Synergistic anti-tumor effect of Allocetra-OTS in combination with immune checkpoint inhibitors (ICI)/chemotherapy/CAR-T, through in-vivo reprogramming of macrophages

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**Background & Aim**
ICI revolutionized solid tumor treatment, however, in many tumors only partial response is 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 anti-cancer agents.

**Methods**
Allocetra-OTS is manufactured from enriched mononuclear fractions and induced to undergo stable early apoptosis. In an immunocompetent model, Balb/c mice were inoculated in the peritoneal cavity with AB12 (mesothelioma) stably transduced with plenti-PGK-VS-Luc-Neo for IVIS visualization and treated with anti-CTLA4, anti-PD1, or cisplatin, with or without Allocetra-OTS. Allocetra-OTS was also administered as monotherapy. Kaplan-Meier log rank test was done for survival. CAR-T model was induced in immune-deficient SCID-Bg mice that were inoculated intraperitoneally with human HeLa-CD19 or HeLa-CD19-luciferase cells, followed by treatment with 10^10 cells of Allocetra-OTS or vehicle, and 1x10^6 CD19-CAR-T cells or mock T cells.

**Results**
Anti-CTLA4 stand-alone therapy significantly improved survival from mean 3429 to 44.9 ± 20 days (p<0.05). Allocetra-OTS stand-alone therapy improved survival to 52.3 ± 20 days (p<0.02) and combinational therapy; anti-CTLA4 + Allocetra-OTS improved survival to 86.7 ± 20 days (p<0.0001) with complete cancer remission in 60-100% of mice (Fig. 1 A+B). Similar anti-tumoral effects of Allocetra-OTS were seen in combination therapy with cisplatin (Figure 2) or anti-PD1 (Figure 3). In the CAR-T model, SCID-Bg mice survived 305 ± 20 days (range 27–37) and were sacrificed or died from tumor progression. Results were verified using IVIS of intraperitoneal HeLaCD19-Luc cells. CAR-T cell therapy significantly ameliorated survival to 551 ± 20 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 a survival of 702 ± 20 days (range 48–80, p<0.05 vs CAR-T alone).

**Conclusion**
During intraperitoneal tumor progression, Allocetra-OTS as stand-alone therapy was effective and in combinational therapy with either anti-CTLA4, anti-PD-1, cisplatin or CAR-T, significantly reduced tumor size and enabled complete remission in up to 100% of treated mice.

**Figure 1.** A. Balb/c mice (female, 7 weeks) were IP inoculated with 0.1x10^6 luciferase-expressing AB12 tumor cells (AB12-luc) on day 0. IP On days 12, 15, 19, and 22 mice were treated IP with 20µg anti-CTLA4 (Bioxell) and/or 5x10^6 Allocetra-OTS cells. Mice were monitored daily for clinical score and survival. B. IVIS imaging on day 81. IVIS was performed at days 6, 11, 18, 25, 39, 53, 67 and 81 (Perkin Elmer, Lumina III).

**Figure 2.** 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 IP with 20µg anti-CTLA4 (Bioxell) and/or 5x10^6 Allocetra-OTS cells (OTC) or vehicle alone. Survival analysis was done using Kaplan-Meier Log Rank test. Survival data were compared with the log-rank test using the GraphPad Prism software.

**Figure 3.** 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 IP with 20µg anti-CTLA4 (Bioxell) and/or 5x10^6 Allocetra-OTS cells (OTC) or vehicle alone. Survival analysis was done using Kaplan-Meier Log Rank test. Survival data were compared with the log-rank test using the GraphPad Prism software.

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