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


Strengthening a 2021 Cancer Signatures Program by Proposing the Use of PD-1 Antibody Toripalimab to Reroute the Tumor Microenvironment

1. **Background**

   - Toripalimab (tori) is a PD-1-targeting humanized IgG4 monoclonal antibody that blocks the interaction of PD-1 with 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 Fc loop of PD-1, which differentiates it from other anti-PD-1 mAbs.

2. **Toripalimab Exhibits 42-fold Higher Binding Affinity for PD-1 Compared to Pembrolizumab**

   - **A**. Biacore sensorgrams of PD-1 binding to covalently immobilized tori, PD-1 was immobilized in triplicate for 1 min. in a range from 0.00-1.00 μM with dissociation followed for 5 min. In 5 μM. **B**. Sensorgrams of PD-1 binding to covalently immobilized pembrolizumab (Pembro). PD-1 was immobilized in triplicate for 1 min. in a range from 0.00-20.00 μM with dissociation followed for 5 min. All sensorgrams were globally fit biexponential to a 1:1 binding model, indicating a 1:1 stoichiometry. (C). Average dissociation and kinetic rate constants (Kd, k on, and k off) from 3 replicates experiments for PD-1 binding to tori and pembrolizumab.

3. **Toripalimab Exhibits Enhanced T-Cell Recruitment and Activation In Staphylococcal Enterotoxin B (SEB)-Mediated T-Cell Activation of Human PBMC**

4. **Toripalimab Exhibits Enhanced Intracellular Activation In CD3/CD28-Mediated T Cell Activation of Human CDB-1 T-Cells**

   - Naive CDB-1 T cells from 7 human healthy donors were activated with PD-1 antagonist anti-PD-1 mAb and human anti-CDC-1 (0.5 μg/mL) immobilized on the plate surface. 10 μg/mL of isotype control antibody (Ctrl), pembrolizumab, or tori was added in duplicate wells. IFN-γ levels in cell culture supernatant were quantified on Day 3 of activation using ELISA.

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

   - **A**, **B**. Pathway analysis. Jurkat T-cell lines expressing the SHIP or SHIP2 signaling assay system were co-cultured with PD-1 mAbs and/or increasing doses of isotype Ab (Ctrl), pembrolizumab, pembrolizumab, cemiplimab, or nivolumab (dose range 0.01-10 μg/mL). Chemoluminescence signal detected as FLU indicates SHIP or SHIP2 recruitment to PD-1. **A**. Schematic representation of the experimental system. **B**. Representative dose response curves for SHIP and SHIP2 recruitment in the Jurkat-CD28 (A) and SHIP2 signaling cell lines, respectively. **C**. Graphical representation of the EC50 values calculated from dose response curves from 2 independent experiments. Data are shown as mean ± SD. **D**. 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 ≤ 5% and the PD-L1 TPS ≤ 10% subgroups. **B**. NSCLC for PD-L1 TPS ≤ 1% and the PD-L1 TPS ≤ 10% subgroups. **C**. ENET for PD-L1 TPS ≤ 1% and the PD-L1 TPS ≤ 10% subgroups. **D**. Kaplan-Meier estimates of OS are shown to compare pembrolizumab treatment at 24 hr.

6. **Conclusions**

   - **A**, **B**. Toripalimab, an anti-PD-1 mAb, in combination with chemotherapy shows clinical efficacy irrespective of PD-L1 status.
   - **C**, **D**. Toripalimab has high binding affinity to PD-1 (~12-fold higher than pembrolizumab).
   - **E**. 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 pembrolizumab.

These findings support the use of toripalimab in patients with PD-L1+ tumors, highlighting its potential as a valuable addition to the anti-PD-1 therapeutic landscape.