Conditional Immune Activation with Daiad-2/15: a Targeted, Hyperpotent IL-2/IL-15 Agonist with an Engineered Logic Gate

Abstract #851

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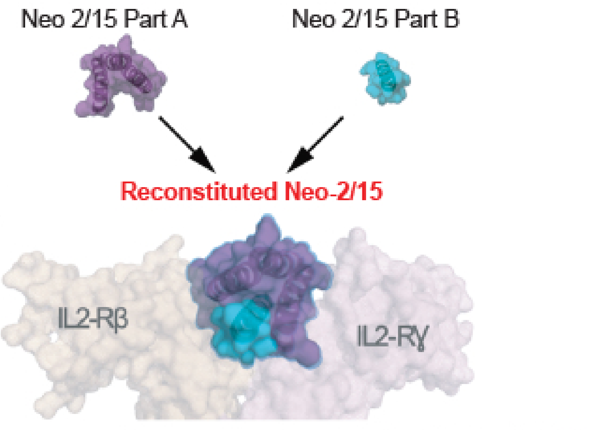
1. Abstract

Although high-dose interleukin-2 (IL-2) has been approved for the treatment of certain cancers since 1992, widespread use has been limited due to systemic toxicity, including cytokine release syndrome and vascular leak syndrome. Versions of IL-2 with reduced potency have been studied but reduce efficacy as well as toxicity. We have developed Daiad-2/15 as a de-novo designed, highly potent IL-2/IL-15 agonist (Neo-2/15) that is targeted and conditionally activated to deliver anti-tumor activity to the tumor microenvironment without systemic immune activation. A logic gate was engineered by splitting Neo-2/15 into two separate pieces, one comprising three alpha helices and the other one helix. The individual domains are unable to bind or activate the IL-2 receptor complex but recombine into Neo-2/15 when the two pieces are brought into close proximity. By fusing each portion of the cytokine mimetic to a single domain antibody, we created two distinct modules, which together constitute Daiad-2/15. We have developed Daiad-2/15 variants that target antigens on the surface of specific T cells, leading to IL-2R signaling and activation in a target-dependent manner. In vitro, Daiad-2/15 treatment activates T cells that express the targeted antigen, with a 10,000-fold lower threshold for signaling compared to antigen-negative T cells. Furthermore, Daiad-2/15 does not trans-activate target-negative cells, even when present in an admixed culture. Finally, Daiad technology enables an 'AND' logic gate by targeting each of the inactive domains to different targets, such that immune cell activation and proliferation only occur when both targets are present on the same cell. Together, our data suggest that Daiad-2/15 could potentiate the systemic delivery of a highly potent immune agonist to specific immune cells within the tumor microenvironment and draining lymph nodes in the absence of significant systemic toxicity.

2. Neo-2/15 split peptides

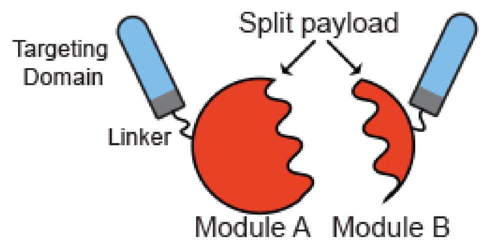
- Neo-2/15 can be divided into Part A (3 helices) and Part B (1 helix)
- Neither piece can bind and activate IL2R signaling unless reconstituted
- At high concentration and in the presence of IL2R, binding activity is reconstituted



Quijano-Rubio et al, Nat Biotech 2022

3. Daiad-2/15 design

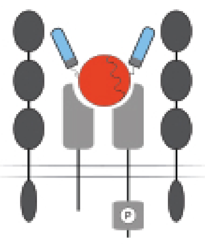
- Module A and Module B each consist of one part of the split cytokine mimetic and one targeting domain that recognizes cell surface antigens on exhausted T cells
- The payload and targeting domain of each module are connected by a hydrophilic, flexible, non-cleavable peptide linker



4. Daiad-2/15 for cis-targeting of immune effector cells

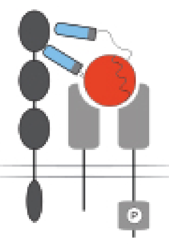
Single Antigen

Modules A and B can use the same targeting domain for antigens that are expressed at high density



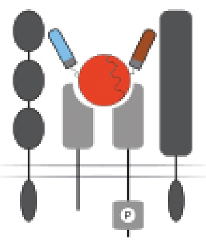
Single Antigen Biparatopic

Two different epitopes on the same antigen can be targeted to activate IL-2 signaling in a density-independent manner



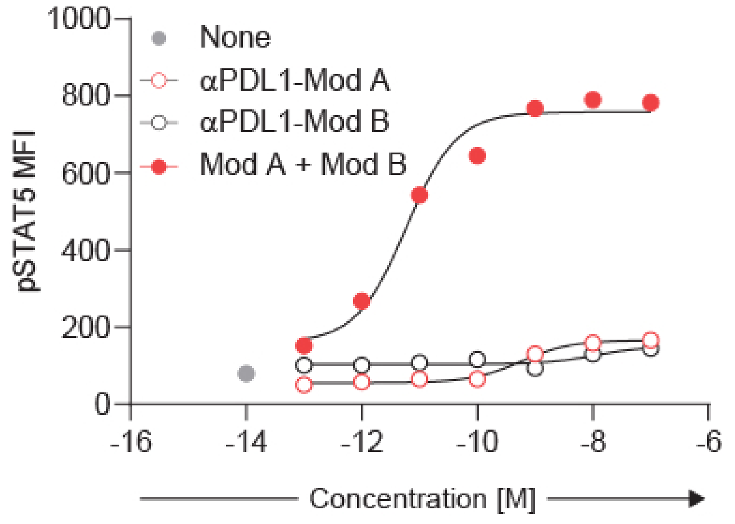
Dual Antigen

Two different antigens can be targeted to improve specificity of targeted cells. Activity is dependent on co-expression of both antigens on the cell surface



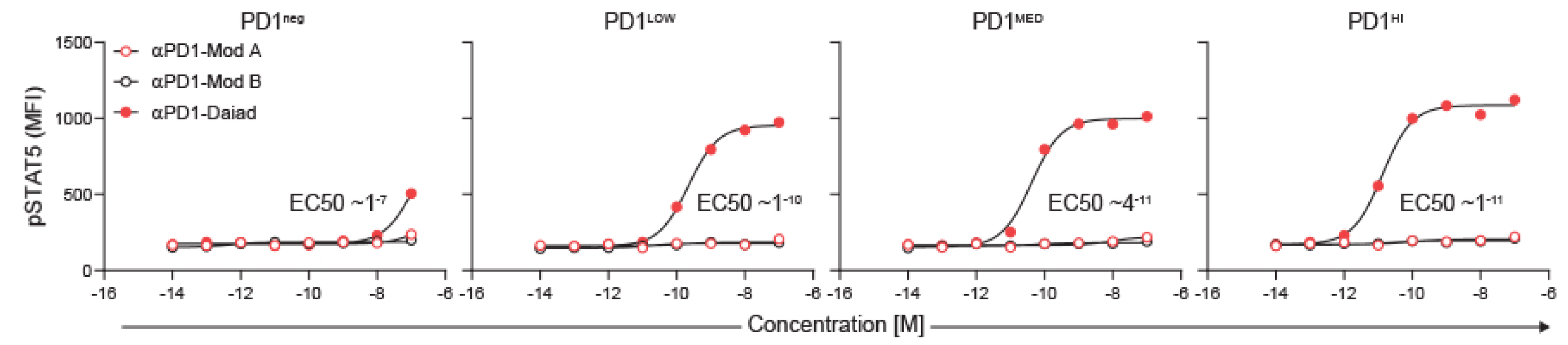
5. Daiad-2/15 activates primary T cells

- Module A and Module B were each targeted to murine PDL1 using a mPDL1 specific VHH
- Primary human T cells were engineered to express mPDL1
- Cells were stimulated with Module A alone, Module B alone, or both together
- STAT5 phosphorylation was used to evaluate IL2R signaling
- Signal transduction occurs at pM concentrations when both modules are present



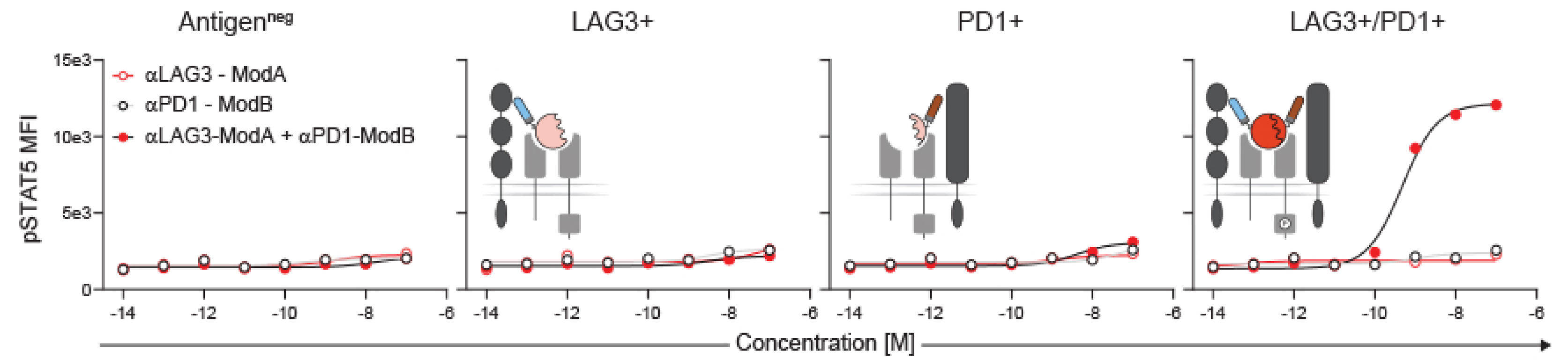
6. Daiad-2/15 activation is antigen dependent

- Generated Daiad-2/15 modules fused to anti-human PD1 nanobody
- Treated HH cells engineered to over-express human PD1 with αPD1 Daiad-2/15
- Antigen-negative cells had no pSTAT5 activation at Daiad-2/15 concentration less than 10^{-7} M
- PD1+ cells were activated at ~10,000-foldt lower Daiad-2/15 concentration



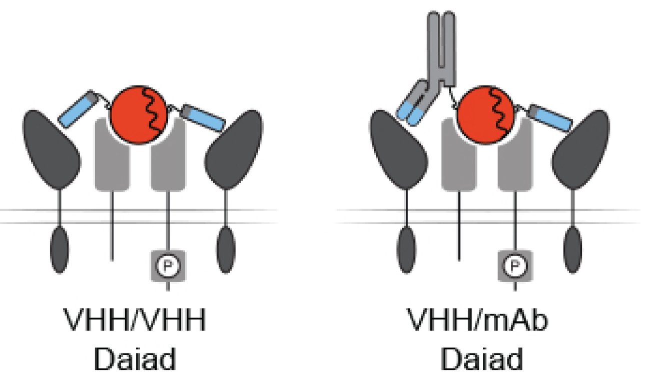
7. Daiad-2/15 with dual antigens creates a logic gate

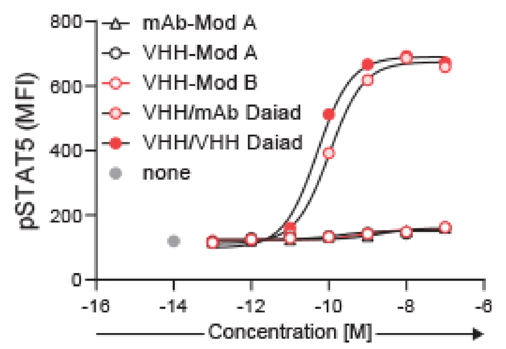
- HH cells were engineered to express LAG3, PD1, or both antigens, confirmed by FACS
- Anti-LAG3 Module A and anti-PD1 Module B were tested for the ability to initiate signaling
- IL2R-dependent pSTAT5 was detected only when both antigens were expressed
- 'AND' logic gate increases selectivity of targeted cells



8. Daiad-2/15 is compatible with multiple targeting domains

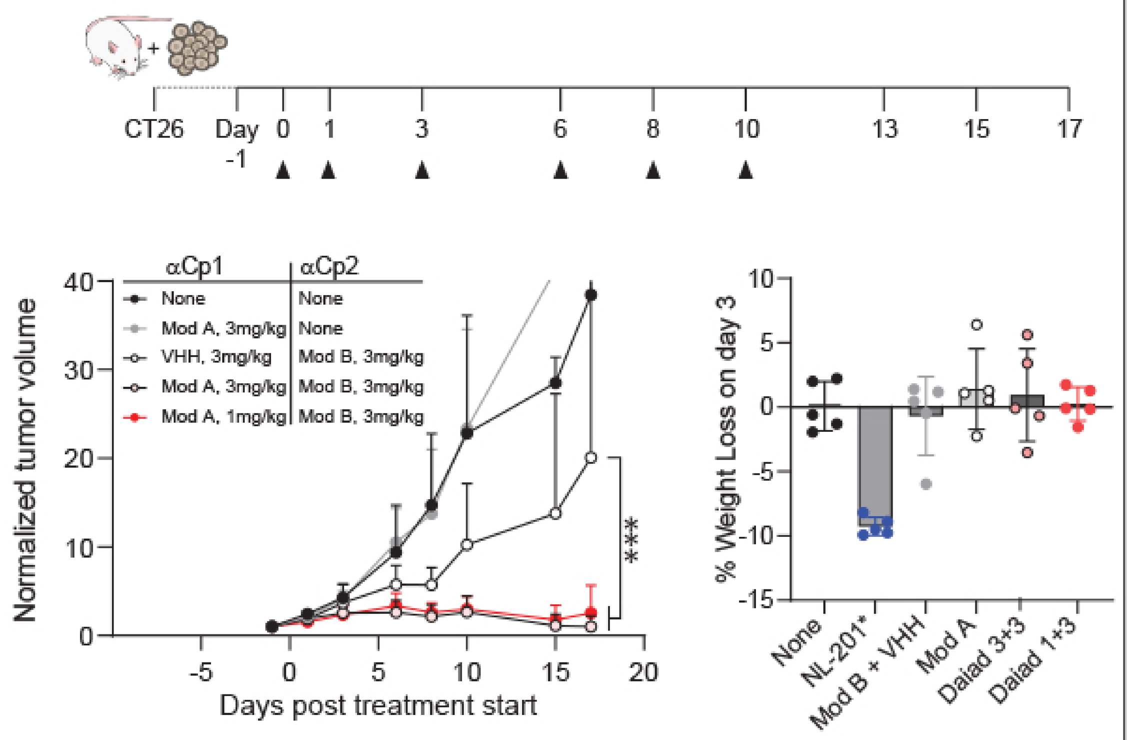
- Engineered PD1-specific Daiad-2/15 module A with either a PD1-specifc VHH or a full length αPD1 antibody with one Fab arm replaced by module A
- The ability of Module A/B pairs to activate antigen-positive cells was tested with each Module A format
- No significant difference in pSTAT5 activation with either VHH or mAb versions of Module A





9. Daiad-2/15 is active and safe in a syngeneic tumor model

- CT26 tumors were treated with Daiad-2/15 fused to checkpoint (Cp) specific nanobodies. All molecules contained an HSA-specific VHH for half-life extension
- NL-201* (0.3mpk) was used as a positive control. Mice died on D5 due to toxicity.
- 40% of animals in 1/3 mpk or 3/3 mpk cohorts had unmeasurable tumors after 3 weeks
- No evidence of weight loss or behavior changes with Daiad-2/15 up to 3mpk



*Mortales et al, Canc Imm Res 2023

10. Summary

- Daiad-2/15 is a novel approach to targeted delivery of highly potent immune agonists for the treatment of cancer
- Flexible, robust design enables an 'AND' gate to improve specificity
- Cis-activation of exhausted T cells abrogates systemic toxicity
- In vivo data demonstrates well-tolerated monotherapy activity
- StradBio plans to advance Daiad-2/15 program into clinical testing

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