Plinabulin: Evidence for an Immune-mediated Mechanism of Action

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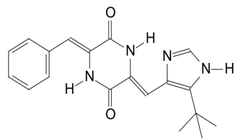
Abstract

The purpose of this study was to investigate the evidence for an immune-mediated mechanism of action for plinabulin. Plinabulin, a novel compound for use in oncology, is an inhibitor of tubulin polymerization (Nicholson et al, 2006) and is in Phase 3 trials for NSCLC (Bazhenova, 2015). The methods for the *in vitro* and *in vivo* studies are described in Martin et al (2014) and Müller et al (2014). Recent data from *in vitro* studies demonstrate that plinabulin enhances immune responses as (1) it increases dendritic cell maturation as evidenced by elevated levels of CD40, MHCII, CD80 and CD86 and (2) the release of pro-inflammatory cytokines such as IL-1beta, IL-6 and IL12p40. These increases were similar to those observed with LPS. Paclitaxel and etoposide were inactive in these assays. In the MC-38 syngeneic colon tumor model in the C57BL/6 immunocompetent mouse, plinabulin (7.5 mg/kg, IP, BIW) significantly enhanced the therapeutic effect of combined PD-1/CTLA-4 inhibition on tumor volume, with a maximal effect occurring between days 13-20 after initiation of treatment. When tumor weight was measured at necropsy, plinabulin significantly enhanced the effect of both PD-1 inhibition and combined PD-1 + CTLA-4 inhibition. In this regard, evidence has been provided that agents which interfere with tubulin polymerization (e.g. ansamytocin-P3, dolastatin-10), but not microtubule stabilizing agents (e.g. docetaxel, paclitaxel) promote anti-tumor immunity as demonstrated by dendritic cell maturation and enhanced T-cell activation both *in vitro* and *in vivo*. In addition, the microtubule depolymerizing agents act synergistically with immune checkpoint inhibitors (Martin et al, 2014, Müller et al, 2014). These data indicate an immune mediated mechanism of action of plinabulin and provide the basis for initiating a clinical trial of plinabulin with the PD-1 mAb Nivolumab in metastatic NSCLC (Yeh et al, 2015).

Structure of Plinabulin



Materials and Methods

In Vitro Markers for cell maturation (CD40, CD80, CD86, MHC II) were measured by FACS analysis in the SP37A3 immature mouse dendritic cell (DC) cell line after 20 hours of incubation with the test compounds. The release of pro-inflammatory cytokines (IL-1β, IL-6, IL 12p40) was quantified by ELISA. The assays were performed as described by Martin et al (2014) and Müller et al (2014). *In vivo* tumor volumes (MC-38 model); 10 mice per group, initial tumor size were measured twice weekly. At necropsy tumors were weighed and bisected on the vertical axis. One-half was collected for FACS analysis. The *in vitro* studies were performed at the Univ. of Basel and the *in vivo* studies at Crown Biosciences.

References

Bezhenova et al, IASLC (2015); Martin et a, Cancer Immunol Immunother, (2015); Muller et al, Cancer Immunol Rs (2015); Nicholson et al,, Anti-Cancer Drugs, (2006); Yeh et al, IALSC, (2015)

Figure 1. Phenotypic Markers of SP37A3 Immature Mouse Dendritic Cell Maturation (FACS Analysis)

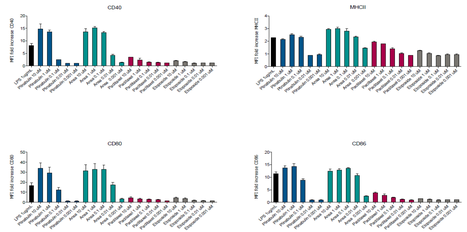
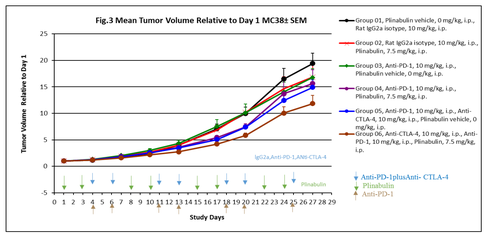


Figure 3. A: Mean Tumor Volumes over Time Relative to D1 in C57BL/6 Mice Implanted with MC-38 Colon Tumor Cells; B: Mean Tumor Volumes at Necropsy Outliers in Groups 5 and 6 Removed)



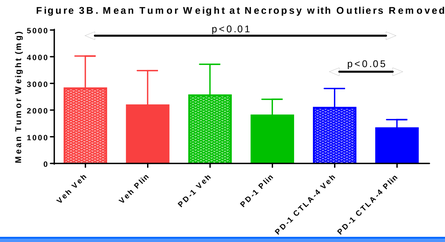


Figure 2. Cytokine Concentrations in Plinabulin-Treated SPA37A3 Mature Dendritic Cells

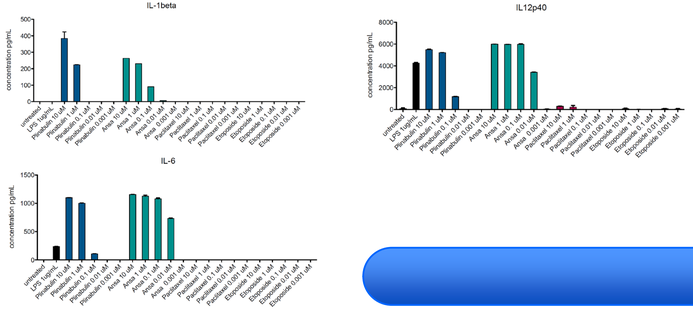
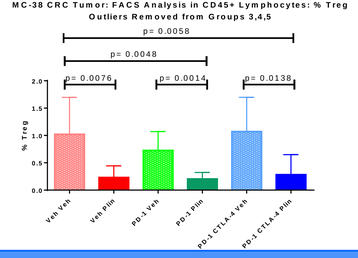
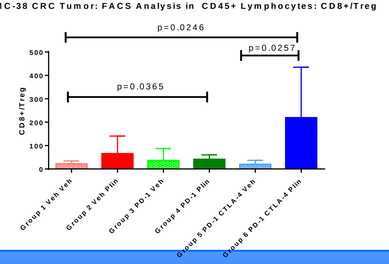
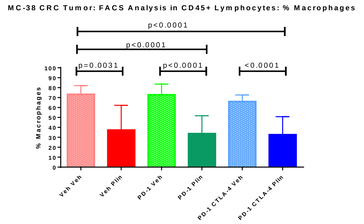


Figure 4. FACS Analysis of Tumors Removed at Necropsy CD45+ Lymphocytes A: % Treg Cells ; B Ratio CD8+/Treg; C % Macrophages







Results and Discussion

In Vitro, Plinabulin enhances immune responses by increasing dendritic cell maturation and subsequent cytokine release, increasing antigen load, In vivo, this translates to an augmented anti-tumor effect of immune oncology (IO) agents such as anti-PD-1 and anti-CTLA-4 antibodies. The latter is demonstrated by both effects on tumor growth and by the decrease in immune-suppressant cells such as Treg cells and a relative increase in immune responses such as cytotoxic T-cells (CD8+/Treg). Furthermore, it appears that the affects of plinabulin on the tumor microenvironment are not limited to the T-cell population as noted by the decrease in % macrophages associated with plinabulin either alone or in combination with IO agents. These findings support clinical trials for the combination of plinabulin with immune oncology agents in different forms of cancer.