A novel anti-PD1-IL-15 immunocytokine potentiates anti-tumor T cell activity of PD-1 checkpoint blockade and IL-2/15R-beta-gamma agonism

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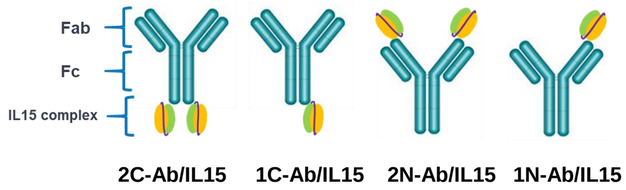
BACKGROUND

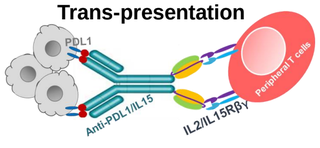
- IL-15 is a key cytokine that promotes CD8+ T, NK, and NKT cell proliferation by binding to the IL-2/15Rβγ receptor complex expressed on these cells.
- IL-15 has demonstrated clinical activity in cancer patients without evidence of any Treg stimulation (1, 2). However, its short half-life and systemic toxicity limit its usefulness.
- Kadmon has established an IL-15 fusion protein platform to extend the IL-15 serum half-life and direct its action to the tumor microenvironment, which would increase its activity and lower its toxicity.
- The platform includes four different formats in which one or two IL-15 complexes are fused to either C-terminal or N-terminal of the IgG.



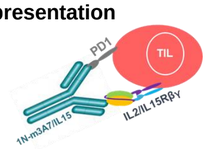
2C-Ab/IL15 1C-Ab/IL15 2N-Ab/IL15 1N-Ab/IL15

- The most advanced candidate of this platform is our clinical anti-PD-L1/IL-15 fusion protein, KD033 (3, 4) .
- We hypothesized that the orientation of the IL-15 fusion to the IgG could have specific benefits to the selected target/IL15 combinations. N-terminal fusion could work better for when the target and IL-2/15Rβγ are co-expressed on the same cells.

Trans-presentation



Cis-presentation



- Single IL-15 fused to anti-PD1 at the N-terminus (1N-anti-PD-1/IL-15) exhibited the lowest stimulation in cell-based assays. Surprisingly, it demonstrated strong anti-tumor activity in the PD-1 resistant Lewis lung model LL/2. We believe that this activity is s result of a longer IL-15 half-life and maximizing the bi-functionality of this fusion through simultaneous blockade of PD-1 and stimulation of IL-2/15Rβγ.

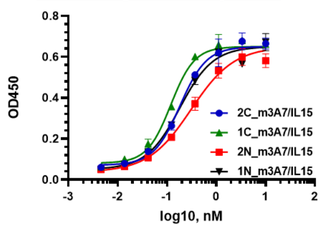
METHODS

- The four formats of fusion antibodies that contain IL-15 and m3A7 (Mouse PD-1 antibody) or DP47 (non-targeting antibody) were engineered and expressed transiently in CHOk1 with at least 95% of monomer after purified by Protein A and SEC-HPLC
- mPD-1 binding was examined by the standard ELISA, Biacore T200 and Guava® easyCyte™. Blockings of mPD-1 binding to mPD-L1 were evaluated by competition ELISA and Promega PD1/PD-L1 blockade assay
- Stimulation of IL-15 was examined by the proliferation of the IL-15 dependent mouse lymphocyte CTLL2 and mouse spleen cells
- *In vivo* efficacy of fusion antibodies was evaluated by LL/2 murine lung cancer syngeneic model in C57BL/6 Mice

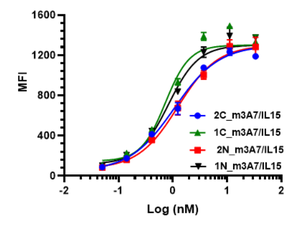
RESULTS

RESULT 1: anti-mPD-1/IL15 fusion antibodies bind to PD-1 and block PD-1 binding to PD-L1

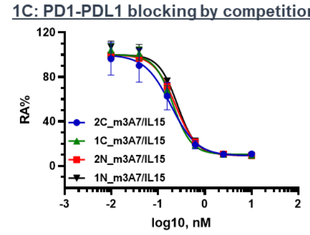
1A: Binding to mPD-1 by ELISA



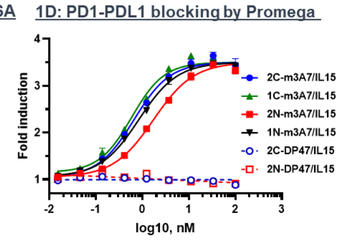
1B. Binding to mPD-1 transfected HEK293



1C: PD1-PDL1 blocking by competition ELSA



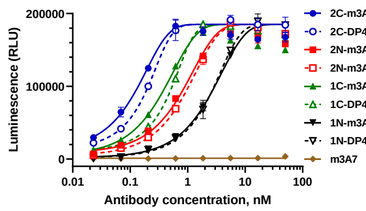
1D: PD1-PDL1 blocking by Promega



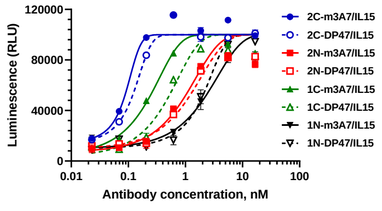
- 2C-, 1C, 2N-, 1N-m3A7/IL15 had similar binding affinity to both soluble and cell surface mPD1 (Fig. 1A and 1B).
- 2C-, 1C, 2N-, 1N-m3A7/IL15 demonstrated comparable blockade of soluble (Fig. 1C) and cell surface mPD1 (Fig. 1D) to PD-L1.
- Affinity kinetics by Biacore analysis for 2C-, 1C, 2N-, 1N-m3A7/IL15 were 0.25, 0.28, 0.30 and 0.46nM, respectively.

RESULT 2: anti-mPD-1/IL15 fusion antibodies stimulate IL2/15Rαβγ+lymphocyte proliferation

2A. Proliferation of CTLL2

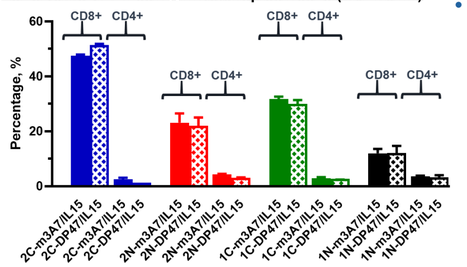


2B. Proliferation of C57BL6 spleen cells



- 2C-, 1C, 2N-, 1N-m3A7/IL15 can induce the proliferation of IL-2-dependent mouse Lymphocyte CTLL2 (Fig. 2A) and C57BL6 mouse spleen cells (Fig. 2B) with the following potencies: 2C>1C>2N>1N
- Only CD8+ T cells were expanded in mouse spleen cells (Fig. 2C)
- Activity is predominantly driven by IL-15: no significant differences are observed between anti-mPD1 fusions and non-target fusions (Fig. 2A, 2B and 2C)

2C. CD8+/CD4+ T cell % in total spleen cells (Ab=1.5nM)



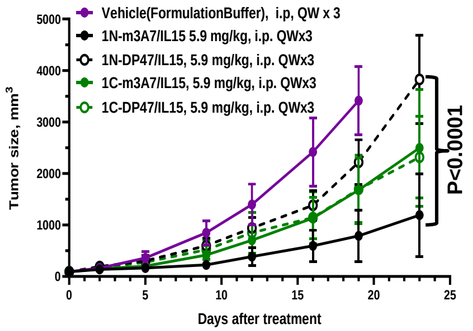
RESULT 3: 1N-anti-mPD-1/IL-15 fusion antibody shows strong bi-functionality by inhibiting tumor growth in a PD1-PDL1 resistant LL/2 murine lung cancer syngeneic model

3A. Tumor growth inhibition (TGI) after 2C-, 1C-, 2N-, and 1N- m3A7/IL15 treatment

Name		Antibody (D17)		2 x IL15 fusions		1 x IL15 fusions	
		RMP-14	m3A7	2C (D17)	2N (D16)	1C (D16)	1N (D16)
Dose	mg/kg	10	10	3.3	3.3	5.9	5.9
	nmol/kg	66.7	66.7	17.5	17.5	35.0	35.0
Frequency, route		BIW, IP		QW, IP		QW, IP	
Tumor growth inhibition, %		-4.9±36.6	9.6±34.9	56.4±25.2	46.7±21.4	53.1±16.7	75.4±12.5

- Non-fused m3A7 and commercial control mPD-1 antibody RMP1-14 showed minimal impact on tumor growth, all m3A7/IL-15 fusions exhibited significant tumor growth inhibition (Table 3A)
- 1N-m3A7/IL-15 fusion, which showed lowest stimulation in vitro had the strongest anti-tumor activity in vivo (Fig. 3A and 3B)
- 1N-m3A7/IL-15 was more efficacious than the non-targeted IL15 fusion 1N-DP47/IL15 (Fig. 3B)

3B. Tumor growth treated by 1N and 1C IL15 fusion antibodies



CONCLUSIONS

- Single IL-15 N-terminal fusion antibody 1N-m3A7/IL-15 showed strong PD1/PDL1 blockade, a modest stimulation of CD8+ T cells *in vitro*, and strong tumor growth inhibition *in vivo*. Further PK/PD studies with this fusion protein are ongoing; data will be submitted to future meetings.
- Our PK data (not shown) demonstrated that by lowering IL-15 stimulation on any fusion protein, the serum half-life increased significantly.
- Lower IL-15 potency and the skewed IL-15 activity solely toward TILs and other PD1-expressing cells in the tumor microenvironment through PD1 targeting may mitigate systemic toxicities, allowing:
  1. The increase of the fusion antibody concentration to reach clinically meaningful doses and a favorable therapeutic index
  2. Maximum bi-functionality potential with both PD1 blockade and IL-15 stimulation

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Disclosure: All authors are employed by Kadmon Corporation, LLC
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