ADXS-PSA immunotherapy increases the magnitude and quality of prostate cancer-antigen-specific T cell responses in patients with metastatic castration-resistant prostate cancer

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INTRODUCTION

- Active immunotherapies, such as ADXS-PSA, are designed to generate tumor antigen-specific T cell effectors that recognize and kill tumor cells.
- ADXS-PSA, a highly attenuated *Listeria monocytogenes* (*Lm*)-based immunotherapy that targets prostate-specific antigen (PSA), is currently being evaluated as a treatment for metastatic castration-resistant prostate cancer (mCRPC) in the phase 1/2 KEYNOTE-046 trial as a monotherapy (Part A, presented here) and in combination with KEYTRUDA® (pembrolizumab) (Part B, ongoing).[1]
- Advaxis' *Lm*-based immunotherapies act by stimulating innate immunity through multiple mechanisms including the STING pathway, by reducing the numbers and activities of immunosuppressive cells in the tumor microenvironment, and by inducing the generation of antigen-specific T cells that infiltrate and destroy the tumor.[2]
- Because tumor antigen-specific T cell responses may be linked to the clinical efficacy of active immunotherapies, we quantified the frequency of functional prostate cancer antigen-specific T cells in the peripheral blood of mCRPC patients participating in Part A of the KEYNOTE-046 trial by ELISpot analysis.

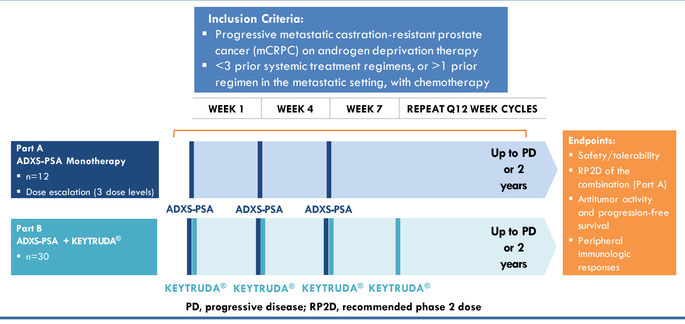
OBJECTIVES

- Evaluate the changes in the frequency and functionality of prostate cancer antigen-specific T cells in mCRPC patients undergoing treatment with ADXS-PSA monotherapy (Part A).
- Determine whether any changes in the frequency and functionality of prostate cancer antigen-specific T cells were associated with clinical activity.

MATERIALS AND METHODS

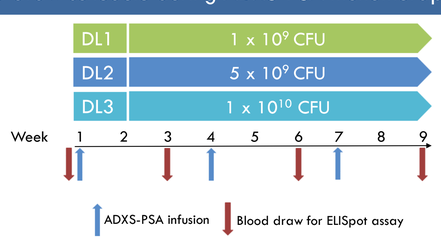
- The KEYNOTE-046 trial (NCT02325557) is a phase 1/2 evaluation of ADXS-PSA alone (Part A), and in combination with KEYTRUDA® (pembrolizumab) (Part B), in the treatment of mCRPC. The study design for KEYNOTE-046 trial is summarized in **Figure 1**.[1]

Figure 1. Study design for KEYNOTE-046 trial



- ELISpot assays were performed on cryopreserved peripheral blood mononuclear cells (PBMCs) isolated at multiple time points from 9 mCRPC patients who received 3 doses of ADXS-PSA monotherapy in the Part A ADXS-PSA dose-determining stage of the KEYNOTE-046 trial (**Figure 2**) and on cryopreserved PBMCs from 2 age-matched (>60 years of age) healthy male donors.
- Cryopreserved unfractionated PBMCs were thawed and rested overnight at 37° C prior to *ex vivo* stimulation. The ELISpot assay protocol followed the protocol outlined by Janetzki et al. for assay harmonization.[3]
- The reactivity of both $CD4^+$ and $CD8^+$ T cells was assayed to peptides derived from PSA, the target antigen of ADXS-PSA, as well as to peptides derived from prostatic acid phosphatase (PAP), prostate-specific membrane antigen (PSMA), prostate stem cell antigen (PSCA), and prostein in order to determine the extent of antigen spreading after ADXS-PSA treatment. All peptide mixes contained peptides that were 15 amino acids in length with 11 amino acid overlap.
- Secretion of IFNγ, TNFα and the cytolytic granule granzyme B was measured using the 3-color fluoroSpot kit (Cellular Technology Limited, Cleveland, OH).
- Statistical analyses were perfomed using GraphPad Prism software.
- RECIST v1.1 criteria were applied to assess the antitumor response (ie, clinical activity) of ADXS-PSA monotherapy. Of the 9 patients who received 3 doses of ADXS-PSA monotherapy, 4 patients achieved, and 5 patients did not achieve, clinical activity. All patients who achieved clinical activity had stable disease.

Figure 2. Blood draw schedule during ADXS-PSA monotherapy



RESULTS

- We have taken a comprehensive approach to monitor the frequency and functionality of prostate cancer antigen-specific T cells in the peripheral blood of ADXS-PSA-treated mCRPC patients.
  - ♦ First, we assayed T cell reactivity to peptides from ***multiple prostate cancer antigens*** to assess the ability of ADXS-PSA to induce or enhance T cell responses to prostate cancer antigens that are ***not*** expressed by the *Lm*-based vector as a measure of antigen spreading.
  - ♦ Second, we measured the secretion of ***multiple T cell effector molecules***
    - ∗ to obtain an accurate estimate of the frequency of functional prostate cancer antigen-specific T cells, as a significant proportion of prostate cancer antigen-specific T cells may be overlooked when only one effector molecule is measured.[4]
    - ∗ to quantify multifunctional T cells (ie, T cells with the ability to produce 2 or more effector molecules). T cell multifunctionality, or T cell quality, has been shown to correlate with a protective immune response against various infectious agents and to be associated with favorable outcomes to a number of active and passive immunotherapies.[4-9]
    - ∗ because IFNγ secretion alone has been shown ***not*** to correlate with tumor regression.[10]
- Quantitative analysis of PSA-specific T cells at baseline detected ***pre-existing***, functional PSA-reactive T cells in 8/9 mCRPC patients prior to ADXS-PSA treatment as well as in 2 age-matched healthy male donors. ***As predicted, assaying for IFNγ secretion alone underestimated the frequency of pre-existing PSA-specific T cells in mCRPC patients*** (**Figure 3**).
- Changes in the frequency of PSA-specific T cell responses were observed in mCRPC patients during the first 9 weeks of treatment (**Figure 4**).
  - ♦ 78% of patients (7/9) exhibited increases in the PSA-specific T cell response on ADXS-PSA treatment, including the 1 patient with no detectable response at baseline. The 2 patients whose PSA-specific T cell response decreased after treatment had non-stable disease.
  - ♦ ***56% of patients (5/9) had a >3-fold increase above baseline in the magnitude of the PSA-specific T cell response***. Of these 5 patients, 3 had stable disease and 2 had non-stable disease.
- Changes in the functionality of PSA-specific T cells were observed in mCRPC patients after 3 doses of ADXS-PSA (**Figure 5**).
  - ♦ ***78% of the patients (7/9) exhibited increases in the frequency of multifunctional PSA-specific T cells after 3 doses of ADXS-PSA***. The 2 patients whose PSA-specific T cell responses decreased after treatment also exhibited decreases in the frequency of multifunctional PSA-specific T cells
  - ♦ 44% of the patients (4/9) exhibited increases in the ***proportion*** of multifunctional PSA-specific T cells after 3 doses of ADXS-PSA. Of these 4 patients, 2 had stable disease and 2 had non-stable disease.
- To determine the extent of antigen spreading after ADXS-PSA treatment, we measured T cell reactivity to peptides derived from PAP, PSMA, PSCA and prostein, all of which are prostate cancer antigens that are ***not*** expressed by ADXS-PSA.
  - ♦ Pre-existing PAP-, PSMA-, PSCA- and prostein-specific T cells were detected in all 9 mCRPC patients prior to ADXS-PSA as well as in 2 age-matched healthy males donors (**Figure 6**).
  - ♦ Changes in the frequency of PAP-, PSMA-, PSCA- and prostein-specific T cells were observed in mCRPC patients during the first 9 weeks of treatment (**Figure 7**).
    - ∗ ***100% of patients (9/9) exhibited increases in the frequency of T cell reactive to at least one other well known prostate cancer antigen, indicative of antigen spreading.***
  - ♦ Changes in the functionality of PAP-, PSMA-, PSCA- and prostein-specific T cells were observed in mCRPC patients after 3 doses of ADXS-PSA (**Figure 8**).
    - ∗ ***100% of patients (9/9) exhibited an increase in the frequency of multifunctional PAP-, PSMA-, PSCA– or prostein-specific after 3 doses of ADXS-PSA.***

Figure 3. IFNγ secretion alone underestimates the frequency of pre-existing PSA-specific T cells in mCRPC patients

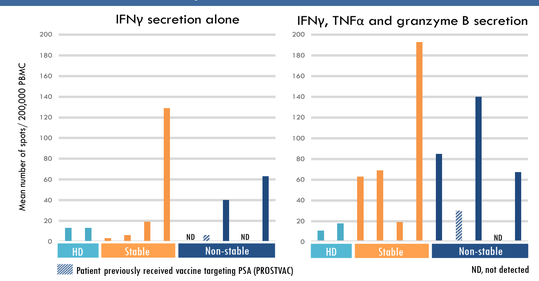


Figure 4. Changes in the magnitude of the PSA-specific T cell response on ADXS-PSA treatment

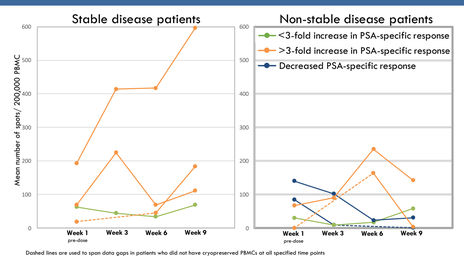


Figure 5. Changes in the functionality of PSA-specific T cells after 3 doses of ADXS-PSA

Figure 6. Magnitude of pre-existing PAP-, PSMA-, PSCA- and prostein-specific T cell responses in mCRPC patients and in age-matched healthy males donors

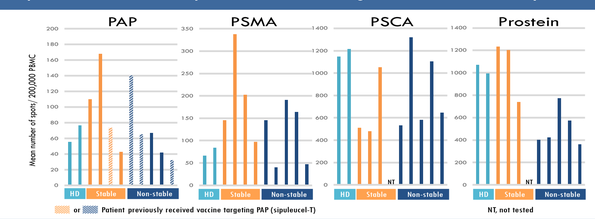


Figure 7. Changes in the magnitude of PAP-, PSMA-, PSCA- and prostein-specific T cell responses on ADXS-PSA treatment

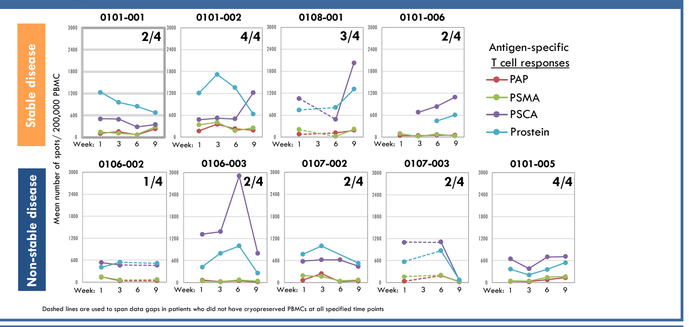
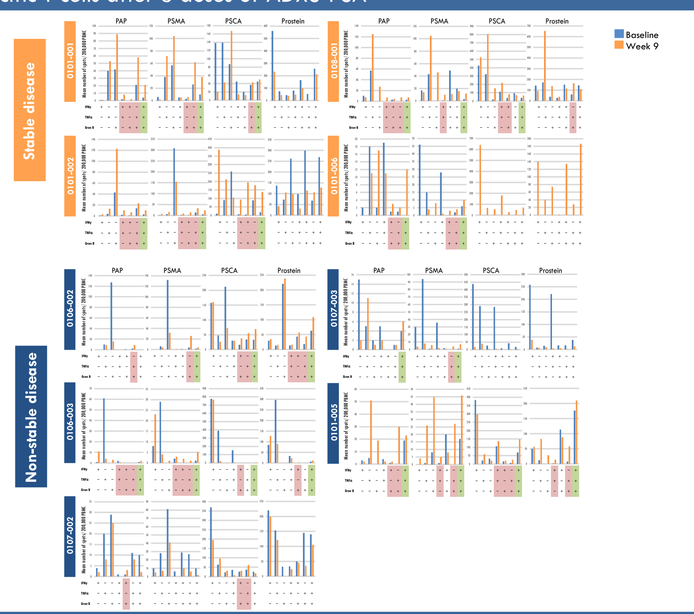


Figure 8. Changes in the functionality of PAP-, PSMA-, PSCA- and prostein-specific T cells after 3 doses of ADXS-PSA



SUMMARY AND CONCLUSIONS

- In Part A patients, ***ADXS-PSA induced multifunctional T cell responses to a broad range of prostate cancer antigens***:
  - ♦ 56% of patients (5/9) exhibited a >3-fold increase above baseline in the magnitude of the PSA-reactive T cell response
  - ♦ 100% of patients (9/9) exhibited increases above baseline in the frequency of T cells reactive to at least one other well known prostate cancer antigen, which is indicative of antigen spreading
  - ♦ 100% of patients (9/9) exhibited increases in T cell multifunctionality to at least 1 of the 5 prostate cancer antigens tested.
- No significant differences in the magnitude and quality of prostate cancer antigen-specific T cell responses were observed between stable and non-stable disease ADXS-PSA-treated mCRPC patients.
- Based on the findings in the ADXS-PSA monotherapy arm, further investigation is merited in the ongoing KEYTRUDA® combination arm (Part B).

ACKNOWLEDGMENTS

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REFERENCES

1. Haas NB, et al. Phase I-II study of ADXS31-142 alone and in combination with pembrolizumab in patients with previously treated metastatic castration-resistant prostate cancer (mCRPC): the KEYNOTE-046 trial. *J Immunother Cancer*. 2015;3(Suppl 2):P153.
2. Wood LM, Paterson Y. Attenuated *Listeria monocytogenes*: a powerful and versatile vector for the future of tumor immunotherapy. *Front Cell Infect Microbiol*. 2014; https://doi.org/10.3389/fcimb.2014.00051.
3. Janetzki S, et al. Guidelines for the automated evaluation of Elispot assays. *Nat Protocol*. 2015;10:1098-1115.
4. Seder RA, et al. T cell quality in memory and protection: implications for vaccine design. *Nat Rev Immunol*. 2008;8:247-258.
5. Perales MA, et al. Phase I/II study of GM-CSF DNA as an adjuvant for a multipeptide cancer vaccine in patients with advanced melanoma. *Mol Ther*. 2008;16:2022-2029.
6. Yuan J, et al. CTLA-4 blockade enhances polyfunctional NY-ESO-1 specific T cell responses in metastatic melanoma patients with clinical benefit. *Proc Natl Acad Sci USA*. 2008;105:20410-20415.
7. Carpenito C, et al. Control of large, established tumor xenografts with genetically retargeted human T cells containing CD28 and CD137 domains. *Proc Natl Acad Sci USA*. 2008;106:3360-3365.
8. Imai N, et al. Tumor progression inhibits the induction of multifunctionality in adoptively transferred tumor-specific CD8+ T cells. *Eur J Immunol*. 2009;39:241-253.
9. Imai N, et al. Glucocorticoid-induced tumor necrosis factor receptor stimulation enhances the multifunctionality of adoptively transferred tumor antigen-specific CD8 T cells with tumor regression. *Cancer Sci*. 2009;100:1317-1325.
10. Malgulne AM, et al. ELISPOT assay for monitoring cytotoxic T lymphocytes (CTL) activity in cancer vaccine clinical trials. *Cells*. 2012;1:111-126.