Modulation of immune gene expression by intra-tumoral oncolytic adenovirus ONCOS-102 is associated with clinical response in anti-PD-1 refractory/resistant melanoma

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INTRODUCTION

Defining molecular parameters of clinical response to immuno-modulatory compounds may lead to development of novel biomarkers, identification of new therapeutic targets and development of more efficacious drug combinations in cancer treatment. We recently completed a pilot study of ONCOS-102, a granulocyte-macrophage colony stimulating factor (GM-CSF)-expressing oncolytic adenovirus (Ad5/3-D24-GMCSF), for therapeutic efficacy and capacity to remodel tumor micro-environment in combination with pembrolizumab in patients (pts) with non-resectable, stage III-IV, anti-PD-1 resistant/refractory (R/R) melanoma (NCT03003676).

OBJECTIVE

The primary objective of the study was to assess the safety of ONCOS-102 in combination with pembrolizumab either given sequentially (Part 1) or combined (Part 2) for patients with aPD-1 resistant melanoma. Secondary objectives included overall response rate by RECIST 1.1 and the immune activation in tumor mass and peripheral blood, as well as correlation of immune markers and transcriptional landscape with clinical outcome.

METHOD

This was a phase 1 prospective, open-label, multi-center pilot safety study. Patients with advanced or unresectable melanoma who had experienced progression of disease despite prior PD-1 blockade were eligible as long as they had at least 1 lesion measurable by RECIST 1.1. The study consisted of two parts **(Fig. 1)**: In study part 1, n=9 pts (of which only 8 were eligible for efficacy) received three intra-tumoral (i.t.) injections of ONCOS-102 every 3 days during the first week of treatment. Pembrolizumab was administered i.v. at 10 mg/kg from day 22 every 3 weeks for 6 months. In study part 2, n=12 pts received additional injections of ONCOS-102 at day 15 and then every 3 weeks along with pembrolizumab. On-treatment tumor biopsies were collected at day 22 and 64 for comparison to the paired baseline samples. Total RNA sequencing followed by differential gene expression analysis using DESeq2 was conducted on n=17 pts to disclose dynamic transcriptome changes associated with ONCOS-102 administration and clinical response.

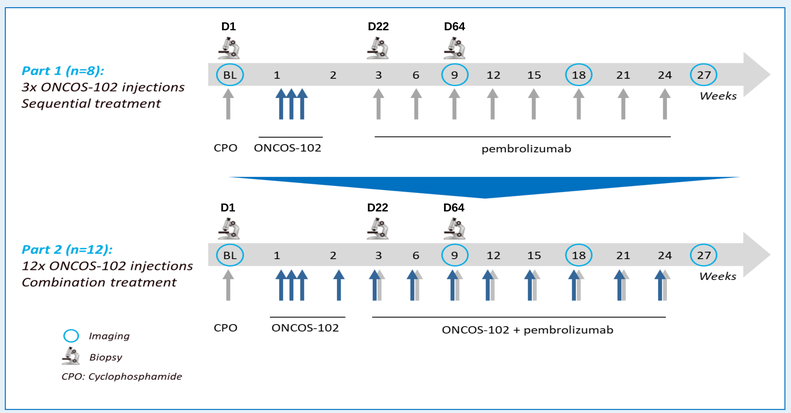

Figure 1: Treatment schedule. In Part 1, patients received 3 intratumoral doses of ONCOS-102 followed by up to 8 sequential doses of pembrolizumab Q3W. In Part 2, patients received 4 intratumoral doses of ONCOS-102 followed by up to 8 doses intratumoral ONCOS-102 in combination with pembrolizumab Q3W. CPO priming was a once only i.v. bolus of 300 mg/m^2 1-3 days prior to initiating ONCOS-102 treatment. Tumor biopsies were performed at baseline (D1), day 22 (D22, week 3; following completion of ONCOS-102 in Part 1; ONCOS-102 ongoing in Part 2), and day 64 (D64, week 9; following 2 doses of pembrolizumab [all patients]; ONCOS-102 ongoing in Part 2 only).

RESULTS – Clinical efficacy

Twenty patients were evaluable for efficacy. Across the study, 7 of 20 patients (35%) achieved a best objective response (BOR) during the treatment period using both RECIST 1.1 and irRECIST, including 1 patient with a complete response in recurrent in-transit lesion. In Part 1, 3 patients had CR or PR during the study according to RECIST 1.1 and irRECIST resulting in a BOR of 38%. In Part 2, 4 of 12 patients had CR or PR during the study according to RECIST 1.1 and irRECIST resulting in a BOR of 33% **(Fig 2A-B)**. Reduction in size of non-injected lesions was seen in 12/36 (33%) of target non-injected lesions with examples of complete disappearance of lesions in two patients (data not shown).

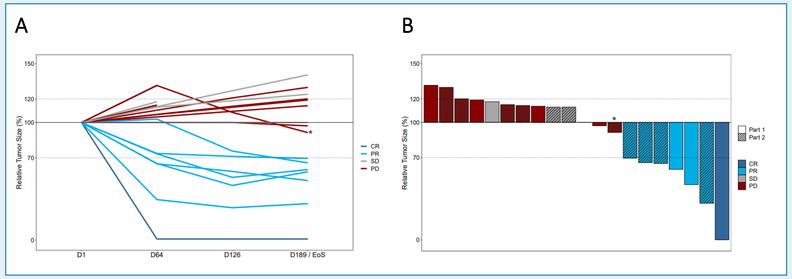

Figure 2: Relative change (percent) in tumor size. A) Spider plot of showing percent change in tumor size from Day 1 until end of study (Day 189). **B)** Waterfall plot for all evaluable patients (n=20) by best response (RECIST 1.1). Asterisk denote patient with pseudo-progression followed by regression. CR, complete response; D, day; EoS, end of study; PR, partial response; PD, disease progression; SD, stable disease.

RESULTS – Immune cell infiltration

Analysis of immune cell subsets in biopsies (multiplex immunohistochemistry (mIHC)) taken at baseline and on day 22 and/or day 64 of the same tumours indicated immuno-stimulatory effects of ONCOS-102 in combination with anti-PD1. CD4+ **(Fig. 3A),** CD8+ **(Fig. 3B)**, and CD8+GrzB+ **(Fig. 3C)** T-cells infiltration appeared to be more prominent in tumour samples from patients with disease control (CR, PR and SD) compared to patients with PD.

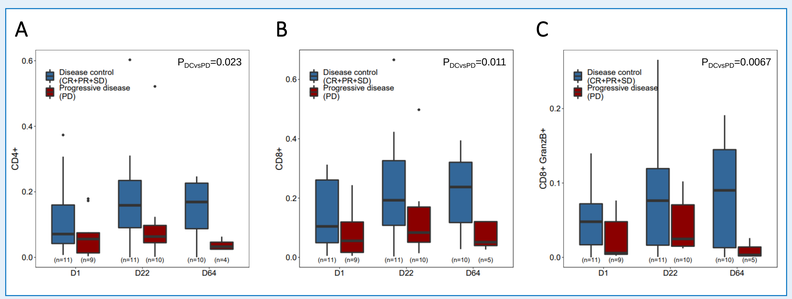

Figure 3: Change in tumor immune cell profile over time (immunohistochemistry). A-C) Boxplot of CD4+ (A), CD8+ (B) and CD8+GranzB+ positive cells (C) grouped by timepoint and clinical response (disease control [PR, CR and SD]) and disease progression [PD]). P-values are calculated using a two-way ANOVA.

RESULTS – Transcriptome profile

Modelling change in gene expression between disease control patients (CR+PR+SD) and progressors (PD) across time shows similar tendency at D22 with notable increase in expression of immune-related groups of genes in both patient categories (**Fig. 4A**). In fact, progressors exhibit higher upregulation of certain pathways compared to disease control patients at D22, suggesting a promising early response in most patients. Comparing D64 with baseline (D1) expression, disease control patients maintain high expression of immune-related pathways, whereas progressors show declining levels (**Fig. 4B)**, consistent with the IHC analysis. The expression-signature of individual genes within cytotoxicity shows persistent upregulation in disease control patients particularly at day 64 compared to progressors (**Fig. 4C**), and, consistently, quantification of viral particles from tumor biopsies indicates continuous viral replication in the disease control group (**Fig. 4D**)

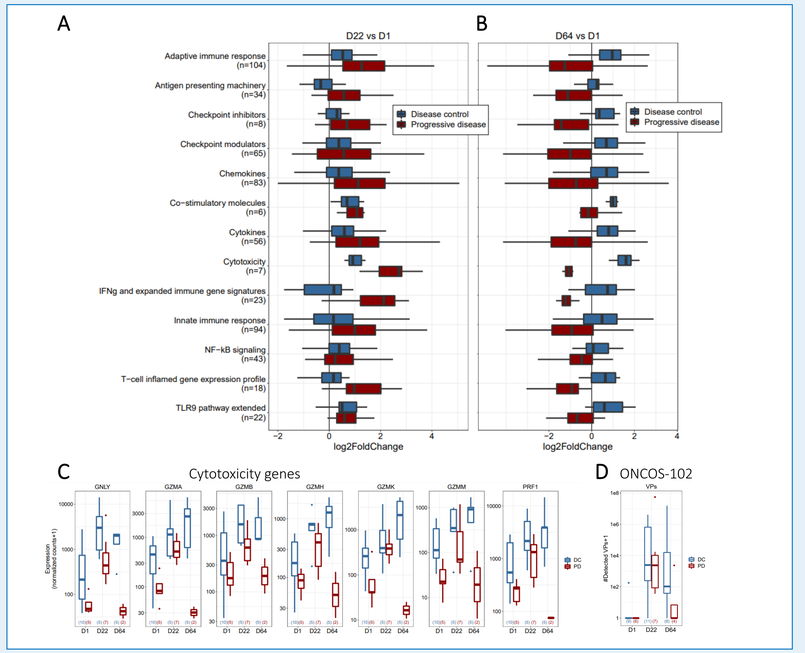

Figure 4: Gene expression profiles between disease control and progressors. A-B) Boxplots depicting overall change in gene expression (log 2-fold change) in select immunological pathways (specified in Supplementary Table 2) between day 22 and day 1 (A), or between day 64 and day 1 (B) grouped by disease control patients (CR+PR+SD, blue) and progressive disease (PD, red). **C)** Boxplot of normalized (using DESeq2) expression for genes classified as cytotoxicity genes color-coded by clinical outcome as in (A). The numbers in parenthesis below represent number of samples for each group/day. **D)** Boxplot of quantified viral particles (VPs) by qPCR color-coded as in (A).

CONCLUSION

Clinical response of ONCOS-102 in PD-1 R/R melanoma tumors is associated with pro-inflammatory modulation of the TME, efficient viral gene expression and strong, sustained activation of immune-related genes. These genes may be targets for future combinations of ONCOS-102 and immune-modulators beyond PD-1/PD-L1 inhibitors.

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