Identification and Characterization of a Potent Anti-Human TIM-3 Antagonist

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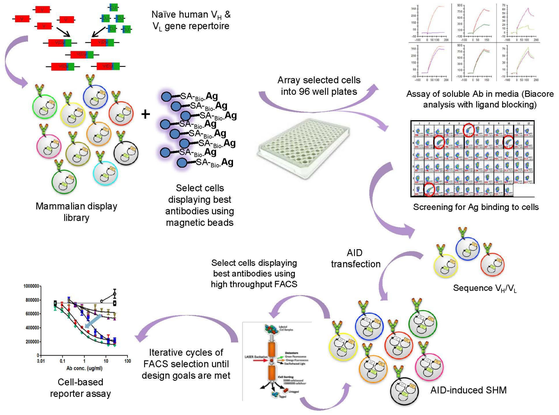
Abstract

The activation of anti-tumor immunity through the blockade of immune checkpoints has become one of the more promising approaches to tumor therapy. Significant clinical activity in a number of settings has been shown through the blockade of PD-1 or CTLA-4, although there remains room for improving the efficacy of these agents. TIM-3 (T-cell immunoglobulin and mucin-domain containing-3) has been reported to play a role as an additional immune checkpoint which may limit anti-tumor T cell responses. To identify potential therapeutic molecules that could enhance the activity of anti-PD-1 therapy in patients, we have generated a panel of human anti-human TIM-3 antibodies using SHM-XEL™, which combines mammalian cell display of human IgG with somatic hypermutation *in vitro* to select and mature antibodies with desired biological activities. Potent anti-TIM-3 antagonist antibodies, with pM affinities for human TIM-3 were identified. These antibodies enhanced T cell function at low nanomolar concentrations as measured by direct cytokine production *in vitro*, representing the most potent anti-TIM-3 antibodies known. In addition, anti-TIM-3 antibodies augment T cell activation in a dendritic cell/T cell mixed lymphocyte reaction. Assays were developed to enable the evaluation of simultaneous inhibition of multiple checkpoint molecules which demonstrated that combination of anti-TIM-3 therapeutic candidates with a novel anti-PD-1 antibody increased specific human T cell activation over that seen with blockade of a single checkpoint alone. Finally, the activity of anti-TIM-3 antibodies was tested in several syngeneic tumor models, including MC38. Anti-TIM-3 alone showed some inhibition of established MC38 tumor growth but was less potent than anti-PD-1 alone, while the combination of both antibodies resulted in sustained tumor regressions. These data suggest that monotherapy with anti-TIM-3 and combination immunotherapy with anti-TIM-3 and anti-PD-1 is worthy of clinical evaluation.

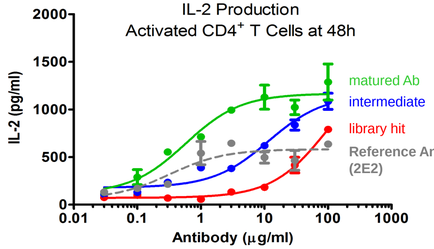


ABEL library screen and maturation overview.

Cells expressing surface-displayed, fully-human antibodies that bind to human TIM-3 were identified from a screening campaign of the ABEL library using magnetic beads coated with huTIM-3 extracellular domain. A panel of antibodies was isolated that bound specifically to TIM-3. Cells displaying anti-human TIM-3 antibodies were then transfected with activation-induced cytidine deaminase (AID) to initiate somatic hypermutation (SHM). Iterative rounds of FACS sorting with decreasing concentrations of antigen were performed to identify mAbs with increased affinity for TIM-3 and improved potency in functional assays.

Maturation of Anti-TIM-3 Antibodies

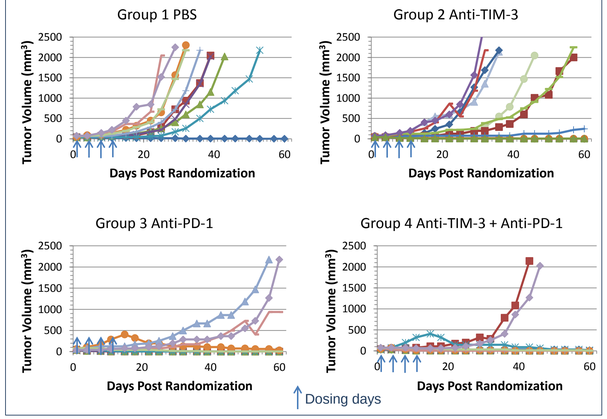
Maturation of the initial library hit antibodies was demonstrated by binding studies using Biacore as well as binding to TIM-3 presented on the surface of a CHO cell line. In addition antibodies with improved binding properties were tested in functional assays. Functional assays included measurement of IL-2 secretion from activated CD4+ T cells, and a dendritic cell/T cell MLR.



Reference antibody 2E2 was purchased from BioLegend. The antibody is described in Hastings *et al.,* (2009) *Eur. J. Immunol*. **39,** 2492-2501.

Activity in Syngeneic Tumor Models

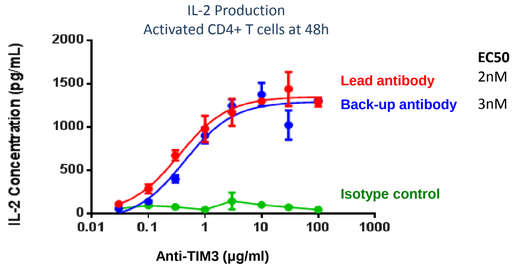
Surrogate antibodies recognizing mouse PD-1 (RMP1-14) and mouse TIM-3 (RMT3-23) were purchased from BioXcel, and tested alone and in combination in the MC38 syngeneic tumor model. MC38 tumor cells (1 x10^6 s.c.) were implanted into C57Bl/6 mice and grown for 10 days. Mice with tumors measuring 40-90 mm^3 were randomized (day of randomization designated day 1) to 4 groups of 10 animals/group and dosed with each antibody at 10mg/kg on days 1, 4, 8 and 11. Tumor volumes were measured twice weekly until reaching 2000 mm^3 which was designated as endpoint and mice were sacrificed. Each line represents the tumor growth for an individual animal.



Anti-TIM-3 Candidates are Potent *in vitro*

Matured antibodies were characterized to meet stringent requirements for therapeutic antibody development. This included assessment of "developabilty" criteria as well as functional potency across assays. Developabilty criteria included thermal stability, expression level, absence of problematic sequence motifs (Variable-region N-linked glycosylation sites, free cysteines, high-likelihood sites for deamidation, isomerization etc.). In addition, high affinity binding to cynomolgus monkey TIM-3 was selected for to facilitate preclinical studies.

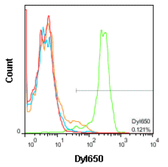
Lead and back-up antibodies with potent antagonistic activity were identified that met all criteria for further development.



	Biacore KD		Tm (Thermofluor analysis)	Non-specific binding	Purity Size Exclusion Chromatography
	Human TIM-3	Cyno TIM-3			
Lead Ab	50 pM	190 pM	72ºC	None detectable	>97%
Back-up Ab	<50pM	1.5nM	71ºC	None detectable	>97%

TIM-3 expression on activated human CD4+ T cells

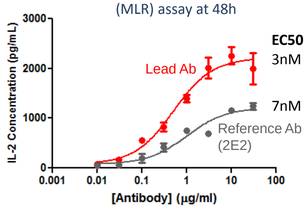
TIM-3 is expressed on activated T-cells. $CD4^+$ T-cells (red line) do not express TIM-3 on the cell surface after purification from human blood. However, 48 hours after culturing with DCs, TIM-3 is highly expressed on the T cells (green line). Levels expressed are subject to donor to donor variation. T cells were also cultured for 48 hours without DCs as a control (blue line). Isotype control staining of activated cells is also shown (orange line). PD-1 is expressed on a subset of $CD4^+$ T-cells without DC co-culture and can be upregulated at 24 and 48 hours in the MLR (data not shown). TIM-3 staining used PE conjugated rat anti-human TIM-3 (R&D Systems #344823).



Anti-TIM-3 Candidates Demonstrate Potent Activity in a Dendritic Cell / T Cell MLR and Have Increased Activity in Combination with anti-PD-1

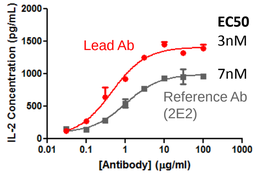
In vitro Comparison of Lead and Reference Antibodies

IL-2 Production
Mixed lymphocyte reaction (MLR) assay at 48h



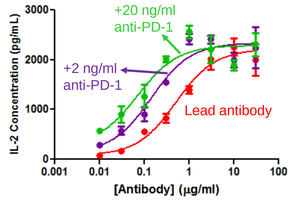
Isolated peripheral blood monocytes from a human donor were differentiated into dendritic cells (DC's) and then mixed with $CD4^+$ T-cells isolated from a second donor. IL-2 levels were measured after 48 hours.

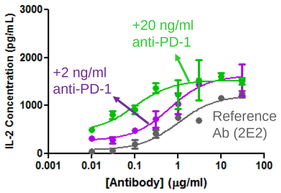
IL-2 Production
Activated CD4+ T cells at 48h



Peripheral blood $CD4^+$ T-cells were isolated and partially activated with plate bound anti-CD3 and CD28. IL-2 levels were measured after 48 hours incubation with antibody.

Mixed lymphocyte reaction (MLR) assay in Combination with Anti-PD-1





Anti-TIM-3 increases IL-2 secretion both alone and in combination with an anti-PD1 antagonist mAb at 48 hours in the MLR assay. Anti TIM-3 mAb alone or in combination with 2ng/ml (purple line) or 20ng/ml (green line) of anti-PD-1 antagonist mAb.

	EC50 Values	
	Lead Ab	2E2 reference
anti-TIM-3 alone	3nM	7nM
+2ng/ml anti-PD-1	0.93nM	4nM
+20ng/ml anti-PD-1	0.47nM	0.7nM

Conclusions

- A panel of high affinity anti-human TIM-3 antibodies has been generated using the ABEL library and in vitro somatic hypermutation
- A lead and a back-up antibody have been identified with potent activity in a $CD4^+$ T cell assay and an MLR
- Combination with anti-PD-1 leads to improved activity in an MLR
- Surrogate anti-PD-1 and anti-TIM-3 antibodies have activity in the MC38 tumor model
- This data suggests co-blockade of PD-1 and TIM-3 is worthy of further investigation