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DR. PHILLIP FROST
DEPARTMENT OF
DERMATOLOGY AND
CUTANEOUS SURGERY

AILERON

Temporary cell cycle arrest by ALRN-6924 selectively protects human scalp hair follicles and their epithelial stem cells from taxane-induced toxicity

Jennifer Gherardini[1], D. Allen Annis[2], Jeremy Cheret[1], Manuel Aivado[2], and Ralf Paus[1,3,4]

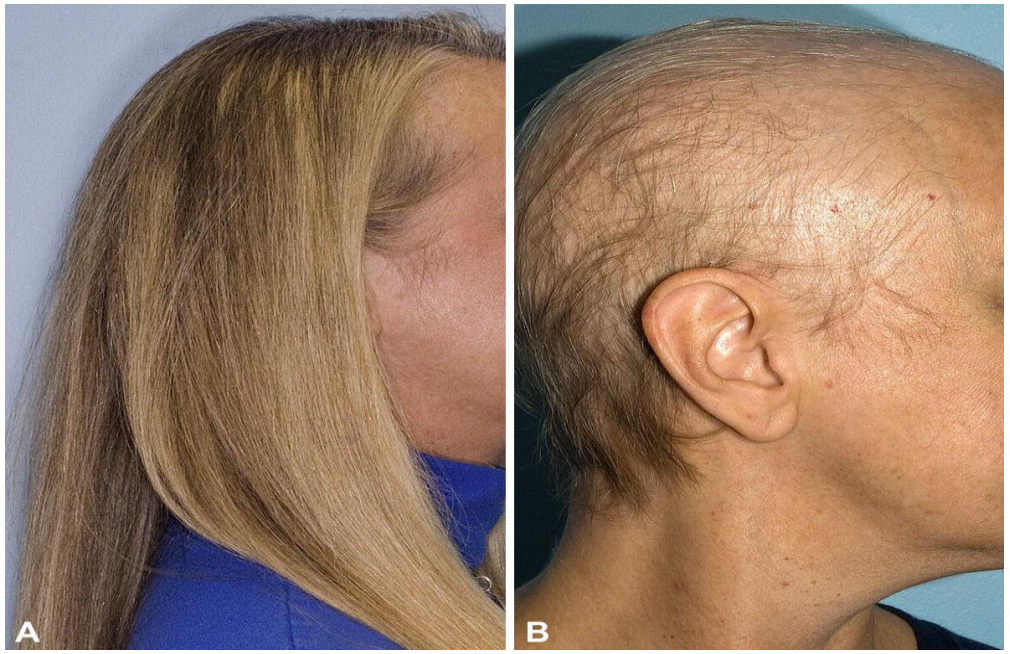
[1] Dr. Phillip Frost Dept. of Dermatology & Cutaneous Surgery, University of Miami Miller School of Medicine, Miami, FL, USA; [2] Aileron Therapeutics, Inc., Boston, MA, USA; [3]Monasterium Laboratory, Münster, Germany; [4] CUTANEON, Hamburg, Germany.

Ralf Paus, MD, DSc, FRSB
Professor of Dermatology, Univ Miami Miller School of Medicine
& Emeritus Professor, University of Manchester, UK

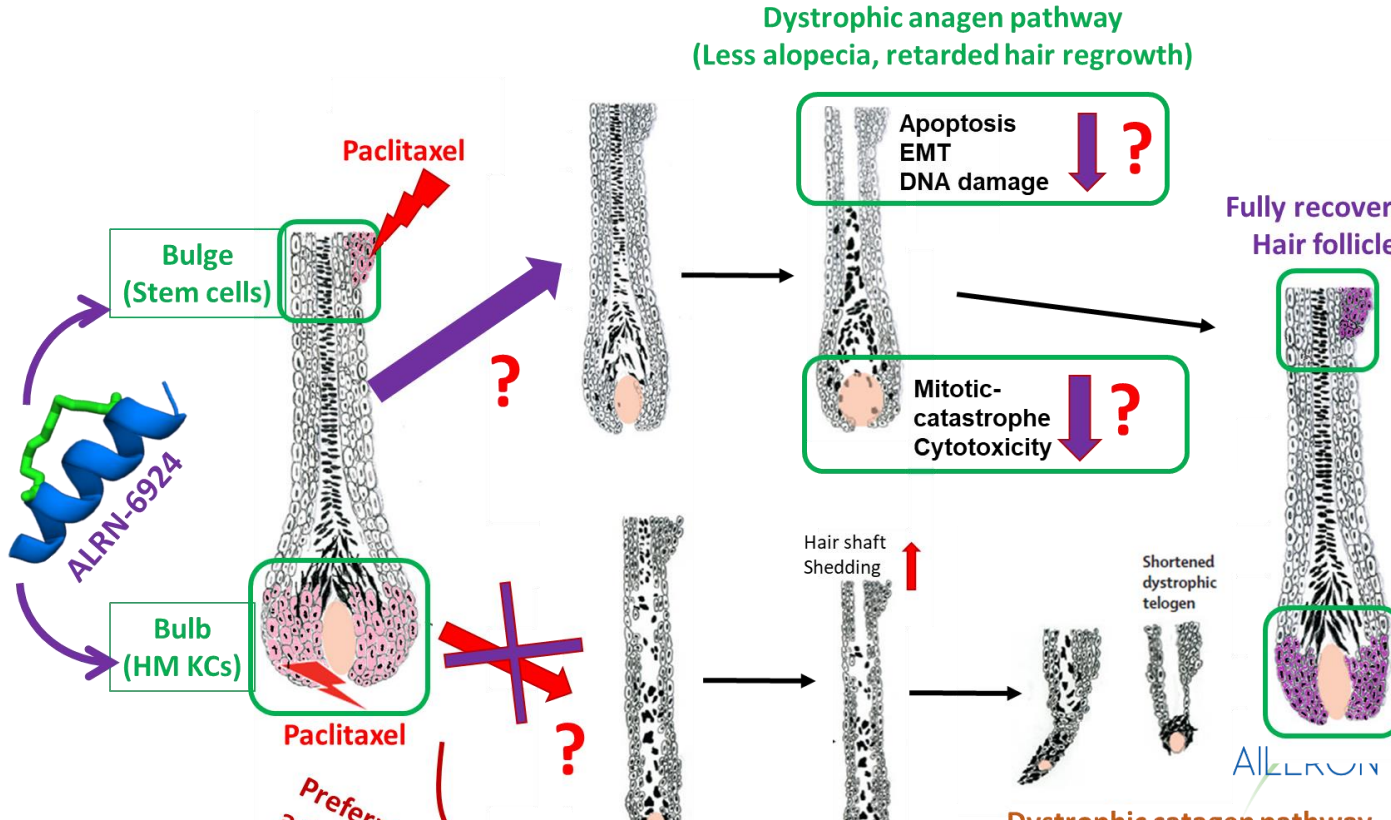
Conflict of interest : Study supported by a **research grant from AILERON Therapeutics**, for which MA serves as CEO, DAA as CSO, and RP as consultant

Chemotherapy-induced alopecia mediated taxanes

- **Chemotherapy-induced alopecia is one of the most devastating, and often life-changing, adverse effects of cancer therapy that can persist long-term, namely under taxane therapy**
- **Permanent alopecia: paclitaxel (PTX) ca. 10%, docetaxel <25%**

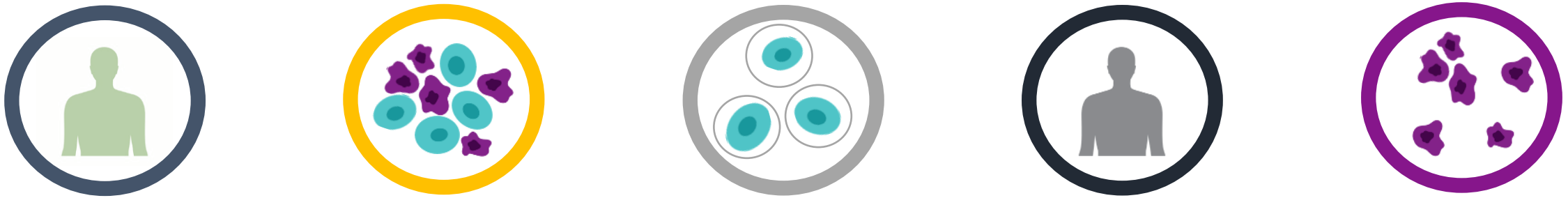


Chon et al., JAAD 2012.



STRATEGY: **Target healthy cells with normal p53, but not cancer cells, by treating only cancer patients with documented mutant p53** (=many types of cancer)

ALRN-6924, inhibitor of MDMX and MDM2, activates normal p53, thereby upregulating p21. This arrests the cell cycle in normal, but not p53-mutant cancer cells



Patient with p53-mutant cancer receives ALRN-6924 before chemotherapy

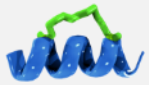
IV administration; 1-hour infusion

→ ALRN-6924 activates normal p53 in healthy cells

→ Activated normal p53 upregulates p21, which pauses cell cycling in healthy cells

→ Patient with p53-mutant cancer receives chemotherapy

→ Chemotherapy's attack on cancer cells with mutant p53 is uninterrupted



ALRN-6924

Selective chemoprotection of healthy cells (=always normal p53)

No protection of p53-mutant cancer cells

- p53 = most common cancer mutation†
- > 50% of all cancer patients have p53 mutation†

†Hoe, K., et al. **Nat. Rev. Drug Discov.**, 2014.

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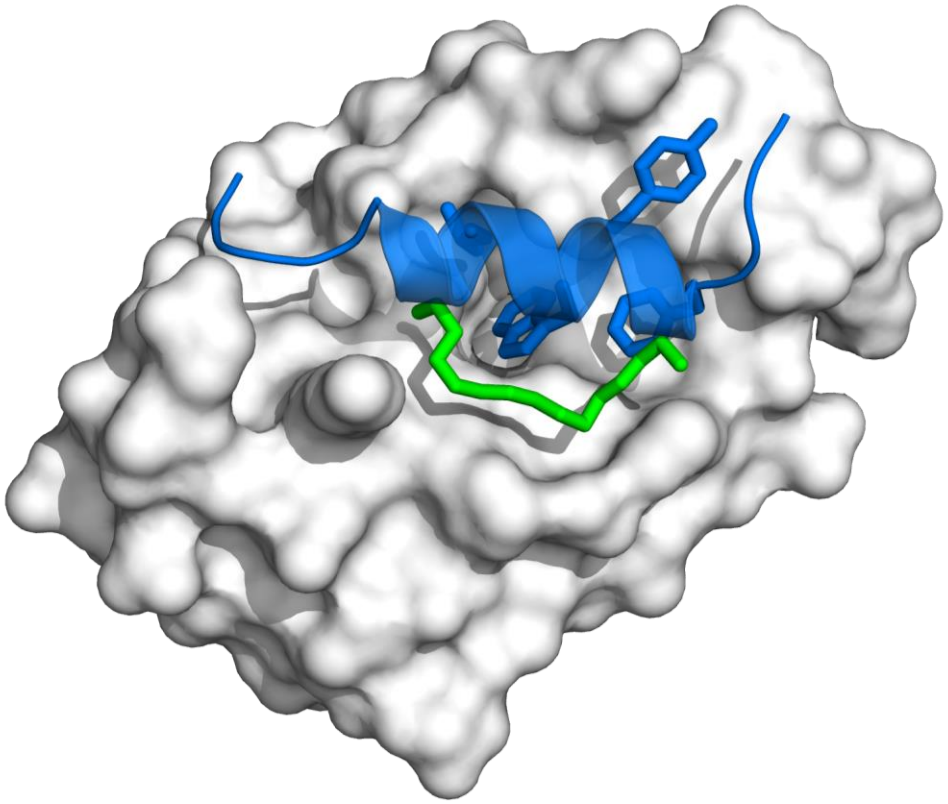
ALRN-6924: Structure & key design properties



ALRN-6924·MDMX
co-crystal structure

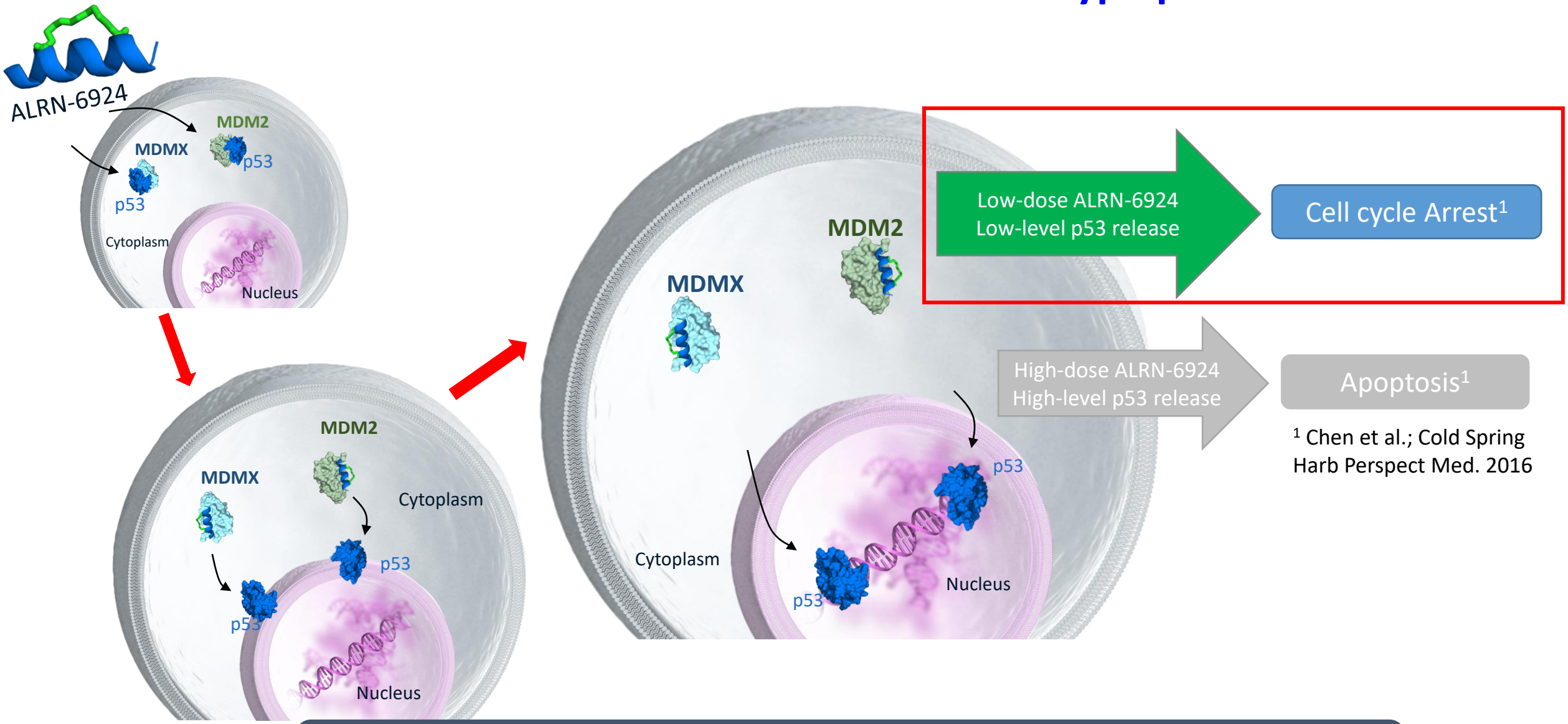
Structure based on the N-terminal α-helical domain of p53, with a hydrocarbon staple and other chemical modifications to ensure:

- **I** Protection from proteolytic cleavage
- **II** Permeation of cell membranes and enhanced cell entry
- **III** High affinity binding to its targets (MDMX and MDM2)
- **IV** Preclinical and clinical on-target, on-mechanism effects

Binding Affinity

K_d, nM	MDM2	MDMX
Native p53	770	480
ALRN-6924	13.7	8.9

ALRN-6924 Mechanism Of Action In Wild-type p53 Cells



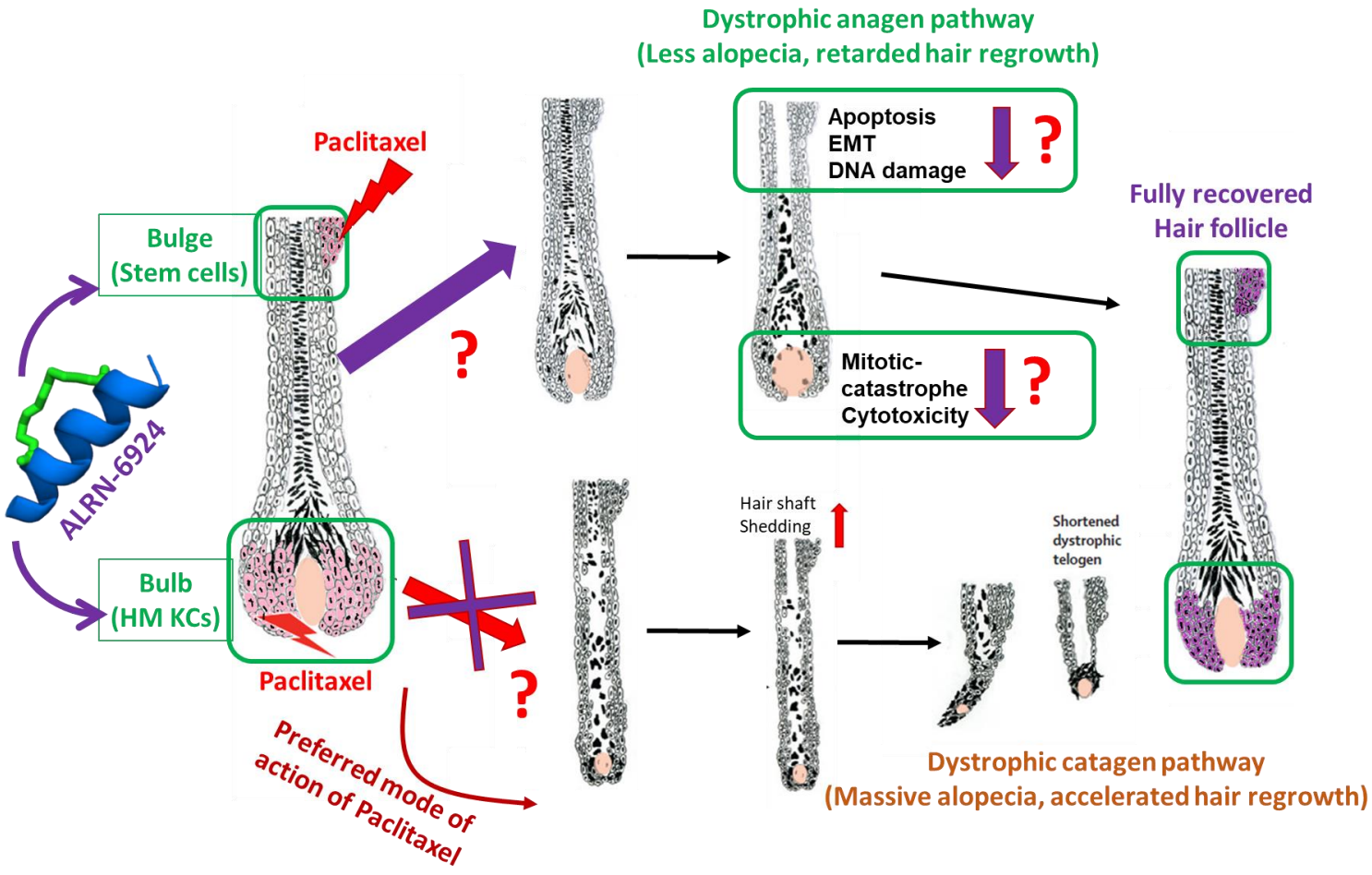
[1] Chen et al.; Cold Spring Harb Perspect Med. 2016

ALRN-6924 is a decoy that mimics p53 and selectively binds to MDMX + MDM2, releasing p53 to induce cell cycle arrest

Questions addressed

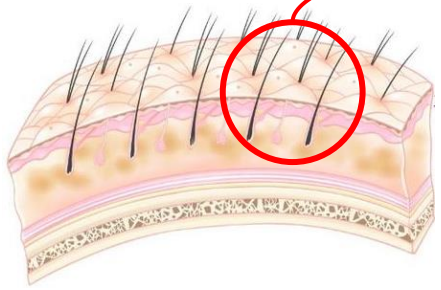
Does ALRN-6924:

- prevent general HF toxicity induced by paclitaxel (Taxol)?
- promote the dystrophic anagen pathway of HF repair after chemotherapy?
- prevent/reduce HF epithelial stem cell damage (apoptosis, DNA damage, EMT) induced by paclitaxel - and thus lower the risk of permanent alopecia?



Experimental Design

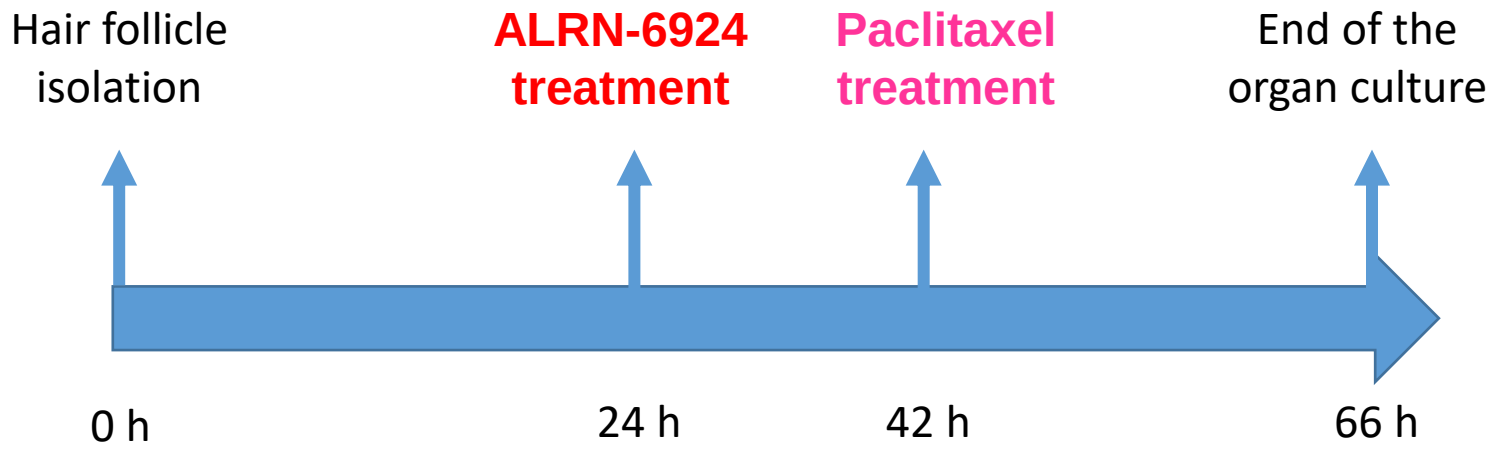
Anagen VI HFs



serum-free anagen scalp HF organ culture from healthy donors

Experimental groups:

- Vehicle
- Paclitaxel (PTX) 100 nM
- ALRN-6924 1 µM
- Paclitaxel 100 nM + ALRN-6924 1 µM

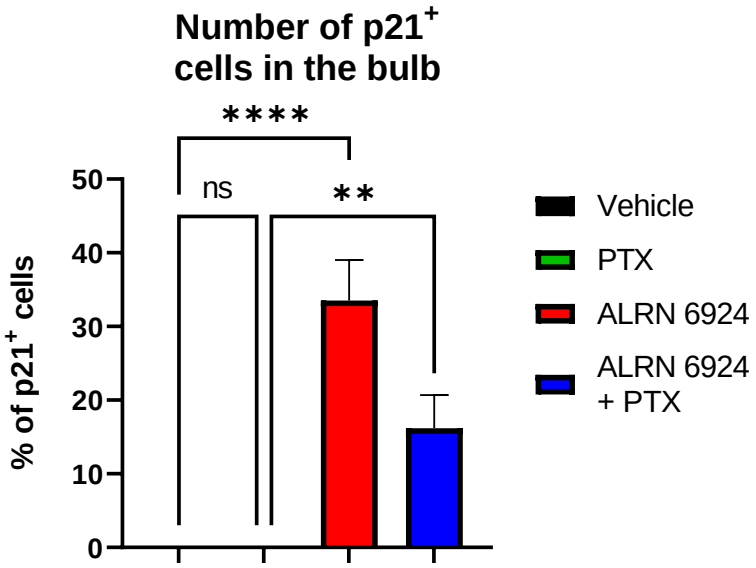


Readout parameter:

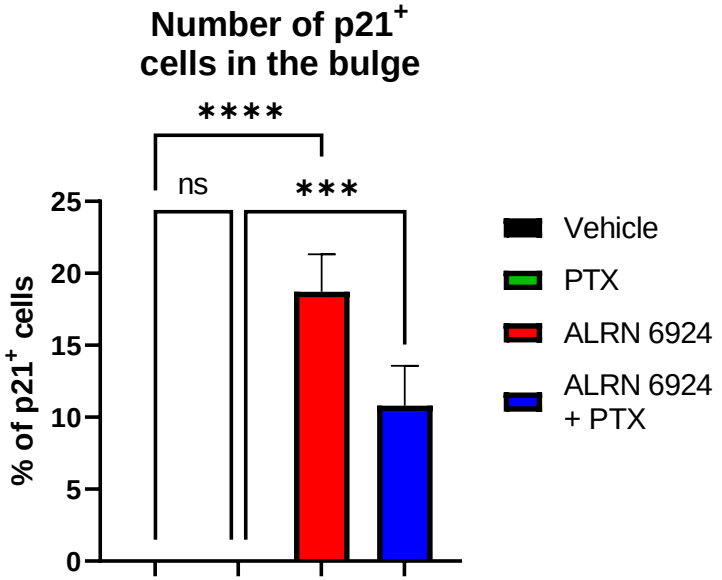
- **Warthin–Starry:** HF morphology and Melanin clumping (cytotoxicity)
- **Ki-67/Activated caspase 3:** Proliferation/apoptosis and hair cycle progression
- **p21$^{Waf1/Cip1}$:** p53 activation (Waf1/Cip1 is induce by p53)
- **Keratin 15/Ki-67:** Keratin 15^{+} stem cell proliferation
- **Keratin 15/Cas 3:** Keratin 15^{+} stem cell proliferation
- **Keratin 15/γH2AX:** DNA damage of stem cells
- **Keratin 15/vimentin:** EMT induction in stem cells

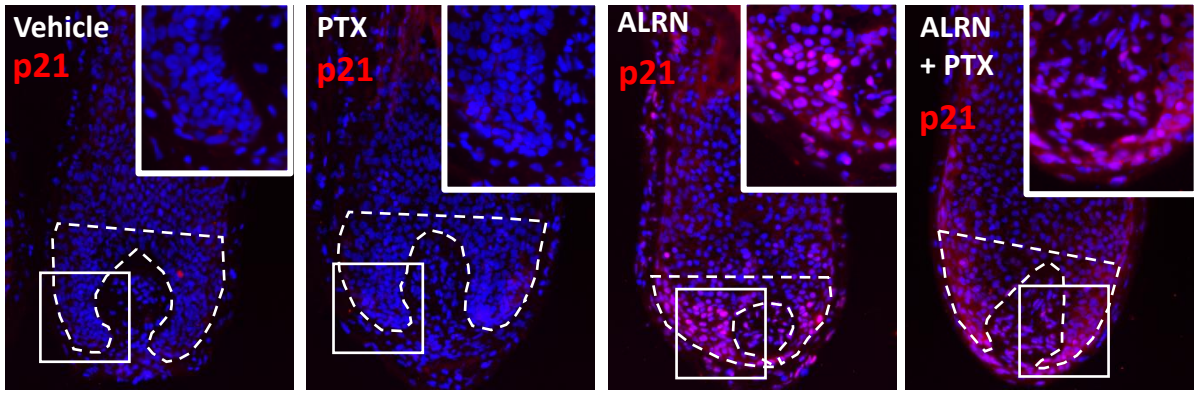
Bodo et al. AJP 2007, Langan et al. EXD 2015, Purba et al. EMBO Mol Med 2019

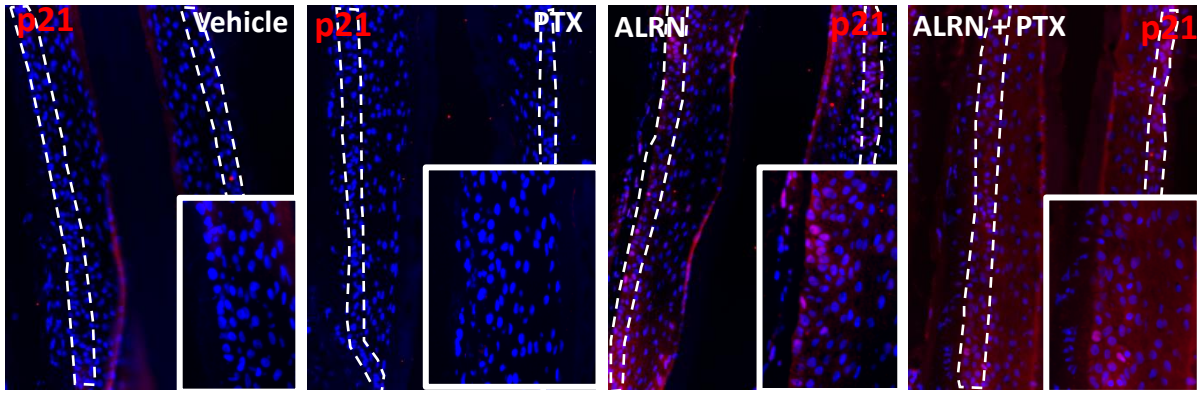
ALRN-6924 significantly enhances p21 expression in the anagen hair matrix bulb and bulge of human scalp HFs *ex vivo*



This shows the expected p53 activation







Mean +/- SEM; n=13-15 HFs from **3 donors**; Student's *t* test; **p<0.01; ***p<0.001; ****p<0.0001. White dottedareas: p21 evaluation area

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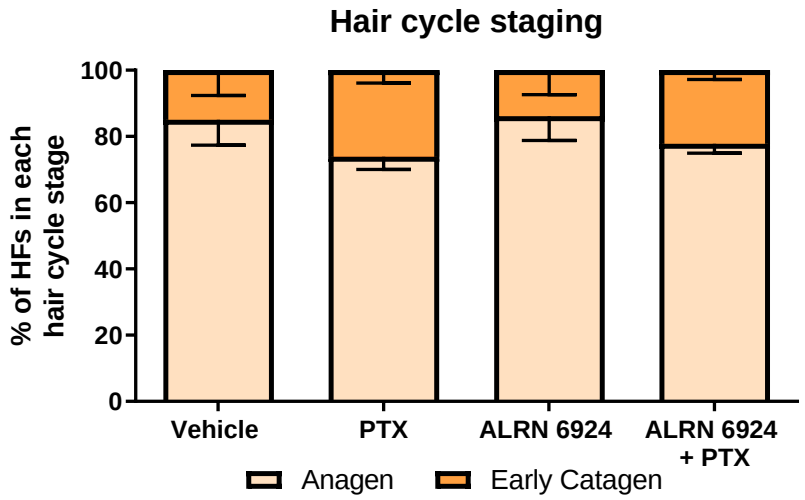
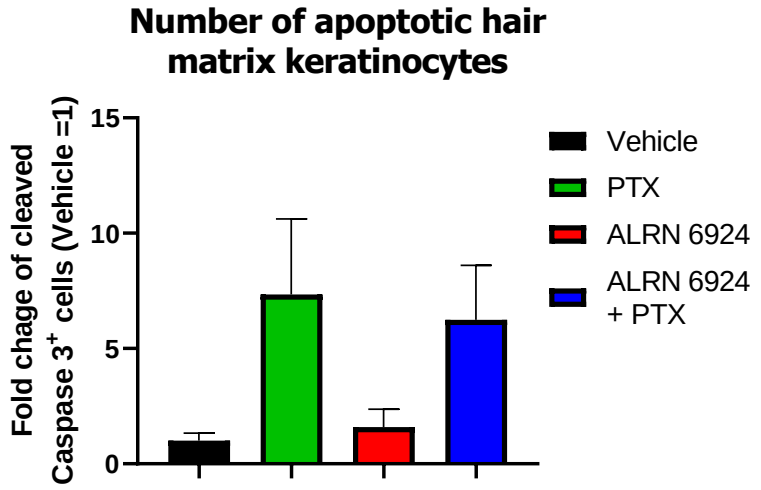
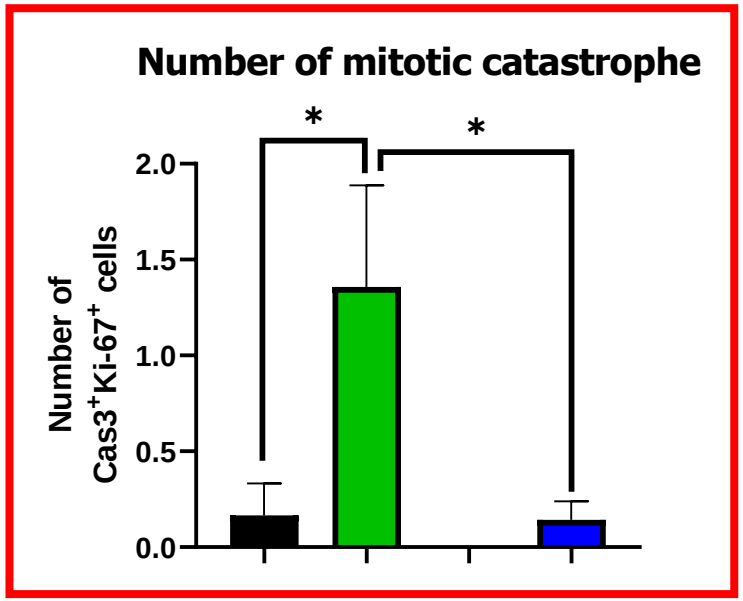
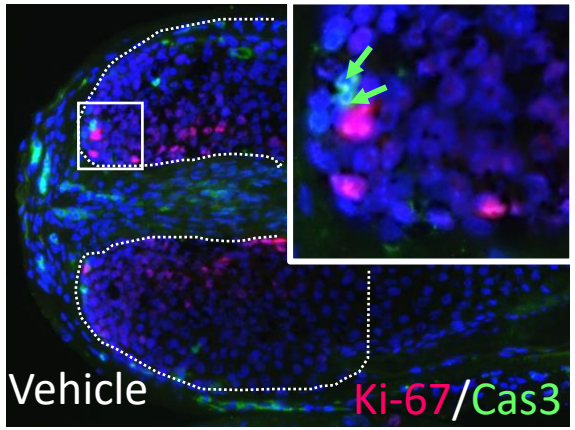
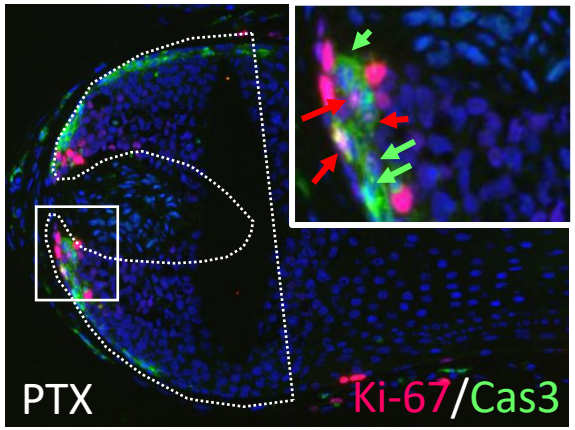
ALRN-6924 does not protect from PTX-induced apoptosis in the hair matrix, but prevents PTX-induced mitotic catastrophe, - without inducing catagen!

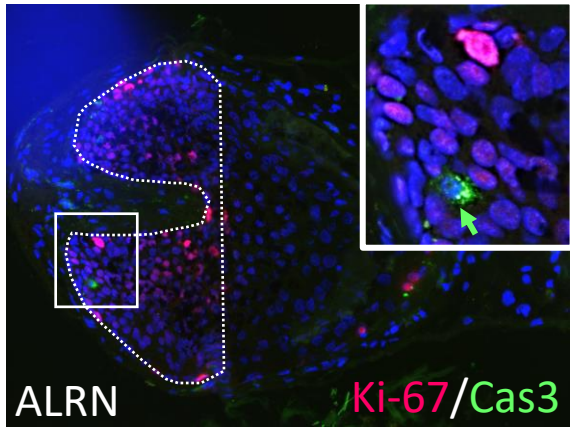


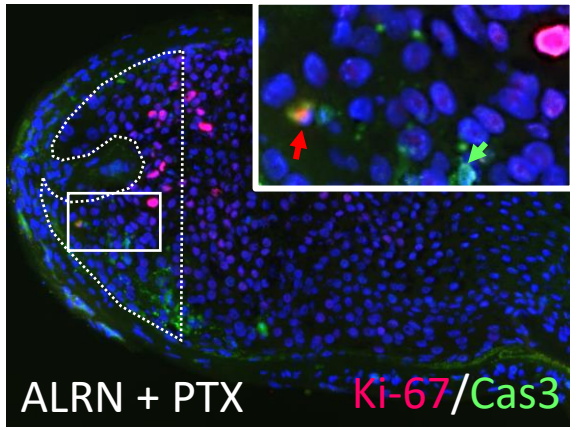












Mean +/- SEM; n=12-14 HFs from **3 donors**; Mann–Whitney test, *p<0.05. Green arrows: cleaved-caspase-3 positive cells; red arrows: Mitotic catastrophe (Ki-67/Caspase-3 double positive cells)

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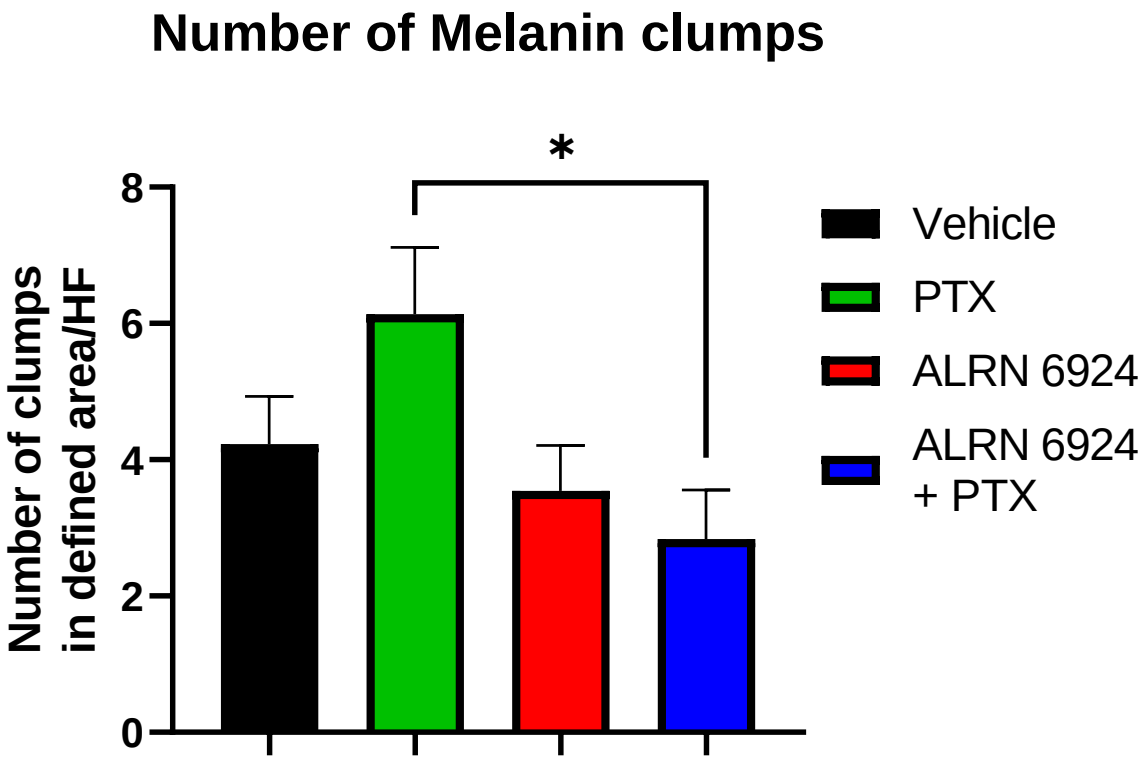
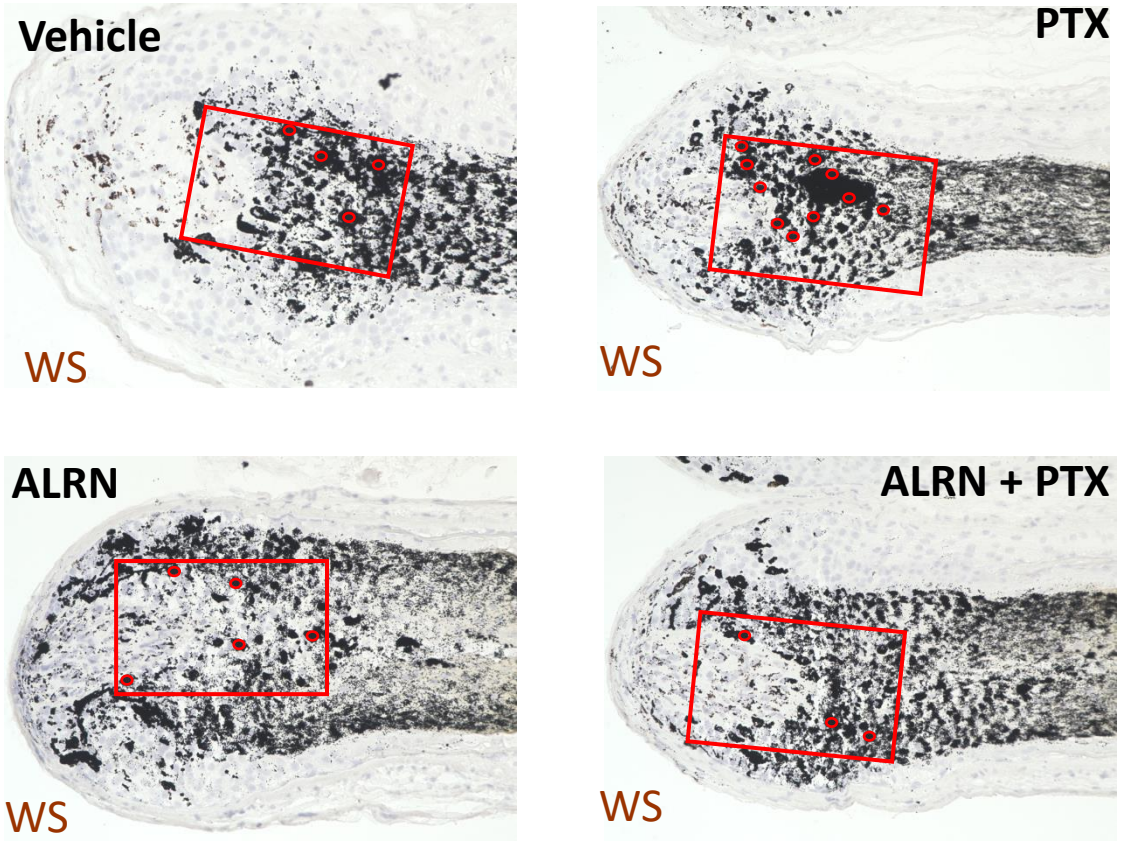
ALRN-6924 itself does not promote melanin clumping, but prevents PTX-induced melanin clumping

Melanin clumping is a very sensitive sign of HF cytotoxicity and dystrophy

Hendrix et al. *JID* 2005, Bodo et al. *Am J Pathol* 2007, Piccini et al., BJD 2021



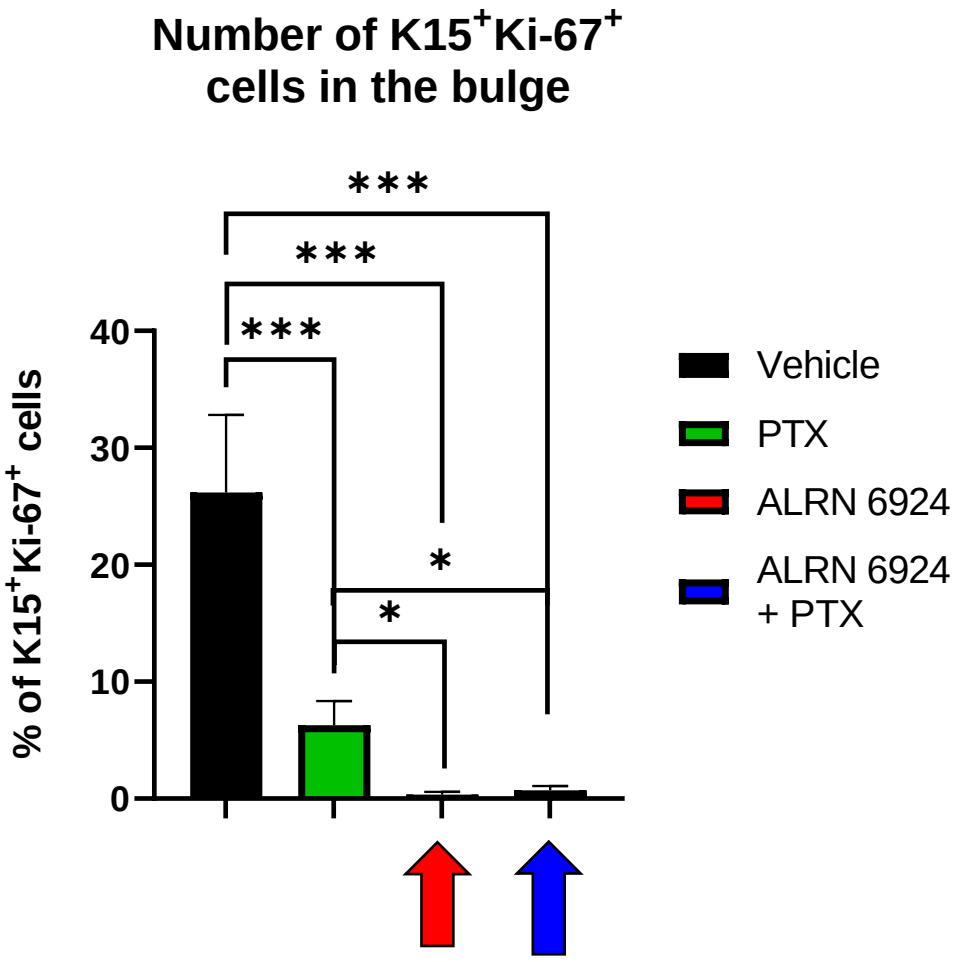


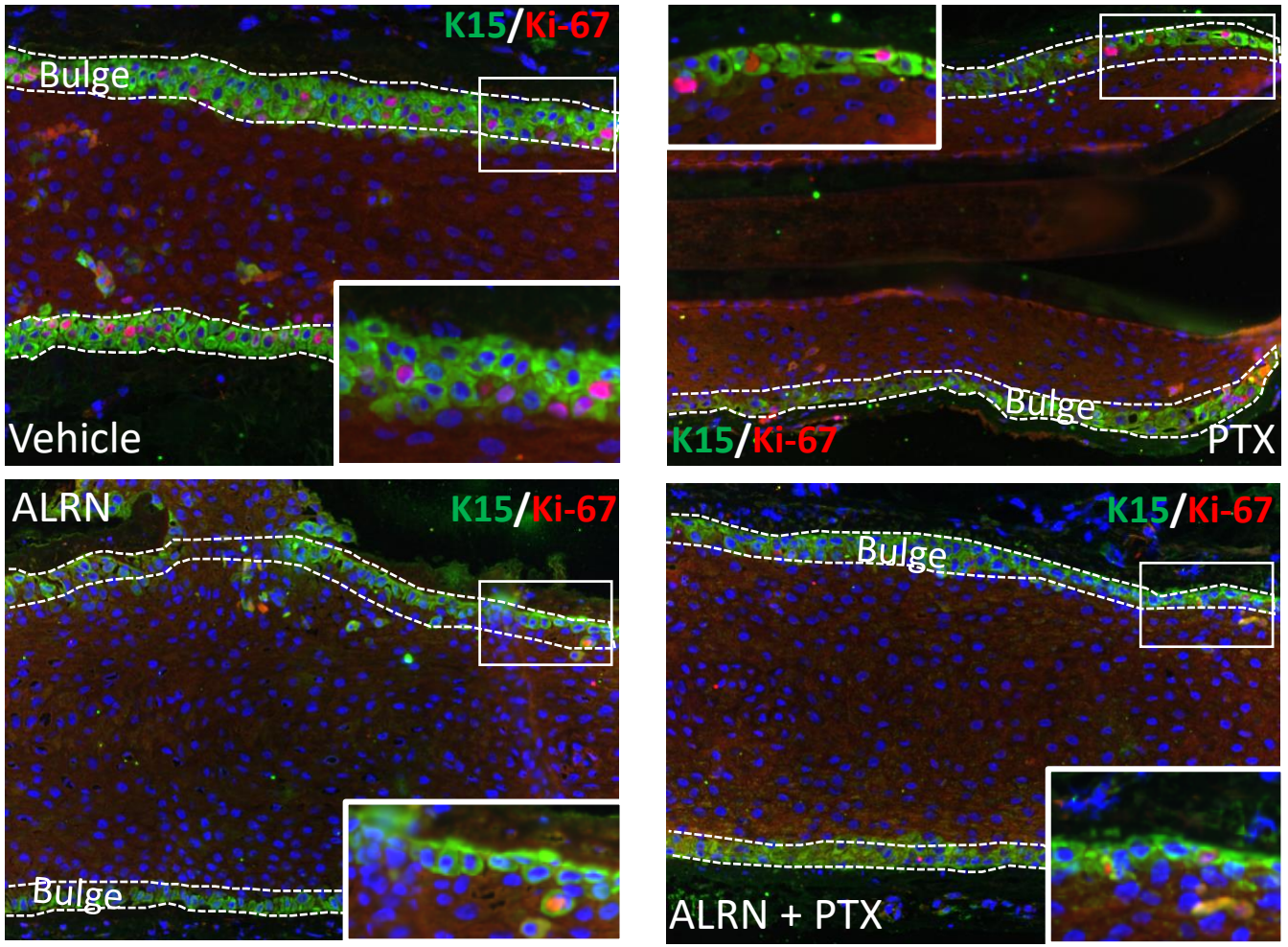
⬭ : Melanin clump

Mean +/- SEM; n=11-15 HFs from **3 donors**; Student's *t*-test, *p<0.05.

ALRN-6924 significantly reduces proliferation of K15$^+$ cells, suggesting cell cycle arrest of HF epithelial stem cells.

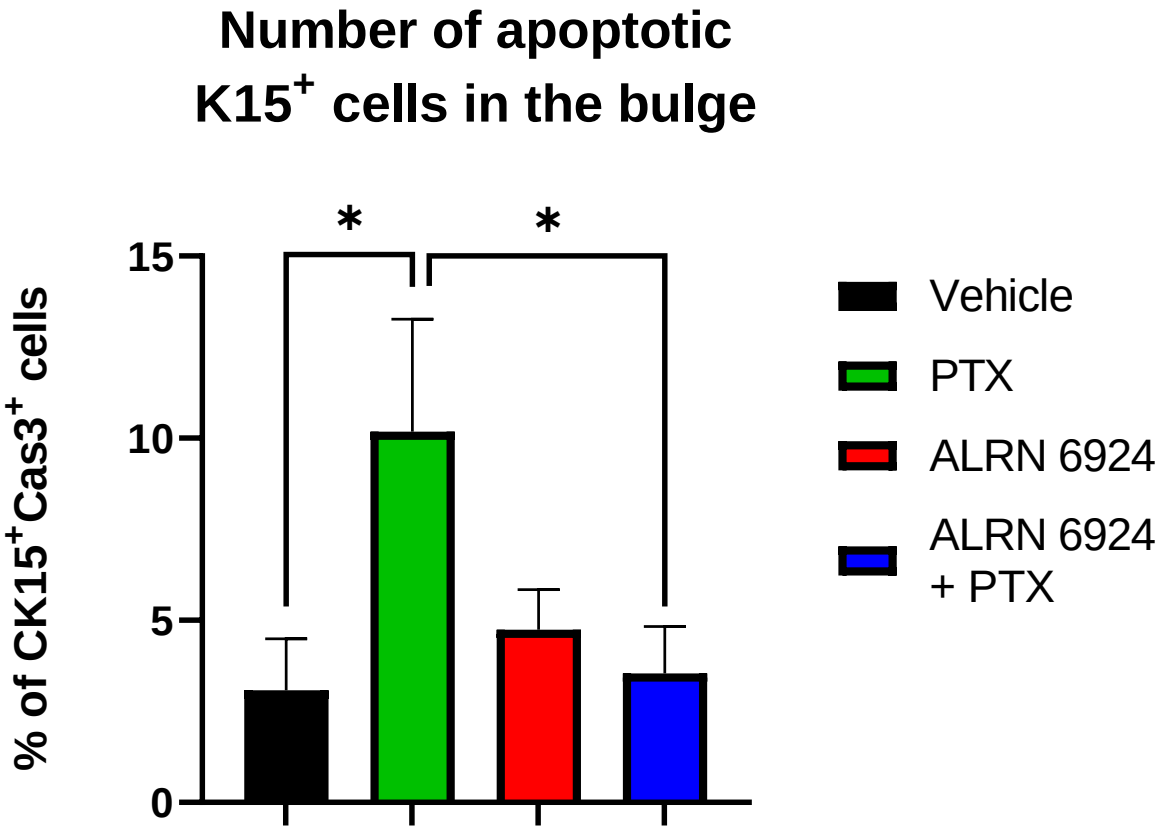
Keratin 15 (K15) HF stem cells marker

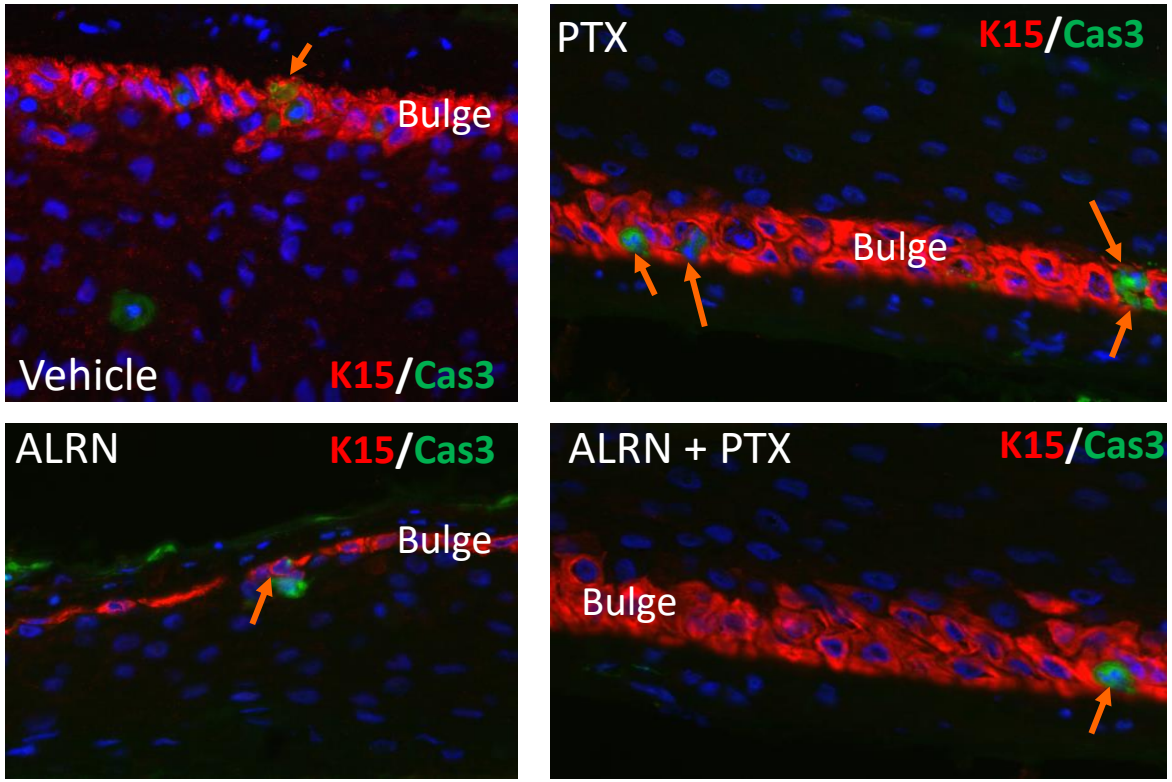




Mean +/- SEM; n=12-15 HFs from **3 donors**; Mann–Whitney test, **p<0.01; ***p<0.001. White dotted areas: Ki-67 evaluation area.

ALRN-6924 itself does not promote apoptosis of K15$^+$ cells, but prevents apoptosis induction by PTX

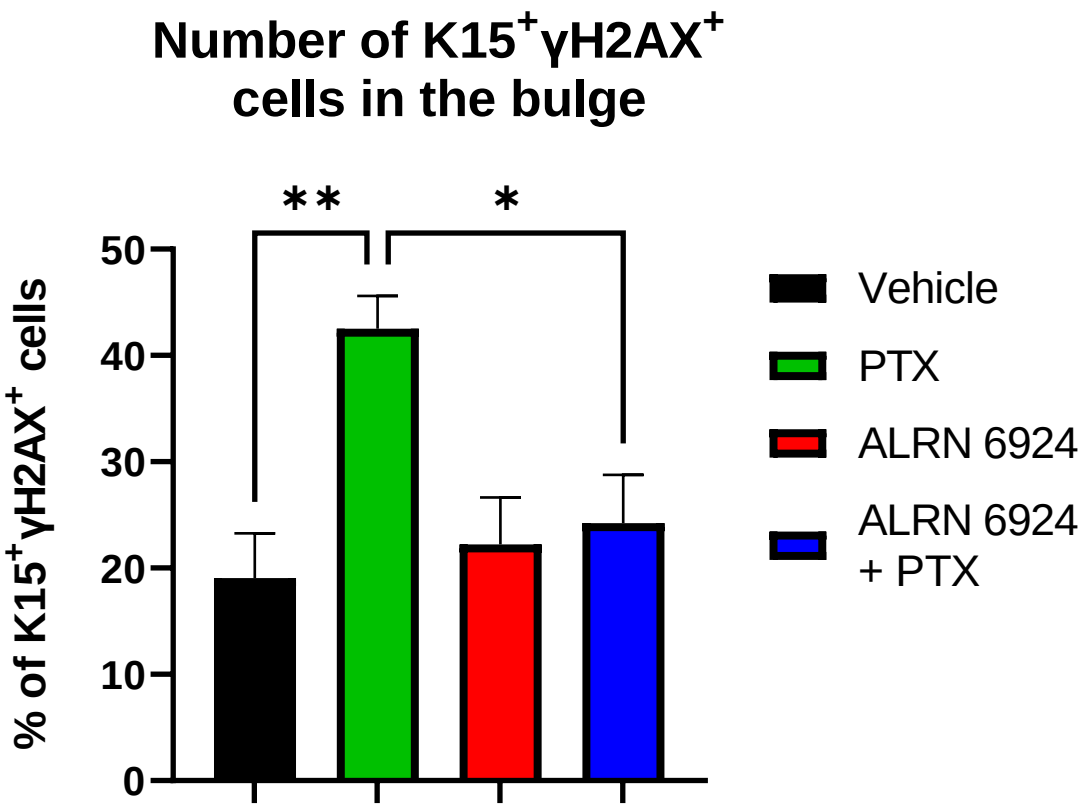


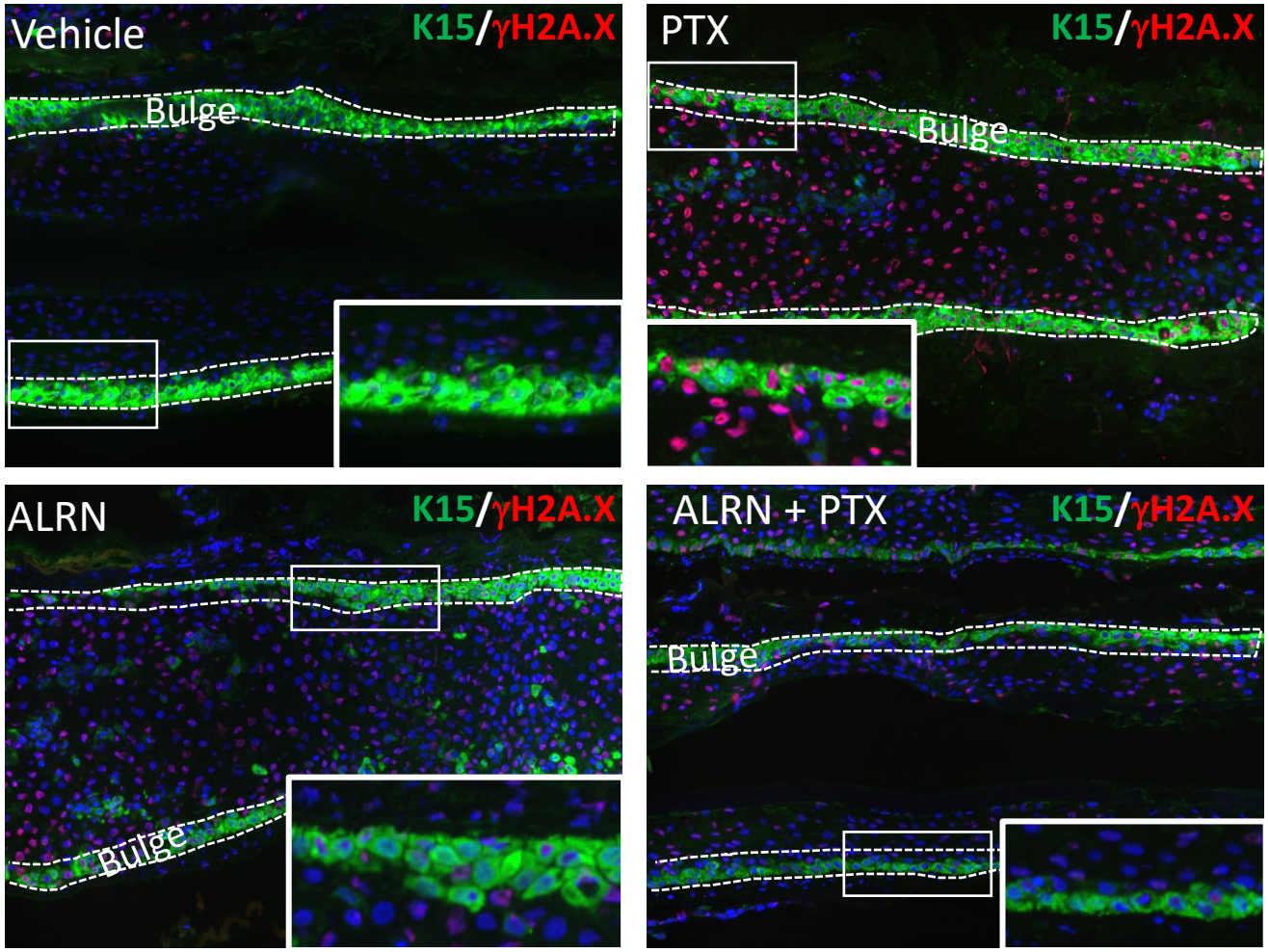


Mean +/- SEM; n=13-15 HFs from **3 donors**; Student's *t* test, *p<0.05; **p <0.01.

ALRN-6924 protects K15⁺ progenitor/stem cells from PTX-induced DNA damage

γH2AX is detected at DNA double strand breaks, indicating for DNA damaged



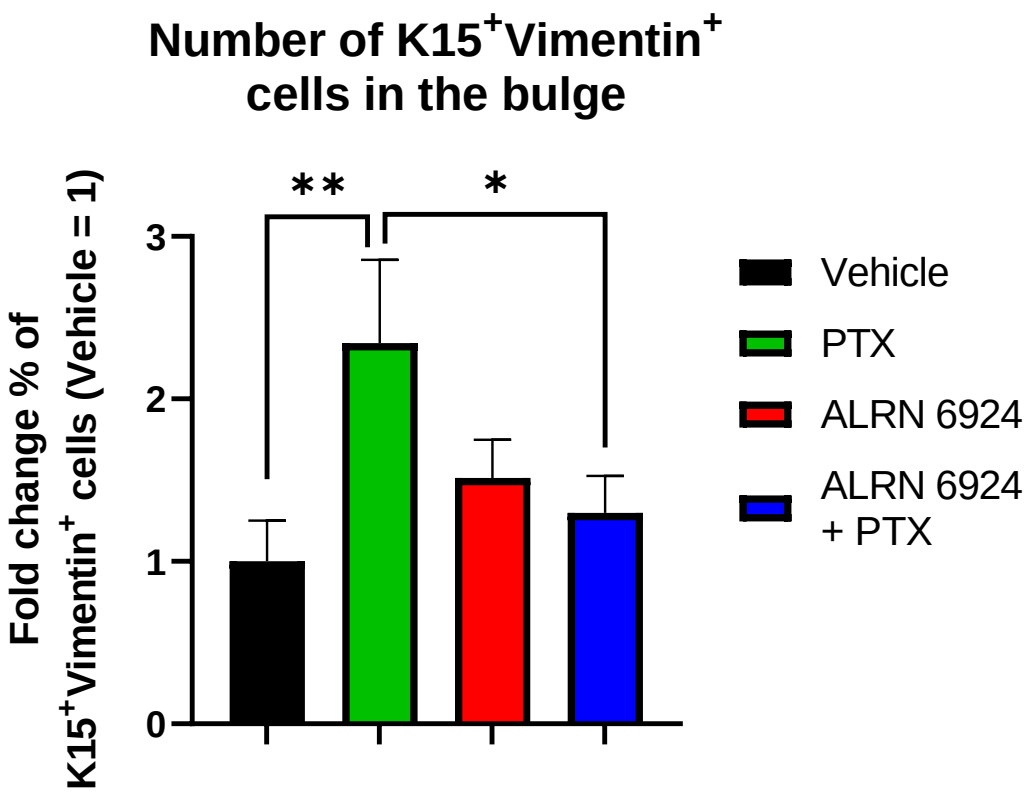


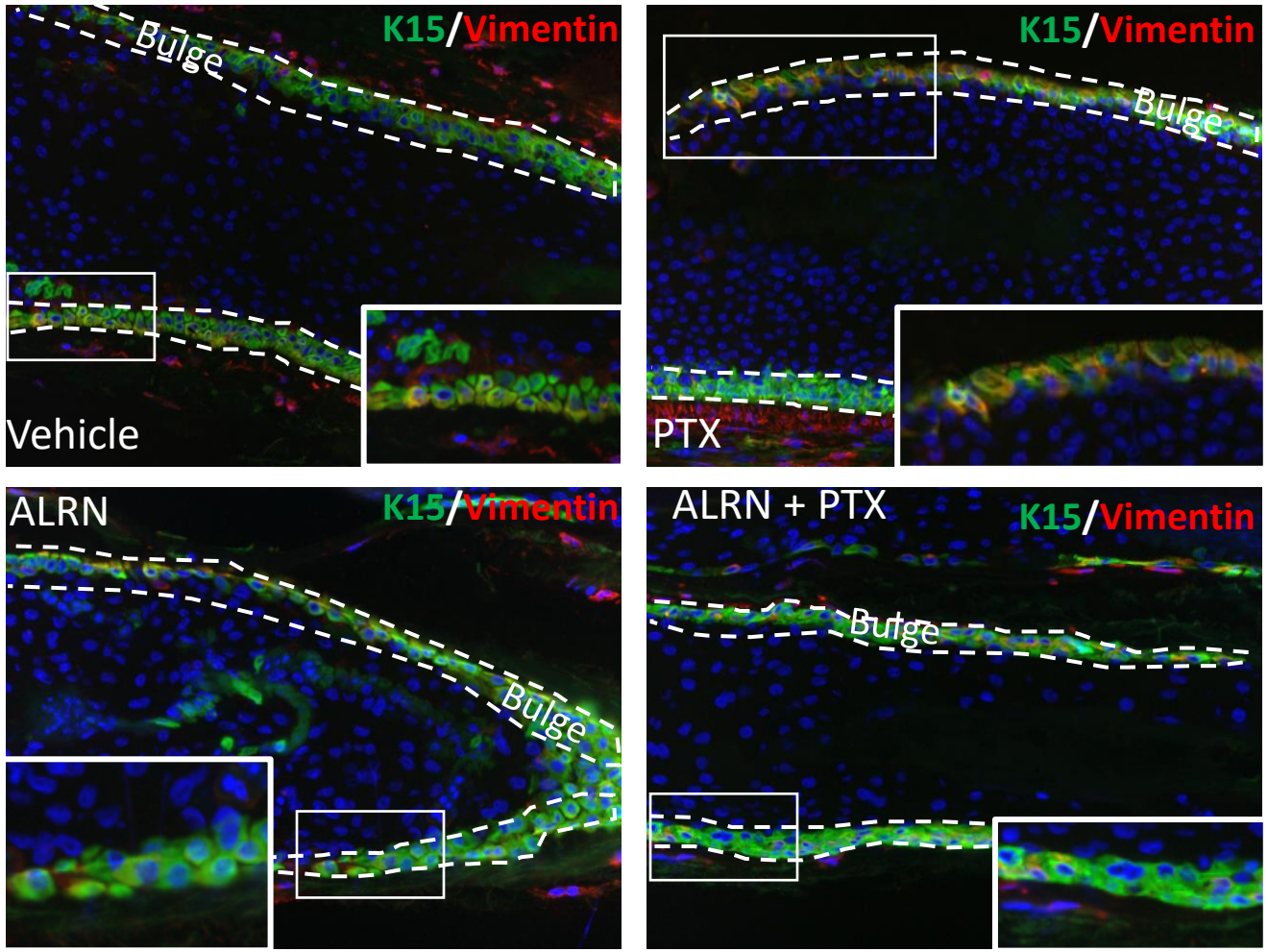
Mean +/- SEM; n=13-15 HFs from **3 donors**; Student's *t* test *p<0.05, **p<0.01. White dotted areas: K15⁺γH2AX⁺ evaluation.

ALRN-6924 protects K15⁺ keratinocyte from PTX-induced EMT

Expression of vimentin (=mesenchymal marker) by epithelial cells is a sign of pathological epithelial-mesenchymal transition (EMT)

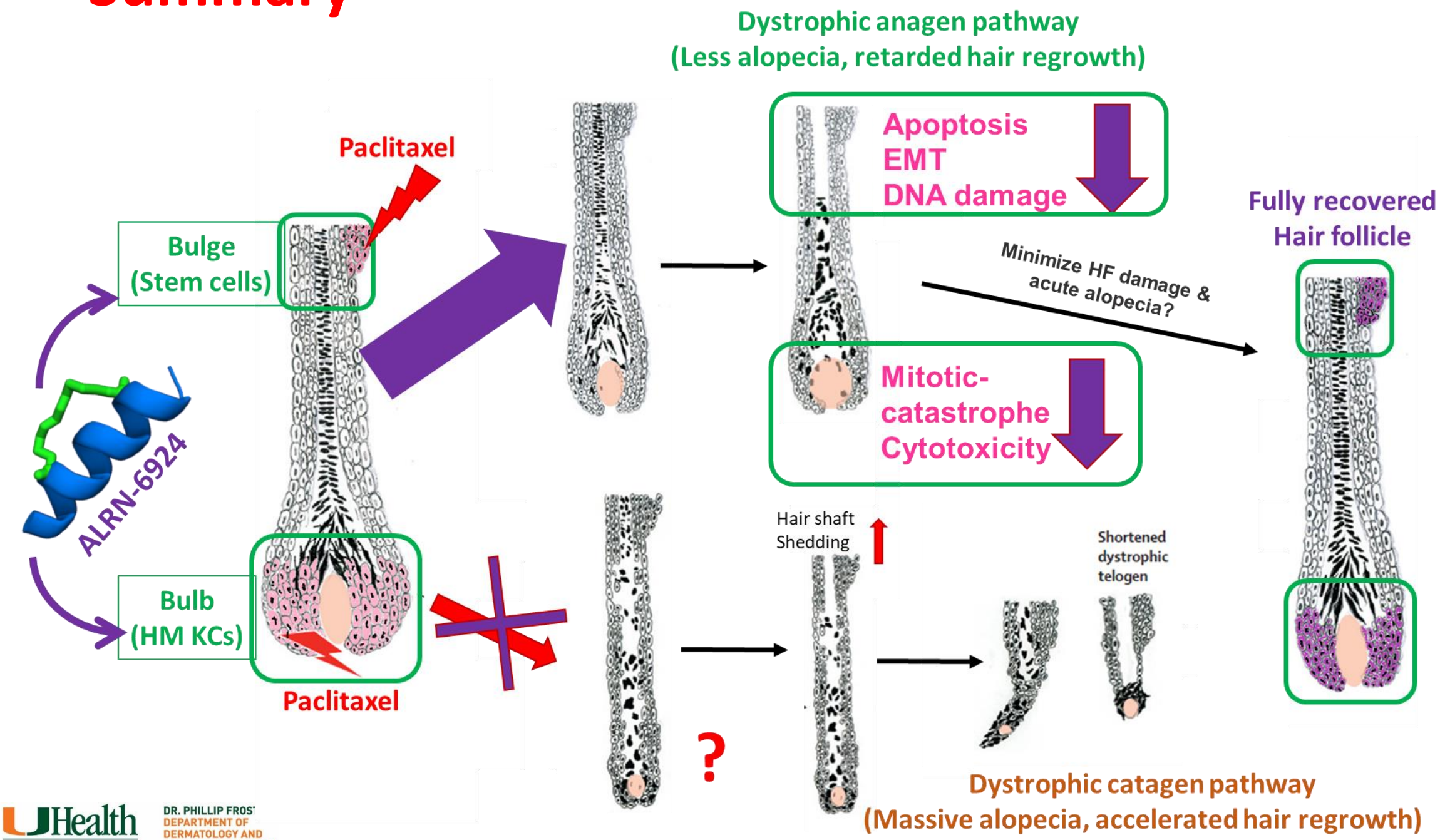
Imanishi et al. JID 2018, Cheret et al. JEADV 2020, Piccini et al. BJD 2021.





Mean +/- SEM; n=12-14 HFs from **3 donors**; Student's *t* test, *p<0.05; **p <0.01. White areas: K15⁺Vimentin⁺ evaluation.

Summary



Conclusions & Perspectives

- **ALRN-6924 promises to reduce or even prevent PTX-induced HF toxicity also *in vivo***
- ALRN-6924 does not induce premature catagen *ex vivo* and is thus unlikely to cause telogen effluvium
- **ALRN-6924** inhibits PTX-induced mitotic catastrophe in the hair matrix, indicating that it may **favor a mild form of dystrophic anagen.** This justifies the expectation that temporary cell cycle arrest by ALRN-6924 will reduce acute alopecia after PTX.
- **Most importantly, ALRN-6924 significantly reduces PTX-induced HF stem cells apoptosis, DNA damage and EMT *ex vivo*** and thus promises to **reduce the incidence and degree of permanent alopecia after taxane therapy.**

These *ex vivo* data support our working hypothesis that ALRN-6924 can SELECTIVELY protect healthy HFs and their stem cells against permanent taxane-induced alopecia.

Acknowledgments

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