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Toripalimab, a Next-Generation Designed Anti-PD-1 Antibody for Treatment of Nasopharyngeal Carcinoma

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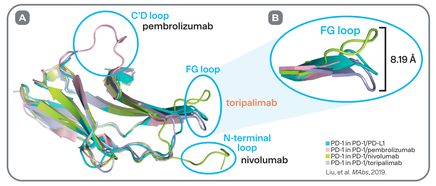
Coherus

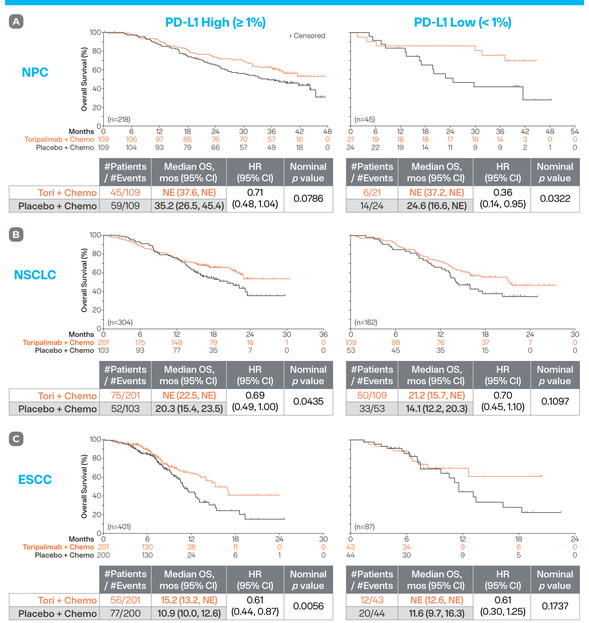
Background

- Toripalimab (tori) is a PD-1 targeting humanized IgG4 monoclonal antibody that blocks the interaction of PD-1 to its ligands PD-L1 and PD-L2.
- Toripalimab is approved by the FDA for metastatic or recurrent nasopharyngeal carcinoma (NPC) as first-line treatment in combination with chemotherapy or as second- or third-line monotherapy treatment.
- Toripalimab binds to the FG loop of PD-1, which differentiates it from other anti-PD-1 mAbs.



(A) Comparative structural conformations[1] of PD-1 when bound to either native PD-L1 (blue) or various PD-1 targeting monoclonal antibodies (pembro = pink; nivo = green; tori = lilac) with **(B)** magnification of the PD-1 FG loop.

Toripalimab in Combination With Chemotherapy Shows Clinical Efficacy Irrespective of PD-L1 Status



	#Patients / #Events	Median OS, mos (95% CI)	HR (95% CI)	Nominal *p* value
Tori + Chemo	45/109	NE (37.6, NE)	0.71 (0.48, 1.04)	0.0786
Placebo + Chemo	59/109	35.2 (26.5, 45.4)		

	#Patients / #Events	Median OS, mos (95% CI)	HR (95% CI)	Nominal *p* value
Tori + Chemo	6/21	NE (37.2, NE)	0.36 (0.14, 0.95)	0.0322
Placebo + Chemo	14/24	24.6 (16.6, NE)		

	#Patients / #Events	Median OS, mos (95% CI)	HR (95% CI)	Nominal *p* value
Tori + Chemo	75/201	NE (22.5, NE)	0.69 (0.49, 1.00)	0.0435
Placebo + Chemo	52/103	20.3 (15.4, 23.5)		

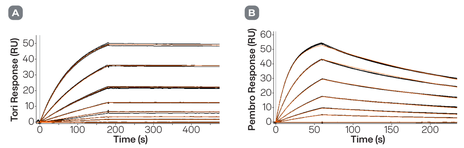
	#Patients / #Events	Median OS, mos (95% CI)	HR (95% CI)	Nominal *p* value
Tori + Chemo	50/109	21.2 (15.7, NE)	0.70 (0.45, 1.10)	0.1097
Placebo + Chemo	33/53	14.1 (12.2, 20.3)		

	#Patients / #Events	Median OS, mos (95% CI)	HR (95% CI)	Nominal *p* value
Tori + Chemo	56/201	15.2 (13.2, NE)	0.61 (0.44, 0.87)	0.0056
Placebo + Chemo	77/200	10.9 (10.0, 12.6)		

	#Patients / #Events	Median OS, mos (95% CI)	HR (95% CI)	Nominal *p* value
Tori + Chemo	12/43	NE (12.6, NE)	0.61 (0.30, 1.25)	0.1737
Placebo + Chemo	20/44	11.6 (9.7, 16.3)		

Kaplan-Meier estimates of OS are shown to compare the tori + chemotherapy arm with the placebo + chemotherapy arm in **(A)** NPC for PD-L1 TPS ≥ 1% and the PD-L1 TPS < 1% subgroups, **(B)** NSCLC for PD-L1 TPS ≥ 1% and the PD-L1 TPS < 1% subgroups, **(C)** ESCC for PD-L1 CPS ≥ 1% and the PD-L1 CPS < 1% subgroups. Numbers of patients at risk at indicated time points are shown below the x axis.

Toripalimab Exhibits ~12-fold Higher Binding Affinity for PD-1 Compared to Pembrolizumab



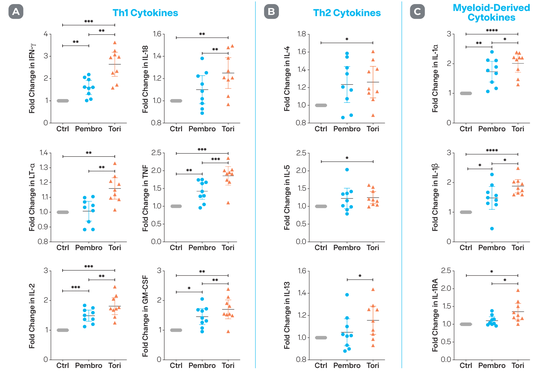
C

Interaction	k_a [$M^{-1}s^{-1}$]	k_d [s^{-1}]	K_D [nM]
tori/PD-1	$1.99(0.54)\times10^6$	$2.03(0.34)\times10^{-3}$	0.103(0.029)
pembro/PD-1	$2.72(0.09)\times10^6$	$3.40(0.05)\times10^{-3}$	1.25(0.02)

Numbers in parentheses are 95% confidence intervals for N=3.

(A) Biacore sensorgrams of PD-1 binding to covalently immobilized tori. PD-1 was injected in triplicate for 3 min. in a range from 0.93-59.5 nM with dissociation followed for 5 min. **(B)** Sensorgrams of PD-1 binding to covalently immobilized pembro. PD-1 was injected in triplicate for 1 min. in a range from 0.63-20.3 nM with dissociation followed for 3 min. All sensorgrams were globally fit (red lines) to a 1:1 interaction model including a term for mass transport. **(C)** Average dissociation and kinetic rate constants (k_a, k_d, and K_D) from 3 replicate experiments for PD-1 binding to tori and pembro.

Toripalimab Exhibits Enhanced Th1-Mediated Response in Staphylococcal Enterotoxin B (SEB)-Mediated T-Cell Activation of Human PBMC

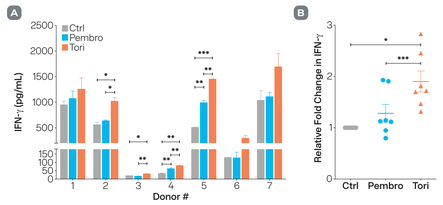


PBMC from nine human healthy donors were cultured with 100 ng/mL SEB in the presence of 3.3 μg/mL anti-PD-1 mAb: pembro, tori, or isotype antibody control (Ctrl) in triplicate. After 3 days, cell supernatants were collected to examine levels of IFN-γ and IL-2 by ELISA and several other Th1 **(A)**, Th2 **(B)**, and myeloid-derived **(C)** cytokines by Luminex. Graphs indicate fold change (mean ± SEM, n = 9) in cytokine secretion in the presence of pembro or tori relative to Ctrl. Statistical analysis was performed using one-way ANOVA with Tukey's multiple comparisons tests.

Abbreviations: cemi = cemipilimab; CI = confidence interval; CPS = combined positive score; ctrl = control; DTC = dissociated tumor cells; EA = enzyme acceptor; EC_{50} = half-maximal effective concentration; ED = enzyme donor; ESCC = esophageal squamous cell carcinoma; FDA = US Food and Drug Administration; GM-CSF = Granulocyte-macrophage colony-stimulating factor HR = hazard ratio; IFN-γ = interferon gamma; IL = interleukin; K_a = association rate constant; k_d = dissociation rate constant; K_D = dissociation equilibrium constant; LT-α = lymphotoxin alpha; mAb = monoclonal antibody; NE = not estimated; NES = normalized enrichment score; nivo = nivolumab; NPC = nasopharyngeal carcinoma; NSCLC = non-small cell lung cancer; OS = overall survival; PBMC = peripheral blood mononuclear cell; PD-1/2 = programmed cell death protein 1/2; PD-L1/L2 = programmed cell death protein ligand 1/2; pembro = pembrolizumab; RLU = relative luminescent unit; RNA = ribonucleic acid; RU = relative units; SEB = staphylococcal enterotoxin B; SHP1/2 = Src homology region 2 domain-containing phosphatase-1/2; TCR = T-cell receptor; TNF = tumor necrosis factor; tori = toripalimab; TPS = tumor proportion score.

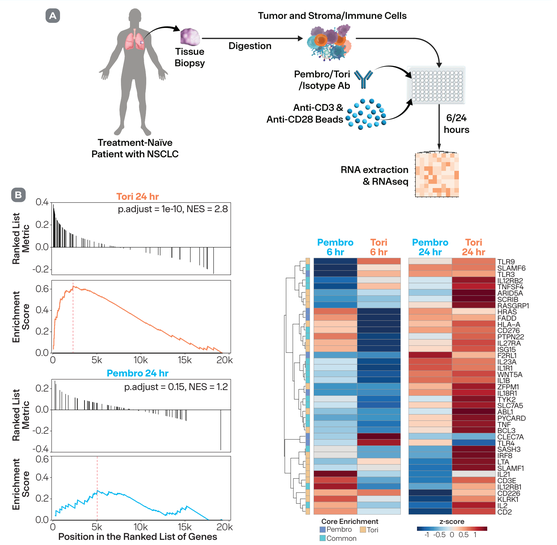
Statistics: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$; when not shown, comparisons are not statistically significant.

Toripalimab Exhibits Enhanced IFN-γ Secretion in CD3/CD28-Mediated T Cell Activation of Human CD8⁺ T Cells



Naïve CD8⁺ T cells from 7 human healthy donors were activated with human anti-CD3 (0.5 μg/mL) and human anti-CD28 (0.5 μg/mL) immobilized on the plate surface. 10 μg/mL of isotype control antibody (Ctrl), pembro, or tori was added in duplicate wells. IFN-γ levels in cell culture supernatant was quantified on Day 3 of activation using ELISA. **(A)** IFN-γ levels. **(B)** Fold change in concentration of IFN-γ relative to the Ctrl. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test **(A)** and paired t-test **(B)**.

Toripalimab Positively Modulates Genes Associated with IFN-γ Gene Signature in NSCLC Dissociated Tumor Cells With Different Kinetics and Higher Intensity Compared to Pembrolizumab



(A) Schematic diagram of expression profiling of DTC treated with tori, pembro, or isotype control antibody. **(B)** Leading edge plots comparing "Positive regulation of IFN-γ production" by tori (top) and pembro (bottom); and heatmap of the core-enrichment genes of the pathway after tori and pembro treatment.

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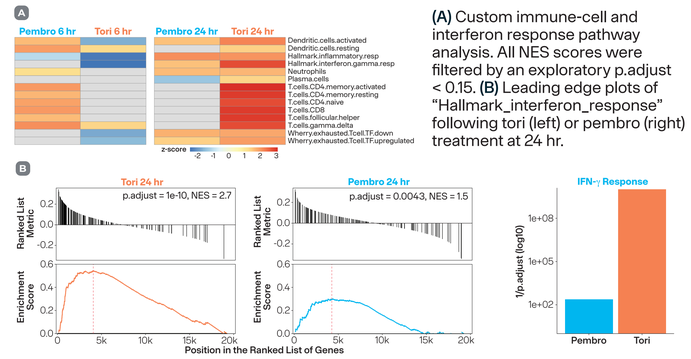
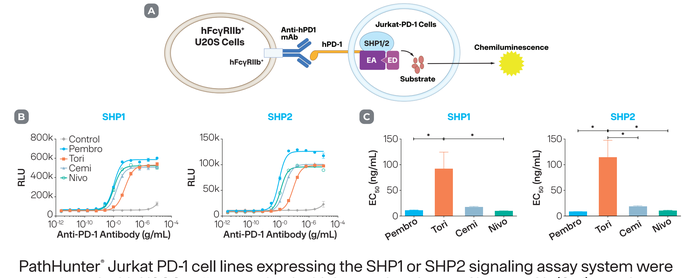
Reference: 1) Liu H, Guo L, Zhang J, Zhou Y, Zhou J, Yao J, Wu H, Yao S, Chen B, Chai Y, Qi J, Gao GF, Tan S, Feng H, Yan J. Glycosylation-independent binding of monoclonal antibody toripalimab to FG loop of PD-1 for tumor immune checkpoint therapy. *MAbs*. 2019 May/Jun;11(4):681-690.

Toripalimab Induces an Elevated IFN-γ Gene Signature in NSCLC DTC With Different Kinetics and Higher Intensity Compared to Pembrolizumab



(A) Custom immune-cell and interferon response pathway analysis. All NES scores were filtered by an exploratory p.adjust < 0.15. **(B)** Leading edge plots of "Hallmark_interferon_response" following tori (left) or pembro (right) treatment at 24 hr.

Toripalimab Exhibits the Lowest Potential for Partial Agonism Among Other Commercial Anti-PD-1 mAbs



PathHunter® Jurkat PD-1 cell lines expressing the SHP1 or SHP2 signaling assay system were co-cultured with U2OS cells opsonized with increasing doses of isotype Ab (Ctrl), pembro, tori, cemipilimab, or nivolumab (dose range 0.01-10 μg/mL). Chemiluminescence signal detected as RLU indicates SHP1 or SHP2 recruitment to PD-1. **(A)** Schematic representation of the experimental system. **(B)** Representative dose response curves for SHP1 and SHP2 recruitment in the Jurkat-PD-1 SHP1 and SHP2 signaling cell lines, respectively; **(C)** Graphical representation of the EC_{50} values calculated from dose response curves from 2 independent experiments. Data are shown as mean ± SEM.

Conclusions

- Toripalimab, an anti-PD-1 mAb, in combination with chemotherapy shows clinical efficacy irrespective of PD-L1 status.
- Toripalimab has high binding affinity to PD-1 (~12-fold higher than pembrolizumab).
- Toripalimab promotes a stronger Th1-mediated response in vitro in human PBMC compared to pembrolizumab.
- Toripalimab induces an elevated IFN-γ gene signature in NSCLC dissociated tumor cells at the 24-hour time point with different kinetics and higher intensity compared to pembrolizumab.
- Toripalimab exhibits the lowest potential for partial agonism by recruiting the lowest levels of SHP1 and SHP2, negative regulators of T cell activation, when compared to other commercial anti-PD-1 antibodies.

These findings present toripalimab as a next-generation anti-PD-1 checkpoint inhibitor that warrants future multi-regional clinical trials to evaluate its efficacy in combination with chemotherapy or other immunotherapy agents in multiple cancer types.