Immunogenicity and disease control induced by a multi-neoantigen vaccine (ADXS-503) in patients with metastatic non-small-cell lung cancer who have progressed on pembrolizumab

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Background

Non-small-cell lung cancer (NSCLC) resistant to checkpoint blockade: current challenges

- Patients with non-small cell lung cancer (NSCLC) who progress on PD-1/PD-L1 blockade represent an unmet need with limited treatment options.
- Re-challenge with anti-PD-1/PD-L1 antibodies or with double checkpoint blockade (CPB) ± radiotherapy is associated with a low objective response rate (ORR) (≤13.5%; partial responses [PRs] only) and disease control rate (DCR) of up to 45%.[1,2]

ADXS-503 immunotherapy

- ADXS-503 is an off-the-shelf, live attenuated *Listeria monocytogenes* (*Lm*) immunotherapy developed to restore sensitivity to PD-1 blockade and/or enhance responsiveness to pembrolizumab without adding toxicity in patients with NSCLC progressing on PD-1/PD-L1 blockade.
- ADXS-503 is bioengineered to secrete an antigen-adjuvant fusion protein (tLLO-503) consisting of a truncated fragment of listeriolysin O (tLLO) fused to 22 tumor antigens commonly found in NSCLC (Table 1).
- ADXS-503 induces innate and adaptive immunity, generating T-cells that target multiple tumor-associated antigens (TAAs), and inducing antigen spreading[3,4] (Figure 1).

Combination of ADXS-503 with PD-1/PD-L1 blockade

- Published preclinical and clinical data have shown the safety, tolerability, and synergistic activity of the combination of ADXS *Lm*-based immunotherapies with a PD-1 blocking antibody.[3,5]
- ADXS-503-101 is an ongoing open-label, multicenter, phase 1 trial (NCT03847519), designed to evaluate the safety, tolerability, and preliminary clinical and immunological activity of ADXS-503 alone and in combination with anti-PD-1 antibody therapy, in patients with NSCLC.
- **This poster describes the correlation between clinical outcomes and immune responses induced by the addition of ADXS-503 to NSCLC patients progressing on pembrolizumab therapy who are being evaluated in Part B dose-level 1 (DL1) of the study.**

Table 1. Antigens expressed by ADXS-503 to induce adaptive immunity

Hotspot peptides	
Gene	Hotspot
KRAS	G12C
KRAS	G12V
KRAS	G12A
EGFR	L858R
KRAS	G12D
U2AF1	S34F
BRAF	V600E
PIK3CA	E545K
TP53	R158L
EGFR	L861Q
TP53	R273L

Proprietary TAA peptides	
TAA	HLA allele
CEACAM5	A*02:01
CEACAM5	A*24:02
CEACAM5	A*03:01
CEACAM5	B*07:02
STEAP1	A*02:01
STEAP1	A*24:02
RNF43	B*07:02
MAGE-A6	A*03:01
NY-ESO1	A*02:01
MAGE-A4	B*07:02
GAGE1	B*07:02

TAA: tumor-associated antigens (oncofetal and cancer testis)

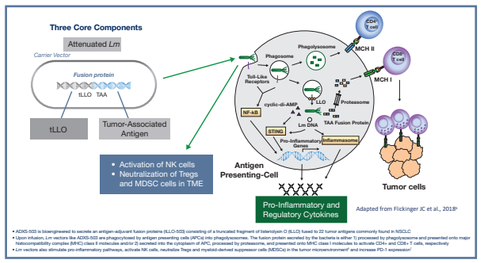
ADXS-503 harbors highly prevalent antigens in NSCLC

A. 11 antigens derived from somatic hotspot mutations (HSMs):
- HSM selection is based on prevalence.

B. 11 oncofetal (OFA) and cancer testis (CTA) antigens that are overexpressed/differentially expressed in NSCLC:
- Proprietary, sequence-optimized peptides of these TAAs (also referred to as heteroclitic peptides), were generated by modifying their anchor-residue positions in order to increase their binding affinity to MHC class I molecules.

ADXS-503 potentially elicits T-cell responses in all patients with NSCLC as 42% express ≥1 hotspot antigen and >90% express ≥1 TAAs targeted by ADXS-503.

Figure 1. Innate and adaptive immune responses triggered by recombinant *Lm*-based immunotherapies, like ADXS-503



- ADXS-503 is bioengineered to secrete an antigen-adjuvant fusion proteins (tLLO-503) consisting of a truncated fragment of listeriolysin O (tLLO) fused to 22 tumor antigens commonly found in NSCLC
- Upon infusion, *Lm* vectors like ADXS-503 are phagocytosed by antigen presenting cells (APCs) into phagolysosomes. The fusion protein secreted by the bacteria is either 1) processed by phagolysosome and presented onto major histocompatibility complex (MHC) class II molecules and/or 2) released into the cytoplasm of APC processed by proteasome and presented onto MHC class I molecules for activated CD8+ T cells, respectively
- *Lm* vectors also stimulate pro-inflammatory pathways, activate NK cells, neutralize Tregs and myeloid-derived suppressor cells (MDSCs) in the tumor microenvironment[6] and increase PD-1 expression[7]

Hypothesis and objectives

Hypothesis

- Adding on ADXS-503 to patients failing pembrolizumab as last therapy could stop clinical progression by restoring sensitivity to PD-1 blockade and/or by enhancing responsiveness to pembrolizumab without adding toxicity.

Study exploratory objectives

For Part B presented in this poster:

- To evaluate correlates of immune response and biomarkers in peripheral blood from patients with progressive disease (PD) or with clinical benefit (i.e., stable disease [SD] or PR).

Methods

Study design

- Part B DL1 is evaluating patients with metastatic NSCLC with PD on initial scan while on pembrolizumab alone as last therapy and who receive ADXS-503 as an add-on therapy.
- Treatment administration for Part B DL1:
  - ADXS-503 (DL1) 1x10[8] intravenously as an add-on to pembrolizumab every 3 weeks for up to 2 years;
  - Pembrolizumab 200 mg IV every 3 weeks for up to 2 years.
- The correlation between clinical outcomes and immune responses induced by ADXS-503 as an add-on therapy to NSCLC patients progressing on pembrolizumab therapy is reported in this poster.

MSD cytokine assay

- Serum cytokine levels are measured to evaluate general patterns and types of immune stimulation and signaling consistent with systemic immunity. Cytokines are evaluated pre- and 2 hours post-infusion on week (W) 1, W4, W7, and W10.

Two 17-color flow cytometry immunophenotyping assays

These assays evaluate NK and T-cell proliferation and activation of lymphocytes as reported elsewhere:[4]

- Two panels were tested in peripheral blood mononuclear cells (PBMCs) at baseline, W2, W5, W8, W25, and end of therapy (EOT);
- Panel 1 (16-color) for T/T-mem/T-prolif/T-regs and Panel 2 (15-color) for T/NK/NK T-cell;
- For the purpose of this analysis, only patients with SD lasting >20 weeks are considered SD.

In-vitro stimulation FluoroSpot testing

This assay was conducted to detect and quantitate T-cell responses against TAAs included in ADXS-503 and also against neoantigens not included in the construct (i.e., antigen spreading):

- Peptides used in four tested pools in this analysis included: Pool 1, hotspot mutations; Pool 2, sequence optimized/heteroclitic TAAs; Pool 3, wild-type TAAs; and Pool 4, antigen spreading (i.e., antigens not included in the ADXS-503 construct) (Table 2);
- PBMCs were tested at baseline, W2, W5, W8, W25, and EOT;
- TNFα, IFNγ, and Granzyme B secretion detection data were generated.

Next-generation sequencing of tumor tissue and cell-free DNA (cfDNA) to document neoantigens, tumor mutational burden, and microsatellite instability in each patient is in progress.

Table 2. Peptides used in tested pools in the FluoroSpot assay

Pool 1 - Hotspot mutations	Pool 2 – optimized TAAs	Pool 3 – wild-type TAAs	Pool 4 – antigen spreading
KRAS (G12C, G12V, G12A, G12D)	CEACAM5 (A*02:01, A*24:02, A*03:01, B*07:02)	CEACAM5 (A*02:01, A*24:02, A*03:01, B*07:02)	KRAS (G12R, G12S, G13D)
EGFR (L858R, L861Q)	STEAP1 (A*02:01, A*24:02)	STEAP1 (A*02:01, A*24:02)	TP53 (V157F, G245V, G245C)
U2AF1 S34F	RNF43 B*07:02	RNF43 B*07:02	PIK3CA E542K, H1047R)
BRAF V600E	MAGE-A6 A*03:01	MAGE-A6 A*03:01	EGFR A289V
PIK3CA E545K	NY-ESO1 A*02:01	NY-ESO1 A*02:01	
TP53 (R158L, R273L)	MAGE-A4 B*07:02	MAGE-A4 B*07:02	
	GAGE1 B*07:02	GAGE1 B*07:02	

Three pools containing neoantigens included in ADXS-503 (i.e., HSM, heteroclitic-optimized and wild-type TAAs), and one pool with other antigens not included in the construct (i.e., antigen spreading), were tested for CD8+ T-cell activation by FluoroSpot in 10 patients.

Results

Disposition and baseline characteristics: Part B DL1

- 14 patients progressing on pembrolizumab as last therapy have received ADXS-503 as an add-on therapy (Table 3).
- 14 patients were evaluable for safety and efficacy and up to 11 were eligible for immune correlative work:
  - The full clinical data set is presented simultaneously by Gerstner G et al. at this 2022 ASCO Annual Meeting (abst. 9038).[8]

Safety

- Only grade 1 and 2, transient and reversible "flu-like" adverse events have been reported in Part B.[8]
- No grade 3 events or additional immune-related toxicities have been observed for the combination of ADXS-503 with pembrolizumab.

Clinical activity

- The ORR is 14% and the DCR is 36% in 14 evaluable patients per RECIST v 1.1 criteria (Table 4):
  - Two durable PRs sustained for 694 and 318 days;
  - Three durable SDs sustained for 448, 175, and 117 days;
  - Two additional patients had SD for 64 and 51 days (not included in the DCR evaluation shown in Table 4).

Immune correlative data: MSD cytokine assay

- Transient increased secretion of cytokines after each infusion of ADXS-503 is indicative of immune stimulation in all 11 patients tested (Fig. 2A):
  - Pro-inflammatory and regulatory cytokines are secreted by antigen-presenting cells (APCs) in response to the phagocytosis of *Lm* bacteria[8]
  - The cytokine release induces a mild and transient "flu-like" syndrome within hours of infusion;[8]
  - Interestingly, IL-10 serum levels increase up to 100X in patients with durable clinical benefit but only up to 10X in those with PD (Fig. 2B). Long-lasting tumor immunity has been observed with IL-10 therapy in pre-clinical and clinical studies[9], so induction of IL-10 may also play an important role in the durability of clinical benefit in these patients;
  - Periodic stimulation with IFNγ, TNFα, and IL-6 and other cytokines is also relevant for the sustained proliferation and activation of NK cells and pre-existing cytotoxic and memory lymphocytes that induce the cell-mediated anti-tumoral response in patients with clinical benefit (see under flow cytometry results and Figs. 3A–I).

Table 3. Demographics and baseline characteristics (Part B DL1)

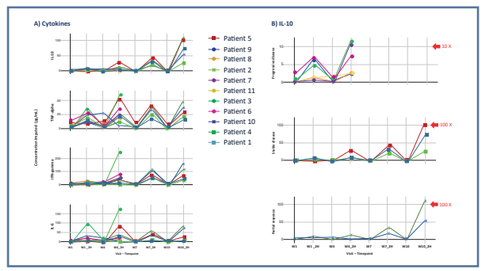
	Part B (n=14)
Median age, years (range)	67 (55–87)
Male, n (%)	7 (50)
ECOG PS, n (%)	
0	4 (29)
1	10 (71)
Missing	0
Race	
Caucasian	7 (50)
Non-Caucasian	3 (21)
Not reported	4 (29)
Smoking status, n (%)	
Current	2 (14)
Former	9 (64)
Never	3 (21)
Histology, n (%)	
Squamous	2 (14)
Non squamous	12 (86)
Brain metastases, n (%)	4 (29)
Median lines of prior systemic therapy (range)	2 (1–6)
Previous thoracic radiotherapy, n (%)	1 (7)
PD-L1 score, n (%)	
<1%	4 (29)
1–49%	3 (21)
≥50%	7 (50)
Not reported	0

[1]ECOG PS, ECOG performance status 1
CPI, checkpoint inhibitor

Table 4. Clinical activity (Part B DL1)[8]

Outcome, n (%)	Patients (n=14)
Best overall response	
Complete response	0
Partial response	2 (14)
Stable disease	3 (21)
Progressive disease	9 (58)
Objective response rate (RECIST v1.1)	2 (14)
Disease control rate*	5 (36)

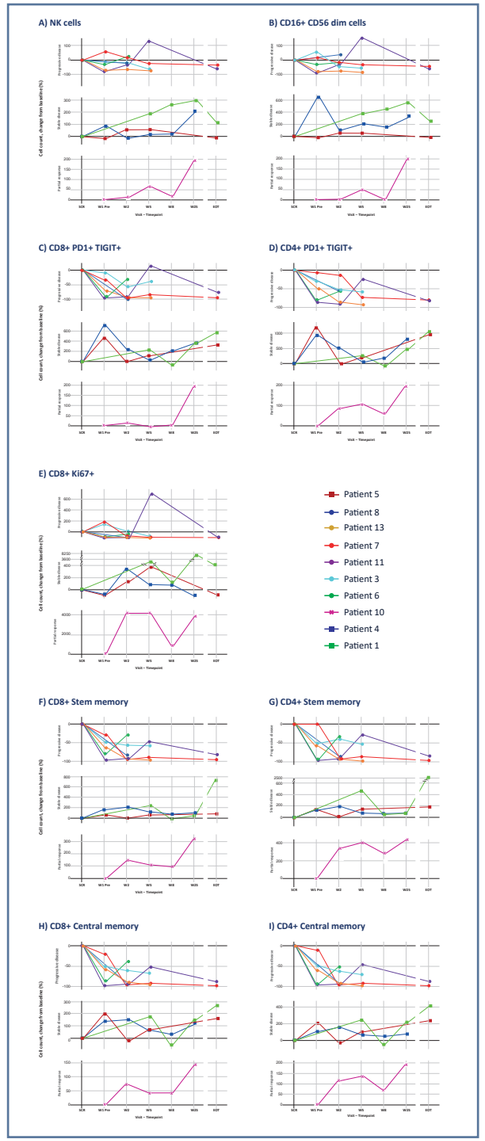
Figure 2. ADXS-503 in combination with pembrolizumab induces transient elevation of cytokines after infusion



Immune correlative data: FluoroSpot

- Efficient priming for neoantigens in ADXS-503 generated specific CD8+ T-cells against hotspot mutations, and/or sequence optimized heteroclitic OFAs/CTAs and/or wild-type OFAs/CTAs and/or other antigens not included in the ADXS-503 construct (i.e., antigen spreading; Table 5):
  - Patients with a durable PR (patients 2 and 10) or SD (patients 1, 4 and 5) showed reactivity of CD8+ T-cells against neoantigens in two or more pools of the assay within 2 months of starting therapy:
    - PBMCs from two patients harboring *KRAS G12V* mutations (patients 1 and 5) reacted against antigens in Pool 1 (i.e., hotspot mutations) and achieved prolonged SD;
    - Interestingly, all five patients with clinical benefit showed reactivity in the pool testing wild-type TAAs and three of the five patients showed antigen spreading, suggesting these may be important mechanisms by which ADXS-503 exerts anti-tumoral activity (Table 5);
  - Conversely, the presence of a hotspot mutation in the tumor (and represented in ADXS-503) did not necessarily correlate with clinical benefit: patient 3 (*EGFR L858R*) and patient 9 (*KRAS G12C*) both had PD;
  - Of the six patients with PD, three did not generate CD8+ T-cells against tested antigens; two patients reacted against one pool only and one patient reacted against two pools.

Figure 3. ADXS-503 in combination with pembrolizumab induces elevation of NK and T-cell counts in patients with clinical benefit as shown by flow cytometry



Immune correlative data: flow cytometry

- Patients with clinical benefit (i.e., those with SD or PR) showed proliferation and activation of NK and T-cells after the addition of ADXS-503 (Figs. 3A–I):
  - Increased NK cell counts in patients with clinical benefit suggest that PD may be associated with NK anergy/exhaustion that could have been reversed by adding ADXS-503 (Fig. 3A):
    - Of particular interest is the CD16+ CD56dim NK cell subset, whose counts were elevated in patients with clinical benefit (Fig. 3B). These cells are known to possess enhanced lytic ability against tumor cells in melanoma patients undergoing PD-1 blockade.[11]
  - Up-regulation of PD-1 and TIGIT in CD8+ and CD4+ T-cells from patients with clinical benefit suggests cell activation (Figs. 3C and 3D):
    - Interestingly, the frequency of circulating PD-1+TIGIT+ CD8+ T-cells has also been associated with clinical responses and OS in NSCLC patients undergoing PD-1 blockade.[12]
  - Proliferation and emergence of cytotoxic and memory T-cells in patients with clinical benefit suggest that ADXS-503 boosts CD8+ and CD4+ T-cell populations:
    - Upregulation of Ki67 shows proliferation of existing CD8+ T-cells that may have been exhausted (Fig. 3E);
    - Memory cell population counts, particularly those of stem and central memory cells, were also increased in patients with clinical benefit (Figs. 3F–I). These two memory cell populations may play a relevant role in the persistent antitumor immunity in these patients;[13]
    - Naïve CD8+ and CD4+ T-cell counts did not change throughout the course of therapy regardless of the clinical outcome (data not shown), which suggest that clinical benefit in these patients may be derived from reversing exhaustion of existing T-cell clones.
  - Elevations of NK, CD8+, and CD4+ T-cell populations were also observed between weeks 2 and 25 in patient 2 who achieved a PR and who is still on therapy beyond 694 days (data not shown; baseline samples were not available).

Table 5. Timing of T-cell responses to antigens in ADXS-503 and antigen spreading in patients receiving ADXS-503 + pembrolizumab (Part B DL1)

PID#	Pool 1 Hotspot mutations	Pool 2 Heteroclitic TAAs	Pool 3 Wild-type TAAs	Pool 4 Antigen spreading	Genetic Aberrations in Tumor Samples	BOR / DOR (days)
Patient 1	W5	n.d.	W8	W5	*KRAS G12V*	SD / 448
Patient 2	W8	W25	W25	n.d.	–	PR / 694
Patient 3	n.d.	n.d.	n.d.	n.d.	*EGFR L858R*	PD / –
Patient 4	W2	n.d.	W5	W25	–	SD / 117
Patient 5	W2	W2	W2	n.d.	*KRAS G12V*	SD / 175
Patient 6	n.d.	n.d.	W2	n.d.	–	PD / –
Patient 7	W2	n.d.	W2	n.d.	–	PD / –
Patient 8	n.d.	n.d.	n.d.	n.d.	–	PD / –
Patient 9	n.d.	n.d.	n.d.	n.d.	*KRAS G12C*	PD / –
Patient 10	n.d.	n.d.	W5	W5	–	PR / 319
Patient 11	n.d.	n.d.	n.d.	EOT	–	PD / –

IFNγ, TNFα, or Granzyme B secretion detection data are shown. Representative plots have been presented previously.[10]
The earliest timepoint after the start of ADXS-503 therapy at which CD8+ T-cells were generated against neoantigens is shown (represented in weeks).
BOR, best overall response; DOR, duration of response; n.d., not detected

Conclusions

- Adding on ADXS-503 to patients progressing on pembrolizumab as last therapy may prevent further clinical progression in select patients:
  - ORR 14% and DCR 36% in 14 evaluable patients with durable PRs (n=2) and SDs (n=3);
  - SD of 2 months' duration observed in two other patients.
- As reported by Gerstner GJ, et al.[8], patients who achieve clinical benefit upon addition of ADXS-503 to pembrolizumab include those with PD-L1 expression ≥50% and secondary resistance to pembrolizumab.
- From this immune correlative analysis, it is suggested that ADXS-503 has pleiotropic effects that lead to durable clinical benefit in select patients progressing on pembrolizumab through:
  - Periodic pro-inflammatory and anti-tumoral effect of cytokines that support innate and adaptive immunity;
  - Central role of NK cells in tumor control, which would differentiate ADXS-503 from other vaccine platforms;
  - Induction of proliferation and activation of pre-existing CD8+ T-cells that may have been exhausted and are now able to react to hotspot mutation antigens, other TAAs, and antigen spreading.
- Enrollment in Part B DL1 will continue up to a total of 18 patients to further evaluate whether ADXS-503 can achieve an ORR of ≥20% in patients progressing on pembrolizumab therapy.

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