C4 Therapeutics

Conquering Disease
With Targeted
Protein Degradation

Development of AchillesTAG degradation systems and their application to control CAR-T activity

C4 Therapeutics team

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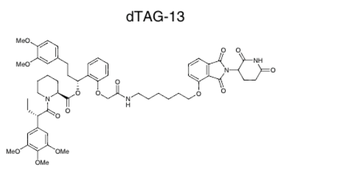
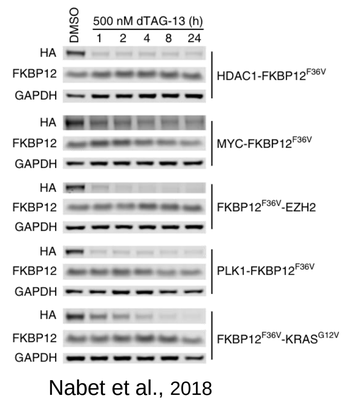
BACKGROUND

In recent years, several chemical genetic approaches have emerged that enable the rapid and acute control of target protein homeostasis. These technologies include the auxin-inducible degron (AID)[1], dTAG system[2], HaloTAG PROTACS[3,4], small-molecule-assisted shutoff (SMASh)[5], cryptic degrons[6], and the Shield system[7]. These sophisticated technologies have elevated the understanding of target biology in both *in vitro* and *in vivo* settings and exemplified the power of rapid target protein modulation. With these technologies as an inspiration, we aimed to develop additional small molecular degradation tags that would allow for the pharmacologic control of protein homeostasis via heterobifunctional molecule mediated target degradation. We developed two non-overlapping aTAG systems that provide the flexibility in choice of aTAG properties and the opportunity to multiplex aTAG systems within the same context to further explore complex biology. Each aTAG system comes with a suite of degrader molecules with a range of *in vivo* properties that allow for optionality to meet the needs of a given *in vivo* application. We applied these aTAG systems as a solution to the increasing demand of mechanisms to control Chimeric Antigen Receptor T cell (CAR-T) activity. We show that pharmacologic control of a chimeric antigen receptor (CAR) via targeted protein degradation (mediated by an aTAG) provides a cell-sparing, reversible, and tunable approach.

AchillesTAG DEGRADATION SYSTEMS

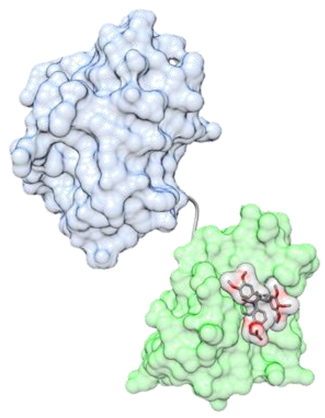
Engineering novel degradation tags: aTAG





Nabet et al., 2018

Exemplified with FKBP12* dTAG

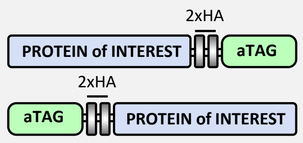


Introduce an *Achilles'* heel
into the protein of interest
rendering the overall chimera degradable
in a cereblon-dependent fashion

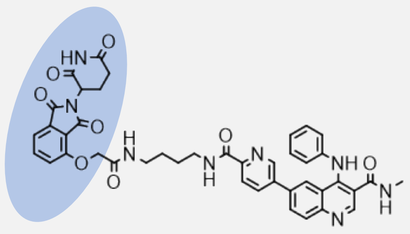
AchillesTAG = aTAG

Development of novel aTAG systems

DESIGN CRITERIA

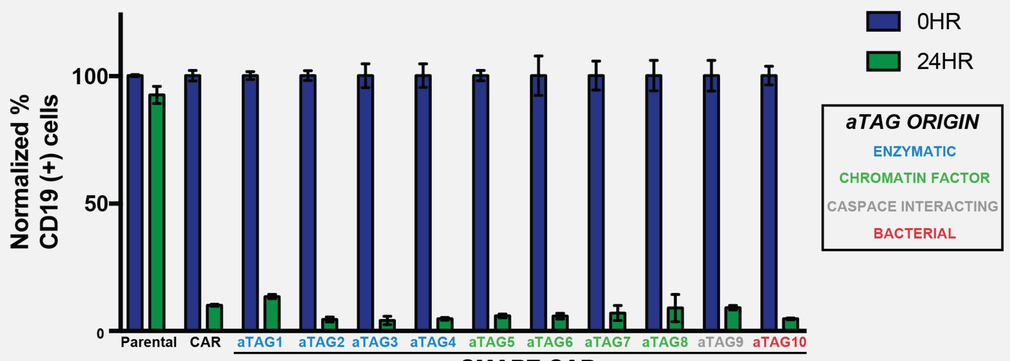


DEGRADATION DOMAIN
Small (<20kDa)
Readily 'ligandable'

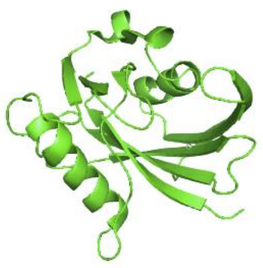


DEGRADER MOLECULES
In vitro potency (DC_{50} < 10nM)
Enabling *in vivo properties*
Clean off target profile

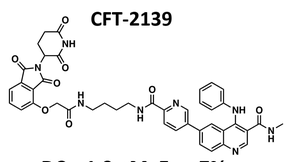
aTAG FUNCTIONAL SCREENING



Two high fidelity aTAG systems

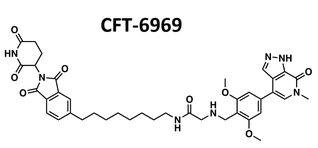


aTAG1 BASED on MTH1 (NUDT1)
Tag size = 17 kDa
Physiologic role: Hydrolysis of 8-oxo-dGTP
$NUDT1^{-/-}$ mice show no phenotype
Acute inhibition or degradation shows no cellular phenotype



DC_{50} 1.9 nM, E_{max} 7%

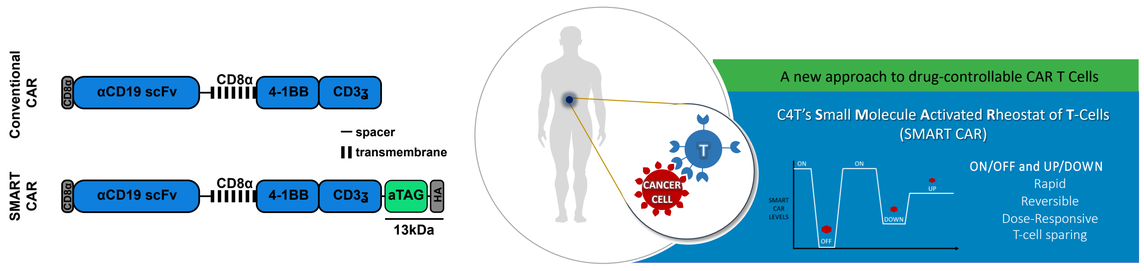
aTAG2 BASED on BRD9
Tag size = 12.5 kDa
Physiologic role: ncBAF complex
Conditional dependency in some AML cells

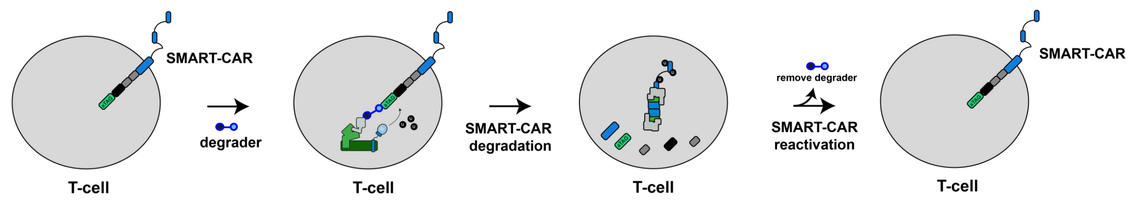


DC_{50} 0.3 nM, E_{max} 2%

aTAGs APPLIED TO CAR-T: SMART-CAR

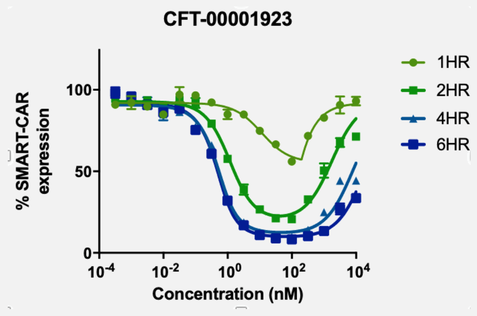
SMART-CAR concept



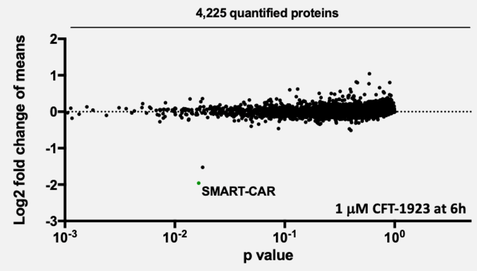


Degradation control

Targeted SMART-CAR degradation

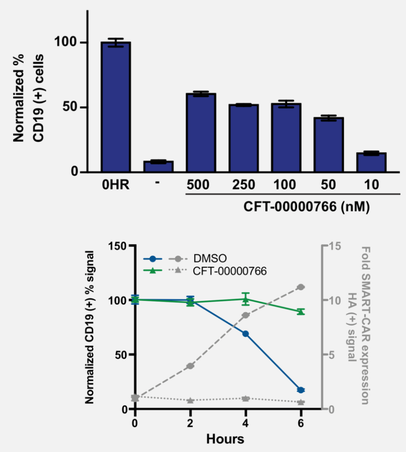


Selective degradation

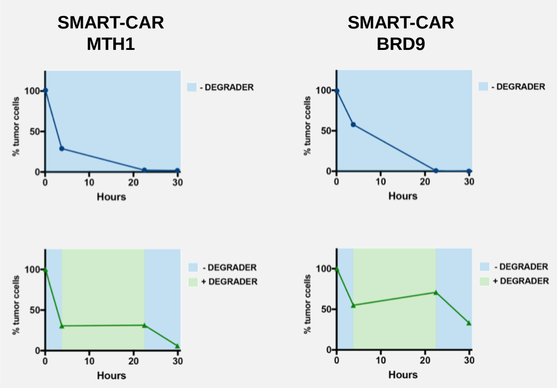


Chemical control of target tumor cell killing

Dose and kinetic control

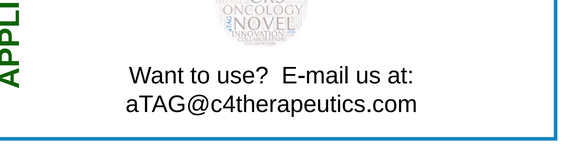


Rheostat function

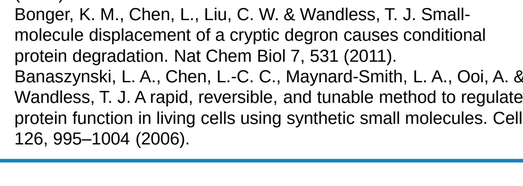


Functional control of SMART-CAR expressing primary T-cells

Degrader based control of SMART-CAR expressing primary T-cells



Degrader based control of cytokine production



OPEN SOURCE

TOOL-KIT

APPLICATIONS

TWO aTAGS + MULTIPLE DEGRADERS
Highly potent and rapid acting
In vitro and *in vivo* applicability

Want to use? E-mail us at:
aTAG@c4therapeutics.com

REFERENCES

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