LB101, a Conditionally Tetravalent PD-L1xCD47 Bispecific Monoclonal Antibody, Combines Tumor Microenvironment Targeted Delivery (PD-L1) and a Single Biological High-Potency Effector (CD47)

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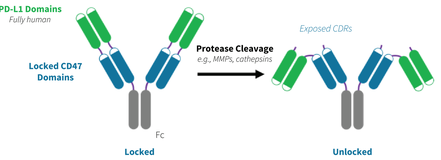
Background

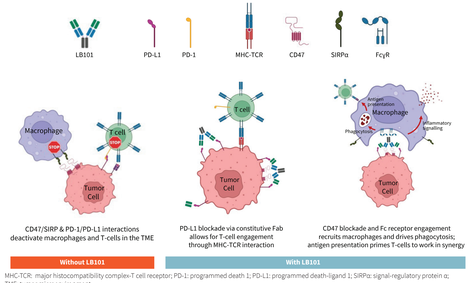
- Immune cell activation in the tumor microenvironment (TME) is a proven approach but is often limited by a narrow therapeutic index[1]
- The LockBody® platform is an innovative pharmacological construct for widening the therapeutic index of immune cell activation. Platform features include:
  - Predictable, tunable, tumor-specific activation via a stacked Fab design of constitutive and contingent Fabs linked by natural immunoglobulin G (IgG) hinges
    - Targeting to the tumor and enriching into the TME with the constitutive Fab against a membrane antigen
    - Minimization of sink effects and off-tumor toxicity for the contingent Fab, which is sterically locked
    - Unlocking of the contingent Fab by cleavage of a natural variant IgG hinge, which has naturally evolved for high serum and native tissue stability, yet with proven susceptibility to TME-specific proteases
  - Use of fully human IgG sequences for the entire construct
    - Predictable, consistent pharmacokinetics
    - Reduced risk of antibody-dependent antigenicity
    - High developability and manufacturability
- LockBody® LB101 is a conditionally tetravalent bispecific monoclonal antibody with constitutive programmed death-ligand 1 (PD-L1) and contingent CD47 binding domains (**Figure 1**)
  - The 2 domains are linked by 2 natural IgG hinges. The construct is designed to fully lock CD47 interactions in the periphery. The natural IgG hinges are specifically degraded in the PD-L1+ TME, thereby activating the CD47 blockade to induce antibody-dependent cellular phagocytosis (ADCP)[2] (**Figure 2**)
  - This is a preclinical study conducted to evaluate the pharmacodynamic properties of LB101 and its efficacy and tolerability in a fully immune mouse tumor model (MC38)

Figure 1. Structure of Engineered LB101 Antibody With 3 Modular Components in its Locked and Unlocked Forms



CDR: complementarity-determining region; MMP: matrix metalloproteinase; PD-L1: programmed death-ligand 1.

Figure 2. Mechanism of Action of LB101 Antibody



MHC-TCR: major histocompatibility complex-T cell receptor; PD-1: programmed death 1; PD-L1: programmed death-ligand 1; SIRPα: signal-regulatory protein α; TME: tumor microenvironment.

Methods

PD-1/PD-L1 cell-based antagonism assay

- The PD-1/PD-L1 cell-based bioassay (Promega, according to manufacturer's instructions) was used to measure the potency of IgG1 isotype, atezolizumab (anti–PD-L1), and LB101 (digested and undigested with MMP12) from 100 nM to 0.01 nM dilutions

Phagocytosis potency assay

- A549 cells were labelled using a green carboxyfluorescein succinimidyl ester (CFSE) cell tracer dye and incubated with antibodies (IgG1 isotype, CD47 IgG4, LB101 [digested or undigested with MMP12])
- Isolated CD14+ peripheral blood mononuclear cells were added to labeled cancer cells after antibody incubation, fixed, blocked with an Fc receptor-binding inhibitor monoclonal antibody, and then incubated with an Alexa Fluor 647 (AF647) conjugated antihuman CD11b antibody
- Cells were analyzed by flow cytometry (BD Fortessa) and the percentage of CFSE+ cells from the CD11b+ population was calculated and plotted

In vivo efficacy analyses in human PD-L1 (hPD-L1)+ MC38 syngeneic tumor-bearing C57B6/hPD-L1/hPD-1 mice

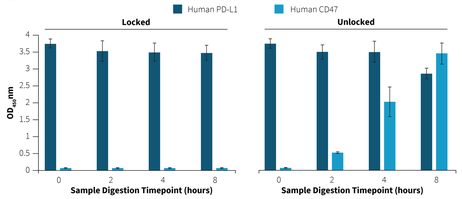
- In in vivo multidose efficacy studies, IgG1 isotype, atezolizumab, and LB101 were each dosed 6 times (on days 0, 3, 6, 9, 12, and 15) in C57B6 mice transgenic for hPD-L1 and hPD-1, bearing tumors generated by subcutaneous inoculation with the mouse cancer cell line MC38 (stably transfected with hPD-L1)
- Dosing began once tumors reached >100 mm^2, and volumes were measured by caliper measurements

Results

Target binding and bioactivity in locked and unlocked states

- LB101 exhibited PD-L1 binding in its locked form, while CD47 binding occurred with the unlocked form (**Figure 3**)

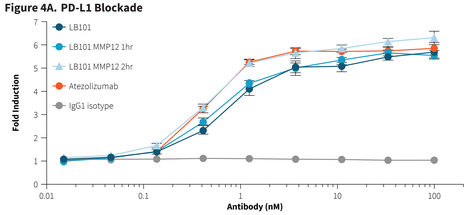
Figure 3. LB101 Binding to PD-L1 and CD47 After MMP Activation



MMP: matrix metalloproteinase; PD-L1: programmed death-ligand 1.

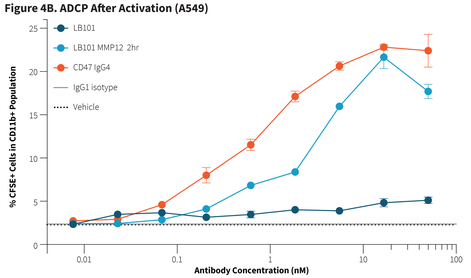
- In its locked form, LB101 exhibited anti–PD-L1 assay potency similar to atezolizumab (**Figure 4A**). In its unlocked form, CD47 binding by LB101 led to strongly enhanced ADCP (**Figure 4B**)

Figure 4A. PD-L1 Blockade



hr: hour; IgG1: immunoglobulin G1; MMP12: matrix metalloproteinase 12; PD-L1: programmed death-ligand 1.

Figure 4B. ADCP After Activation (A549)

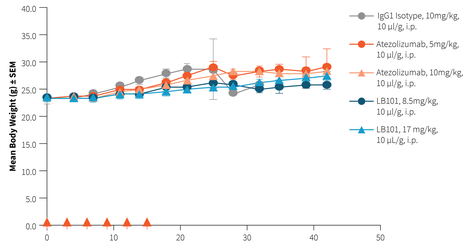


ADCP: antibody-dependent cellular phagocytosis; CFSE: carboxyfluorescein succinimidyl ester; IgG1: immunoglobulin G1; MMP12: matrix metalloproteinase 12

In vivo multidose efficacy and tolerability of LB101

- In the MC38 mouse model, systemically administered LB101 monotherapy delivered at equimolar dose to atezolizumab led to no anemia, weight loss, or overt toxicity (**Figure 5**)
  - No animals were lost during the study and no lethargy, as would be associated with anemia, was observed
  - Mean weights were highly similar across all groups with 1 animal showing mild loss in the isotype and LB101 17 mg/kg groups, respectively

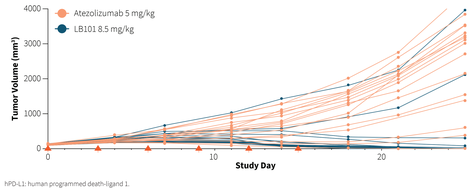
Figure 5. Absolute Body Weight Change (g) Over 42 Days for All Treatment Groups



i.p.: intraperitoneal; IgG1: immunoglobulin G1.
Note: Orange arrows indicate dosing every 3 days (Q3d x 6) at days 0, 3, 6, 9, 12, and 15.

- LB101 monotherapy exhibited greatly improved efficacy and durability of response (14/16 tumors eradicated) over isotype control IgG (0/16) and atezolizumab (1/16) (**Figure 6A-C**)

Figure 6A. LB101 vs Atezolizumab in Head-to-Head Challenge in Fully Immune Syngeneic Model (hPD-L1-MC38): Individual Tumor Volume Curves



hPD-L1: human programmed death-ligand 1.

Figure 6B. LB101 vs Atezolizumab in Head-to-Head Challenge in Fully Immune Syngeneic Model (hPD-L1-MC38): Average Tumor Volumes

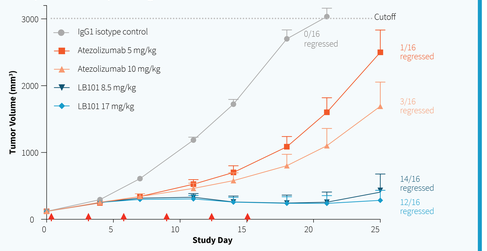
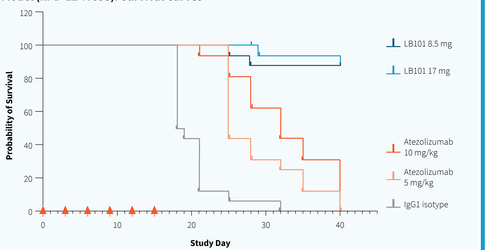


Figure 6C. LB101 vs Atezolizumab in Head-to-Head Challenge in Fully Immune Syngeneic Model (hPD-L1-MC38): Survival Curves

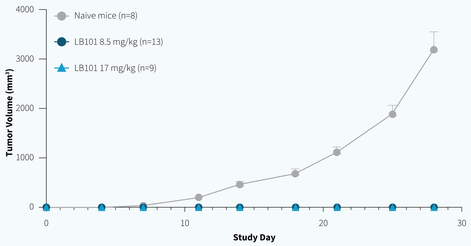


hPD-L1: human programmed death-ligand 1; IgG1: immunoglobulin G1.
Note: Orange arrows indicate dosing every 3 days (Q3d x 6) at days 0, 3, 6, 9, 12, and 15.
LB101 8.5 mg/kg is equimolar to atezolizumab 5 mg/kg.

Rechallenge with LB101

- In rechallenge experiments, all naïve mice developed tumors rapidly while none of the mice from groups with prior LB101-induced regressions exhibited tumor growth (**Figure 7**)

Figure 7. LB101 MC38 Rechallenge Study



Acknowledgements

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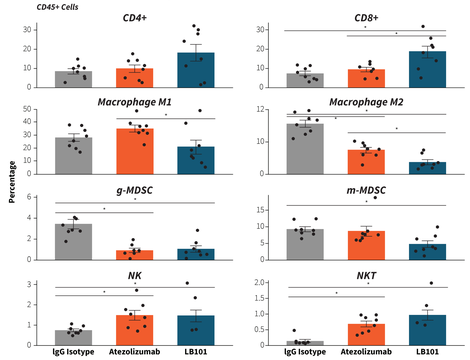
Effect of LB101 on immune cell composition in tumors and spleen

- LB101 induced a significant increase in CD4+ and CD8+ T cells and natural killer (NK) cells, and reduction in immunosuppressive myeloid-derived suppressor cells and macrophages compared with IgG isotype in tumors (**Figure 8**). No effect of LB101 was observed on immune cell composition in the spleen

Figure 8. Increased CD4+ and CD8+ T Cell and NK Cell Infiltrates and Decreased MDSC and Macrophages in the LB101 Group



IgG: immunoglobulin G; g-MDSC: granulocytic myeloid-derived suppressor cells; m-MDSC: monocytic myeloid-derived suppressor cells; NK: natural killer; NKT: natural killer T cell.
Note: *Indicates statistical significance.

CONCLUSIONS

- Single-agent LB101 immunotherapy delivered systemically in a difficult-to-treat syngeneic mouse model resulted in PD-L1 directed, local tumor-specific CD47 effector engagement leading to significant tumor regressions, compared with equimolar dose of atezolizumab, without overt toxicity
- LB101 treatment induced an increase in effector T cells and innate NK cells while decreasing immunosuppressive immune cells and macrophages when compared with atezolizumab
- The modular LockBody platform provides facile access to many permutations:
  - Targeting Fabs: HER2, PD-L1, and others
  - Effector Fabs: CD47, CD3, and others
  - Hinges: variants with tunable rates of cleavage
- Future directions will focus on developing LB101 as a single-agent therapeutic (IND expected late 2022) and exploring the potential of LockBody technology in improving the therapeutic index of other anticancer biological effectors through contingent activation in the TME

References

1. Hegde PS, et al. *Immunity*. 2020;52:17-35. **2.** Brezski RJ, et al. *Proc Natl Acad Sci U S A*. 2009;106:17864-17869.

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