A novel human anti-PD1/IL15 bi-functional protein with robust anti-tumor activity and low systemic toxicity

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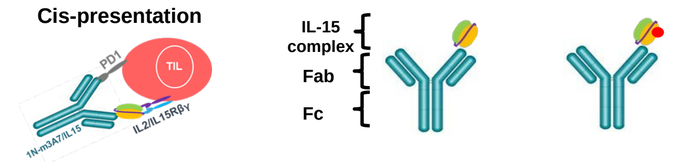
SITC 2020
Abstract ID: 573

BACKGROUND

- Recombinant IL-15 is a key cytokine promoting CD8+ T, NK, and NKT cell proliferation and has demonstrated clinical activity in cancer patients without evidence of Treg activation [1, 2]. However, its short half-life and systemic toxicity limit clinical utility.
- Kadmon has generated an IL-15 fusion protein platform to extend the serum half-life and direct its immune stimulatory activity to fight tumors [3].
- A novel asset of this platform is an anti-PD1/IL-15 bi-functional protein. To test the bi-functionality hypothesis of this fusion protein in murine models, four different formats of the surrogate bi-functional proteins were engineered by fusing mouse IL-15 complex to a anti-mouse PD1 antibody (m3A7).
- The single IL-15 complex fused to N-terminus of anti-mPD1 antibody (1N-IL-15/m3A7)[figure 1B] showed significant anti-tumor activity primarily due to cis -presentation to PD1 and IL2Rβγ co-expressed on TILs (figure 1A). The cis-presentation potentially maximizes the bi-functionality of PD1 blockade and stimulation [4].
- Here, we generated a novel therapeutic anti-human PD1 (38B2) antibody fused to the IL-15 complex. Furthermore, a point mutation in IL-15 was introduced to the bi-functional protein to lower its stimulation and reach a therapeutic dose of anti-PD1 (figure 1C). The PD1 functions, stimulations and anti-tumor activities of IL-15/38B2 fusion antibodies were evaluated *in vitro* and *in vivo*.



1A: cis-presentation of fusion antibody to the TIL

1B: 1N-IL-15/m3A7 (38B2 or DP47, negative control)

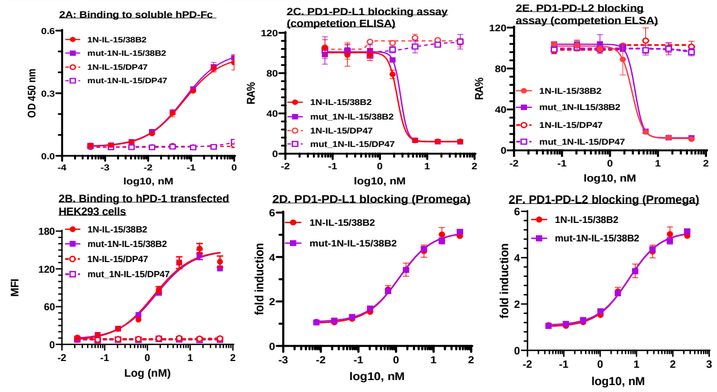
1C: mut-1N-IL-15/38B2 (DP47). A point mutation was introduced in IL-15

METHODS

- 1N-IL-15/38B2, 1N-IL-15/DP47 (non-targeting negative control antibody), mut-1N-IL-15/38B2 and mut-1N-IL-15/DP47 were engineered and expressed transiently in CHOk1 with at least 95% of monomer after purified by Protein A and SEC-HPLC.
- PD-1 binding activities were examined by the standard ELISA, Biacore T200 and Guava® easyCyte™. Blockings of PD-1 binding to PD-L1 or PD-L2 were evaluated in competition ELISA and Promega PD1/PD-L1 or PD-L2 blockade assay.
- Stimulating activities of IL-15 fusions were measured by the proliferation of the IL-2/-15 dependent CTLL2 (mouse lymphocyte) and M07e (acute human megakaryoblastic leukemia), mouse spleen cells and human PBMC.
- *In vivo* efficacy was evaluated in hPD1/hPDL1 transgenic BALB/c mice bearing hPD-L1-CT26 tumors. The fusion antibodies (once per week) and controls (twice per week) were injected (intraperitoneal) when the tumor size reached approximately 100mm^3 .

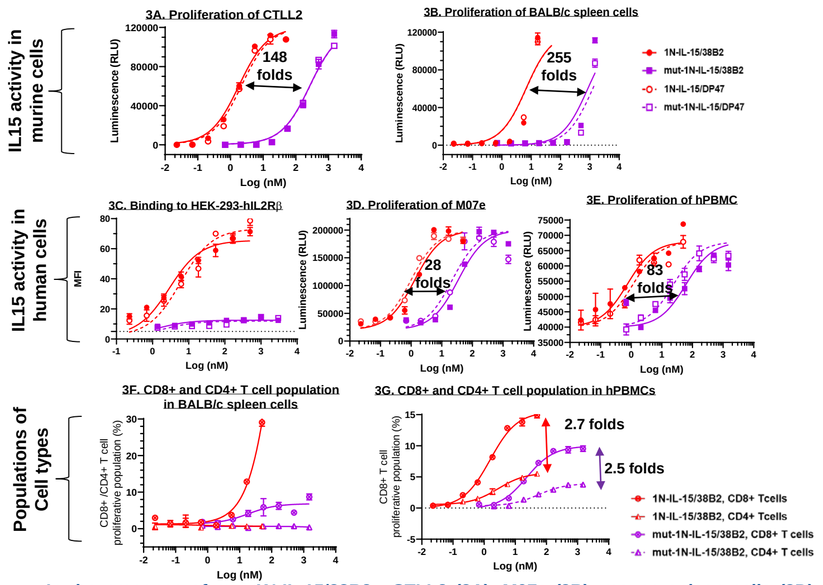
RESULTS

RESULT 1: 1N-IL-15/38B2 and mut-1N-IL-15/38B2 Showed Comparable Binding and Blocking Activities



- 1N-IL-15/38B2 and mut-1N-IL-15/38B2 bound strongly to soluble PD1 (2A) and to PD1 overexpressing human cell line (2B).
- 1N-IL-15/38B2 and mut-1N-IL-15/38B2 blocked the hPDL-1 or hPDL-2 binding to hPD1 in both competition ELISA (2C or 2E) and Promega blockade assay (2D or 2F).
- 1N-IL-15/38B2 and mut-1N-IL-15/38B2 did not change 38B2 CDRs and hence did not impact PD1 blocking activities.
- Binding affinity measured by Biacore for 1N-IL-15/38B2 and mut-1N-IL-15/38B2 were 0.08 and 0.18nM respectively

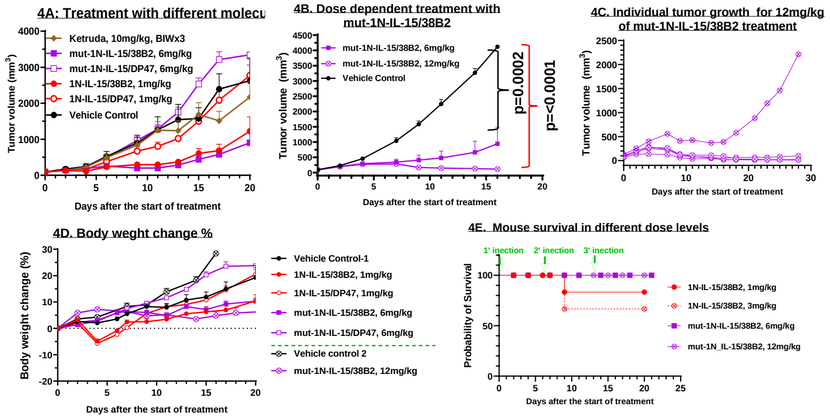
RESULT 2: mut-1N-IL-15/38B2 Showed Significant Potency Decrease in Proliferating hPBMCs, CTLL2 , Mouse Spleen and M07e Cells



- In the presence of mut-1N-IL-15/38B2, CTLL2 (3A), M07e (3B), mouse spleen cells (3D) and hPBMCs (3E) grew slower than in the presence of wild type 1N-IL-15/38B2. Fold decreases in potency between wild type and mutated fusions were 148, 255, 28 and 83 respectively. Binding of mut-1N-/38B2 to hIL2Rβ transfected HEK293 diminished (3C).
- No differences were observed between PD1 and non-targeting fusions in binding to hIL2Rβ (2C) or proliferation/stimulation of human (3D and 3E) and mouse lymphocytes (3A and 3B).
- As expected, 1N-IL-15/38B2 and mut-1N-IL-15/38B2 induced a better dose response in proliferating CD8+ T cells than CD4+ T cells in both mouse spleen (3F) and hPBMCs (3G).

RESULTS

RESULT 3: Both 1N-IL-15/38B2 And mut-1N-IL-15/38B2 Exhibited Strong Anti-tumor Activity; mut-1N-IL-15/38B2 Was Safer At High Doses



- In pembrolizumab resistant hPDL1-CT26 model, bi-functional fusions of both 1N-IL-15/38B2 and mut-1N-IL-15/38B2 exhibited strong antitumor activity (4A).
- Anti-tumor efficacy of mut-1N-IL-15/38B2 was dose dependent (4B). 6mg/kg of mut-1N-IL-15/38B2 was comparable to 1mg/kg of 1N-IL-15/38B2 (4A). 12mg/kg, QW x 3 weeks of mut-1N-IL-15/38B2 demonstrated tumor regression (4B and 4C).
- No weight loss or mortality was observed with mut-1N-IL-15/38B2 when dosed at 12mg/kg, but significant weight loss and mortality were observed with 1N-IL-15/38B2 when dosed at ≥1mg/kg (4D and 4E).
- Interesting, non-targeting 1N-IL-15 fusion did not show any anti-tumor efficacy. Interestingly, as expected significant weight loss (4D) and mortality were observed when dosed ≥1mg/kg (data not shown).

CONCLUSIONS

- Early data showing robust anti-tumor activity with surrogate 1N-IL-15/m3A7 in a mouse Lewis Lung LL2 model was confirmed with the therapeutic bi-functional antibodies in a pembrolizumab resistant hPDL1-CT26 colorectal model.
- Our experiments demonstrated that mut-1N-IL-15/38B2:
  1. Retained full PD1 blocking activities and exhibited significant potency decrease in proliferating IL2Rβγ expressing cells
  2. Induced tumor regression in a hPD1-CT26 model at 12 mg/kg highlighting the bi-functionality of this fusion when it bound to PD1 and IL2Rβγ co-expressed on TILs
- Mutated IL-15 lowered potency/stimulation which resulted in lower toxicity and increase therapeutic treatment window.
- These promising results warrant further investigation of this bi-functional in the clinic.

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