Genetic and Molecular Profiling of ICOS hi CD4 T Cells Demonstrates Clonal Expansion of Th1 Effector Cells Following Vopratelimab (JTX-2011) Treatment in Subjects With Solid Tumors

ABSTRACT# 4053

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ABSTRACT

Background: Inducible T cell Co-stimulator (ICOS) is a costimulatory molecule expressed primarily on T lymphocytes that is upregulated upon cell activation. Vopratelimab (JTX-2011) is a first-in-class ICOS agonist antibody whose primary mechanism of action is the stimulation of primed CD4 T effector cells. Clinical and biological activity of vopratelimab was assessed in the advanced solid tumor setting in the Phase I/II ICONIC trial (NCT02904226). Clinical responses to vopratelimab were associated with the emergence of an ICOS hi CD4 T cell population, which was subsequently demonstrated to be due to activity of vopratelimab and not a PD-1/L-1 inhibitor.

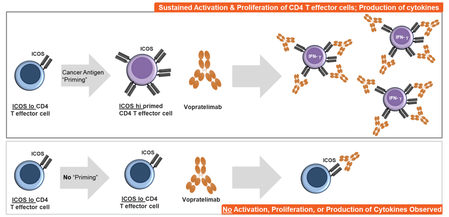
Methods: Assessment of phenotype and function of ICOS hi CD4 T cells was conducted using serial collections of peripheral blood mononuclear cells (PBMCs), whole blood, and tumor samples from a subset of evaluable subjects treated with vopratelimab at 0.1mg/kg and 0.3mg/kg monotherapy and in combination with nivolumab. Biological activity was assessed through ex vivo functional assays, flow cytometry-based profiling, and genomic analysis including assessment of changes in T cell clonality.

Results: Flow cytometry based phenotyping revealed ICOS hi CD4 T cells as T effector cells of primarily the Th1 lineage. Analysis of changes in clonal abundance in the peripheral blood T cell receptor repertoire (TCR) identified significant on-treatment expansion of clones in 18/22 (~82%) of subjects following vopratelimab treatment, including those from monotherapy. Expanded clones detected in the periphery were tumor-associated clones present in archival tumor samples, suggesting that vopratelimab may function to enhance cell-mediated anti-tumor immunity. In independent experiments using tetanus based priming of donor PBMCs, ex vivo stimulation by soluble vopratelimab was active only if ICOS hi CD4 T cells were already present, with vopratelimab inducing potent polyfunctional cytokine responses characterized by a 4-fold average increase in both IFNγ and TNFα.

Conclusion: Emergence of a distinct ICOS hi population of peripheral CD4 T cells correlates with response to vopratelimab treatment. Independent characterization of ICOS hi CD4 T cells induced by recall antigens demonstrate potent effector function induced by vopratelimab, but only if the cells were already ICOS hi, which is hypothesized to contribute to biological activity. The emergence of this population is being used to guide clinical development by informing sequencing of combination approaches that maximize the potential for a combination agent to induce ICOS hi CD4 T cells, followed by subsequent agonism with vopratelimab.

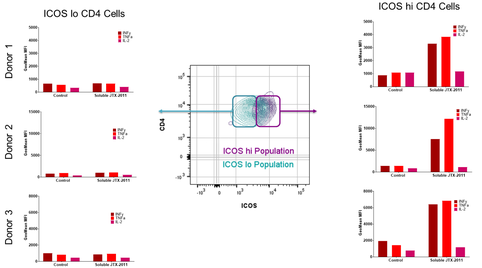
MECHANISM OF ACTION

Vopratelimab is an ICOS agonist of IgG1 isotype



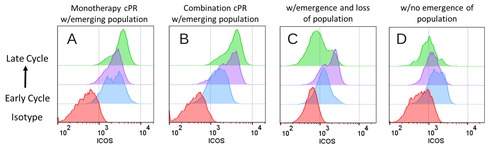
ICOS expression is low on resting CD4 T effector cells and increases with antigen stimulation. Soluble vopratelimab is capable of inducing activation, proliferation, and cytokine production from primed but not resting CD4 T effector cells.

Figure 1: Agonism by Soluble Vopratelimab Induces a Polyfunctional Cytokine Response in Antigen-Specific ICOS hi, but not ICOS lo CD4 T Cells



PBMCs from healthy donors were stimulated to induce an ICOS hi CD4 population using tetanus toxoid as a model recall antigen (representative plot in inset). Following 24hr of stimulation with antigen, cells were washed to remove stimulus and rest the CD4 T cells. Following washing, soluble vopratelimab (JTX-2011) was added, and intracellular cytokine production was assessed by flow cytometry following 6hr incubation in the presence of brefeldin A.

Figure 2: Emergence and Persistence of ICOS hi CD4 T Cells Correlates with Clinical Response in Subjects Treated with Vopratelimab



Emergence of ICOS hi CD4 T cells was assessed longitudinally in PBMCs from subjects treated with vopratelimab using flow cytometry. A) Emergence of ICOS hi CD4 T cells is detected in a subject with confirmed PR treated with 0.3mg/kg vopratelimab monotherapy. B) Emergence of ICOS hi CD4 T cells is detected in a subject with confirmed PR treated with 0.1mg/kg vopratelimab in combination with nivolumab. C) ICOS hi CD4 T cells emerged and were subsequently lost in a subject with stable disease treated with 0.3mg/kg vopratelimab in combination with nivolumab. D) No ICOS hi CD4 T cells were detected in a subject with primary target progression treated with vopratelimab at 0.3mg/kg in combination with nivolumab. All response data are based on investigator assessment.

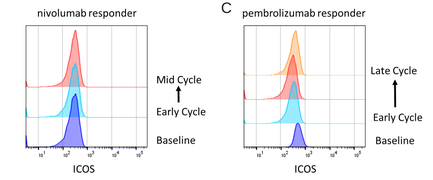
See Poster #CT189: Improved Progression-Free and Overall Survival (PFS/OS) in Patients (pts) with Emergence of JTX-2011 (vopratelimab) Associated Biomarker (ICOS high CD4 T cells) on the ICONIC Trial

Figure 3: Emergence of ICOS hi CD4 T Cells are Due to the Activity of Vopratelimab and Not PD-1 Inhibition

A

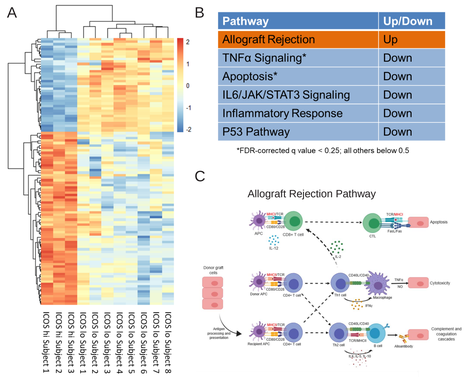
ICOS hi CD4 Analysis from anti-PD-1/L1 Treated Subjects

Indication Type	# of Subjects Profiled	# of Known Responders to PD-1i Treatment	% of Subjects with ICOS hi Cells Observed
Melanoma	12	1	0
Lung	64	5	0
Other	1	0	0



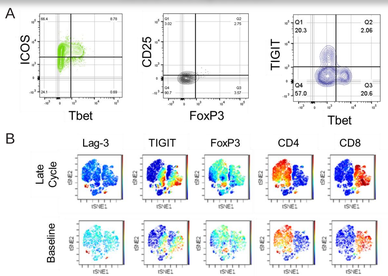
Samples from subjects receiving standard of care PD-1 inhibitor treatment were obtained from a commercial biorepository. In total, PBMCs from 77 subjects were assessed and compromised primarily lung cancer and melanoma. A summary of samples tested in shown in table (A). B) Longitudinal flow profile of a NSCLC subject who responded to nivolumab shows no induction of ICOS hi CD4 T cells. C) Longitudinal flow profile of a NSCLC subject who responded to pembrolizumab shows no induction of ICOS hi CD4 T cells. Histograms are arranged in chronological order, starting with baseline profiles for each responder.

Figure 4: Transcriptional Profiling of Isolated ICOS hi vs ICOS lo CD4 T Cells Demonstrates Enrichment of Effector Pathways



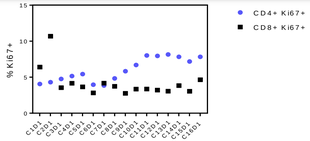
Transcriptional analysis of purified CD4 T cells from subjects with treatment-emergent ICOS hi cells vs CD4 T cells from reference cancer patients not displaying the cell population were performed using Nanostring human immunology panel. A) Patient and donor CD4+ T cell samples form distinct clusters when applying unsupervised clustering using Pearson's correlation coefficient. Depicted in this gene expression heatmap are genes that are significantly differentially expressed genes (FDR adjusted p value < 0.05) across these two clusters and define key components of the transcriptional differences between the CD4 T cell populations. B) Gene set enrichment analysis demonstrated trends towards modulation of several pathways in ICOS hi cells relative to ICOS lo CD4 T cells. Pathways with overall FDR corrected q-values below 0.5 are shown. C) Allograft rejection pathway and representative genes are shown.

Figure 5: Increase in Activation but not Exhaustion in Tbet+ non-Treg CD4 and CD8 T Cells in Subjects with Emergent ICOS hi Population



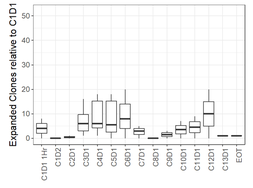
Immunophenotype of peripheral blood T cells was assessed by flow cytometry at various time points pre- and post-treatment with vopratelimab alone or in combination with nivolumab. A) Expression of lineage and activation markers were assessed in a subject with a late-cycle uniform ICOS hi population (subject shown in second panel of Figure 2). B) Baseline and on-treatment analysis of a gastric cancer subject with an investigator-assessed cPR using tSNE clustering algorithm demonstrated global reduction in LAG-3 expression and increase in TIGIT expression on non-Treg cells (FoxP3-) following vopratelimab treatment. Red indicates high expression and blue indicates low expression of indicated markers.

Figure 6: Biphasic Proliferation of CD8 and CD4 T cells is Observed in Vopratelimab Treated Subjects with Emergent ICOS hi CD4 T Cells



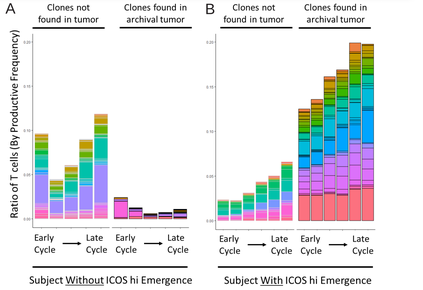
Immunophenotype of peripheral blood T cells was assessed by flow cytometry at various time points pre- and post-treatment with vopratelimab alone or in combination with nivolumab. Longitudinal analysis of average Ki-67 staining in subjects with confirmed investigator-assessed PRs to vopratelimab treatment demonstrate early and late proliferation of total CD8 and total CD4 T cells, respectively. Means of 4 subjects profiled longitudinally are shown.

Figure 7: Polyclonal Expansion of TCR Repertoire is Observed Following Vopratelimab Treatment



T cell receptor repertoire clonality was assessed on peripheral T cells using the Adaptive Biotechnologies immunoSEQ® assay. Longitudinal profiling of vopratelimab induced changes in peripheral clonality demonstrate bi-phasic expansion of the circulating repertoire. Average of 22 subjects with available longitudinal samples is shown.

Figure 8: Tumor Associated Clones Expand Only in Subjects With Emergent ICOS hi CD4 T cells; Expansion of Bystander Clones is Observed Regardless of ICOS hi Emergence



T cell receptor repertoire clonality was assessed on peripheral blood and archival tumor tissue using the Adaptive Biotechnologies immunoSEQ® assay. A) Longitudinal profiling of bystander and tumor-associated clones demonstrates indiscriminate polyclonal expansion of TCR clones in a representative subject without ICOS hi cell emergence. B) In a subject with ICOS hi emergence, clonal expansion was also observed, with greater expansion of tumor-associated clones relative to bystander. Clonal expansion in peripheral blood has not been associated with anti-PD-1 responses[1].

SUMMARY

Strong pharmacodynamic evidence of vopratelimab activity has been observed:

- The presence of ICOS hi CD4 T cells tracked with clinical response and is due to activity of vopratelimab but not anti-PD-1/L1 inhibition

ICOS hi CD4 T cells are phenotypically distinct from ICOS lo cells as demonstrated by both flow cytometry and NGS profiling.

- ICOS hi CD4 T cells are enriched in effector pathways, including those associated with allograft rejection
- ICOS hi CD4 T cells are not enriched in Tregs

TCR clonality assessment on-treatment suggests clonal expansion, with greater expansion of tumor associated clones in subjects displaying ICOS hi CD4 T cell phenotype

- Vopratelimab treatment results in expansion of de novo T cell clones regardless of ICOS hi CD4 T cell emergence

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