Silica-Based Scaffold Delivery of ATT-01 (CpG & GM-CSF Loaded Silica Scaffold) Induces Durable Remission in AML Murine Models via T- Cell Immunity

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Poster #4833

Background: AML is a heterogenous hematological malignancy characterized by the rapid proliferation of myeloid progenitor cells in the bone marrow and peripheral blood. Despite advancements in treatment, AML remains associated with a poor prognosis and high rates of relapse, highlighting the need for novel therapeutic strategies. Differentiation therapy has emerged as a promising approach for treating specific subtypes of leukemia. This therapeutic strategy aims to drive malignant blast cells towards terminal differentiation, restoring normal hematopoiesis and immune function. Recent studies using a cryogel scaffold system demonstrated the potential to achieve complete remission and stimulate a vaccine-like immune response in preclinical AML murine models. Notably, these findings suggest that the scaffold formation can provide localized delivery of immune-modulatory agents and serve as a platform for enhancing anti-tumor immunity. Building upon the promising results of the cryogel system, here we explored the efficacy of an alternative injectable silica-based scaffold, named ATT-01, consisting in Mesoporous Silica Rods (MSR) loaded with CpG and Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF). Our hypothesis was that ATT-01 is that ATT-01 can effectively induce the differentiation of the AML blasts into antigen-presenting cells (APCs), activating anti-tumor T-cells immunity.

***Methods*:** AML was induced via tail vein injection of AML cells (C1498-eGFP, and WEHI-3) to establish a robust leukemia model. Mice received a combination of cytarabine (ara-C) and doxorubicin (a standard chemotherapy regimen) for 5 days post-AML induction to reduce tumor burden prior to the immunotherapeutic intervention with ATT-01. ATT-01 was administered subcutaneously on day 14 post-induction to slowly release the loaded factors (CpG and GM-CSF), enhancing their bioavailability and effectiveness within the tumor microenvironment. Mice were euthanized at specified endpoints for immunological analysis. Primary organs (spleen, bone marrow, and peripheral blood) were collected for quantification of immune cell populations. Flow Cytometry was employed to assess cellular differentiation and activation, focusing on CD8+ T cell populations and other immune markers. To determine the role of specific immune cell types in mediating therapeutic effect of ATT-01, mice were treated with monoclonal antibodies targeting CD8+ T cells, CD4+ T cells, and NK cells prior to the C1498 therapeutic model. The depletion allowed for the evaluation of the immune mechanisms underpinning the observed anti-tumor efficacy.
In vitro assays were conducted using THP-1 cells (human monocytic leukemia cell line) and murine WEHI-3 AML cells to investigate the differentiation effects of ATT-01 on myeloid cells. ATT-01 was co-cultured with the THP-1 and AML cells to assess its ability to induce differentiation in APCs and stimulate immune responses. Immune phenotyping was performed via Flow Cytometry to evaluate the expression of key immune markers and the functionality of the APCs. The capability of these APCs to process and present AML-derived antigens to T-cells was also assessed.

Figure 1. ATTimmune Platform Showing Local Immune Cascade Resulting in Systemic Anti-tumor Immunity

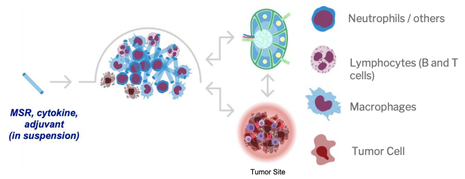

FIG. 1 shows the forming of a large immune structure after subcutaneous injection of mesoporous silica rods (MSR) and the trafficking of immune cells to and from lymph nodes and tumors. The structure includes MSR, neutrophils, lymphocytes, macrophages, and other immune cells, and tumor cells.

Figure 2. ATT-01 Induces Persistent and Long-Duration Survival in Leukemic Murine Tumor Models

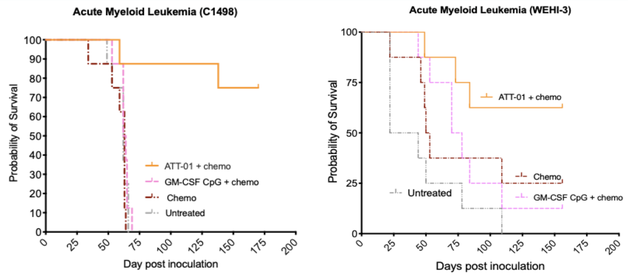

Fig. 2 shows the survival rates of mice challenged with two murine leukemia cell lines and treated with different therapeutics. The combination of chemotherapy and ATT-01 significantly improves compared to other treatments.

Figure 3. ATT-01 Enhances the Development of Protective Memory Response

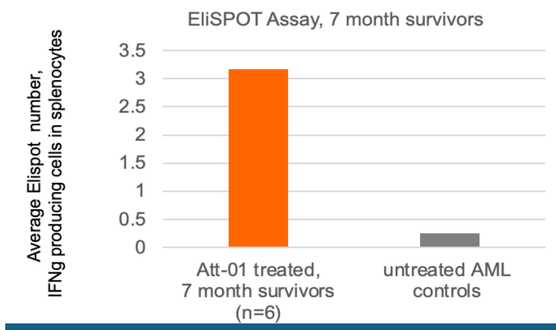

Fig.3 Mice survived for 6+ months were tested for functional immune protection. IFN-γ+CD8+T cells were measured in the spleen of the surviving mice. ELISPOT analysis results suggest the protective memory response of the ATT-01 treatment.

Figure 4. Histological and immunological analysis of mice treated with ATT-01

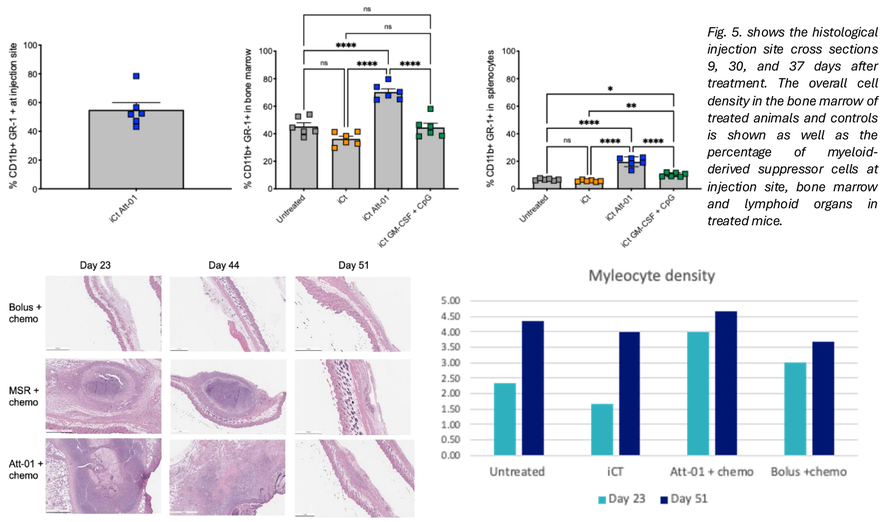

Fig. 5. shows the histological injection site cross sections 9, 30, and 37 days after treatment. The overall cell density in the bone marrow of treated animals and controls is shown as well as the percentage of myeloid-derived suppressor cells at injection site, bone marrow and lymphoid organs in treated mice.

Figure 5. Depletion of effectors T-cells in Att-01 treatedleukemic mice

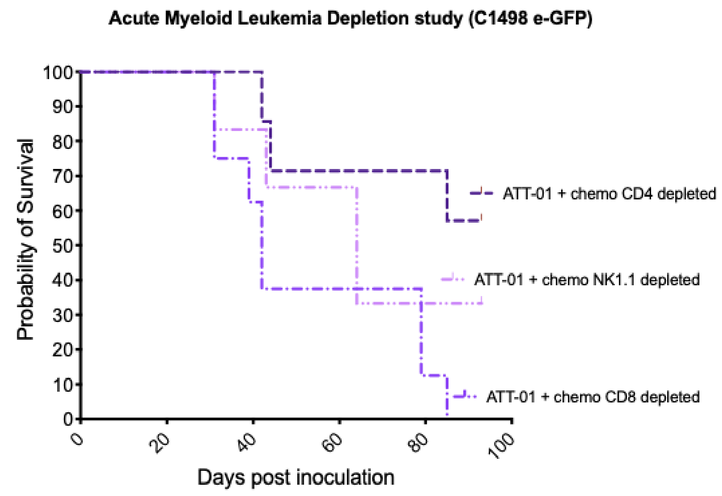

Fig. 5 show the overall survival for mice bearing AML tumor treated with ATT-01 and that were administered depleting antibodies

Figure 6. Differentiation of Murine and Human AML Cell Lines by ATT-01

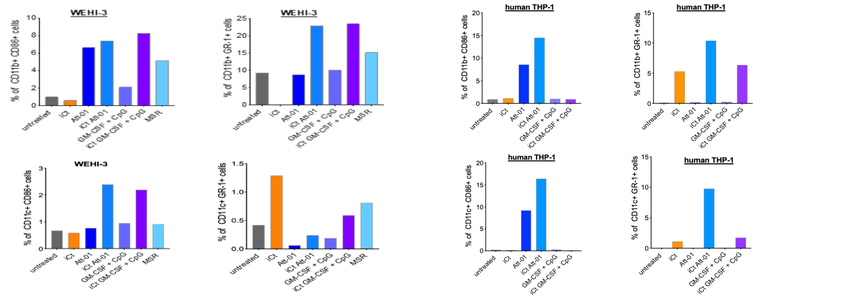

Fig. 6 shows the differentiation as shown by increased myeloid immune markers of the urine WEHI-3 and human THP-1 AML cell lines when treated with chemo, ATT-01, ATT-01 + chemo, GM-CSF + CpG and GM-CSF CpG + chemo as monotherapy or in combo with chemo agents. Untreated cells are used as control. Harvest after 24 hours culture.

Results: Mice treated with the combination of chemotherapy (ara-C and doxorubicin) followed by ATT-01 administration demonstrated a significant increase in complete remission (CR) rates compared to controls. In the C1498 model, a CR was observed in 80% of treated mice. Similarly, in the WEHI-3 model, the combination treatment resulted in a notable CR percentage of 75%. These results highlighted the critical role of ATT-01 in the efficacy of the therapeutic approach. In addition, ex vivo analysis showed elevated levels of IFNg, indicating robust T cell activation in response to the ATT-01 therapy.
Depletion of CD8+ T cells prior to ATT-01 administration suppressed this effect, confirming the necessity of T cell-mediated immunity in achieving remission. In vitro analysis of murine and human AML cells in co-culture with ATT-01 showed phenotypic changes towards an antigen-presenting cell (APC) phenotype. Increased expression of co-stimulatory molecules was observed, indicating their potential to activate T cells.