Molecular Insights on Safety and Anti-tumor Activity of Anti-CTLA-4 Monoclonal Antibody ONC-392

Yang Liu[1], Yan Zhang[2], Xuexiang Du[2], Mingyue Liu[2], Xianfeng Fang[3], Libing Mu[3], Vadim Tevetnitsky[1], Martin Devenport[1], Pan Zheng[1]

[1]OncoC4, Inc. Rockville, MD20850, USA; [2]Institute of Human Virology, University of Maryland School of Medicine, Baltimore, MD20212, USA; [3]AcroImmune, Ltd, Guangzhou, China

Background: Anti-CTLA-4 antibodies have brought about limited clinical benefit because severe toxicity limits dosing levels and/or duration. By screening for antibodies with higher anti-tumor activity but lower autoimmunity it was revealed that key the better safety and preclinical efficacy is preservation of CTLA-4 for immune tolerance and intratumorial Treg depletion. Our work established that, independent of blocking activities, mAbs that preserve CTLA-4 recycling maintain the physiological immune tolerance checkpoint function while allowing more efficient and selective elimination of tumor-infiltrating regulatory T cells, resulting in highest efficacy and lowest toxicity and was thus developed for clinical testing of all antibodies tested. The antibody with best safety and efficacy profile, ONC-392 was developed for clinical testing. The first-in human studies showed that ONC-392 is safe and well tolerated. Remarkably, ONC-392 was well tolerated among patients who has received repeated dosing of 3.0 mg/kg and 10.0 mg/kg of ONC-392. The molecular and cellular characterization of ONC-392 will be presented.

Method: In vitro binding and disassociation were determined between pH 4.0-7.0. The intracellular traffic of both antibodies and CTLA-4 molecules were visualized by confocal microscopy. The binding to human and mouse FcγRI, IIA, IIB, and III (A), FcRn as well as mouse FcγRIV were evaluated by surface plasmon resonance (SPR). Depletion of regulatory T cells in tumor and lymphoid tissues were determined by flow cytometry.

Conclusions: Unlike other clinical anti-CTLA-4 antibodies, ONC-392 preserves CTLA-4 recycling and thus Treg function in the peripheral tissues. The higher cell surface CTLA-4 allows more efficient Treg depletion in the tumor microenvironment. In addition, despite reduced binding to mouse activating FcγRI, III/IV, ONC-392 was more effective in intratumor Treg depletion in the mice. Therefore, lacking negative signaling from FcγRIIB may also contribute to its anti-tumor activity.

Results:

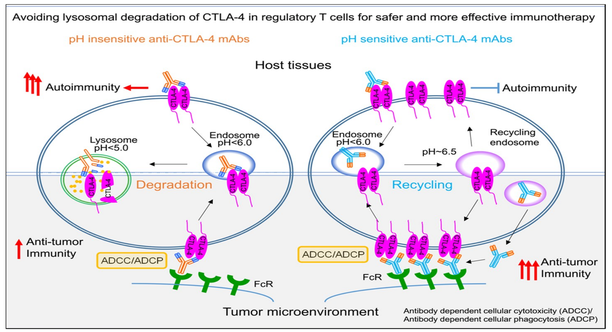

Figure 1: New insights on CTLA-4 targeting: preserving the CTLA-4 checkpoint for safer and more effective cancer immunotherapy.

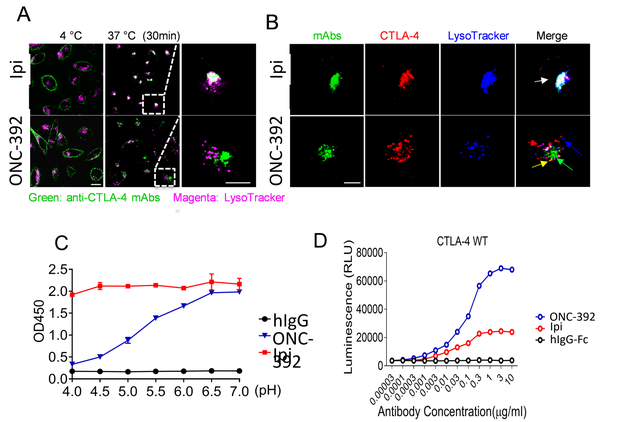

Figure 2: ONC-392 is a pH-dependent anti-CTLA-4 antibody that does not induce lysosomal CTLA-4 degradation. (A) Ipilimumab (Ipi) or ONC-392 were labeled with AF488 and incubated with CHO-hCTLA-4 and further stained with lysotracker. Co-localization between AF488 labeled anti-CTLA-4 mAbs and lysosomes. Scale bar: 10μM. (B) Co-localization of AF488 labeled anti-CTLA-4 mAb Ipilimumab but not ONC-392, lysosomes and orange-fluorescence protein (OFP) tagged CTLA-4 Scale bar: 10μM. (C) ONC-392 binding CTLA-4 in a pH-dependent manner. (D) Superior ADCC activity of ONC-392 over Ipilimumab.

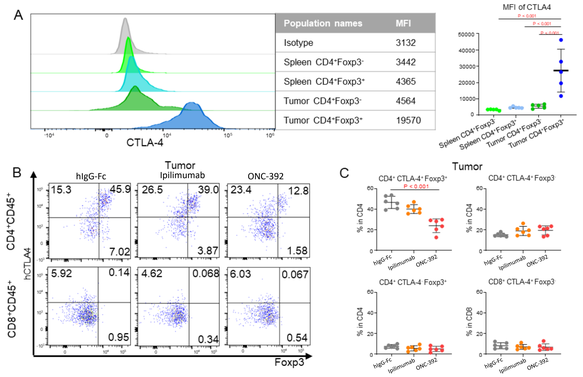

Figure 3: Improving Treg depletion in tumor microenvironment without jeopardizing selectivity of anti-CTLA-4 antibodies. (A) Comparing cell surface CTLA-4 levels among different T cell subsets. (B and C) Selectivity of Treg depletion at 24 hours post anti-CTLA-4 treatment. (B) Representative flow cytometry profiles depicting the proportion of different subpopulations among single-cell suspensions prepared from tumor tissues (upper panel) and spleen tissues (lower panel) on day 15. (C) Summary data of the percentage on different subpopulations from tumor tissues (upper panel) and spleen tissues (lower panel).

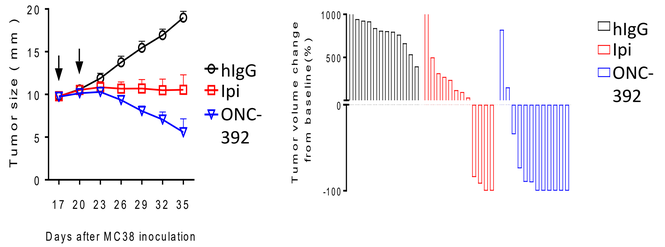

Figure 4: ONC-392 causes rejection of large established tumors.
MC38 tumor-bearing-*Ctla4*$^{h/h}$ male mice (n=12) were i.p. treated with either control hIgG, Ipilimumab or ONC-392 at 30 mg/mouse on day 17 and day 20 after tumor inoculation. The data are from day 17 to day 35 when some mice in control group has reached tumor size endpoint. The line graphs in the left panel show mean tumor sizes as mean diameters ± S.E.M. The waterfall graphs on the right show either grow (above X-axis) or regression (underneath X-axis) of individual tumors. All data from 2 independent experiments are included. The tumor volume immediately prior to antibody treatment was defined as baseline for the waterfall graph.

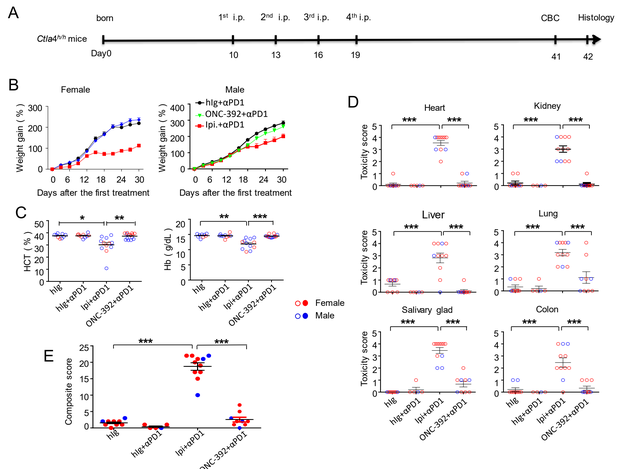

Figure 5: Safety studies of ONC-392 in young *CTLA4*$^{h/h}$ mice.
(A) Time-line of antibody treatment and analysis. (B) Comparing ONC-392 with Ipilimumab for their combination toxicity when used during perinatal period using growth retardation as readouts. (C) Ipilimumab but not ONC-392 induced anemia when used in combination with anti-PD-1 antibody. (D) Pathology scores of internal organs and glands after the mice were treated with either control of given combination of immunotherapeutic drugs. (E) Composite pathology scores. Blue circles represent scores of male mice and the red scores represent female mice used.

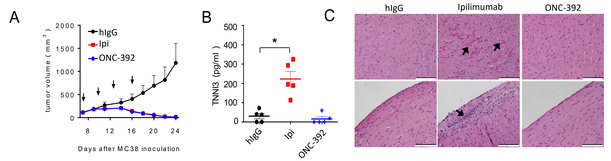

Figure 6: Safety of ONC-392 and Ipilimumab in 6-7 week-old young adult tumor-bearing mice. (A-C) MC38-bearing young male mice (7-weeks old) were inoculated with MC38 tumor cells and treated intraperitoneally with either control hIgG, Ipilimumab or ONC-392 on days 7, 10, 13 and 16 after tumor cell challenge. (A) Tumor volumes over time. (B) Serum TNNI3 levels on day 25 after tumor challenge as determined by ELISA. (C) H&E staining show hyalinization and inflammation in the myocardium. Scale bar 100μm.