Combination of in vivo monitoring and flow cytometry-based immunoprofiling on syngeneic tumor models to assess new immunotherapeutics and decipher their MoA

Syngeneic tumor models were becoming invaluable for preclinical development and evaluation of immuno-based therapies in the presence of a functional immunocompetent system. Explicyte is providing a series of syngeneic mouse models including subcutaneous and orthotopic models, which were fully characterized in terms of their responsiveness to immune checkpoint inhibitors (ICI) and their immune infiltration features using a high throughput-compatible flow cytometry platform.

While the potential of various immunological modulators (e.g. ICI) to provide an anti-tumor immune-driven response can be evaluated through *in vivo* monitoring, efficacy of many of these modulators is usually underlied by a strong impact on the immune system, which thus needs to be delineated through relevant models and immunological readouts, in order to see whether the modulation of immune subset responses correlates with the impact of therapeutics on tumor growth. Furthermore, depending on their responsiveness degree to immunotherapeutics, tumor models differ in extent and type of immune cell modulation, while the in vivo tumor responses can be similar. Profiling immune cell function permits for building a complete package with *in vivo* data for a deeper understanding of how a cancer therapy performs.

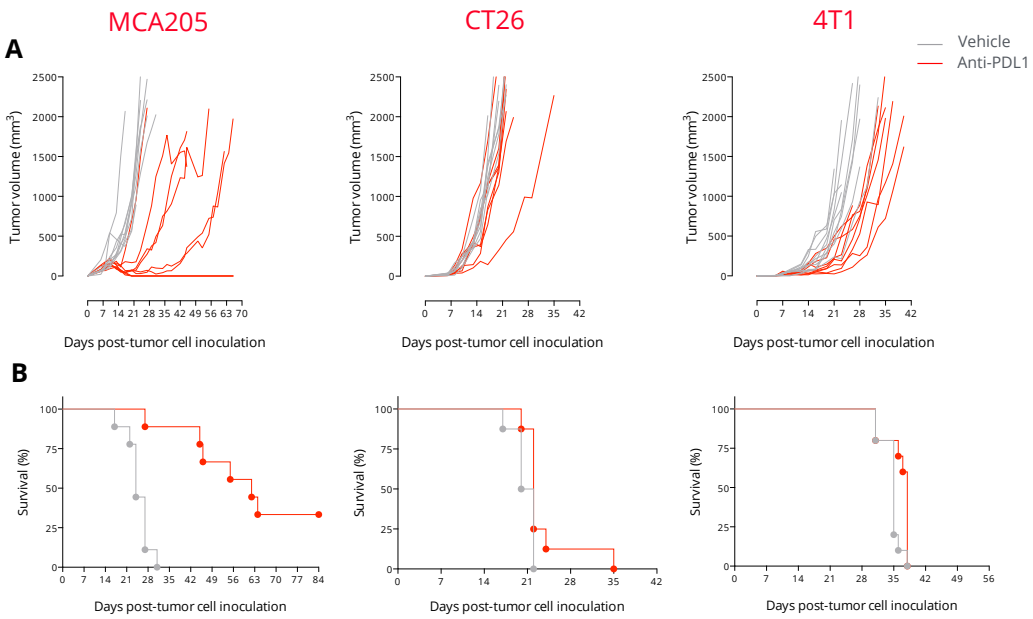

Figure 1: Subcutaneously-implanted MCA205 sarcoma, CT26 colon, and 4T1 breast tumor-bearing mouse models are differentially sensitive to PDL1 blockade.
Mice were subcutaneously implanted with respective tumor cells and exposed to anti-PDL1 antibody. Tumor growth was monitored overtime via calipering. Tumor volume (**A**) and survival (**B**) were then determined.
The three tumor models exhibit differential responsiveness levels to PDL1 blockade, MCA205 tumors being strongly responsive while CT26 and 4T1 models are only partially sensitive.

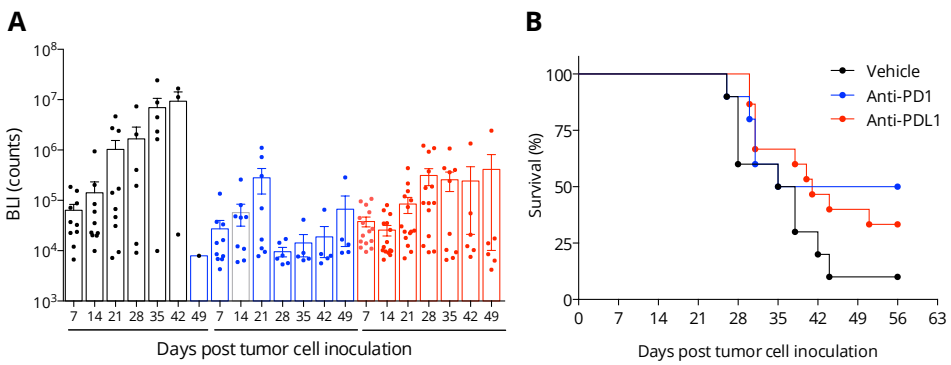

Figure 2: Orthotopic syngeneic GL261 glioblastoma model is responsive to PD1/PDL1 axis blockade.
Mice were OT inoculated with GL261-Luc2 glioma cells and then treated with either Vehicle or anti-PD1 or anti-PDL1 antibodies. Bioluminescence imaging was performed once a week starting from day7 post-tumor inoculation (**A**), on days 7, 14, 21, and 28 post-tumor cell inoculation. Survival was also determined (**B**).

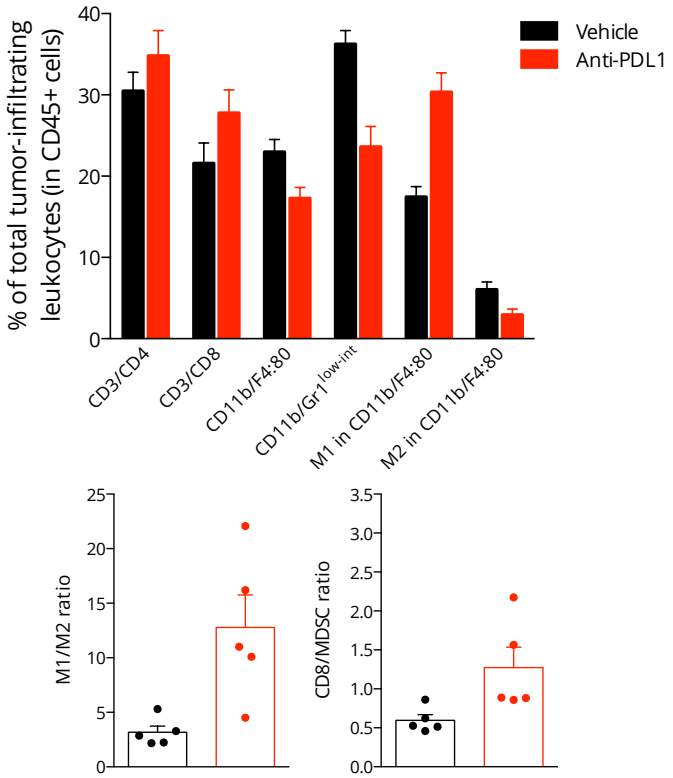

Figure 3: Analysis of PDL1 blockade mechanism of action on the MCA205 tumor-infiltrating leukocytes populations.
Flow cytometry analysis of MCA205 tumors shows infiltration by both lymphocytic (CD4, CD8) and myeloid cells including the immunosuppressive MDSC population (CD11b/Gr1$^{low-int}$) and macrophages (M1 and M2 subsets).
Upon anti-PDL1 treatment, investigation of tumor-infiltrating leukocytic cell subsets showed an increase in CD8 cytotoxic lymphocyte proportion as well as decrease of MDSC cell infiltration. In addition, while vehicle tumors were shown to be infiltrated by M2 macrophages, which helps conferring an immunosuppressive microenvironment, PDL1 blockade was demonstrated to reprogram this macrophage profile by increasing the M1 and decreasing the M2 subset proportions, thereby suggesting an effect on macrophage polarization and proliferation.

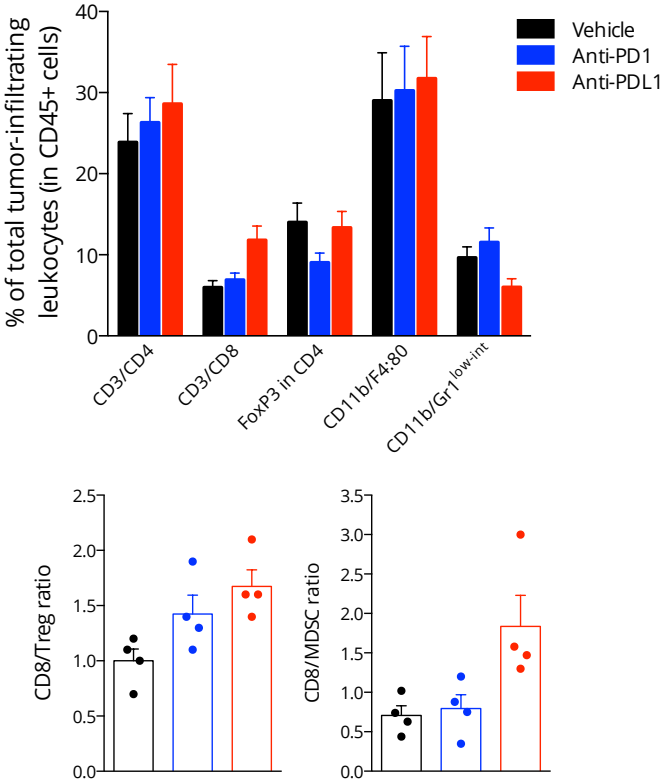

Figure 4: Intracranial glioma tumor-bearing model is characterized by an immunoprivileged microenvironment.
Flow cytometry analysis of GL261 tumors shows infiltration by both lymphocytic (CD4, CD8, and Treg (FoxP3+/CD4+) subsets) and myeloid cells including the immunosuppressive MDSC population (CD11b/Gr1$^{low-int}$).
Those key immune cell subsets were shown to be differentially modulated upon treatment with either anti-PD1 or anti-PDL1 antibody – Treg cell population being mainly decreased upon PD1 blockade, while anti-PDL1 antibody was shown to both increase CD8 and decrease MDSC cell infiltration.

The data presented here on the sensitivity of four syngeneic tumor models to PD1/PDL1 axis blockade and the way this latter can – differentially – affect immune cell populations over these models, provide a good overview of our comprehensive service offerings combining syngeneic tumor models and flow cytometry analysis to characterize the effects on anti-tumor immunity and decipher mechanism(s) of action of novel immunotherapeutics.

To learn more about our models and test your drug(s), contact@explicyte.com

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