Generation of anti-LAG-3 monoclonal antibodies for use in immunotherapy combinations

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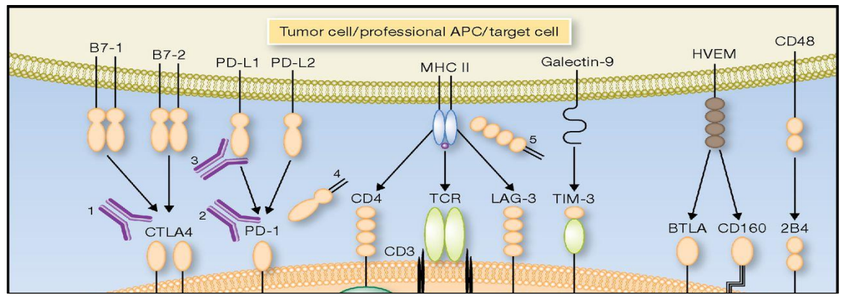
Abstract

Inhibitory immune checkpoints maintain self-tolerance in the normal immune system but can be co-opted in cancer to allow tumor escape from immune surveillance. Validation of immune checkpoint inhibitors has been provided by antibodies (mAbs) that inhibit the CTLA-4 and PD-1 pathways, which have shown significant clinical activity alone and in combination. In mouse models, blockade of other T-cell inhibitory signaling checkpoints such as LAG-3 has also been shown to be effective. Combination anti-PD-1 and anti-LAG-3 therapy was explored *in vivo* in MC38 and Colon26 mouse syngeneic tumor models with mAbs to mouse PD-1 and LAG-3. In the MC38 model, the tumor-free animals were increased from 7/10 in the anti-PD-1 alone arm to 10/10 in the combination. In the Colon26 model, the numbers of tumor-free animals in the anti-LAG-3/anti-PD-1 combination group were 10/12 while anti-PD-1 alone had 3/12 tumor-free animals.

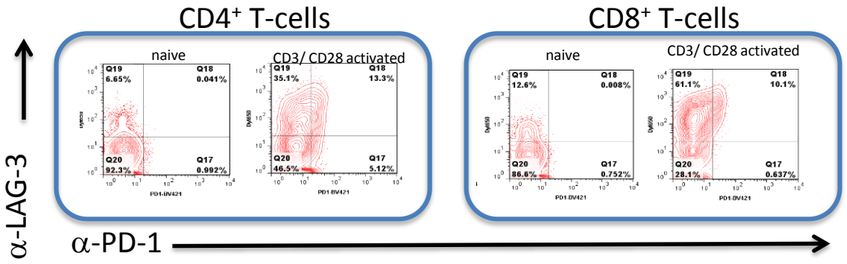
mAbs that inhibit human LAG-3 signaling were identified by screening the AnaptysBio Evolvable Library of fully human mAbs using a soluble LAG-3. We also generated humanized mouse mAbs to LAG-3. The resulting mAbs were matured to high affinity and potency via SHM-XEL™ which uses mammalian cell display of human IgG followed by *in vitro* somatic hypermutation. In *in vitro* cell assays, inhibition of LAG-3 alone demonstrates immune stimulatory activity as evidenced by increased IL-2 secretion in a mixed lymphocyte reaction (MLR). Combination of an anti-LAG-3 antibody with an anti-PD-1 antibody could shift the EC_{50} approximately 17-fold depending upon donor.

Overall, these data support combination cancer immunotherapy with anti-LAG-3 and anti-PD-1.

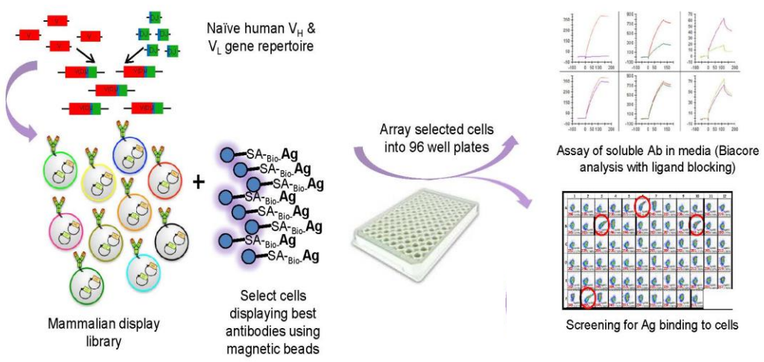


Overview of select immune checkpoint molecules.
PD-1 ligation by PD-L1 or PD-L2 results in membrane proximal decreases in TCR signaling. LAG-3 signaling is dependent on interaction with its ligand, MHC II to inhibit T-cell signaling.
Nirschl and Drake (2013).

PD-1 and LAG-3 are Co-Expressed on Activated T-cells

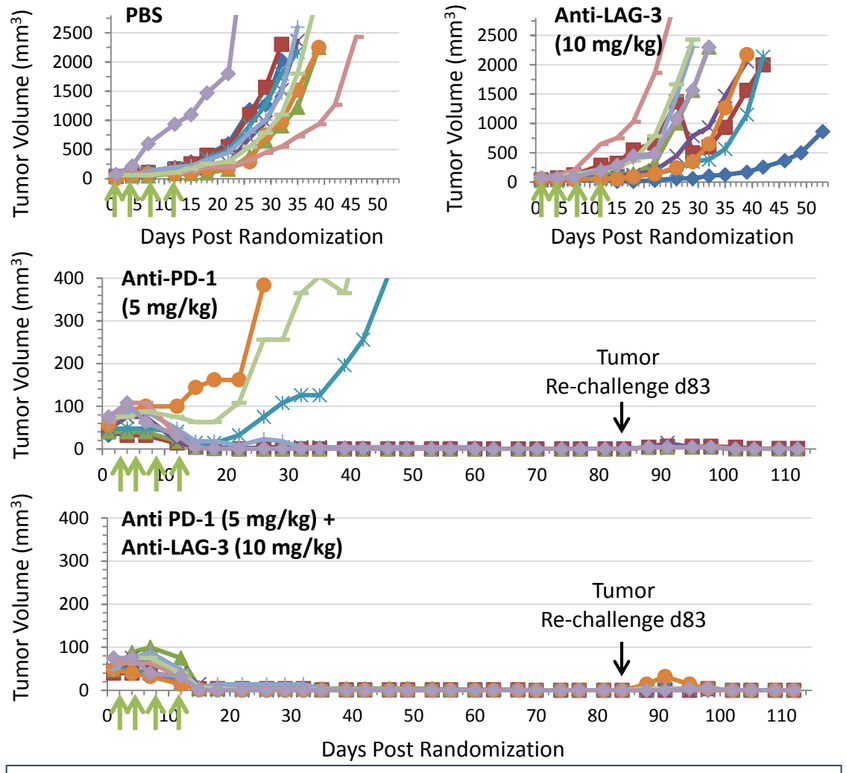


Human donor CD4+ or CD8+ T-cells were isolated and activated using plate-bound anti-CD3 and anti-CD28 mAbs. After 48 hours, cells were stained for PD-1 and LAG-3. Increased levels of PD-1/LAG-3 double positive cells were observed after stimulation compared to naïve T-cells.



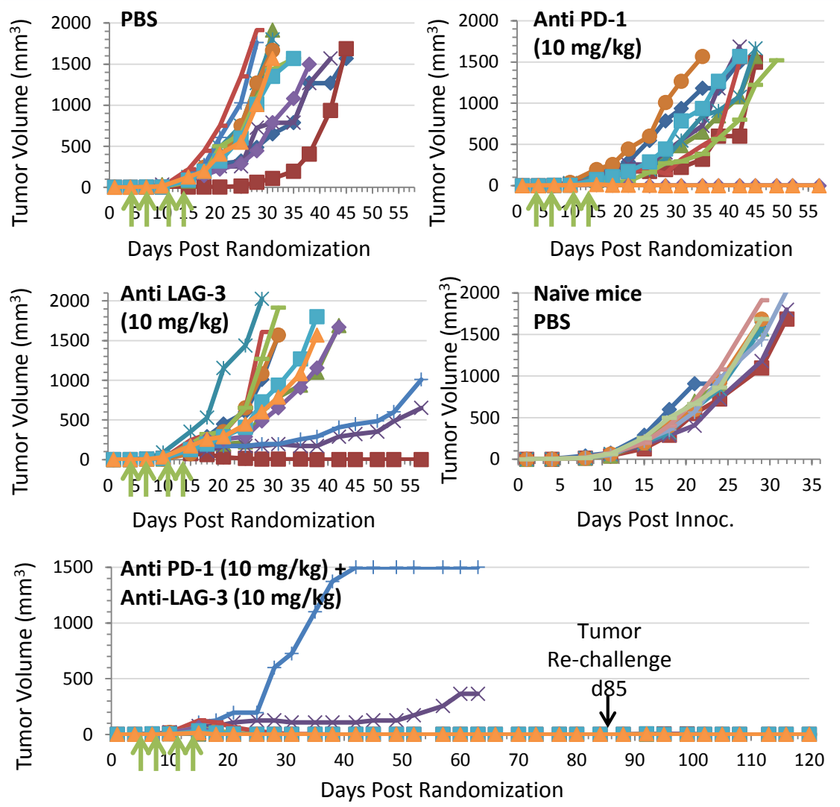
ABEL library screen overview.
Cells expressing surface-displayed, fully-human antibodies (Abs) that bind to soluble human LAG-3 ECD were identified from a screening campaign of the ABEL library using magnetic beads coated with huLAG-3. A panel of fully-human germline antibodies were isolated that bound specifically to LAG-3. The heavy (HC) and light (LC) chains of select hits were retransfected to create a stable cell line displaying the mAb on the cell surface. Activation induced cytodine deaminase (AID) was then transfected to begin somatic hypermutation (SHM). Iterative rounds of FACS sorting with decreasing concentrations of antigen were performed to identify mAbs with increased affinity for LAG-3.

Complete Tumor Regressions with anti-LAG3 and anti-PD1 Combination in the MC38 Syngeneic Tumor Model



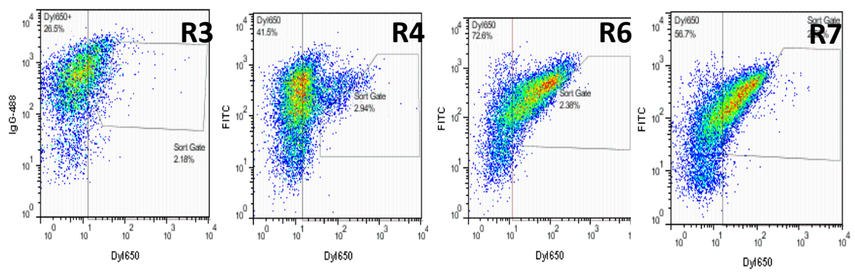
10 animals in each group were implanted with MC38 cells and dosed on d1, d4, d7, and d11 post randomization for size. All 10 animals in the combination group were tumor free compared with 7 animals in the anti-PD-1 (mAb RMP1-14, BioXcell) alone group and none in the anti-LAG-3 (mAb C9B7W, BioXcell) group. Tumor free animals from the anti-PD-1 and combination-treated groups were re-inoculated with 4x the number of MC38 cells. No tumors grew in either re-challenged group compared with 100% tumor take in the control group (not shown).

Complete Tumor Regressions with anti-LAG3 and anti-PD1 Combination in the Colon26 Syngeneic Tumor Model



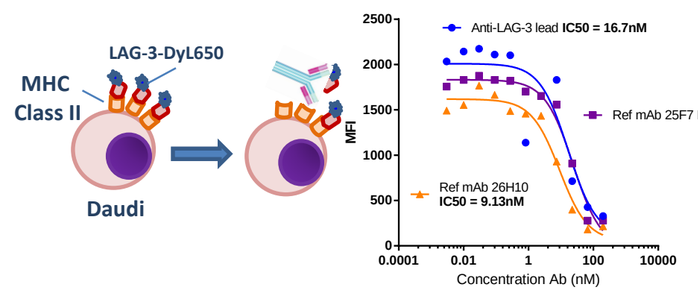
12 animals in each group were implanted with Colon26 cells dosed at d4, d7, d11, and d14. Complete response was observed in 10 out of 12 animals in the combination treated group compared with 3 out of 12 animals in the PD-1 alone group and one in the anti-LAG-3 group. Complete responder animals from the combination group were re-inoculated with the same number of Colon26 cells as initially implanted. No tumors grew in the re-challenged groups compared with 100% tumor take in the control group.

Increased Affinity of Anti-LAG-3 Candidate mAbs Generated by Somatic Hypermutation



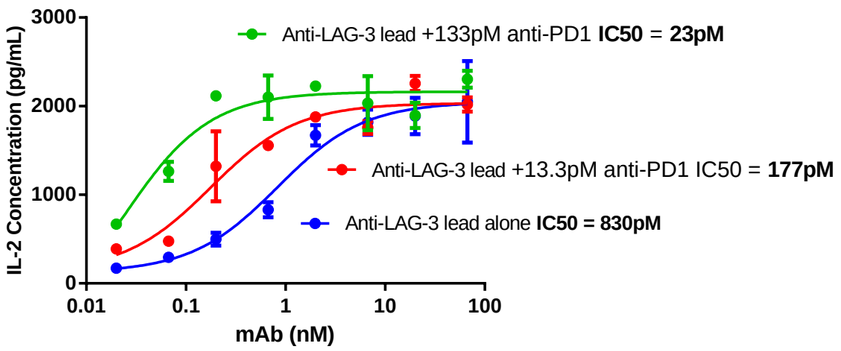
Cells from an evolving population were sorted by flow cytometry following incubation with antigen. The gated population indicated on each FACS plot was expanded after sorting and subjected to additional rounds of cell sorting as well as sequence analysis to identify improving mAb mutations.

Lead Anti-LAG-3 mAb Inhibits the Interaction of Soluble LAG-3 with MHC Class II



To test the ability of an anti-LAG-3 antibody to block the LAG-3/MHC II interaction, an *in vitro* assay was developed using a soluble form of LAG-3 fused to Fc, labeled with DyL650 binding to a Daudi B-cell line, which endogenously expresses MHC II. A dose range of neutralizing antibodies was preincubated with the soluble LAG-3 and analyzed by flow cytometry. The anti-LAG-3 lead can completely inhibit the interaction of soluble LAG-3 with MHCII.

Lead Anti-LAG-3 mAb Potentiates IL-2 Secretion Alone and in Combination with an Anti-PD-1 Antagonist mAb in a Mixed Lymphocyte Reaction



Isolated peripheral blood monocytes from a human donor were differentiated into dendritic cells and then mixed with CD4+ T-cells isolated from a second donor. IL-2 levels were measured after 48 hours. The anti-LAG-3 mAb was combined with an anti-PD-1 mAb. PD-1 was able to decrease the anti-LAG-3 EC50.

Conclusions

- **A high affinity anti-human LAG-3 antibody has been generated by humanization of a mouse monoclonal Ab coupled with *in vitro* somatic hypermutation**
- **LAG-3 inhibition with an anti-LAG-3 antibody displays potent activity alone and in combination with an anti-PD-1 antibody in an MLR assay**
- **Surrogate anti-PD-1 and anti-LAG-3 antibodies have activity in the MC38 and Colon26 tumor models alone and increased anti-tumor activity in combination**
- **These data suggests co-blockade of PD-1 and LAG-3 is worthy of further investigation**