Bispecific Antibody-Based Redirection of Endogenous IL-15 to PD-1+ Cells Enhances Antitumor Activity Over Checkpoint Inhibition Alone

LB 137

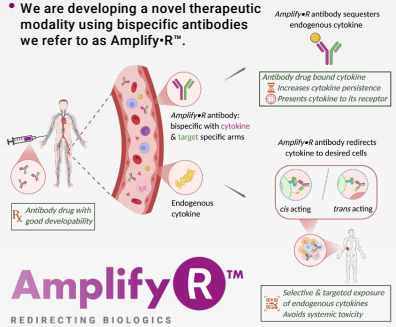
Surjit Dixit[1], Mark H Fogg[1], Stacey Tom-Yew[1], Harsh Pratap[1], Vivian Li[1], Abhishek Mukhopadhyay[1], Yuneivy Cepero Donates[2] and David de Graaf[1]

[1] Reverb Therapeutics, 2405 Westbrook Mall, Vancouver BC V6T 1Z3, Canada;

[2] Human Health Therapeutics Portfolio & Medical Devices Research Centre, National Research Council, 6100 Royalmount Ave, Montreal QC H4P 2R2, Canada.

INTRODUCTION

- Cytokines have the potential to reinvigorate the immune response against tumors, address shortcomings of checkpoint inhibition and expand the reach of immunotherapy.
- Development efforts with recombinant cytokines, their engineered muteins and fusion molecules have encountered challenges.
- We are developing a novel therapeutic modality using bispecific antibodies we refer to as Amplify•R™.



Amplify R™
REDIRECTING BIOLOGICS

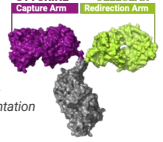
- Amplify•R antibodies engage the naturally available endogenous cytokines *in vivo*, enhance persistence of the bound cytokine, while regulating and redirecting its therapeutic effect to target cells of interest.
- We hypothesize that this modality will overcome limitations such as systemic toxicity, increased immunogenicity, and manufacturing challenges often associated with traditional recombinant cytokine treatment approaches.

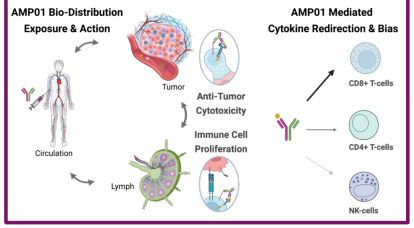
AMP01: DESIGN OF A PD-1 DIRECTED IL-15 AMPLIFY•R™

We have designed a panel of bispecific antibodies capable of co-engaging the T and NK cell stimulating cytokine, IL-15, and the immune checkpoint, PD-1. Data from a representative AMP01 molecule is shown on this poster.

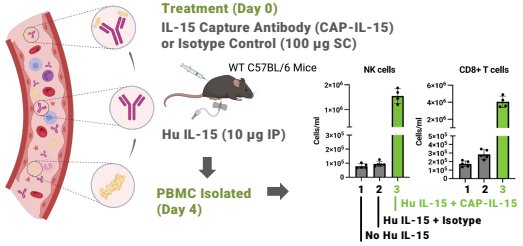
Increase Efficacy
Selective Engagement of CD8+ T-cells
PD-1 mediated avidity for IL-15 receptor engagement can drive preferential activation of tumor primed T-cells.

Reduce Toxicity
Avoid Engagement of NK-cells
Modulation of IL-15 affinity for receptor chains upon Amplify•R mediated presentation can bias against activation of NK-cells.



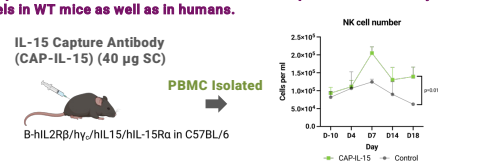


IN VIVO CAPTURE OF CYTOKINE BY ANTIBODY AMPLIFIES CYTOKINE EFFECT



- Treatment of wild type C57BL/6 mice with CAP-IL-15 and Hu IL-15 results in *in vivo* complexation and amplifies cytokine effect, resulting in significant proliferation of IL-15Rβγ expressing T & NK cells

In a second *in vivo* study, CAP-IL-15 was dosed in an engineered C57BL/6 mice, humanized for IL-15, IL-15Rα and IL-2/15Rβγ. Endogenous IL-15 and receptor levels in these humanized mice are comparable to natural cytokine levels in WT mice as well as in humans.



- Treatment (on Day 0) induces significant *in vivo* expansion of cells expressing IL-15Rβγ, and this effect is driven by amplification of naturally available endogenous IL-15

AMP01, A MULTIFUNCTIONAL ANTIBODY: INHIBITS PD-1 ACTIVITY AND INDUCES IL-15 SIGNALING

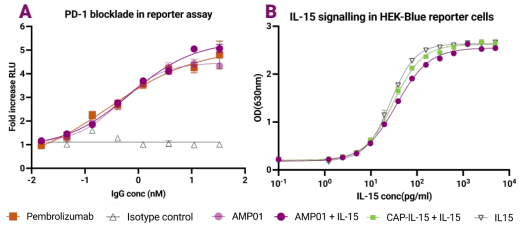


Figure 1. Dose dependent blockade of PD-1 signaling (A) and IL-15 signaling (B) in cell-based reporter assays

A

- AMP01 Amplify•R and control anti-PD-1 clinical benchmark antibodies were evaluated in the Promega™ PD-1/PD-L1 Blockade Bioassay
- Blockade of PD-1 results in an increase in luciferase reporter activity
- Potency of AMP01 molecules with PD-1 binding was identical to the clinical benchmark
- Precomplexing AMP01 molecules with IL-15 did not impact potency in the PD-1 assay

B

- AMP01 Amplify•R and control IL-15 capture (CAP-IL-15) antibodies were precomplexed with the indicated concentration of IL-15 and added to an IL-15Rβγ reporter cell line (Invivogen™ HEK Blue). Level of signaling was determined by SEAP activity
- IL-15 capture and presentation to IL-15Rβγ by AMP01 molecules results in productive IL-15 signaling *in vitro*

AMP01 REDIRECTS IL-15 MEDIATED pSTAT5 SIGNALING TO PD-1 EXPRESSING PRIMARY CELLS

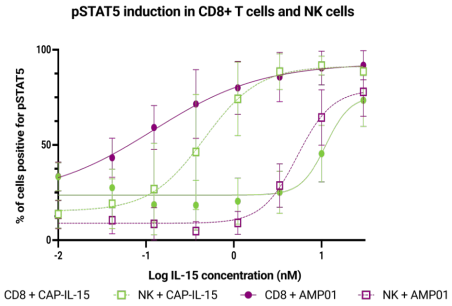


Figure 2. IL-15 dependent pSTAT5 activity in *in vitro* activated PBMC

- Pre-activated PBMC from 3 independent human donors (cultured with CD3/CD28 beads for 48h to induce PD-1 expression) were stimulated for 20 minutes with the indicated concentration of IL-15 pre-complexed with AMP01 Amplify•R or control IL-15 Capture antibody (CAP-IL-15)
- pSTAT5+ population within either CD3+/CD8+ T cells or CD56+ NK cells was determined by flow cytometry
- CAP-IL-15 preferentially induced pSTAT5 in NK cells over CD8+ T cells, with 1.5-fold increased potency
- The AMP01 Amplify•R molecule redirected IL-15 mediated pSTAT5 activity away from NK cells towards CD8+ T cells with greater than 2 log increase in potency
- This demonstrates *in vitro* that the AMP01 molecules can modulate IL-15 activity away from NK cells and towards PD-1 expressing T cells

AMP01 REDIRECTS IL-15 INDUCED PROLIFERATION TOWARDS PRIMARY T CELLS

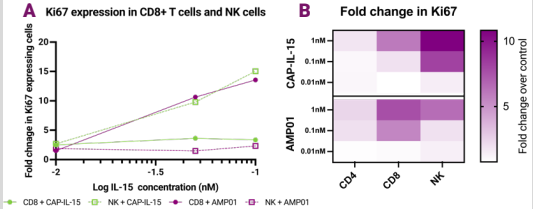


Figure 3. IL-15 dependent proliferation of CD8+ T cells and NK cells

- Human PBMC were cultured for 4 days in the presence AMP01 or control IL-15 capture (CAP-IL-15) antibody precomplexed with the indicated concentration of IL-15. Cell subset proliferation was monitored by Ki67 staining and flow cytometry
- In a representative donor, the CAP-IL-15 antibody preferentially induced Ki67 in NK cells versus CD8+ T cells whereas the AMP01 molecules redirected IL-15 induced proliferation towards CD8+ T cells (A)
- PD-1 mediated redirection of IL-15 induced proliferation was consistent across 3 independent donors as shown in the heatmap (B) of fold change in Ki67 expression in different cell subsets induced by CAP-IL-15 antibody or the AMP01 molecule

AMP01 EFFECTIVELY CONTROLS TUMOR GROWTH IN AN *IN VIVO* MODEL

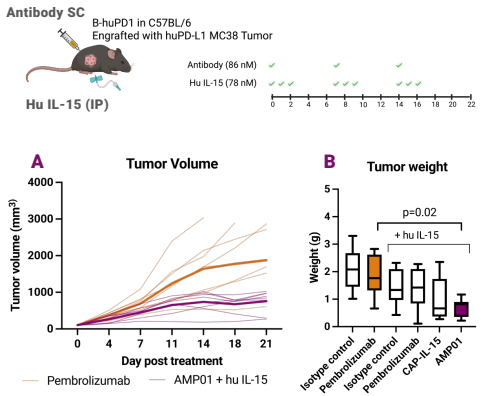


Figure 4. *In vivo* tumor growth (A) and tumor weight at study end point (B)

- AMP01 molecules were evaluated in a humanized PD-1 mouse using a humanized PD-L1-MC38 tumor model that is responsive to checkpoint inhibition treatment
- Mice were treated weekly with equimolar anti-PD-1 dose as either AMP01 Amplify•R™ or control antibodies, in the presence or absence of 3 consecutive daily doses of 1 µg of human IL-15
- Tumor growth was monitored during the duration of the study. AMP01 in the presence of IL-15 effectively controlled tumor volume (A) compared to pembrolizumab
- In addition, tumor weight (B) at sacrifice on day 21 was significantly reduced by AMP01 molecules in the presence of IL-15 compared to pembrolizumab or pembrolizumab with human IL-15

CONCLUSION

In summary, we show that the Amplify•R modality to endogenous cytokine engagement and redirection may be a viable approach in clinic, capable of overcoming limitations encountered with traditional approaches to cytokine therapy. Our lead Amplify•R candidate, AMP01, is progressing towards candidate selection and IND enabling studies.

Contact
Surjit Dixit, PhD
Reverb Therapeutics
surjit@reverbtx.com
https://www.ReverbTx.com