Human anti-PD-L1/PD-1 Antibodies Exhibit Potent Immune Cell Activation

SORRENTO THERAPEUTICS

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ABSTRACT

Abstract: Immunomodulatory antibodies have shown great promise in recent clinical studies. Via inhibition of immunosuppressive signals on cancer cells or direct agonistic stimulation of T cells, a strong sustained anti-tumor immune response could be induced.[1] Disruption of the interaction between PD-L1 and PD-1 has been one target for such antibody therapy.[2,3] Using its proprietary G-MAB® antibody library, Sorrento has identified a number of human anti-PD-L1 and anti-PD-1 monoclonal antibodies (mAbs) and is currently preclinically developing the lead compounds. These mAbs exert strong immunomodulatory activity *in vitro* via immune cell activation and induction of cytokine production as well as anti-tumor activity *in vivo*.

Methods: The human mAbs were identified from Sorrento's G-MAB® library and candidate mAbs were produced as fully human IgG1 antibodies. Characterization of the mAbs included functional *in vitro* studies using mixed lymphocyte reactions (MLR) to measure T cell activation and cytokine production *in vitro*, as well as anti-tumor activity in a syngeneic mouse cancer model.

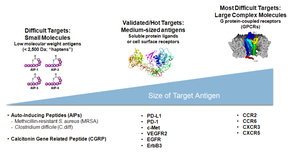
Results: Sorrento has selected two lead human anti-PD-L1 mAbs as well as one PD-1 antibody, all of which possess excellent affinity for their respective cellular target. Functional analyses demonstrated potent T cell activation and increased release of interleukin-2 (IL-2) and interferon-γ (IFN-γ) in MLR assays as well as *in vivo* anti-tumor activity.

Conclusions: The human anti-PD-L1 and anti-PD-1 mAbs identified from Sorrento's G-MAB® antibody library have demonstrated excellent functional characteristics *in vitro* as well as *in vivo*, and thus, might hold potential as future anti-cancer therapeutics.

INTRODUCTION

Sorrento Therapeutics, Inc. (OTCQB: SRNE) is a development stage biopharmaceutical company engaged in the acquisition, discovery, development and commercialization of proprietary drug candidates for the treatment of cancer, inflammation, metabolic disorders, and infectious diseases.

Sorrento has developed and validated its proprietary G-MAB® human antibody library:



Anti-PD-L1 and Anti-PD-1 Antibodies

The Programmed Death 1 (PD-1) inhibitory receptor is expressed by T cells during long-term antigen exposure. Upon ligation with its ligands PD-L1 and PD-L2, whose expression can be elevated within inflamed tissues and the tumor microenvironment, it results in negative regulation of T cell activity. This PD-1/PD-L1 interaction happens during the effector phase of a T-cell response in peripheral tissues. The blockade of this molecular engagement with specific antibodies to PD-1 or PD-L1 has been shown to result in the preferential activation of T cells with specificity for the cancer.[4]

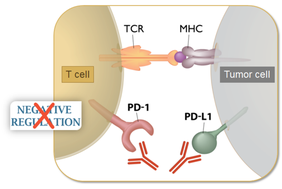


Figure 1. Interaction between PD-L1 and PD-1
(adapted from 4)

In vitro Immunomodulation by Anti-PD-L1 mAbs

To measure the ability of the anti-PD-L1 antibodies to modulate T cell responsiveness purified CD4+ cells were cultured with allogeneic dendritic cells, prepared by culturing monocytes in GM-CSF and IL-4 for seven days. Parallel plates were set up to allow collection of supernatants at day 2 or 3 and day 5 to measure IL-2 and IFNγ respectively using a commercial ELISA kit. The remaining cells of the day 5 culture were assayed for CD25 expression as a measure of cell activation. Genentech/Roche's humanized anti-PD-L1 mAb YW243.55S70 was produced in-house and used as positive control IgG1 and an unrelated STI human mAb was utilized as negative control IgG1 antibody.

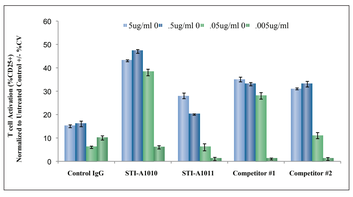


Figure 2. Enhancement of T cell activation by anti-PD-L1 antibodies.
Source data on file.

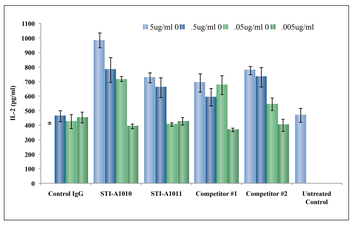


Figure 3. Increased IL-2 release in response to anti-PD-L1 antibodies.
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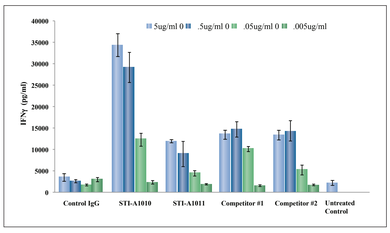

Figure 4. Increased IFN-γ release in response to anti-PD-L1 antibodies.
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In vivo Tumor Growth Inhibition by Anti-PD-L1 mAbs in a Syngeneic Tumor

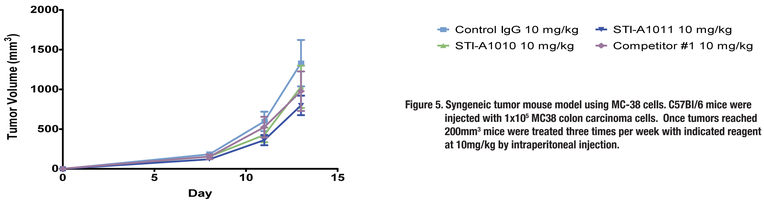

Figure 5. Syngeneic tumor mouse model using MC-38 cells. C57Bl/6 mice were injected with 1x10^5 MC38 colon carcinoma cells. Once tumors reached 200mm^3 mice were treated three times per week with indicated reagent at 10mg/kg by intraperitoneal injection.

In vitro Immunomodulation by Anti-PD-1 mAb

Similar to the studies described for our PD-L1 mAbs, to measure the ability of anti-PD-1 antibody to modulate T cell responsiveness, purified CD4+ cells were cultured with allogeneic dendritic cells, prepared by culturing monocytes in GM-CSF and IL-4 for seven days. Parallel plates were set up to allow collection of supernatants at day 2 or 3 and day 5 to measure IL-2 and IFN-γ, respectively using a commercial ELISA kit. The remaining cells of the day 5 culture were assayed for CD25 expression as a measure of cell activation. The competitor mAbs were produced in-house and used as positive control and an unrelated STI human mAb was utilized as negative control IgG1 antibody.

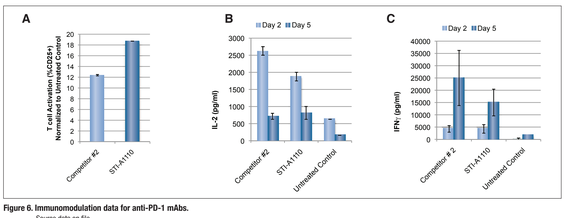

Figure 6. Immunomodulation data for anti-PD-1 mAbs.
Source data on file.

CONCLUSIONS

The human anti-PD-L1 and anti-PD-1 antibodies generated by Sorrento have demonstrated outstanding functional characteristics as potent immunomodulatory molecules in vitro as evident by enhanced T cell activation and enhanced cytokine release as well as in vivo in a syngeneic tumor model. These data indicate that Sorrento's antibodies might possess potential as future anti-cancer therapeutics via recruitment of the patients' immune system against the tumor. Sorrento will continue to develop its anti-PD-L1 and anti-PD-1 antibodies in IND-enabling activities and expects commencement of clinical development in 2015.

REFERENCES

1) Melero I er al.; *Clin. Cancer Res.* 2013 Mar 1; 19(5):997-1008
2) Brahmer JR et al.; *NEJM* 2012 Jun 28; 366(26): 2455-65
3) Topalian SL et al; *NEJM* 2012 Jun 28; 366(26): 2443-54
4) Ribas A; *NEJM* 2012 Jun 28; 366(26): 2517-19