Generation of antagonistic anti-TIM-3 and anti-LAG-3 monoclonal antibodies for potential novel immunotherapy combinations

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Abstract

Among the most promising approaches in the treatment of cancer is the activation of antitumor immunity by blockade of immune checkpoint targets. These inhibitory immune checkpoints are crucial for maintaining self-tolerance in the normal immune system but can be co-opted in cancer to allow tumor escape from immune surveillance. Therapeutic validation has been provided using antibodies that inhibit the CTLA-4 and PD-1 signaling pathways, which have shown significant clinical activity. Interestingly, blockade of other T-cell inhibitory signaling molecules TIM-3 and LAG-3 has also shown to be effective in mouse models of cancer. Potential therapeutic molecules that inhibit the negative signaling of TIM-3 or LAG-3 were identified by screening the AnaptyBio Evoluble Library (ABEL) of fully human germline antibodies. The initial ABEL screened used the soluble extra cellular domains (ECD) of either TIM-3 or LAG-3, followed by screening on cell-surface expressed antigens. The resulting panels of human antibodies were matured to high affinity and potency using SMI-RELIG (which uses single molecule elongation display of human IgG) followed by in vitro somatic hypermutation (SHM). We have explored the activity of these antibodies both as single agents as well as in combination blockade of multiple pathways in in vitro cell-based assays. Inhibition of each pathway in isolation demonstrated immune stimulatory activity as evidenced by increased secretion of IL-2 in a mixed lymphocyte reaction or an activated T-cell assay. Combination of an anti-LAG-3 or an anti TIM-3 antibody with an anti-PD-1 inhibitory antibody could increase IL-2 secretion over that seen with blockade of a single checkpoint alone. The magnitude of this effect was dose cell dependent. These data are suggestive that combination immunotherapy towards these targets is worth of clinical evaluation and may lead to increased efficacy.

Overview of selected 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 in the ligand, MH-1 to inhibit T-cell signaling. TIM-3 reportedly binds to Gal-9, as well as other ligands.

Single agent activity of ABE20515, an anti-PD-1 antagonist mAb, in a mixed lymphocyte reaction assay. Isolated peripheral blood mononuclear cells from a donor human were differentiated into dendritic cells (DC’S) and then mixed with CD4+ T-cells isolated from a second donor. IL-2 was added to each well and after 48 hours and graphed above. ABE20515, a humanized mAb that displays PD-1 antagonist activity, has an EC50 of 20ng/ml.

Anti-LAG-3 and anti-TIM-3 increase IL-2 secretion both alone and in combination with anti-PD-1 antagonist mAbs at 48 hours in the MLR assay. Anti-LAG-3 or anti-TIM-3 mAbs was characterized in the MLR assay alone (blue) or in combination with 20ng/ml human IgG (red) or 20ng/ml green IgG of anti-PD-1 antagonist mAbs. No effect is observed at 24 hours in PD-1 combination with anti-LAG-3 (blue panel). Combination with anti-PD-1 increases the potency of both anti-LAG-3 (middle panel) and anti-TIM-3 (bottom panel). EC50 data are summarized in the table.

References