Anti-mmu-mir-721 as a therapeutic target for immune checkpoint inhibitor-associated myocarditis

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Abstract: Immune checkpoint inhibitors (ICIs) have changed cancer treatment. However, ICIs could lead to undesirable events. Myocarditis is one of the rarest, yet most fatal, with a mortality rate of 60%. Our lab has generated a novel murine model to study the susceptibility to develop ICI-myocarditis. In this study, tumor-bearing cd69-/- mice under anti-PD1 treatment presented exacerbated Th17 responses in peripheral blood, cardiac inflammation and dysfunction. Wild-type mice do not develop the disease under the same settings. Previous work has validated mmu-miR-721 as a specific biomarker for acute myocarditis. In our model, mmu-miR-721 levels are significantly elevated at day 10 of the treatment in plasma of cd69-/- mice. This finding has clinical relevance since the mmu-miR-721 could be a potential biomarker and therapeutic target of ICI-myocarditis.

Th17 cells are the main producers of mmu-miR-721, and it controls RORγt expression by inhibiting PPARγ. Our hypothesis is that in vivo inhibition mmu-miR-721 will produce an overexpression of PPARγ, and subsequently lower expression of RORγt. Therefore, tumor-bearing cd69-/- susceptible mice under anti-PD1 treatment will exhibit lower Th17 responses and the development of ICI-myocarditis will be impaired without affecting tumor regression.

cd69-/- mice were used since our group has described how these animals develop exacerbated Th17 responses and ICI-myocarditis with anti-PD1 treatment. Mice were treated with anti-mmu-miR-721 (single-stranded DNA molecules with four reverse complementary sequences to the microRNA) or scramble the same day as anti-PD1 treatment begin. We analyzed the subsequent immune response in peripheral blood, lymph nodes and heart by flow cytometry, as well as the cardiac dysfunction by echocardiography.

We proposed a novel therapeutic target for ICI-myocarditis. Anti-mmu-miR-721 treatment at the beginning of immunotherapy protects susceptible mice of cardiac inflammation with lower immune infiltration, and lower Th17 and γδ T cells responses in the heart. Additionally, these mice do not generate exacerbated Th17 responses in peripheral blood at day 10 of immunotherapy. Consequently, these animals do not overexpress mmu-miR-721 biomarker levels in plasma 10 days after initiating anti-PD1 treatment, as observed in the scramble and control groups. Echocardiography analysis of mice treated with anti-mmu-miR-721 show preserved cardiac function at the end-point of ICI-myocarditis.

Anti-mmu-miR-721 therapy in tumor-bearing cd69-/- susceptible mice under anti-PD1 treatment reduces cardiac inflammation due to a lower immune infiltration and Th17 responses towards the heart. Moreover, biomarker mmu-miR-721 levels are not elevated in plasma at day 10 when treated with anti-mmu-miR-721. This finding has clinical relevance since the mmu-miR-721 could be a specific therapeutic target of ICI-myocarditis.