Treatment With *Listeria monocytogenes (Lm)*-LLO-based Immunotherapies Causes Reduction Of Immunosuppression In The Tumor Microenvironment

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Listeria monocytogenes (Lm)-LLO immunotherapies

- Attenuated Lm is genetically engineered to secrete antigenic fusion protein (LLO-TA) within the cytoplasm of APCs.
- Non-hemolytic detoxified Listeriolysin O (LLO) possess PAMP-like properties. (Wallecha et al., CVI 2013)
- Unique life-cycle of Lm in APC allows it to cross present naturally, stimulating CD4+ and CD8+ activation.
- Antigen-specific CD8+ T cell responses are detected in the tumors.
- Lm-LLO cancer immunotherapeutic ADXS11-001 is being evaluated in Phase 1 and 2 clinical trials in HPV-associated cancers (cervical, head and neck and anal cancer).
Generation of antigen (Prostate Specific Antigen or PSA)-specific CD8 T cells

Generation of high levels of antigen-specific T cell in the spleen and subsequently their infiltration in the tumors for causing therapeutic tumor regression

Shahabi et al. 2008 Cancer Immunol Immunother 57:1301–1313
Generation of high-avidity antigen (PSA)-specific CTLs in the spleens

The cytotoxic activity of CD8 T cell was reduced in proportion to E:T ratio and maximum specific lysis was observed in 0.1 μM peptide concentration

Shahabi et al 2008 Cancer Immunol Immunother 57:1301–1313
Population of antigen (PSA)-specific CD8\(^+\) T cells vs regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSC)

*Lm*-LLO treatment causes an increase in the frequency of antigen-specific T cells in the spleen and tumors followed by decrease in the Treg (CD4\(^+\)CD25\(^+\)Foxp3\(^+\)) and MDSC (Gr\(^+\)CD11b\(^+\)) in the tumor microenvironment.
Frequency of Treg and MDSC in the blood and tumor

$Lm$-LLO treatment causes reduction in the frequency of Treg (∼2-3 fold) and MDSC (∼2 fold) in the tumor but had no effect on the frequency of these cells in the blood.
Suppressive activity of monocytic (Ly6G\(^{-}\) Ly6C\(^{\text{high}}\) CD11b\(^{+}\)) and granulocytic (Ly6G\(^{+}\) Ly6C\(^{\text{low}}\) CD11b\(^{+}\)) MDSC isolated from tumors

A reduction in the suppressive activity of both monocytic- and granulocytic- MDSC was observed in the *Lm*-LLO treated groups (control *Lm* and *Lm*-LLO-Her2) under both antigen-specific as well as non-specific stimulation.
No change in the suppressive activity of monocytic or granulocytic MDSC in spleens

The suppressive activity of both monocytic- and granulocytic- MDSC isolated from spleens of tumor-bearing mice was similar in the treated and untreated groups.
Relative expression of Arginase I was slightly reduced in the MDSC isolated from tumors treated with *Lm*-LLO immunotherapies. There was no change in the relative arginase I expression in MDSC isolated from the spleens.
Treg from *Lm*-LLO treated tumors fail to inhibit T cell activation

**Antigen-specific stimulation**

**Non-specific stimulation**

Treg (CD4+ CD25+) isolated from *Lm*-LLO treated tumors (control *Lm* and *Lm*-LLO-Her2) were less-suppressive.
Splenic Treg were suppressive after *Lm*-LLO immunization

**Antigen-specific stimulation**

**Non-specific stimulation**

Splenic Treg (CD4⁺ CD25⁺) were suppressive in both antigen-specific as well as non-specific stimulation conditions in different groups.
Reduced expression of IL-10 by Treg isolated from tumors of *Lm*-LLO treated groups

**Treg**

- **Foxp3**
- **IL-10**
- **IL-6**
- **TGF-β**
- **β-actin**

**Tcon**

- **IL-10**
- **IL-6**
- **TGF-β**
- **β-actin**

Lane 1- 100 bp ladder, Lanes 2-4: Spleen Treg (Naïve, Lm or Her2) and Lanes 5-7: Tumor Treg (Naïve, Lm or Her2)
Summary

• *Lm*-LLO immunotherapies cause a reduction in the frequency (~ 2-3 fold) of both Treg and MDSC specifically in the tumors.

• Treg and MDSC isolated from *Lm*-LLO treated tumors exhibit diminished suppressive function. Reduction in the suppressive function of Treg is linked to reduced expression of IL-10 and MDSC is linked to reduced expression of arginase I.

• PAMP-like properties of LLO likely have an effect on MDSC induced suppression.

• Therapeutic regression of tumors by *Lm*-LLO immunotherapies is likely dependent on the generation and infiltration of antigen-specific CD8+(cytotoxic) T cells combined with a reduction of suppressive cells in the tumor microenvironment.

• ADXS11-001 has demonstrated clinical benefit with complete and partial responses including improved survival and prolonged stable disease which may be related to decreased Treg and MDSC activity in the tumor microenvironment.

(Poster #258)