Effect of Aging on the anti-Tumor Activity of GITRL-Fc

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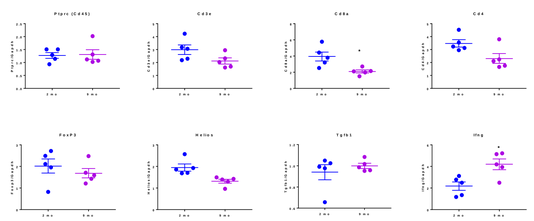
ABSTRACT

Mouse tumor models have been successfully used to generate preclinical data for numerous clinical programs including immunotherapy. Preclinical *in vivo* studies are typically carried out using young mice (often less than 2-month-old) to generate efficacy data, predictive biomarker and pharmacodynamic markers. In notable contrast, the majority of human cancer occurs in adult and older patients. It has become increasingly clear that the immune system of young and old mice is quite different with regards to the relative abundance and functionality of different cell populations. Data generated using young mice could provide a distorted assessment of the potential activity of immuno-oncology drugs in the clinic. Therefore, we tested the activity of GITRL-Fc protein in both young and older mice (>9 months). Previously using extensive young mice experiments, we have shown that GITRL-Fc promoted a robust anti-tumor immune response and enhanced tumor-specific T-cell responses, particularly of the Th1 type, and also led to a reduction in Treg-mediated immunosuppressive activity. Compared to young mice, tumors grew faster in older mice, and peripheral blood of older tumor-bearing mice has fewer T cells and NK cells. The total MDSC population was increased in the blood and spleen of old tumor-bearing mice, with a significantly higher number of G-MDSCs in the blood. On the other hand, old mice had reduced "antigen-presenting cells" (macrophages/dendritic cells expressing MHCII) in the blood. Furthermore, splenocytes from old mice had impaired production of IL-2. GITRL-Fc significantly inhibited tumor growth in both older and younger mice. However, efficacy was more pronounced in young mice which frequently exhibited complete tumor regression. There were fewer tumor-infiltrating immune cells with less CD8 T and NK cells in older mice compared to young mice consistent with faster tumor growth. Interestingly, GITR expression in CD8 T cells in old mice was lower compared with young mice at the tumor site. In old mice, GITRL-Fc (mIgG2a) was still able to deplete Tregs in tumor and increase Tregs in the spleen as has been previously shown with GITRL-Fc in young mice. On the other hand, GITRL-Fc deficient in effector function (mIgG2a (N297A)) did not deplete Tregs in the tumor but did retain some anti-tumor growth activity, indicating a role for GITR signaling in the mechanism of efficacy by GITLR-Fc. In conclusion, the results demonstrate the potential for the aging of the immune system to impact the efficacy observed with immunotherapy agents and highlight the potential benefits of conducting efficacy studies with both young and older mice.

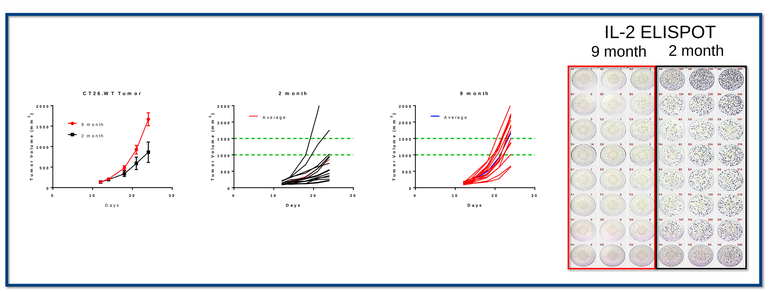
Immune gene expression in 2 month vs. 9 month old non tumor-bearing mice

Older mice have less T cells but have more IFNγ production in blood

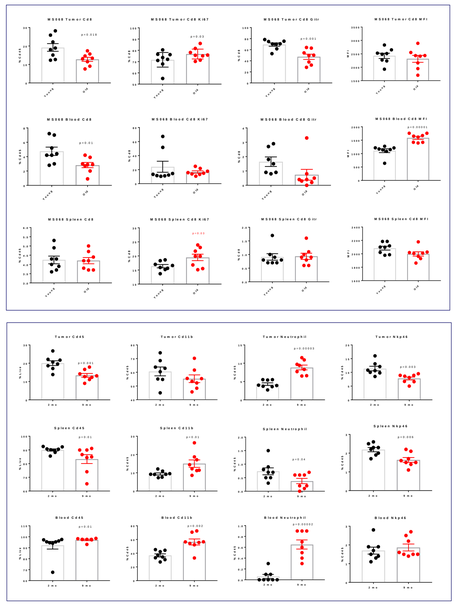


Nine month–old mice have
- Less Cd8 and Cd4 T cells
- A similar number of Tregs
- Less Helio expression
- More IFNγ expression

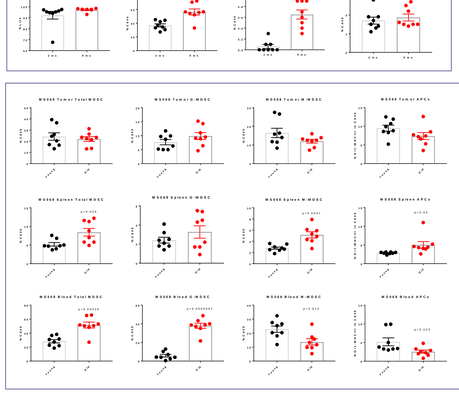
Older mice are more permissive for CT26.WT tumor growth



Immune profiling between 2 and 9 month-old tumor bearing mice



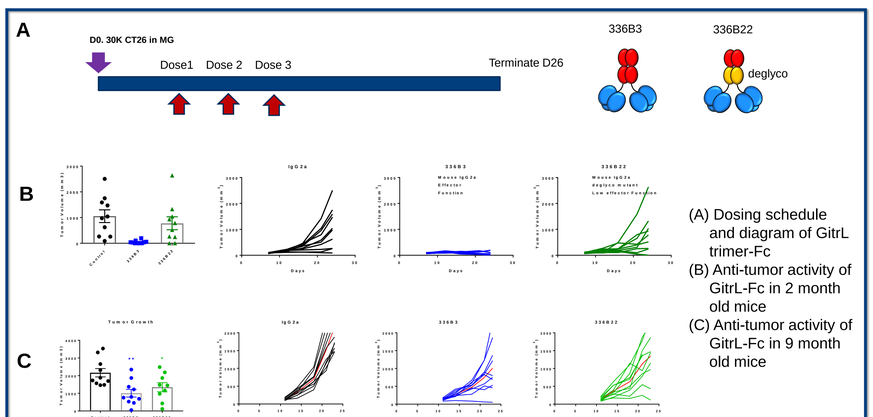
- Nine month–old mice have less Cd8 T cells in CT26.WT tumors.
- A more Ki67+ fraction in Cd8 T cells
- Less Gitr expression in Cd8 T cells
- More MHCI expression in blood but not in tumors and spleen



Nine-month–old mice have
- Less Cd45 cells in CT26.WT tumors and spleens.
- More myeloid cells (Cd11b+) are present in the periphery
- More neutrophils in tumors and blood but less in the spleen
- Less NK cells in tumor and spleen but not in blood

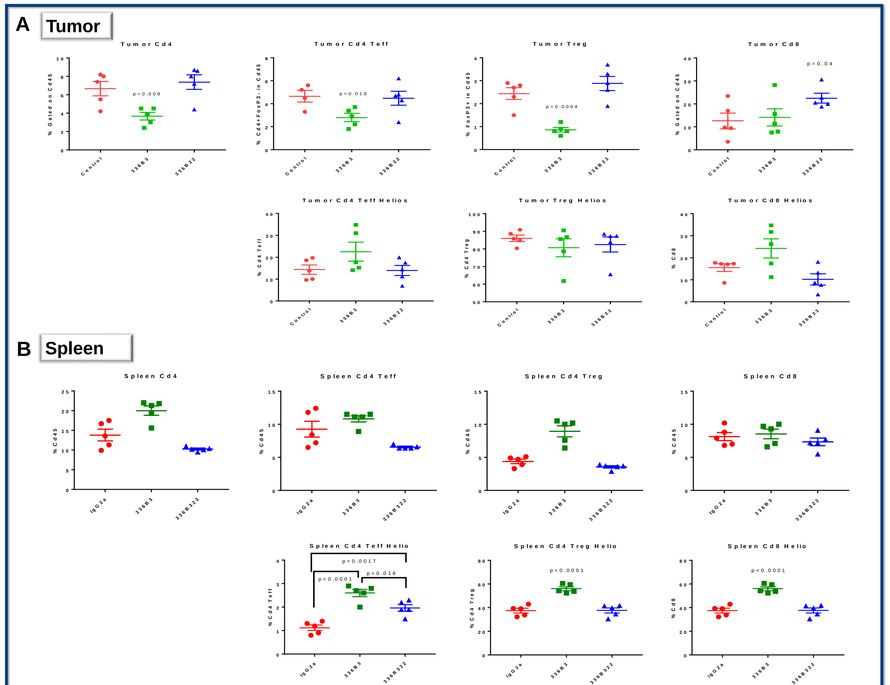
Nine month–old mice have
- More MDSCs in spleen and blood but no difference in tumor
- More G-MDSCs in blood
- More M-MDSCs in spleen and less in the blood.
- More total antigen presenting cells in spleen, but MHCII was expressed less.
- A similar number of antigen presenting cells but a much higher expression of MHCII in the tumor.

Anti-Tumor activity of GITR L-Fc



(A) Dosing schedule and diagram of GitrL trimer-Fc
(B) Anti-tumor activity of GitrL-Fc in 2 month old mice
(C) Anti-tumor activity of GitrL-Fc in 9 month old mice

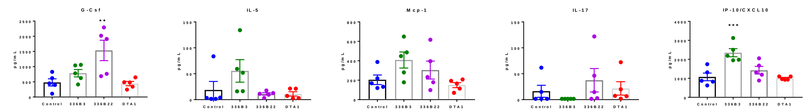
Unlike GitrL-IgG2a Fc (336B3), GitrL-IgG2a(N297A) Fc (336B22) did not reduce Tregs in tumor and did not increase Tregs in spleen



FACS analysis of tumors and spleens of 336B3 and 336B22.

Plasma cytokine/chemokine

336B22 induced G-csf whereas 336B3 induced IL-5, Mcp-1, and IP-10



SUMMARY

- The strong anti-tumor activity of GITRL-Fc previously shown in 2 month-old BALB/c mice was significantly impaired when 9 month-old mice were used.
- GITRL-FC IgG2a (336B3) was still able to deplete Tregs in tumor and increase them in the periphery.
- The main factor contributes to the lower efficacy might be reduced Cd8 T cell infiltration in the tumor in 9-month-old mice compared to 2 month-old mice, and multiple factors might play a role in this process.
- Interestingly, GITRL-Fc with diminished effector function (336B22) was still efficacious, indicating that signaling through GITR is still important for anti-tumor activity.
- Depleting Treg alone may not be enough to elicit strong anti-tumor immunity in the older mice. Therefore, combination approaches resulting in T cells into the tumor might be needed.
- Testing IO agent in both young and old mice would be beneficial since the majority of cancer patients are older and the immune systems are declined in older population.