ImmunoTools *special* Award 2024

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In vitro immunomodulation of the tumor microenvironment for the treatment of specific histological subtypes of soft tissue sarcomas

Introduction

Soft tissue sarcomas (STS) represent a rare and heterogeneous group of malignancies originating from mesenchymal tissues.[1] Disturbingly, 40-50% of STS patients develop metastasis and only 20% of these patients survive beyond five years after diagnosis.[2,3] Current treatment options for these advanced cases are severely lacking. The standard approach for localized disease includes surgery and radiotherapy, while chemotherapy is used for metastatic stages. The response to chemotherapy remains distressingly low, with rates between 15–35% and a median survival of just 12 months.[4–6] These statistics highlight the critical need for new and more effective therapeutic approaches to improve outcomes for patients with advanced STS.

Immunotherapy introduces innovative treatment strategies, with immune checkpoint inhibitors emerging as the most advanced drug class.[7] Clinical trials have shown mixed results in STS.[8] Nivolumab plus ipilimumab achieved a 16% response rate, similar to the 14% with doxorubicin.[9,10] Immune checkpoint molecules are expressed across many STS subtypes, nevertheless, their levels vary greatly.[11,12] Although cytotoxic T cells, which utilize cytotoxic mechanisms such as IFN-γ, play a critical role in tumor eradication, macrophages represent the most predominant immune cell subset within the (TME) of many STS.[13] These macrophages are usually immunosuppressive, thereby promoting tumor progression. In addition to macrophages, myeloid-derived suppressor cells further support tumor growth. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a double-edged sword in cancer immunology, and its neutralization or, conversely, addition is being tested in many clinical trials.[14] The lack of understanding around the effects of GM-CSF in STS is deeply concerning and this gap in knowledge could be a missed opportunity. In fact, treatments that modulate the immune system by targeting T cells and myeloid cells may be especially important for STS patients, yet remain underexplored.

Research aim & hypothesis

Immunomodulation with single agents has led to significant improvements in many cancer cases. However, many patients have not responded, underscoring the need for combinatorial strategies tailored to these patients. Myeloid cells outnumber lymphocytes within the TME of STS, and thus combinatorial approaches aiming at both populations might be crucial for the best outcomes. In the proposed project, I will aim to modify T cells and myeloid cells from freshly isolated STS tumors by *in vitro* modulation of T cell activation/inhibitory pathways and myeloid cells by GM-CSF.

Methods

The tissue samples after surgical resections will be mechanically and enzymatically dissociated to obtain single-cell suspensions of $CD45^+$ and $CD45^-$ cells. These cells will be rinsed with saline solution and red blood cells will be removed. After phenotypic analysis of immune cells by flow cytometry, the isolated cells will be cultured for 24-72 hours, 37 °C, 5% CO_2 at various conditions to evaluate the immunomodulation of tumor-infiltrating immune cells. Briefly, 200 000 cells per well will be stimulated (PMA/ionomycin) and seeded under various conditions with rh GM-CSF and T cell immunomodulator. Control samples will consist of unstimulated samples and a combination of the aforementioned conditions. After the incubation, the phenotype of immune cells will be analyzed by flow cytometry, among analyzed markers will be ImmunoTools anti-human TNF-alpha FITC. Moreover, supernatants will be collected and evaluated by ImmunoTools human Tumor Necrosis Factor alpha ELISA set, and human IFNgamma ELISA set.

The ImmunoTools special Award would be a great opportunity to evaluate targeted modulation of the TME in STS using ImmunoTools rh GM-CSF, potentially unveiling novel insights into T cell and myeloid cell interactions. This exploration could pave the way for therapeutic strategies that offer meaningful improvements for patients with limited treatment options.

References

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