Discovery of a Multispecific CD73/PD-1 Targeting Drug-Fc Conjugate (DFC) for the Treatment of Cancer

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BACKGROUND

The approval of PD-(L)1 axis inhibitors have firmly established immunotherapy as an effective cancer treatment option with reduced adverse effects compared with conventional chemotherapy[1]. However, while anti-PD-(L)1 therapy has demonstrated durable responses, only a small subset of patients respond. One mechanism of tumor escape from anti-PD-1 therapy is via the release of immunosuppressive metabolites such as adenosine. CD73, an extracellular enzyme expressed on immune cells and some tumors, catalyzes the rate limiting step in adenosine production, the conversion of AMP to adenosine[2] (**Figure 1A/B**). As a strategy to improve response rates, we developed a first-in-class multispecific CD73/PD-1 targeting DFC. Herein, we describe CD73/PD-1-001, a dual targeting DFC comprising a multivalent conjugate of a small molecule CD73 inhibitor stably linked to a proprietary human IgG1 Fc-fusion with a PD-1 inhibitor peptide (**Figure 2**). This multispecific DFC has the potential for differentiation and increased therapeutic efficacy compared to approved PD-(L)1 inhibitors.

Figure 1. Immune suppression by PD-1 and CD73 checkpoint pathways (**A**). Release of immune blockade by simultaneous targeting of PD-1 and CD73 (**B**).

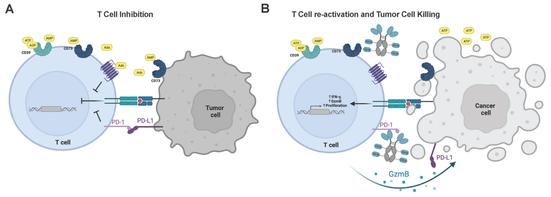
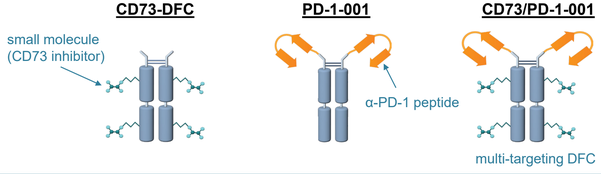


Figure 2. CD73 (left) and PD-1 (center) DFCs. Bispecific CD73/PD-1 DFC (right).



METHODS

Binding of PD-1-001 and CD73/PD-1-001 to biotinylated hPD-L1 was measured by ELISA; test articles were also evaluated in a functional hPD-1 blockade assay (Promega #J1250). CD73 binding was evaluated by surface plasmon resonance (ACROBiosystems). Efficacy was evaluated in transgenic mice expressing human PD-1 and PD-L1 with MC-38 (hPD-L1) tumors (genOway, France) (n=10). MC-38 (hPD-L1) cells were injected at a concentration of 10^6 cells mixed 1:1 with Matrigel (Corning). Treatment was initiated when tumor sizes were 25 – 75 mm^3. Test articles were dosed IP twice weekly for 3 weeks. As a comparator, a pembrolizumab biosimilar (Bio-X-Cell #SIM0010) was included. Tumor volumes were recorded, and statistical analysis was conducted by t-test (Mann-Whitney) or two-way ANOVA. *p≤0.05; **p≤0.01.

RESULTS

In vitro characterization of PD-1-001 and CD73/PD-1-001. The binding affinity of CD73/PD-1-001 for CD73 was determined using a kinetic binding assay. CD73/PD-1-001 was found to have a K_D of 0.66 nM confirming that it binds CD73 with high affinity. Multiple assays were used to confirm that PD-1-001 and CD73/PD-1-001 bind to hPD-1 with high affinity. Binding of both molecules to immobilized hPD-1 was measured by ELISA and found to be virtually identical with average EC_{50}'s from two assays of 0.055 and 0.064 nM for PD-1-001 and CD73/PD-1-001, respectively (**Figure 3**). A cell-based functional hPD-1/hPD-L1 signaling assay was used to compare CD73/PD-1-001 to approved PD-1 inhibitors. In this assay CD73/PD-1-001 was potent, demonstrating comparable activity to pembrolizumab, and approximately 5-fold lower activity compared to nivolumab (**Table 1**).

Figure 3. Binding of DFCs to immobilized hPD-1.

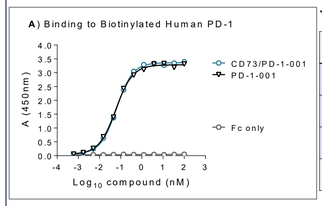


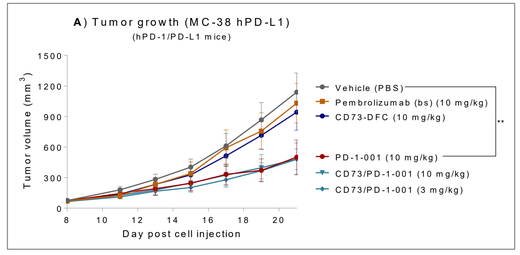
Table 1. hPD-1 Blockade assay

Test article	Target	EC_{50} (nM)
CD73/PD-1-001	CD73/PD-1	33.96
Pembrolizumab*	PD-1	18.10
Nivolumab*	PD-1	7.22

*biosimilar

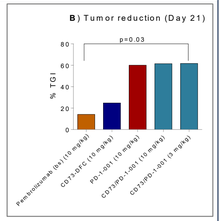
In vivo characterization of PD-1-001 and CD73/PD-1-001 in a humanized mouse model. In transgenic mice expressing hPD-1/L1 a pembrolizumab biosimilar at 10 mg/kg had minimal effects on tumor growth (**Figures 4A/B**). Similarly, a CD73 only targeting DFC (CD73-DFC) did not significantly reduce tumor growth. In contrast, our proprietary anti-PD-1 inhibitor, PD-1-001, dosed at 10 mg/kg significantly reduced tumor growth (p≤0.01). Robust tumor volume reductions were also achieved with the multispecific CD73/PD-1-001 construct at 3 and 10 mg/kg. Notably, the PD-1-001 and CD73/PD-1-001 constructs reduced tumor burden to a similar extent when dosed at 10 mg/kg. The lack of a dose response with CD73/PD-1-001 and the similar performance of PD-1-001 and CD73/PD-1-001 at 10 mg/kg necessitated a follow up study with lower doses to define a minimum efficacious dose and to differentiate PD-1-001 and CD73/PD-1-001.

Figure 4A. Efficacy of test articles in a transgenic mouse model of colorectal cancer.



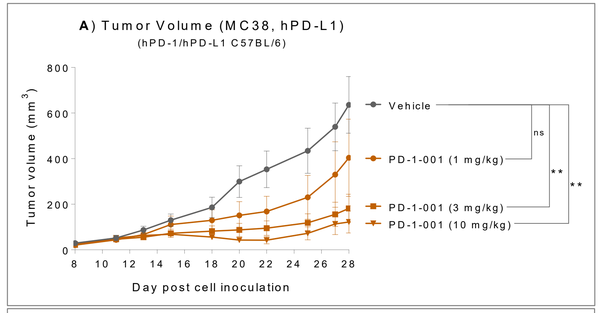
RESULTS

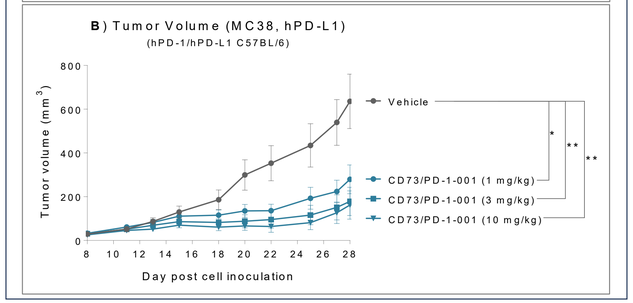
Figure 4B. Percent tumor growth inhibition (TGI) of test articles relative to vehicle at study end (Day 21). A pembrolizumab biosimilar and CD73-DFC generated modest TGIs of 14.2 and 24.8% respectively, which were not significant. However, PD-1-001 and CD73/PD-1-001 generated nearly identical tumor reductions of 60.1 and 61.5% respectively (p≤0.0068). The virtually identical TGIs for CD73/PD-1-001 further suggests the maximum achievable signal in this model has been reached by PD-1-001 and CD73/PD-1-001.



Activity of PD-1-001 and CD73-PD-1-001 in a transgenic mouse tumor model with an expanded dose range. In a follow-up study both DFCs were dose ranged at 1, 3, and 10 mg/kg. At the lowest test concentration (1 mg/kg) the DFCs began to differentiate, with CD73/PD-1-001 achieving a statistically significant reduction in tumor volume (p≤0.05) while PD-1-001 did not (**Figures 5A/B**). This result highlights the exceptional potency of CD73/PD-1-001 and demonstrates the therapeutic benefits of combing two important checkpoint targeting inhibitors on a single multispecific DFC.

Figure 5A/B. Dose range of test articles in a humanized mouse model.

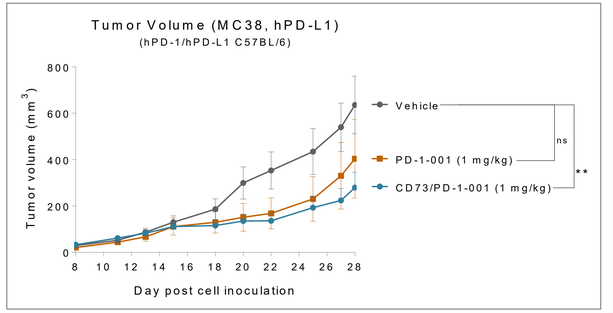




RESULTS

At study end (Day 28) PD-1-001 (1 mg/kg) had a modest TGI of 36.5%, which was not significant. In contrast CD73/PD-1-001 achieved a significant TGI of 56.0% (p≤0.01). At 3 and 10 mg/kg this was further increased to 72.0% and 74.5% respectively. Similarly, PD-1-001 was significantly active at 3 and 10 mg/kg with TGI's of 71.4 and 80.8% respectively. The observation that PD-1-001 and CD73/PD-1-001 are differentiated at 1 mg/kg highlights the improved therapeutic potential of a dual CD73 and PD-1 targeting DFC (**Figure 6**).

Figure 6. Improved activity of CD73/PD-1-001 compared to PD-1 monotherapy.



SUMMARY

PD-(L)1 checkpoint blockade has revolutionized cancer therapy. However, additional tumor immune escape mechanisms, including the adenosine pathway, play potentially important roles in generating resistance to PD-(L)1 therapy. Here we describe CD73/PD-1-001, a dual targeting DFC with potent activity against both validated immune checkpoint pathways.

CD73/PD-1-001 is a potent inhibitor of hCD73 and hPD-1 in vitro. This potency translated into robust efficacy in an in vivo model of colorectal cancer using transgenic mice. CD73/PD-1-001 reduced tumor growth by 56% with a low dose of 1 mg/kg (twice weekly, x3 weeks). At 3 mg/kg TGI increased to a maximal value of > 70%. Additionally, we demonstrated that both checkpoint pathways inhibited by CD73/PD-1-001 contributed to efficacy, highlighting the potential clinical benefits of this first in class multispecific inhibitor.

DISCLOSURE & REFERENCES

All authors are shareholders & employees of Cidara Therapeutics.

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[1]Franzin et. al., 2020; PMID: 33162990

[2]Xia et. al., 2023; PMID: 36859386