on animal survival. Moreover, the number of spontaneous lung metastasis were reduced in mice treated with SFV vectors expressing Gal3 inhibitors. Preliminary mechanistic studies showed an increase of CD3 cells infiltration in tumors treated with SFV-Gal3-N-C12 and SFV-Gal3-N vectors. Despite the antitumor effect observed with SFV-Gal3-N-C12, no protection against tumor rechallenge was observed in cured mice, indicating the lack or insufficient memory immune response generation. These data suggested that this therapeutic approach might benefit from combination with other immunomodulatory strategies. We are currently characterizing the underpinnings of the mechanisms underlying this strategy.

Conclusions In summary, we believe that inhibition of Gal3 using SFV vectors could constitute a potential approach to explore as therapy for pediatric osteosarcoma.

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Ethics Approval Animals were maintained under standard conditions, and all procedures were approved by the Institutional Ethical Committee (CEEA) in accordance with the guidelines of the University of Navarra, approval number 044-21.

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