A single intratumoral injection of Il-12 bound to mesoporous silica rods generates effective anti-tumor immune responses and tumor growth control in multiple syngeneic tumor models

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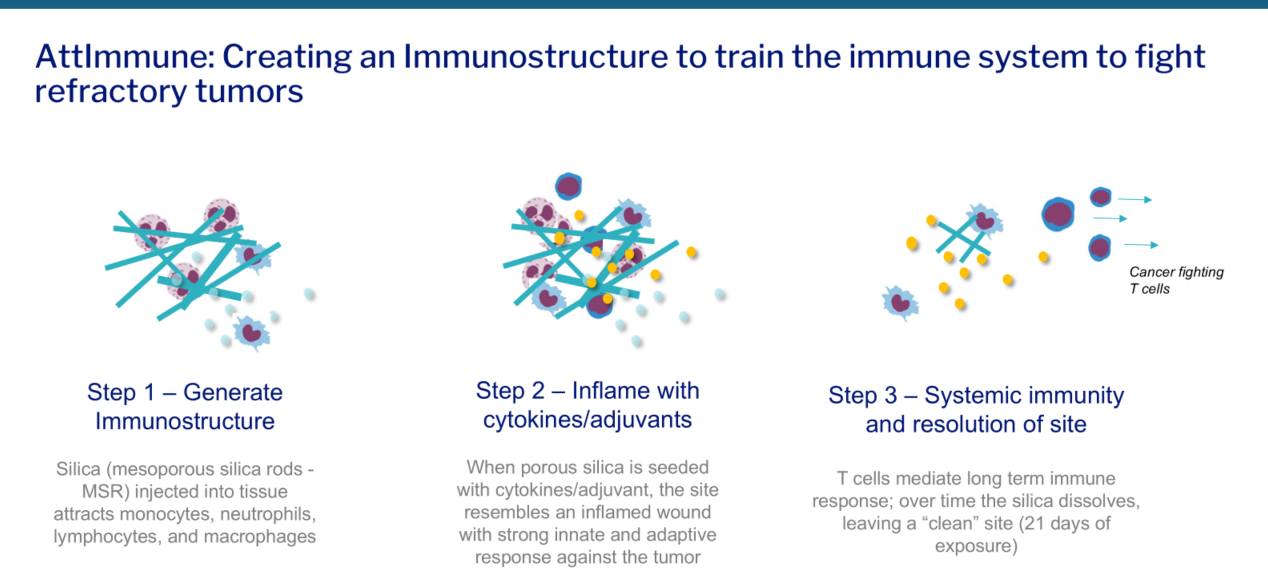
ATTIVARE THERAPEUTICS

Poster #3768

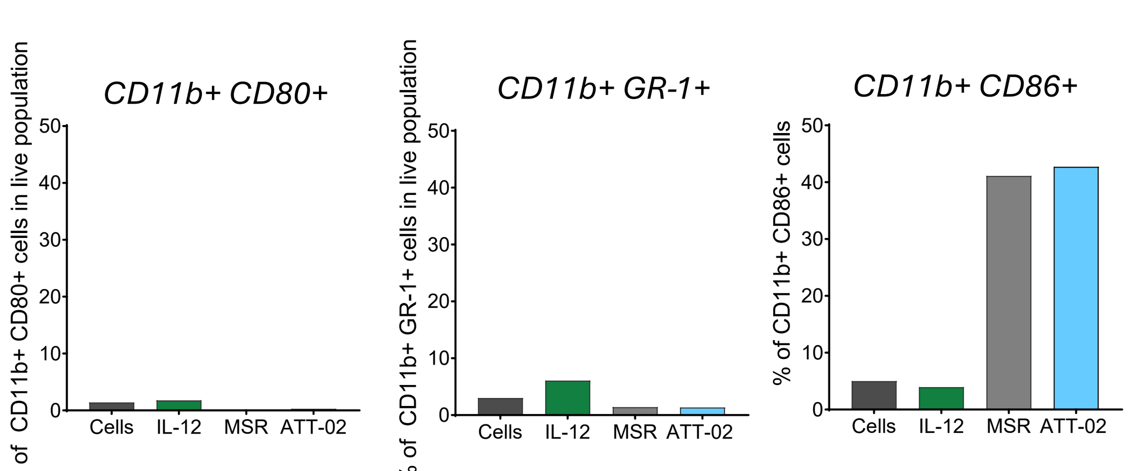
Background: Interleukin-12 (IL-12) is a potent pro-inflammatory cytokine, which has demonstrated significant anti-tumor activity in both preclinical and clinical trials. Unfortunately, the development of IL-12 as a therapeutic has been hampered by severe toxicity in the event of significant systemic exposure. However, as the critical locus of action is within the tumor microenvironment (TME), intralesional IL-12 therapy been actively pursued as an intratumoral therapy. When successfully delivered into the TME, IL-12 increases intratumoral interferon-gamma expression leading to conversion of intratumor myeloid cells from an immunosuppressive state into an immunogenic one, recruitment of CXCR3+ NK and effector T cells, upregulation of antigen presentation and processing machinery and generation of effective anti-tumor immunity. Mesoporous silica rods [MSRs, ATTimmune, Fig. 1] are currently in development as a modular self-adjuvanted vaccine platform, due to their large payload capacity, slow-release kinetics, high biocompatibility and intrinsic proinflammatory nature. In this study, we evaluated the anti-tumor effect of MSR plus IL-12 [ATT-02] as an "in situ" ("antigen-less") vaccine and characterized the immune effects of MSR alone and in in combo with IL-12.

Figure 1. ATTimmune Technology Overview



Methods: Syngeneic mouse models [B16F10, CT26, and EMT-6] were conducted by challenging the mice with tumor cells on one flank. Tumors were treated one time with ATT-02 [Murine IL-12 and MSR] with an intratumoral injection when tumors reached the target size. Abscopal models were conducted by injecting tumor cells [CT26 and B16F10] on both flanks at day zero. Tumors were treated when they reached the target size with an intratumoral dose of ATT-02. Tumors, lymph nodes, and spleens were collected at day 7 and analyzed by flow cytometry for a subset of tumors. *In vitro* work was conducted by co-culturing MSRs with THP-1 cells that had been initially polarized to an "M2" like phenotype. Repolarization ("M2 to M1") and APC functionality of THP-1 cells were assayed using flow cytometric phenotyping and proliferation of co-cultured antigen-specific T- cells.

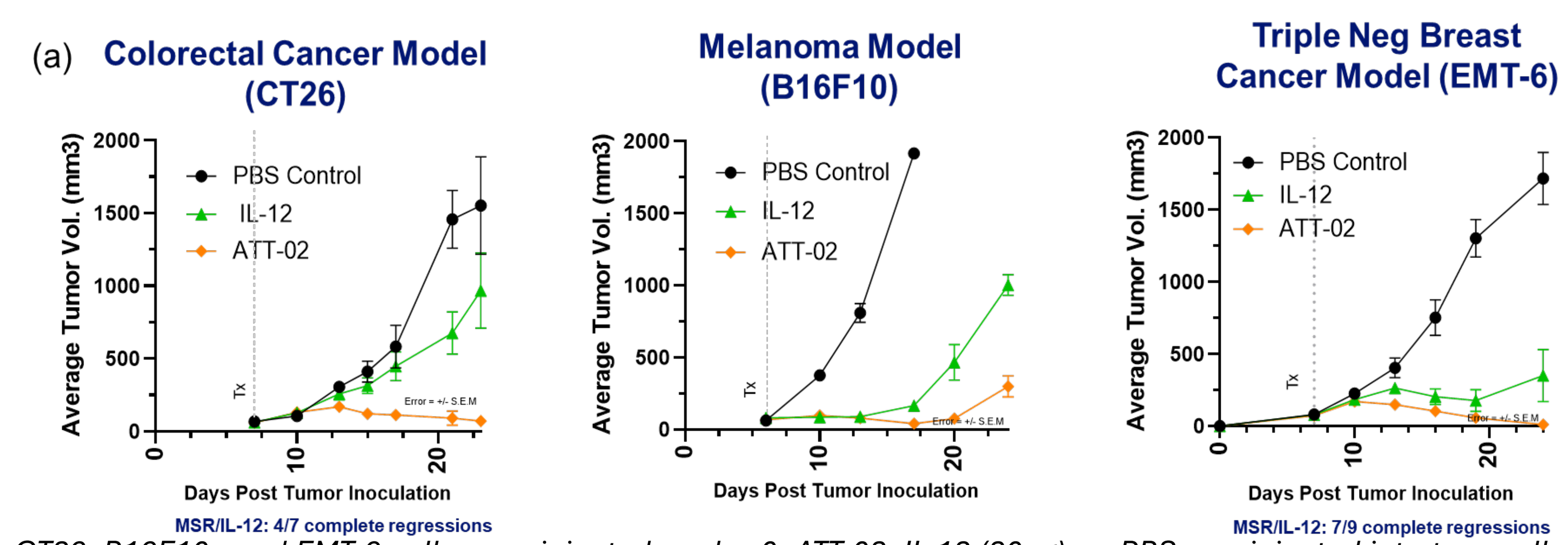
Figure 2. ATTimmune MSR treatment repolarizes macrophages from an "M1" phenotype to an "M2" phenotype



Murine bone marrow cells cultured for 6 days of 10ng/mL murine M-CSF. Exposed to IL-4 (24hours). Exposed to experimental conditions for 24 hours and then ran flow cytometry

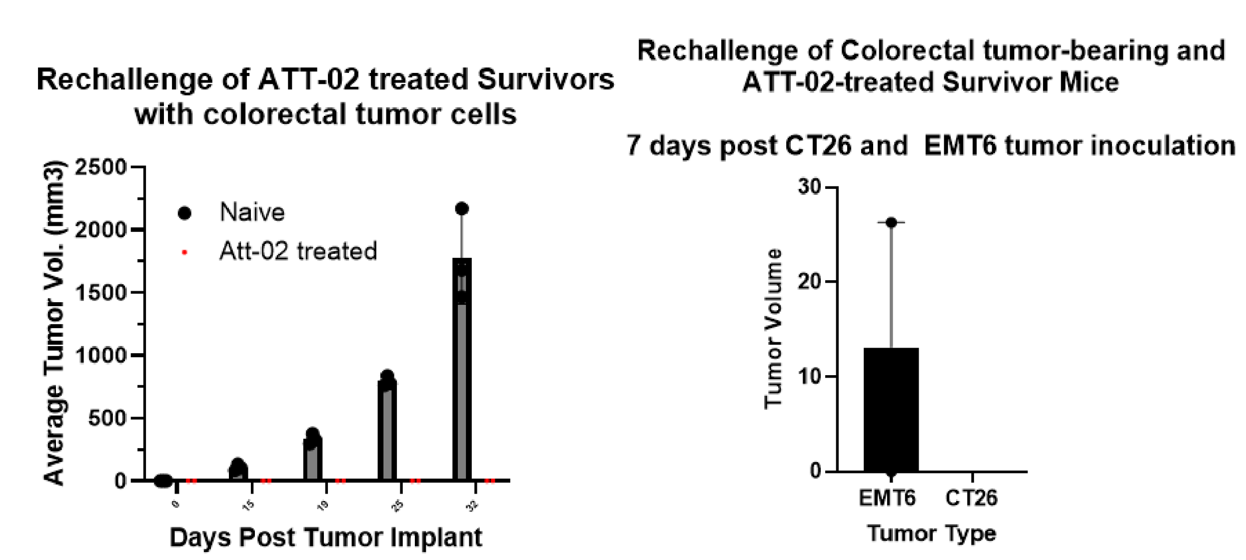
Results: ATT-02 i.t. [MSR, IL-12] generated potent anti-tumor immunity compared to PBS and IL-12 alone in single tumor syngeneic models and abscopal syngeneic models. In addition, ATT-02 can repolarize "M2" macrophages to an "M1" phenotype (Figure 2). Data for the in vivo single tumor models is shown in Figure 3. Complete responses [CRs] were observed in the CT26 and EMT-6 model after a single dose of ATT-02 (Figure 3). Mice that had tumor regression in the CT26 model were rechallenged, as shown in Figure 4. The data for a single injection of ATT-02 in a two tumor "abscopal" CT26 model is shown in Figure 5 (tumor growth and survival). The single dose and multidose data for ATT-02 in B16F10 abscopal model is shown in Figure 6. Interestingly, increasing the amount of IL-12 loaded on the MSR did not

Figure 3. ATT-02 demonstrates single agent efficacy in Multiple Tumor Types



CT26, B16F10 , and EMT-6 cells were injected on day 0. ATT-02, IL-12 (20ug), or PBS was injected intratumorally on day 0. The tumor was treated on the day shown by the dotted line.

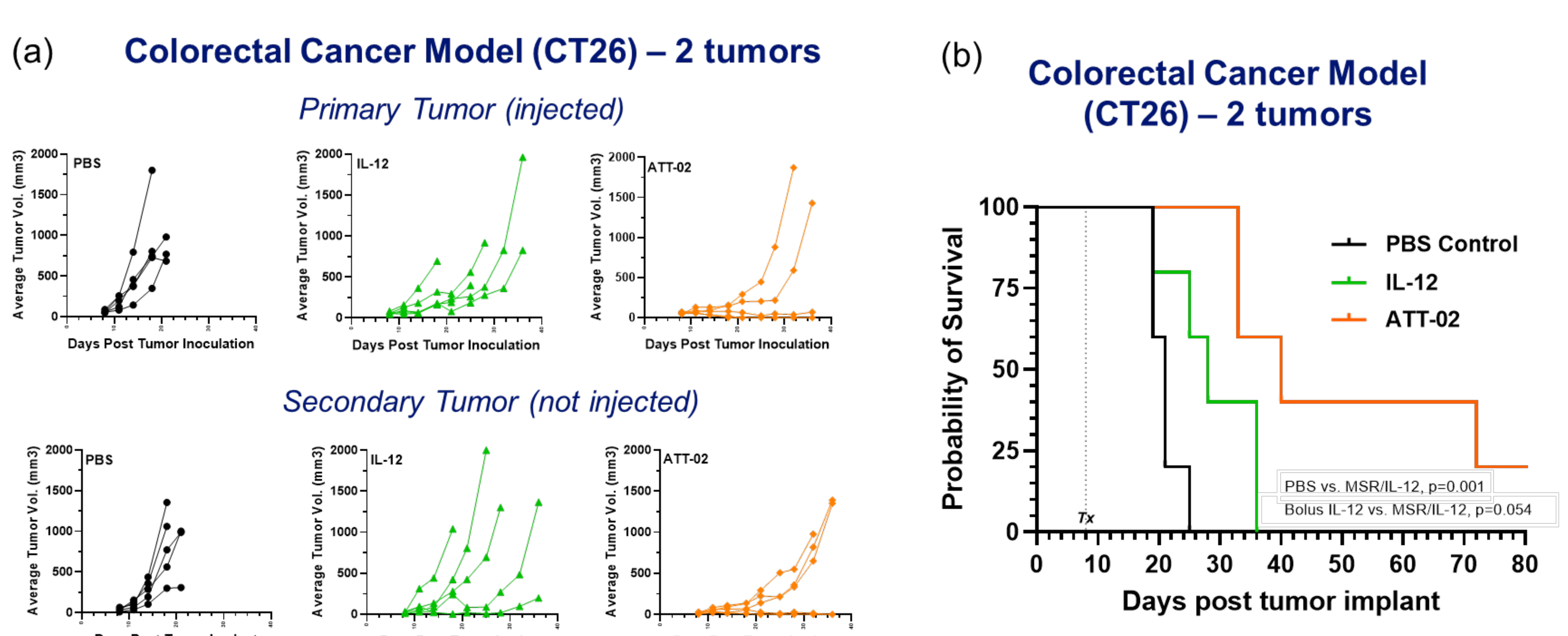
Figure 4. Tumor specific immunity and memory demonstrated by rechallenge



[Left] In the original study, mice were inoculated with CT26 cells on one flank and treated with ATT-02 or ATT-02 plus PD-1 (every 2 days, systemic) when tumor exceeded 50mm3. 65 days after initial tumor inoculation 4 surviving mice that were treated with ATT-02 or ATT-02 plus PD-1 were selected for the re-challenge study. For the rechallenge study, mice received 3M CT26 cells on day 65 of the original study (day 0 of rechallenge study). 3 naive mice were also inoculated with 3M CT26 cells.

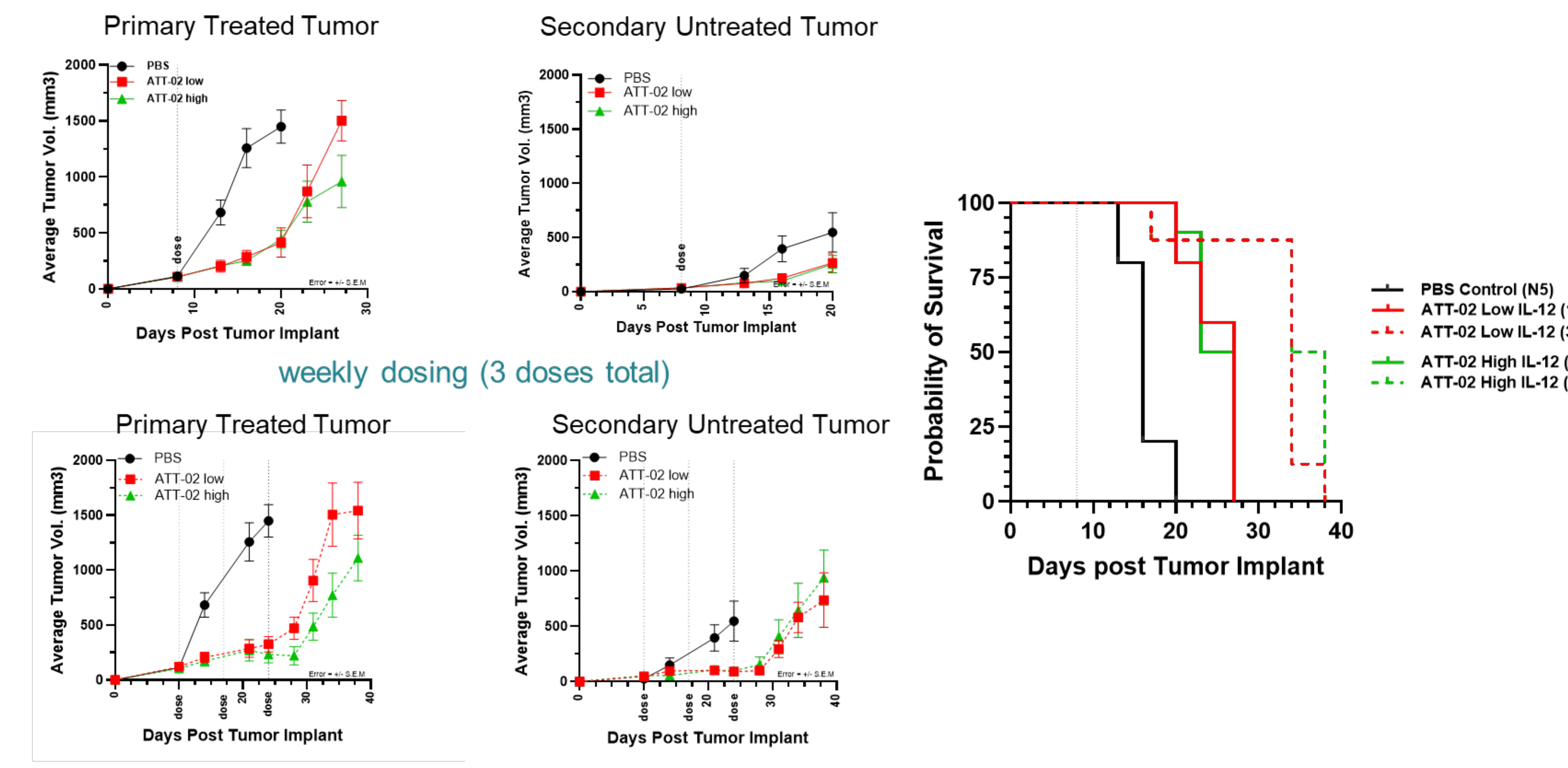
[Right] In the original study, mice were inoculated with CT26 cells on one flank and treated with ATT-02 either intratumorally, peritumorally, or perilymphatically. 67 days after initial tumor inoculation, 9 survivors received 1m CT26 cells in the opposite flank and 0.3M EMT6 cells in the same flank as the original CT26 injection. Tumor volumes were measured at 7 days post inoculation.

Figure 5. A single injection of ATT-02 shows efficacy in the treated and "untreated" tumors (CT26 Model)



CT26 cells were injected on both flanks The primary tumor was treated on day 8 with a single injection of ATT-02, IL-12 (20ug) or PBS. Tumor growth (a) and survival (b) were monitored.

Figure 6. Single dose and multidose ATT-02 demonstrates efficacy in two tumor abscopal model (B16F10)



Mice were injected on both flanks with B16F10 cells on day 0. Mice were treated with PBS or ATT-02 (i.t.) the days shown in the graph (dotted line).

In the B16F10 model, ATT-02 was tested in different dose locations to determine the impact on tumor growth. Interestingly, intratumoral, peritumoral, and perilymphatic dosing all had similar effect on tumor growth (Figure 7).

Figure 7. ATT-02 (single dose) demonstrates efficacy through multiple routes of administration

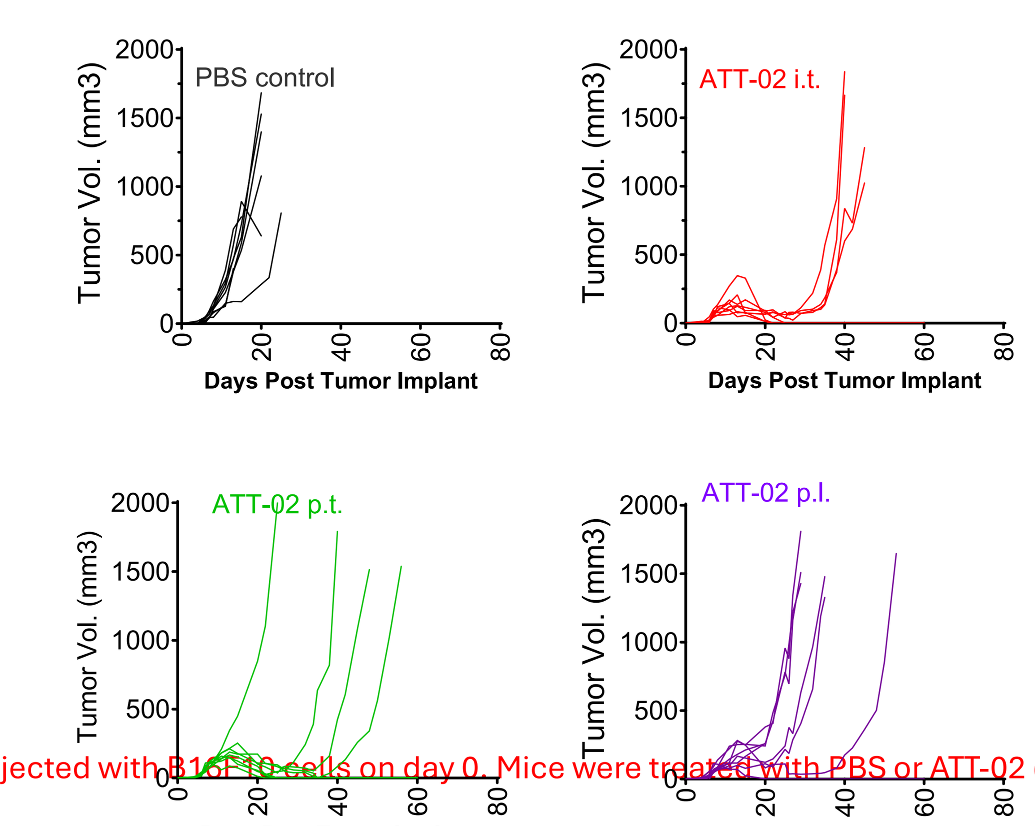


Figure 7. Mice were injected with B16F10 cells on day 0. Mice were treated with PBS or ATT-02 (i.t.) on day 10.

Interestingly, MSR alone had some anti-tumor activity in the B16F10 model with delayed tumor progression (data not shown). We hypothesize that the MSR-related effect enhance IL-12 anti-tumor activity.

Conclusions: Intratumoral injection of ATT-02 (MSR_IL-12) leads to significant anti-tumor immune responses, tumor-specific responses, and tumor growth inhibition in multiple syngeneic tumor models.