129. FOXP3 stapled peptides to deactivate Regulatory T cells

(University of Chicago)

Asset Overview

Product Type	Peptide
Disease Area	Immuno-oncology
Indication	Cancer
Current Stage	HIT to Lead
Target	FOXP3
MoA	Stapled alpha-helical peptides (SAHs) in the likeness of a portion of the native FOXP3 antiparallel coiled-coil homodimerization domain (SAH-FOXP3) block this key FOXP3 protein-protein interaction (PPI) through molecular mimicry.
Brief Description	• Regulatory T cells (Tregs) modulate immune function by preventing autoreactive T cell responses, including those that would normally target cancer cells. Treg activity can significantly limit the efficacy of immunotherapies designed to stimulate CD8+ T cells, and therefore a strategy for reducing Treg activity as an aspect of immunotherapy is highly desirable. • The FOXP3 transcription factor is selectively expressed in Tregs and is necessary for Treg function. The inventors have synthesized stapled alpha helix peptides that inhibit the FOXP3 homo and heterodimerization necessary to form a functional complex. These peptides block FOXP3 signaling, and consequently indirectly inhibit Treg function. • The product is (1) stapled alpha helix peptides that inhibit FOXP3 transcriptional activity and (2) intracellular delivery of the stapled alpha helix peptides using targeted nanoparticles. • In initial proof-of-concept studies, the stapled alpha helix peptides bound to recombinant FOXP3 with nanomolar affinity and reduced expression of FOXP3 regulated genes.
Intellectual Property	US20210094990A1
Publication	Inhibition of FOXP3 by stapled alpha-helical peptides dampens regulatory T cell function. PNAS, (2022)
Inventors	James LaBelle, Rachel Eclov, Gregory Bird, Loren D. Walensky

Highlights

- Lead SAH(229–259)c Constructs Are Cell Permeable and Nontoxic to T Cells.
- Single-Stapled SAH(229–259)c Induces Greater Gene and Protein Expression Alterations in Treg Cells Compared with Double-Stapled SAHs.
- SAH(229-259)c Results in Greater Dampening of Treg Cell–Mediated Immune Suppression Compared with Double-Stapled SAHs.
- SAH(229–259)c Alters Treg Cell mRNA Expression in vivo.
- Lower drug resistance potential than therapeutic antibodies
- Fewer off-target effects than small molecules

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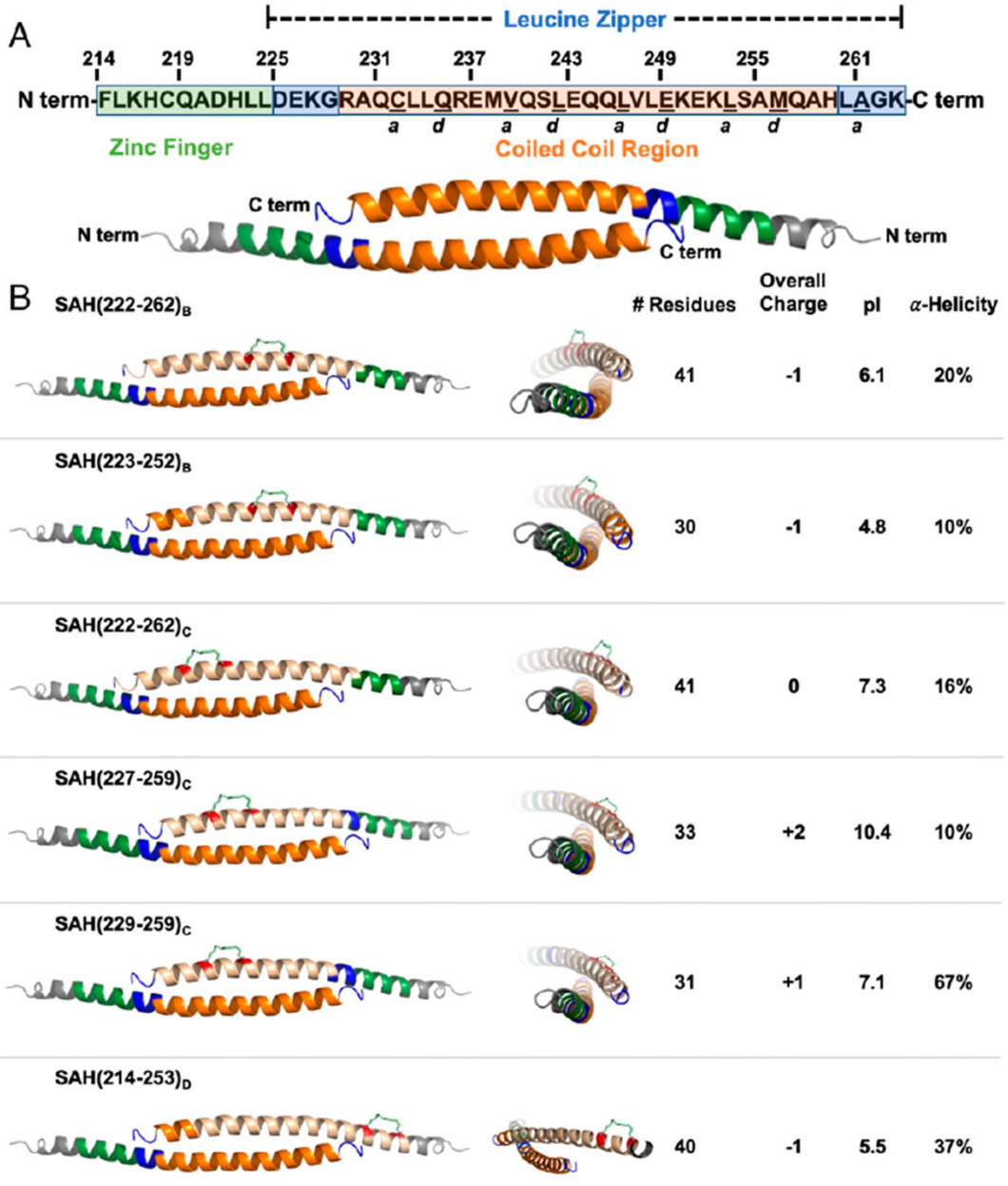
Key Data

Design and synthesis of stapled alpha-helical peptides targeting FOXP3



	# Residues	Overall Charge	pI	α-Helicity
$SAH(222-262)_B$	41	-1	6.1	20%
$SAH(223-252)_B$	30	-1	4.8	10%
$SAH(222-262)_C$	41	0	7.3	16%
$SAH(227-259)_C$	33	+2	10.4	10%
$SAH(229-259)_C$	31	+1	7.1	67%
$SAH(214-253)_D$	40	-1	5.5	37%

(A) Human FOXP3 sequence. Residues F214–K263 represent the entirety of a helix constituting the zinc finger (F214–L224; shown in green) and leucine zipper (D225–K263; shown in blue) regions. Within the leucine zipper, the coiled-coil (R229–H259; shown in orange) represents an area that is highly conserved between human and murine FOXP3. Underlined residues indicate a and d core residues, which bind each other on an identical antiparallel helix and are key contact points for homodimerization. The homodimerized FOXP3 helix is labeled according to the color codes in the sequence above. (B) Design strategy for SAHs targeting FOXP3. SAHs differing in sequence, length, staple position, overall charge, pI, and alpha-helicity are represented. Each three-dimensional structure shown is based on the crystal structure (PDB: 4I1L (26)); the upper helix represents each SAH (shown in tan) and hydrocarbon staple (insertion sites shown in red).

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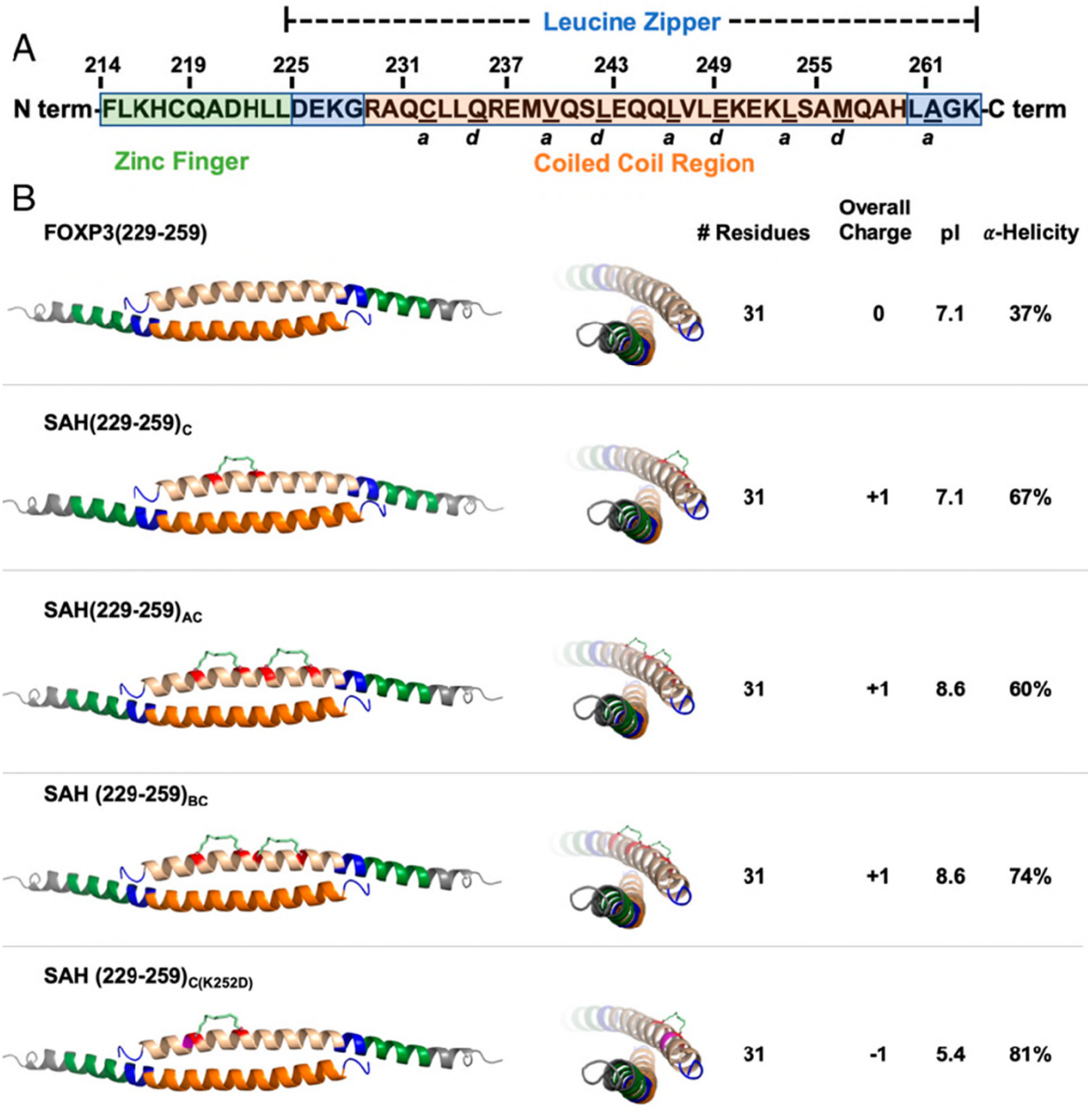
Double stapling of peptides modeled after SAH(229–259)c



	# Residues	Overall Charge	pI	α-Helicity
FOXP3(229-259)	31	0	7.1	37%
$SAH(229-259)_C$	31	+1	7.1	67%
$SAH(229-259)_{AC}$	31	+1	8.6	60%
$SAH(229-259)_{BC}$	31	+1	8.6	74%
$SAH(229-259)_{C(K252D)}$	31	-1	5.4	81%

(A) Human FOXP3 sequence. Residues F214–K263 represent the entirety of a helix constituting the zinc finger (F214–L224; shown in green) and leucine zipper D225–K263; shown in blue) regions. Within the leucine zipper, the coiled-coil (R229–H259; shown in orange) represents an area that is highly conserved between human and murine FOXP3. Underlined residues indicate a and d core residues, which bind each other on an identical antiparallel helix and are key contact points for homodimerization. The homodimerized FOXP3 helix is labeled according to the color codes in the sequence above. (B) Design strategy for SAHs modeled after SAH(229–259)C. SAHs share sequence and length, differing in position and number of staples. These changes influence overall charge, pI, and alpha-helicity. Each three-dimensional structure shown is based on the crystal structure [PDB: 4I1L (26)]; the upper helix represents each SAH (shown in tan) and hydrocarbon staple (insertion sites shown in red).

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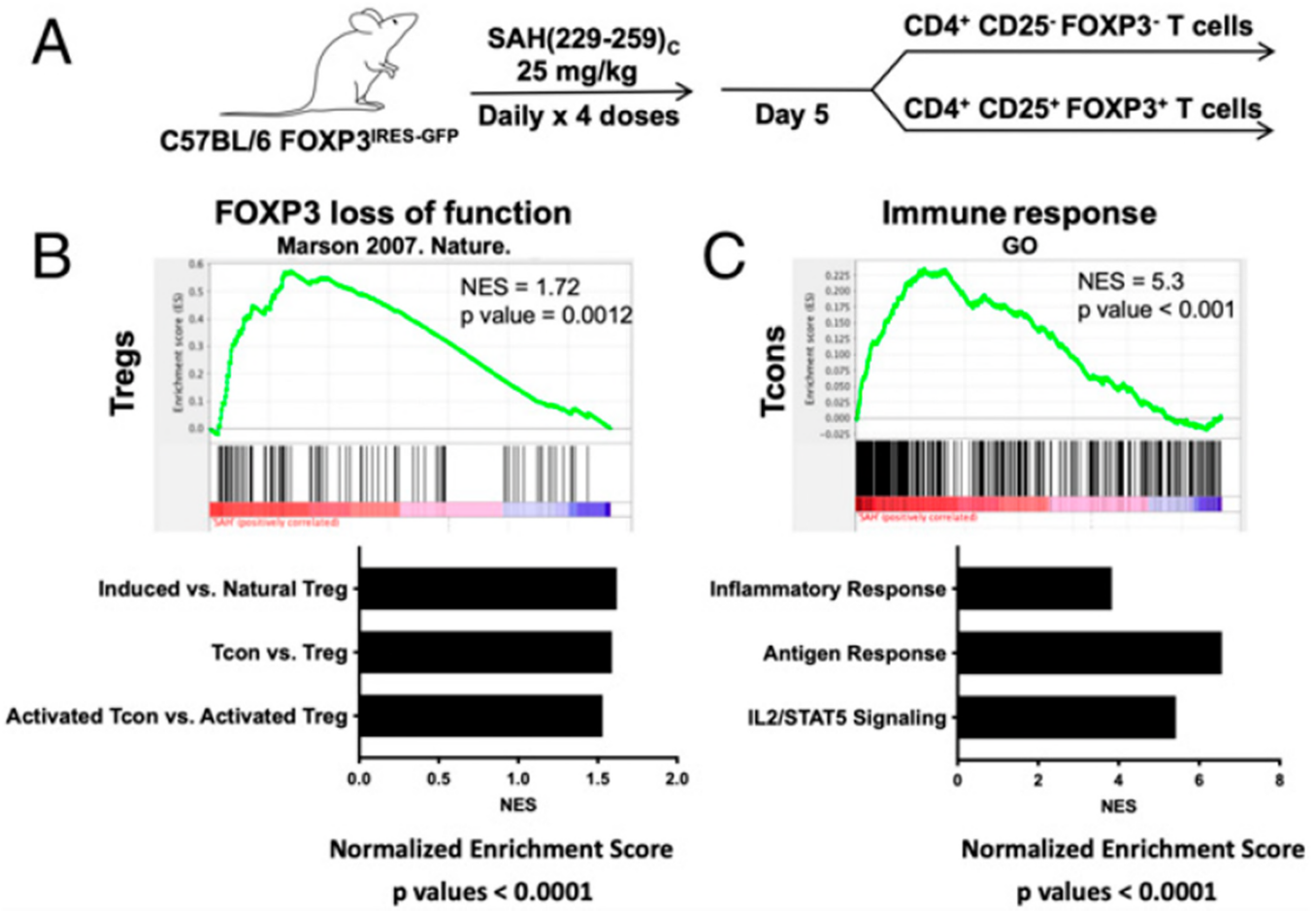
SAH(229–259)c alters Treg cell mRNA expression in vivo



(A) Treatment and isolation strategy of C57BL/6FOXP3-IRES-GFP mice with SAH(229–259)c in preparation for RNA sequencing. (B and C) Treatments of SAH(229–259)C altered global gene expression programs in (B) Treg cells and (C) Tcon cells with P < 0.0001. Following RNA sequencing analysis and subsequent GSEA, Treg cell gene expression mimicked gene sets corresponding to FOXP3 loss, among others. Tcon gene expression alterations mimicked gene sets corresponding to increased inflammatory responses, among others. NES, normalized enrichment score; GO, gene ontology.