Allocetra-OTS, an Early Apoptotic Cellular Therapy Synergize with Chimeric Antigen Receptor (CAR) T Cell Therapy or Immune Check Point Inhibitor Against Peritoneal Solid Tumor

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Background & Aim.

Immune check point inhibitors revolutionized the treatment of solid tumors. Chimeric antigen receptor (CAR) genetically engineered T cells can activate an immune response to a cancer-specific antigen. However, in many tumors only partial responses are achieved. Here we questioned the role of monotherapy and synergistic effect of Allocetra-OTS (Enlivex Therapeutics Ltd), that reprogram macrophages, on solid tumor progression.

Methods.

In an immune-competent model, Balb/c mice were treated with AB12 (mesothelioma) with pLenti-PGK-V5-Luc-Neo and treated with anti-CTLA4 with or without Allocetra-OTS. Mice were monitored daily for clinical signs and peritoneal fluid accumulation and weekly for tumor growth. Kaplan-Meier log rank test was done for survival. In the CAR-T model, HeLa-CD19 cells were stably transduced with pLenti-PGK-V5-Luc-Neo. For CAR preparation, fresh mononuclear cells (MNC) were introduced to CD28+CD3+ beads and transfected with CD19-CAR plasmids. SCID-Bg mice were injected intraperitoneally (IP) with human HeLa-CD19 or HeLa-CD19-luciferase cells, 10×10^6 Allocetra-OTS or vehicle, and 10×10^6 CD19-CAR T cells or mock T cells. For Allocetra-OTS preparation, enriched mononuclear fractions were collected by leukapheresis from healthy eligible human donors and induced to undergo apoptosis as described (Van-heerden et al, Frontiers in Immunology 2021).

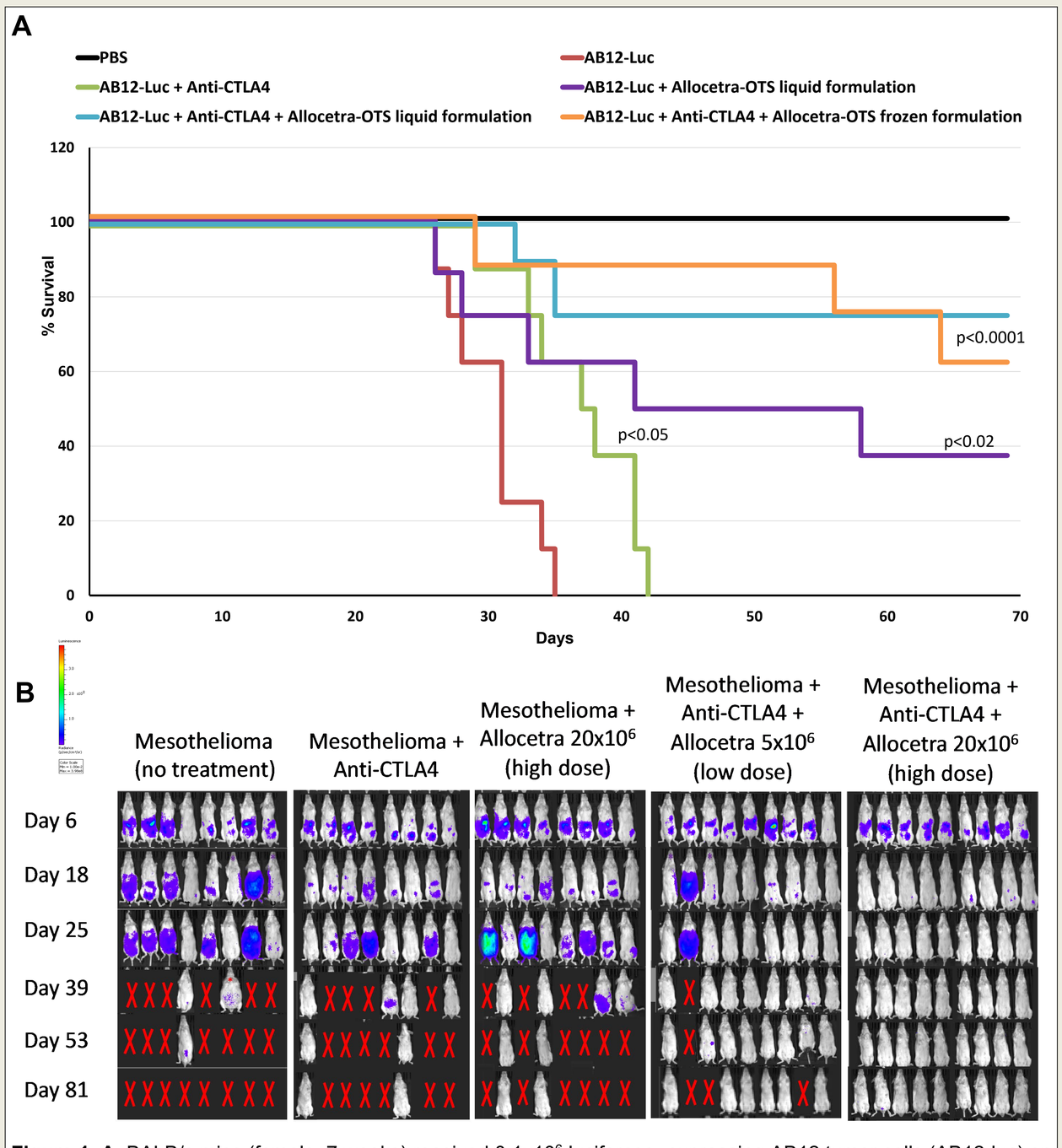

Figure 1. A. BALB/c mice (female, 7 weeks) received 0.1×10^6 luciferase- expressing AB12 tumor cells (AB12-luc) on day 0. On days 12, 15, 19 and 22, mice were treated i.p with 200µg anti-CTLA-4 (BioXcell, BE0164) and / or 20×10^6 Allocetra-OTS cells in 1 hour interval. Mice were monitored daily for clinical score and survival. Mice were sacrificed when reaching score 15. Apart PBS, healthy control (n=4) all groups consisted of 8 mice. **B. In an additional experiment** mice were monitored for tumor imaging by IVIS (Perkin-Elmer, Lumina III). Mice were injected IP with luciferin solution and were imaged 10 minutes after luciferin injection.

Results.

In AB12 model (Figure 1), after 75 days of follow-up, anti CTLA4 therapy significantly ameliorated survival from 26±5 to 38 ±9 days ($p<0.05$). However, Allocetra-OTS monotherapy ameliorated survival to 45 ±12 days ($p<0.02$) and combinational therapy to 75±9 days ($p<0.0001$) with complete remission in 60-75% of mice. Results were visualized using IVIS of intraperitoneal AB12-Luc cells (Figure 1B). In the CAR-T model, SCID mice survived 30±5 days (range 27–37) and were sacrificed according to clinical score or died from solid tumor in the peritoneal cavity after accumulation of bloody peritoneal fluid and clinical deterioration. Kaplan-Meier survival curve is shown in Fig. 2. As shown, CAR T cell therapy significantly ameliorated survival to 55±11 days ($p<0.05$ vs MOCK). However, when mice received co-administration of Allocetra-OTS and CAR T cells, a further significant increase in survival was seen with survival of 70±20 days (range 48-90, $P<0.05$ vs CAR-T alone). Flow cytometry and single cell analysis showed that large peritoneal macrophages (LPM), were associated with antitumor activity (Figure 3).

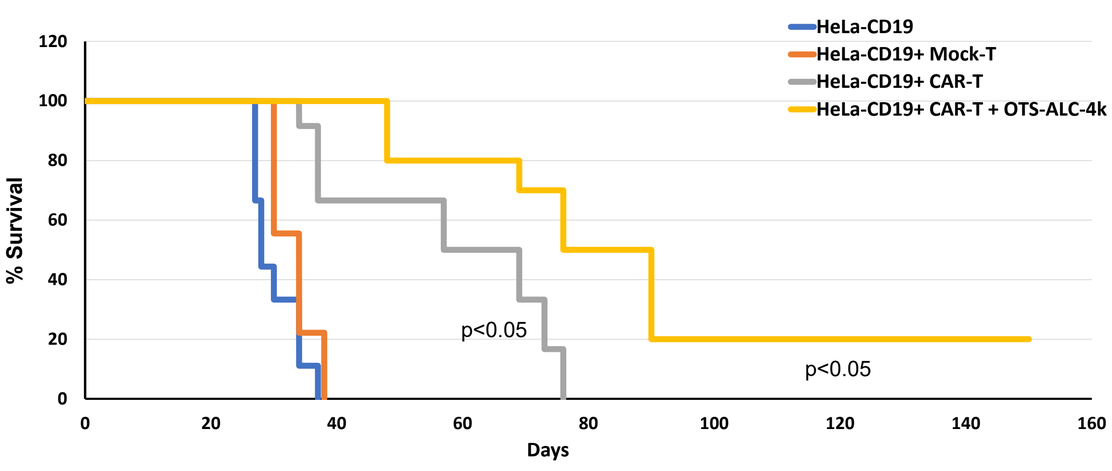

Figure 2. SCID-bg mice (female 7-8wk) were injected with 250,000 HeLa-CD19 cells i.p. om days 1 and 2. 10×10^6 Allocetra-OTS cells were administered i.p. on day 9 and 10×10^6 CAR-T or Mock-T cells were administered on day 10. mice were monitored for 150 days. Results are representative of 5 separate experiments.

Conclusion.

During intraperitoneal tumor progression, Allocetra-OTS as monotherapy was effective and in combinational therapy with CAR or anti-CTLA4 significantly reduced tumor size and enable complete remission in up to 75% of treated mice.

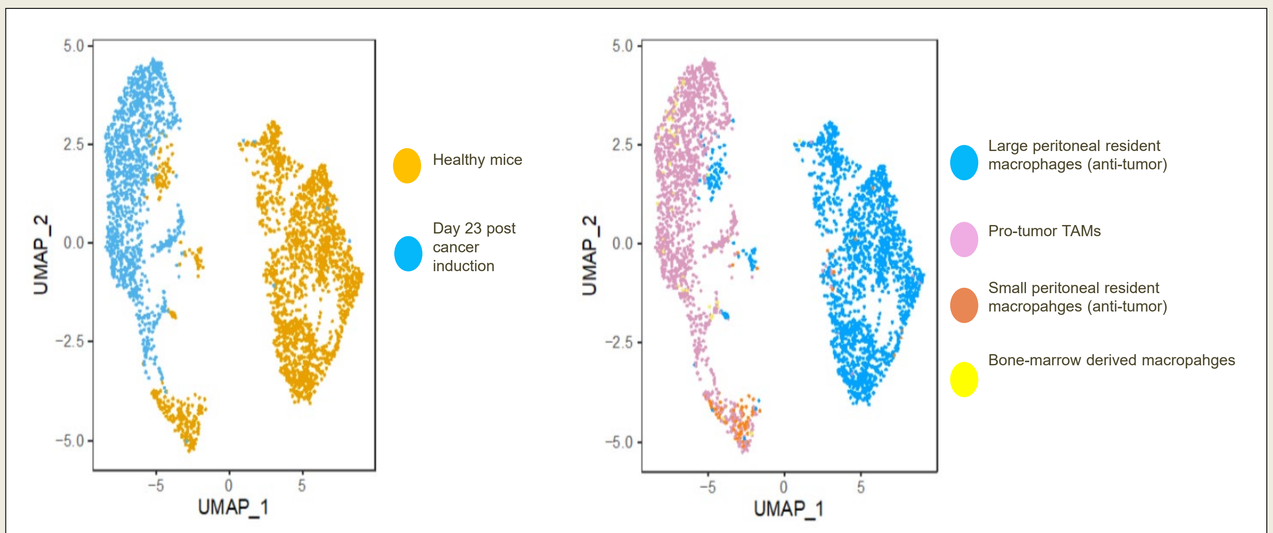

Figure 3. Single cell analysis of macrophages in healthy mice and 23 days following cancer induction. Resident macrophages disappear and tumor associated macrophages (TAMs) are recruited. Targeting macrophages by Allocetra-OTS restores the macrophage population.