Regulated Membrane-Bound IL15 Drives Controlled Expansion of Tumor Reactive Lymphocytes

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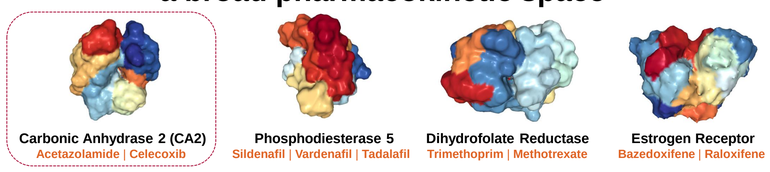
Introduction

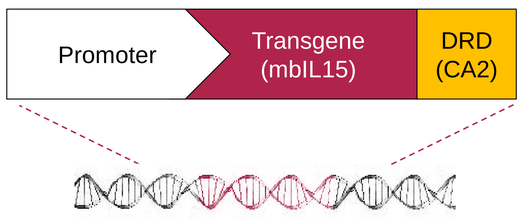
- **Drug responsive domains** (DRD) are human proteins stabilized by FDA-approved drugs
- Tagging a potential therapeutic protein with a **DRD could confer drug-like properties to cell or gene therapy** by controlling timing, level, and activity of protein expression
- Regulating expression of interleukin 15 (IL15) could **increase efficacy of immune cell therapy** while preventing toxicity that could occur from continuous IL15 exposure

How DRD technology works

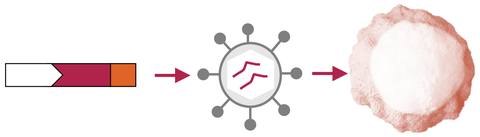
1. DRDs coupled to the drugs that regulate them cover a broad pharmacokinetic space



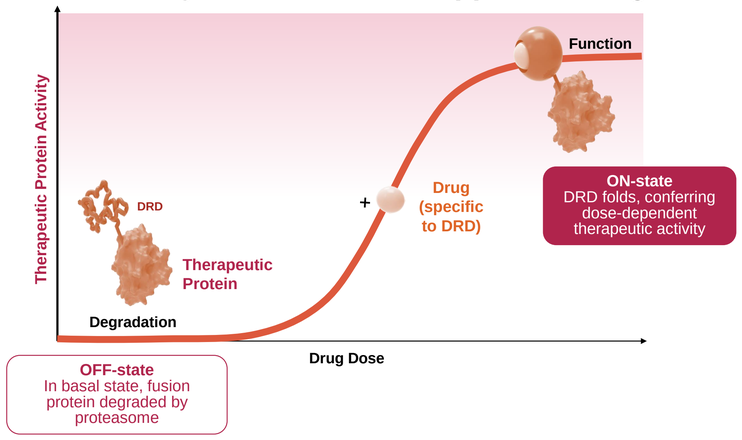
2. Engineer expression cassette encoding therapeutic protein of interest fused to DRD



3. Gene transfer of transgene-DRD expression cassette into cells *ex vivo* or *tissue in vivo*



4. Control the level and timing of protein DRD fusion by addition of FDA-approved drugs

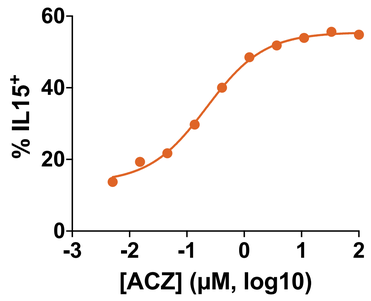


DRD-regulated IL15 controls the level of T and NK cell expansion

Why regulate IL15?

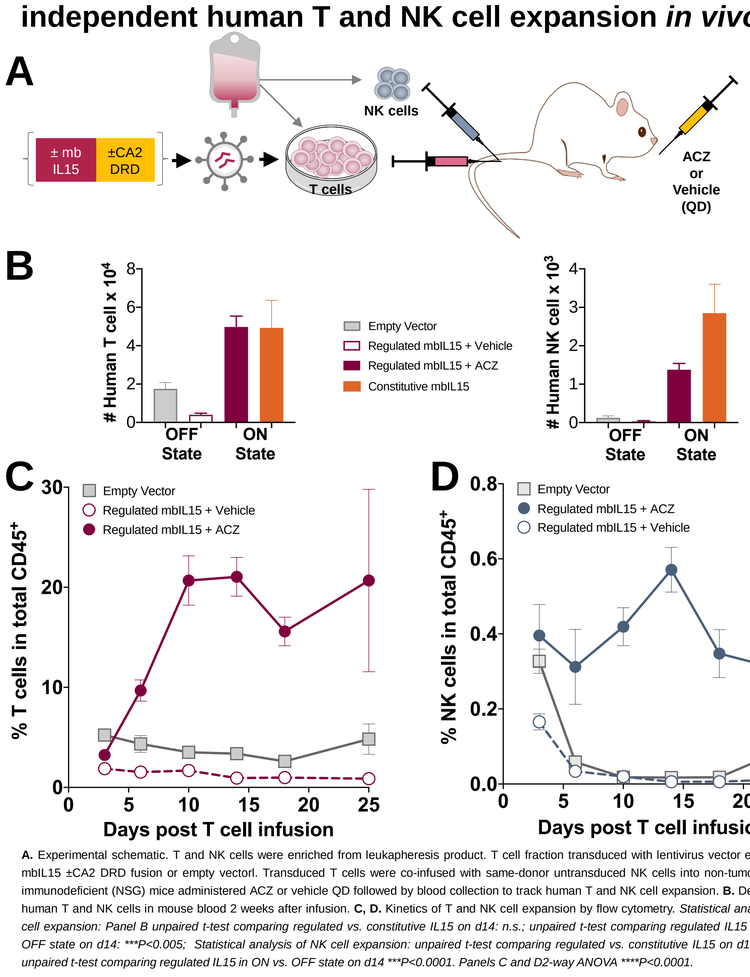
- **Drive toward memory phenotype** to increase durability of response
- **Expand T & NK cells** in presence of tumor & control antigen independent expansion, survival & persistence after tumor clearance
- **Mitigate toxicity** from chronic systemic or constitutive IL15

FIGURE 1. ACZ induces drug concentration-dependent expression of CA2 DRD-regulated membrane-bound (mb)IL15 in transduced T cells



IL15 expression was evaluated by flow cytometry in human T cells 1 week after transduction with mbIL15 CA2 DRD lentivirus vectors

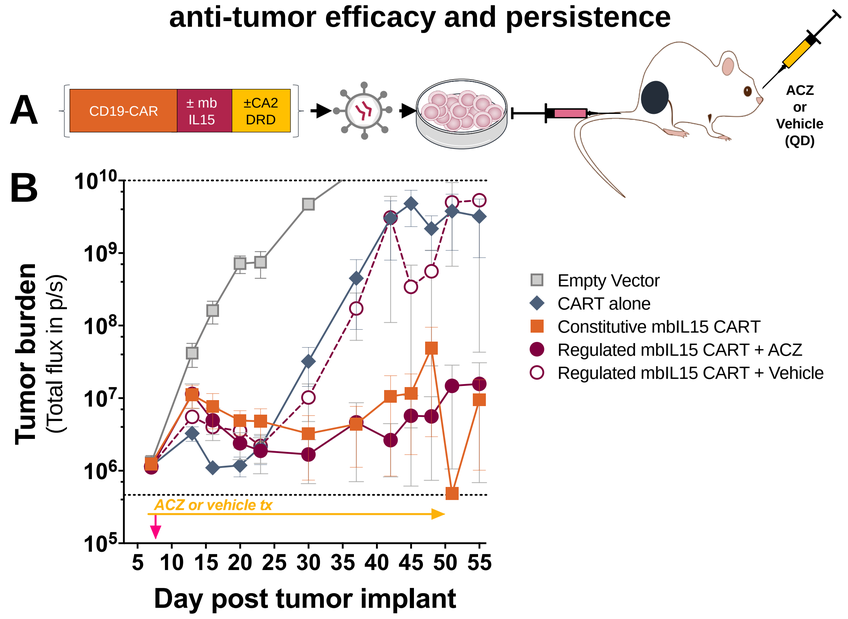
FIGURE 2. ACZ drives regulated IL15-induced antigen-independent human T and NK cell expansion *in vivo*



A. Experimental schematic. T and NK cells were enriched from leukapheresis product. T cell fraction transduced with lentivirus vector expressing mbIL15 ±CA2 DRD fusion or empty vectorl. Transduced T cells were co-infused with same-donor untransduced NK cells into non-tumor bearing immunodeficient (NSG) mice administered ACZ or vehicle QD followed by blood collection to track human T and NK cell expansion. **B.** Detection of human T and NK cells in mouse blood 2 weeks after infusion. **C, D.** Kinetics of T and NK cell expansion by flow cytometry. *Statistical analysis of T cell expansion: Panel B unpaired t-test comparing regulated vs. constitutive IL15 on d14: n.s.; unpaired t-test comparing regulated IL15 in ON vs. OFF state on d14: ***P<0.005; Statistical analysis of NK cell expansion: unpaired t-test comparing regulated vs. constitutive IL15 on d14 P<0.05; unpaired t-test comparing regulated IL15 in ON vs. OFF state on d14 ***P<0.0001. Panels C and D2-way ANOVA ****P<0.0001.*

DRD-regulated IL15 increases CART efficacy and increases survival

FIGURE 3. ACZ regulated mbIL15 increases CART anti-tumor efficacy and persistence



A. Experimental schematic. To evaluate regulation by ACZ, activated human T cells were transduced with lentiviral vectors expressing a human CD19-targeting CAR +/- constitutive or CA2 DRD regulated mbIL15, expanded, frozen, thawed, and 0.3 million CAR+ T cells infused into NSG mice bearing CD19+ luciferase+ Nalm6 tumors. Mice were orally dosed with ligand or vehicle daily and tumor growth monitored by bioluminescent imaging (BLI). **B.** Tumor burden by BLI. **C.** Overall survival analysis of tumor bearing mice. **D.** Detection of human T cells in the peripheral blood of tumor implanted mice after human T cell infusion. *Statistical analysis of Panel B tumor burden 2-way ANOVA ****P<0.0001; Panel C Kaplan Meier estimate of survival and log rank test ****P<0.0001; Panel D Human cell expansion in blood 2-way ANOVA **P<0.01.*

Conclusions

- **DRD-regulatable IL15:**
- **Supports** antigen-independent T cell expansion
- **Increases** bystander NK cell persistence
- **Enhances** anti-tumor activity of CART cells