ANTI-TUMOR THERAPEUTIC EFFECTS IN MICE TREATED WITH *LISTERIA MONOCYTOGENES (Lm)*-LLO IMMUNOTHERAPY IN COMBINATION WITH ANTI–PD-L1

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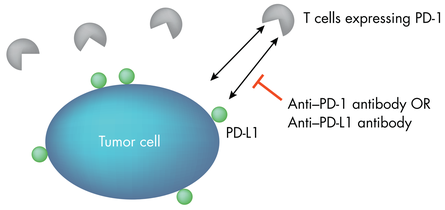
INTRODUCTION

- Published reports have shown that *Lm*-LLO (attenuated *Listeria monocytogenes* with tLLO fusion peptide) immunotherapies can be combined with different modalities such as radiation, chemotherapy, or immunotherapy for the treatment of cancer.[1–3]
- ADXS11-001 is a non-pathogenic, attenuated, and genetically modified *Lm* vector that secretes an HPV-E7 tumor antigen as tLLO-E7 fusion protein; tLLO refers to truncated form of non-hemolytic listeriolysin O protein.[4]
  - This strain is mainly attenuated due to expression of a mutated form of virulence gene transcription activator PrfA that causes a reduction in expression of virulence genes
  - Due to the unique life-cycle of this bacterium, *Lm* vectors are taken up by antigen-presenting cells, where the tumor antigen E7 is cross-presented via both major histocompatibility complex (MHC) class I and II pathways, resulting in E7-specific T-cell response toward HPV-transformed tumors
- Tumors often take advantage of immune tolerance mechanisms, one of which is through receptor/ligand interaction on T cells. Interaction of programmed death receptor-1 (PD-1) expressed on T cells with its ligand, PD-L1, expressed by tumor cells, can dampen T-cell receptor (TCR) signaling and suppress activation and proliferation of T cells, and thereby inhibit the potential infiltration of activated lymphocytes into the tumor (**Figure 1**).
- In a recent study, the combination of ADXS11-001 with an anti–PD-1 blocking antibody significantly inhibited tumor growth and prolonged survival in animals, which supports promising clinical potential.[3]
- The US Food and Drug Administration has recently approved the anti–PD-1 drug pembrolizumab (Keytruda®) for the treatment of melanoma. Similarly, a number of anti–PD-L1 therapies such as BMS-936559, MPDL3280A, and MEDI4736 have shown promising results in clinical trials targeting tumor cells that express PD-L1.

Figure 1. Anti–PD-1 or anti–PD-L1 antibodies inhibit PD-1 interaction with its ligands, increasing TCR signaling, activation, and proliferation



HYPOTHESIS AND STUDY AIMS

HYPOTHESIS

- Combination of *Lm*-LLO immunotherapy with an anti–PD-L1 antibody is synergistic, inhibits the proliferation of tumors, and prolongs survival without exacerbating potential adverse events. The combination of *Lm*-LLO immunotherapy and PD-L1–blocking antibodies is translatable to the clinical setting for the treatment of malignancies.

STUDY AIMS

- To evaluate the therapeutic efficacy of ADXS11-001 (*Lm*-LLO-E7) in combination with anti–PD-L1 antibody in a TC1 mouse tumor model.
- To monitor serum cytokine/chemokine levels associated with ADXS11-001 infusion and evaluate the inhibition of tumor growth and survival.

METHODS AND STUDY DESIGN

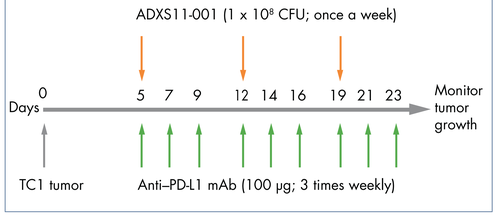
TUMOR MOUSE MODEL

- TC1 cells expressing HPV 16 E7 antigen (1×10^5 cells/mouse) were implanted subcutaneously in 6- to 8-week-old female C57BL/6 mice.

TREATMENT AND DOSE

- ADXS11-001 was injected intraperitoneally at 1×10^8 colony-forming units (CFU)/mouse/dose. The anti–PD-L1 monoclonal antibody (mAb) was obtained from BioXCell and was injected intraperitoneally at a dose of 100 µg/mouse.
- Four different treatment groups (n=12/group) were included in this study:
  - Control group: no treatment
  - ADXS11-001: monotherapy with ADXS11-001 (total of 3 doses)
  - Anti–PD-L1: monotherapy with anti–PD-L1 mAb (total of 9 doses)
  - ADXS11-001 + anti–PD-L1: combination therapy (total of 3 doses for ADXS11-001 and 9 doses for anti–PD-L1)
- Treatment schedule for the combination study is shown in **Figure 2**.

Figure 2. Combination therapy group: treatment schedule



CFU, colony-forming units; mAb, monoclonal antibody.

EXPERIMENTAL READOUT

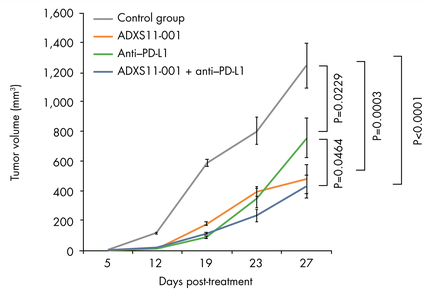
- Tumor growth was monitored every 3–4 days using digital calipers.
- Mice were sacrificed when tumors reached a size of 12 mm in diameter.
- Tumor volume (V) was calculated using the formula $V = (W^2 \times L)/2$, where L is the longest diameter and W is the shorter diameter.
- To assess safety for combination therapy, serum samples were collected at pre-, 2h, and 4h post-treatment on Days 5, 12, and 19.
- The changes in serum cytokine and chemokine levels were assessed using Mouse InflammationMAP (Myriad RBM).

RESULTS

ANTI-TUMOR EFFICACY

- The combination of ADXS11-001 (1×10^8 CFU/mouse) and anti–PD-L1 antibody (100 µg/mouse) was slightly better at controlling TC1 tumor growth compared with anti–PD-L1 antibody monotherapy (**Figure 3**).

Figure 3. Effect of monotherapy versus combination therapy on anti-tumor efficacy using TC1 model



P values calculated using one-way ANOVA test.

IMMUNOLOGIC RESPONSE (CYTOKINES AND CHEMOKINES)

- Serum cytokine and chemokine analysis indicated treatment-related differences in the kinetics of cytokines and chemokines induced after ADXS11-001 monotherapy or combination therapy (ADXS11-001 + anti–PD-L1) during different days of the study.
- Sharp increases in cytokine (interleukin [IL]-2, IL-4, IL-6, and IL-10; **Figure 4**) and chemokine (macrophage-derived chemokine [MDC], macrophage inflammatory protein [MIP]-α, MIP-1β, MIP-2, monocyte chemotactic protein [MCP]-1, 2, 5; **Figure 5**) levels were observed at 2h post-treatment on Day 5 in mice treated with ADXS11-001 monotherapy or combination therapy (ADXS11-001 + anti–PD-L1).
- On Days 12 and 19, although some increases were observed in cytokine (**Figure 4**) and chemokine (**Figure 5**) levels 2h post-treatment, these were less pronounced compared with Day 5.

CONCLUSIONS

- Co-administration of ADXS11-001 with anti–PD-L1 antibody is well tolerated in a preclinical mouse tumor model.
- Combination of ADXS11-001 with anti–PD-L1 antibody does not exacerbate cytokine release compared to ADXS11-001 monotherapy.
- ADXS11-001 in combination with anti–PD-L1 antibody appears to be better at controlling TC1 tumor growth compared to anti–PD-L1 monotherapy.

ONGOING STUDIES

- A phase 1–2 study of ADXS11-001 and MEDI4736 (alone or combination) in cervical or HPV-positive head and neck cancer has been initiated (ClinicalTrials.gov Identifier: NCT02291055).

Figure 4. Pre- and post-treatment mouse serum cytokine levels (Days 5, 12, and 19)

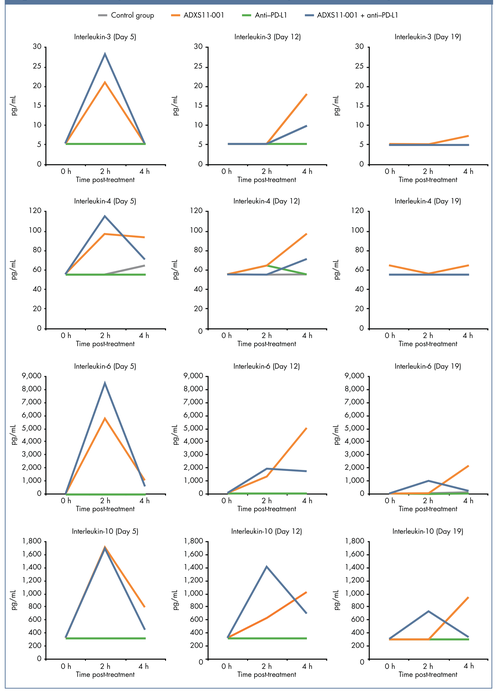
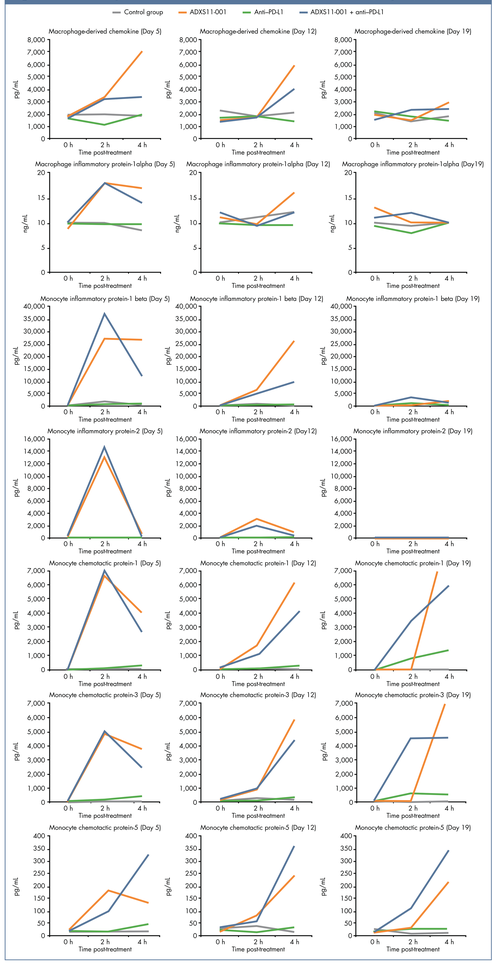


Figure 5. Pre- and post-treatment mouse serum chemokine levels (Days 5, 12, and 19)



REFERENCES

1. Basu P, et al. *J Clin Oncol*. 2014;32(suppl): abstr. 5610.
2. Perez K, et al. *Sexual Health*. 2015;12(1):87. Abstr. 23.
3. Mkrtichyan M, et al. *J Immunother Cancer*. 2013;1:15.
4. Gunn GR, et al. *J Immunol*. 2001;167:6471–9.

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DISCLOSURES

The authors are employees and shareholders of Advaxis, Inc.