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

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During progression of intraperitoneal tumor (mesothelioma, ovarian), Allocetra-OTS as monotherapy was effective and in combinational therapy with either anti-CTLA4, anti-PD-1, or CAR-T, significantly reduced tumor size and enabled complete remission in up to 75% of treated mice

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 investigated the potential anti-cancer activity of Allocetra-OTS, a novel macrophages reprogramming cell therapy (Enlivex Therapeutics Ltd), on solid tumor progression as a monotherapy and in combination with various ant-cancer agents.

Methods.

In two immune-competent models, Balb/c mice were injected, respectively, with AB12 (mesothelioma) and ID8 (ovarian cancer), followed by treatment with anti-CTLA4 (mesothelioma) or anti-PD-1 (ovarian cancer), 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).

Results.

In the mesothelioma immune-competent 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 ovarian cancer model (Figure 2), IP treatment with Allocetra-OTS alone or anti-PD-1 alone provided comparable delayed tumor growth rate. However, a marked synergistic effect was observed in the mice treated with Allocetra-OTS plus anti-PD-1 compared to the single therapy of Allocetra-OTS or anti-PD-1 alone. This study was conducted by the Center for Precision Cancer Modeling, Yale University.

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. 3. 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 4).

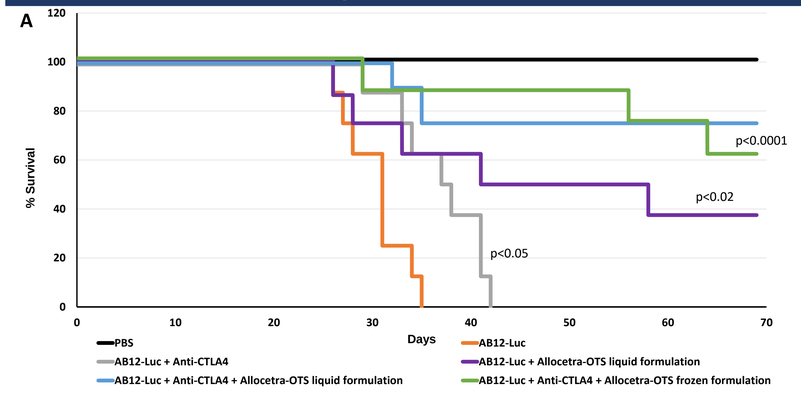

Figure 1. A. BALB/c mice (female, 7 weeks) received $0.1x10^6$ luciferase-expressing AB12 tumor cells (AB12-luc) on day 0. On days 12, 15, 19, and 22, mice were treated i.p with 200µg anti-CTLA-4 (BioXcell, BE0164) and/or $20x10^6$ Allocetra-OTS cells in 1-hour intervals. Mice were monitored daily for clinical score and survival. Mice were sacrificed when reaching a score of 15. Apart from 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.

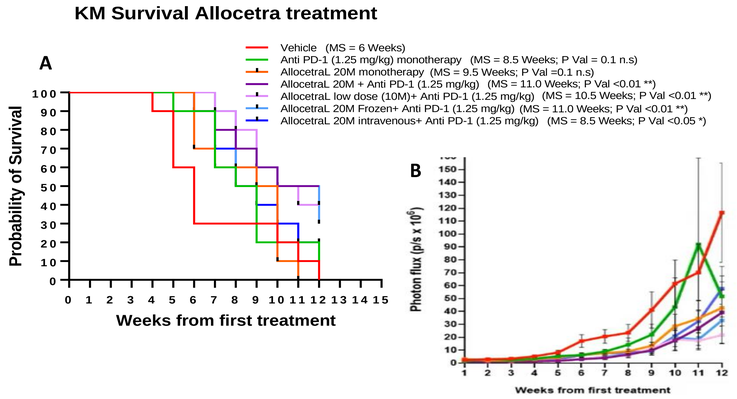

Figure 2A: Simulated Kaplan Meier survival curve showing of survival of mice in each of control and treatment arms. Survival is defined as tumor burden corresponding to a photon flux of <1×107 photons/sec.
Figure 2B: ID8 (ovarian cancer), tumor burden evaluation.

Figure 3. SCID-bg mice (female 7-8wk) were injected with 250,000 HeLa-CD19 cells i.p. on days 1 and 2. $10x10^6$ Allocetra-OTS cells were administered i.p. on day 9 and $10x10^6$ CAR-T or Mock-T cells were administered on day 10. mice were monitored for 150 days. Results are representative of 5 separate experiments.

Figure 4. 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.