Combination of *Listeria*-based human papillomavirus (HPV)-E7 cancer vaccine (AXAL) with CD137 agonistic antibody provides an effective immunotherapy for HPV$^+$ tumors in a mouse model

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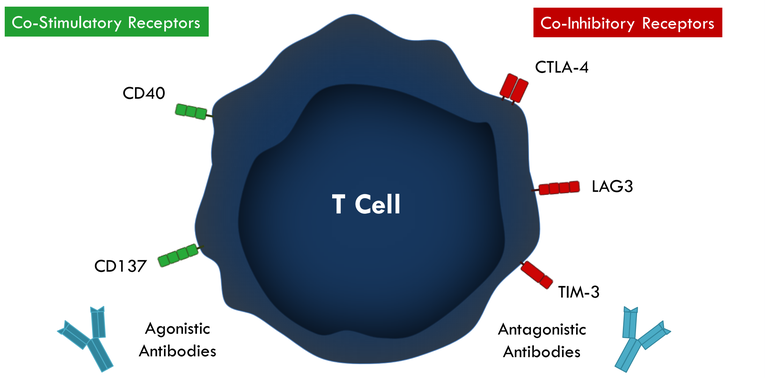
INTRODUCTION

- Axalimogene filolisbac (AXAL) is a live attenuated *Listeria monocytogenes* (*Lm*)-based immunotherapy that expresses the full length E7 protein of human papillomavirus (HPV) 16.
- Advaxis' *Lm*-based immunotherapies act by inducing the *de novo* generation of tumor antigen-specific T cells that infiltrate and destroy the tumor and by reducing the numbers and activities of immunosuppressive regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) in the tumor microenvironment.[1]
- Previous studies have shown that an antagonistic anti-PD-1 antibody as well as antagonistic anti-OX40 and anti-GITR antibodies synergize with AXAL to enhance anti-tumor immunity.[2,3]
- To identify additional antibody-based immunotherapies that may synthesize with AXAL in the treatment of HPV$^+$ cancers, we evaluated the ability of AXAL to control tumor growth, prolong survival, and reprogram the tumor microenvironment in combination with agonistic antibodies of T cell co-stimulatory receptors or with antagonistic antibodies of T cell co-inhibitory receptors in a mouse HPV$^+$ tumor model (**Figure 1**).

Figure 1. T cell targets of antibody-based immunotherapies



Adapted from Mellman I, et al. *Nature.* 2011;480:480-489.[4]

OBJECTIVE

To identify agonistic antibodies of T cell co-stimulatory receptors and antagonistic antibodies of T cell co-inhibitory receptors that synergize with AXAL to enhance anti-tumor immunity in a mouse HPV$^+$ tumor model.

MATERIALS AND METHODS

- Monoclonal antibodies (mAbs) specific for the T cell co-stimulatory and co-inhibitory receptors shown in **Figure 1** were purchased from Bio X Cell (West Lebanon, NH USA). The clone names and the concentration(s) tested for each antibody are listed in **Table 1**.

Table 1. Clone names and concentrations of antibodies used in study

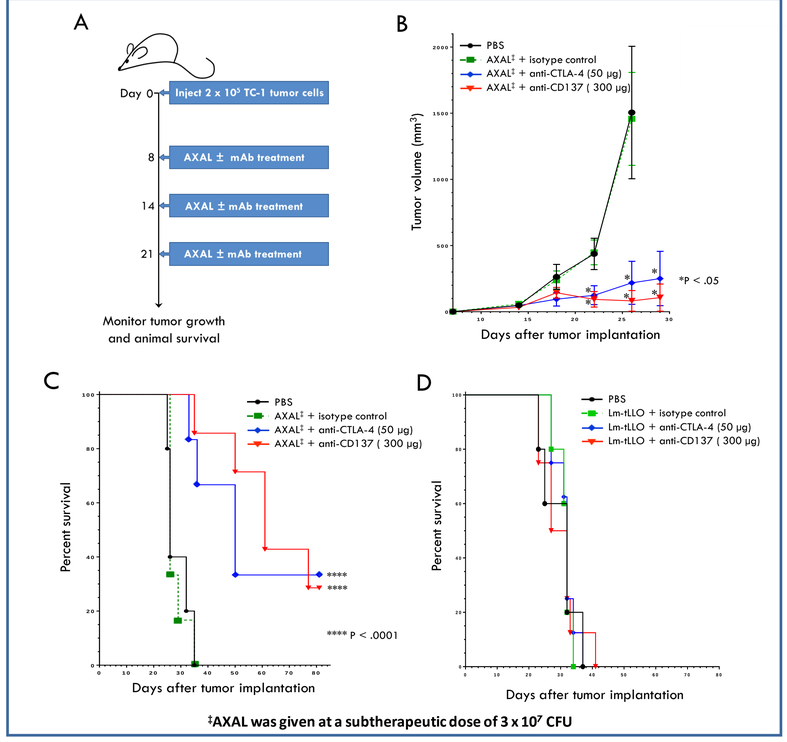
Antibody Target	Clone	Concentration(s) Tested (µg/dose)
CD40	FGK4.5	100
CD137	LOB12.3	200/300
CTLA-4	9H10	50/100
LAG3	C9B7W	200/300
TIM3	RMT3-23	150/250

- TC-1 tumor cells, which are derived from a C57BL/6 lung epithelial cell line, were immortalized with E6 and E7 of HPV 16 and transformed with an activated *ras* oncogene. To establish primary tumors, 2 x 10^5 TC-1 cells were injected subcutaneously in the right flank and allowed to grow for 8 to 10 days prior to the start of treatment.
- To reveal potential synergy between AXAL and antibody-based immunotherapies, a dose titration was performed to determine a subtherapeutic dose of AXAL. A dose range of 3 to 5 x 10^7 CFU was found to exhibit a nominal therapeutic effect. Lm-tLLO, an *Lm* vector that does not express any tumor-associated protein, was used as a vector control.

RESULTS

- To assess the ability of a subtherapeutic dose of AXAL combined with antibody-based immunotherapies to control tumor growth and prolong animal survival, C57BL/6 mice were injected subcutaneously with 2 x 10^5 TC-1 tumor cells and were then treated on days 8, 14, and 21 after tumor implantation with PBS, 3 x 10^7 CFU AXAL, or 3 x 10^7 CFU AXAL plus an agonistic or antagonistic mAb shown in **Table 1** (**Figure 2A**)
  - ♦ Of the 5 mAbs tested, anti-CD137 mAb and anti-CTLA-4 mAb were the most effective at synergizing with AXAL to eradicate established TC-1 tumors and to provide long-term survival (ie, >80 days post tumor implantation). Complete tumor regression was observed in 28% of the mice treated with AXAL + anti-CD137 mAb and in 33% of the mice treated with AXAL + anti-CTLA-4 mAb (**Figure 2B, 2C, and data not shown**).
  - ♦ These anti-tumor effects were dependent upon the expression of the HPV-E7 protein, as mice receiving anti-CD137 mAb or anti-CTLA-4 mAb in combination with the Lm-tLLO vector (which does not express any tumor-associated protein) had survival curves similar to the survival curve of PBS-treated mice (**Figure 2D**).
- To elucidate the mechanism(s) by which AXAL + anti-CD137 mAb and AXAL + anti-CTLA-4 mAb mediate tumor control, phenotypic analysis of the tumor-infiltrating immune cells was performed on day 25, 7 days after the last AXAL and/or mAb treatment (**Figure 3**).
  - ♦ The distribution of immune cell types (all defined as CD45$^+$) within the tumor differed slightly between the two combination therapies (**Figure 3A**).

Figure 2. Combining a subtherapeutic dose of AXAL with either anti-CD137 mAb or anti-CTLA4 mAb enhanced tumor control and prolonged animal survival



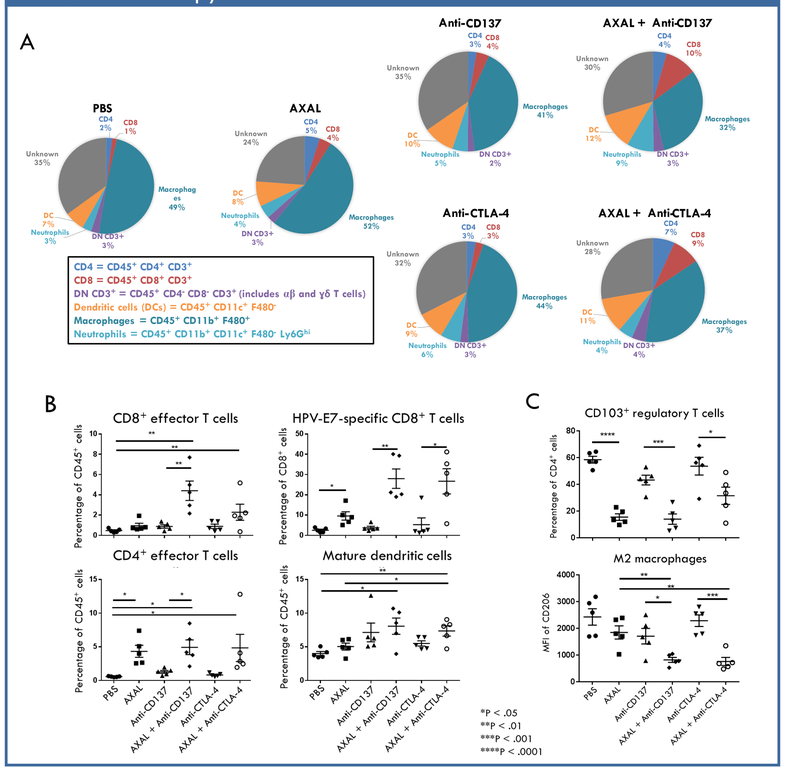
‡AXAL was given at a subtherapeutic dose of 3 x 10^7 CFU

- ∗ With the combination of AXAL + anti-CD137 mAb, there were increases in the percentages of CD8$^+$ T cells, dendritic cells (DCs), and neutrophils compared with AXAL alone, anti-CD137 mAb alone, or PBS treatment.
- ∗ With the combination of AXAL + anti-CTLA-4 mAb, there were increases in the percentages of CD8$^+$ T cells, CD4$^+$ T cells, and DCs compared with AXAL alone, anti-CTLA-4 mAb alone, or PBS treatment.
- ∗ Notably, both AXAL + anti-CD137 mAb and AXAL + anti-CTLA-4 mAb reduced the percentages of total macrophages in the tumor compared with the single-agent therapies.

♦ Quantitation of the relative percentages of effector and suppressor cell subsets in the tumor revealed similarities between the two combination therapies (**Figure 3B and 3C**). Specifically, there were:

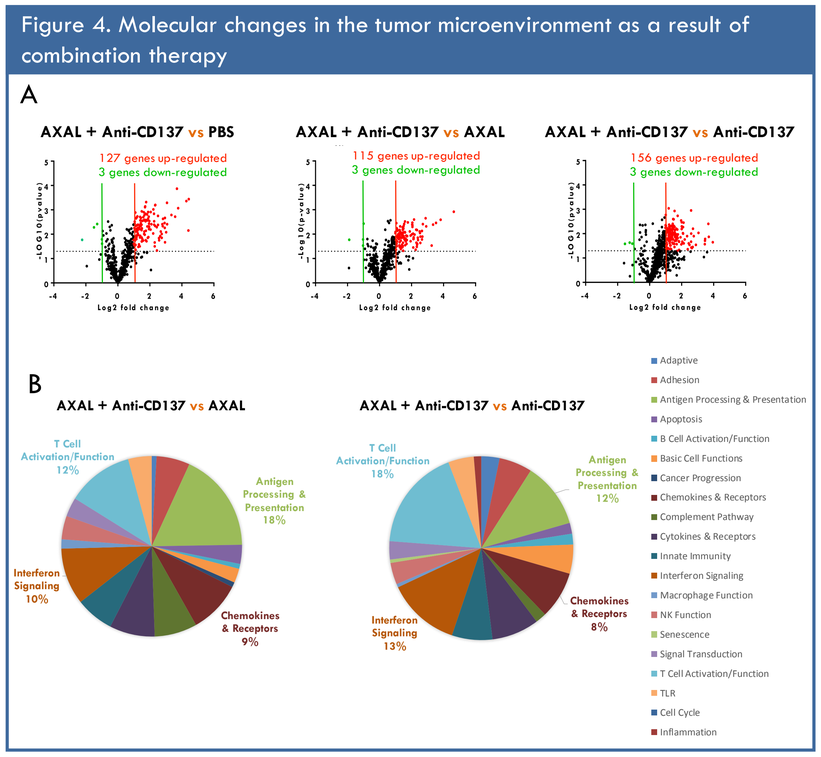
- ∗ Increases in the percentages of total CD8$^+$ effector T cells and HPV-E7-specific CD8$^+$ T cells.
- ∗ Increase in the percentage of total CD4$^+$ effector T cells.
- ∗ Increase in the percentage of mature (ie, CD86$^+$ MHC Class II$^+$) DCs.
- ∗ Decrease in the percentage of CD103$^+$ Tregs cells (defined as CD4$^+$ CD25$^+$ Foxp3$^+$).
- ∗ Decrease in the percentage of immunosuppressive M2 macrophages (based on CD206 expression levels, which are measured by mean fluorescence intensity or MFI).

Figure 3. Cellular changes in the tumor microenvironment as a result of combination therapy



- To investigate further the mechanism(s) by which the AXAL + anti-CD137 mAb combination mediates tumor control, immune-related gene expression profiling of the intact tumor was performed using NanoString Technology.
  - ♦ Volcano plots, which plot significance on the y-axis and fold-change on the x-axis, were used to identify differences in the large gene expression data sets between the following groups (**Figure 4A**):
    - ∗ When AXAL + anti-CD137 mAb was compared to PBS, 127 genes were up-regulated while 3 genes were down-regulated
    - ∗ When AXAL + anti-CD137 mAb was compared to AXAL, 115 genes were up-regulated while 3 genes were down-regulated
    - ∗ When AXAL + anti-CD137 mAb was compared to anti-CD137 mAb, 156 genes were up-regulated while 3 genes were down-regulated
  - ♦ To gain a better understanding of the contribution of each single-agent therapy to the anti-tumor effects observed with the AXAL + anti-CD137 mAb combination treatment, we compared the up-regulated genes in each treatment group by their functional category (**Figure 4B**).
    - ∗ In both comparisons, the top functional categories were T cell activation/function, antigen processing and presentation, chemokines and receptors, and interferon signaling, suggesting that AXAL and anti-CD137 mAb trigger similar pathways involved in anti-tumor immunity.
    - ∗ When AXAL + anti-CD137 mAb was compared to AXAL, which revealed the contribution of anti-CD137 mAb to anti-tumor immunity, **18% of the genes were involved with antigen processing and presentation**, 12% in T cell activation/function, 10% I interferon signaling, and 9% in chemokines and receptors.
    - ∗ When AXAL + anti-CD137 mAb was compared to anti-CD137 mAb, which revealed the contribution of AXAL to anti-tumor immunity, **18% of the genes were involved with T cell activation/function**, 12% in antigen processing and presentation, 13% I interferon signaling, and 8% in chemokines and receptors.

Figure 4. Molecular changes in the tumor microenvironment as a result of combination therapy



CONCLUSIONS

- Subtherapeutic doses of AXAL, anti-CD137 mAb, and anti-CTLA-4 mAb, all of which have nominal therapeutic benefit when given as a single agent, synergize as combinations (ie, AXAL + anti-CD137 mAb and AXAL + anti-CTLA-4 mAb) to provide effective anti-tumor immunity in a mouse TC-1 tumor model.
- Additional studies are underway to determine the mechanisms underlying the synergy between AXAL + anti-CD137 mAb and between AXAL + anti-CTLA-4 mAb.

REFERENCES

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