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DEPARTMENT OF
DERMATOLOGY AND
CUTANEOUS SURGERY

AILERON

Temporary cell cycle arrest in human scalp hair follicles and their epithelial stem cells by ALRN-6924: A novel strategy to selectively protect p53-wildtype cells against paclitaxel and 4-HC-induced alopecia

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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 by paclitaxel (PTX)

Chon et al., JAAD 2012.

- **Chemotherapy-induced alopecia is a highly distressing adverse effect of cancer therapy that can persist long-term, namely under taxane therapy** Paus et al. *Lancet Oncol* 2013

Previously, we have shown that:

I. **PTX induces "mitotic catastrophe", apoptosis, and DNA damage in proliferating hair follicle (HF) stem/progenitor and transit amplifying cells.**

II. **Temporary G1 arrest via the CDK4/6 inhibitor, palbociclib, protects the HF from PTX toxicity**
Purba et al. EMBO Mol Med 2019

CHALLENGE:

→ **How to avoid that tumor cells are also protected ?**

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

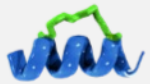
IV administration; 1-hour infusion

→ 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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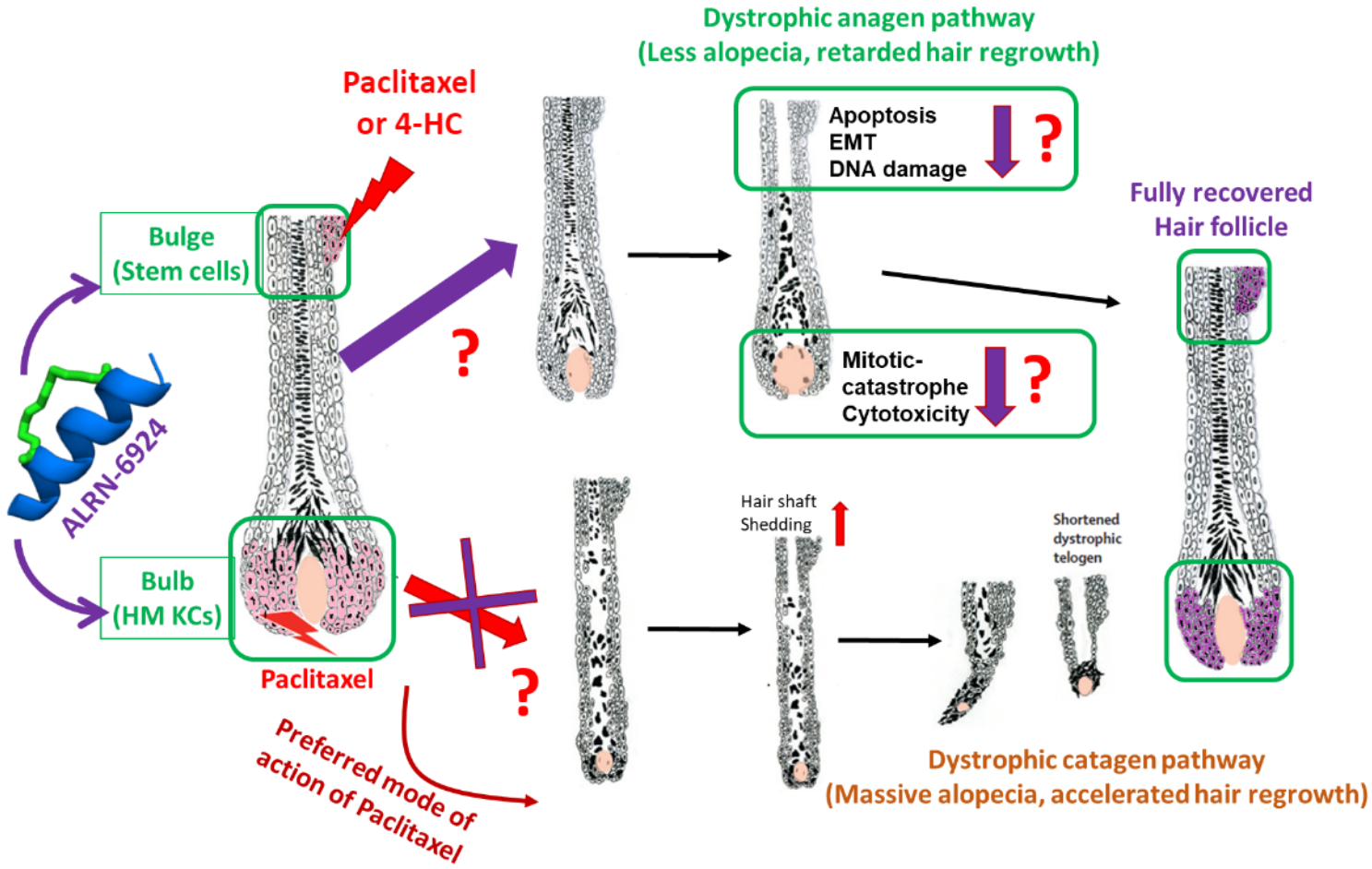
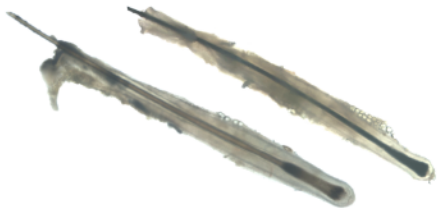
Questions addressed

Does ALRN-6924:

- prevent general HF toxicity induced by paclitaxel (Taxol) or 4-hydro-peroxycyclophosphamide (4-HC)?
- 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?

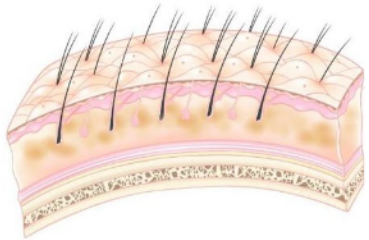


Experimental Design Paclitaxel



Isolated Anagen VI HFs

or



Full thickness hairy scalp skin punches (4mm)

ALRN-6924 (1 µM) treatment

Paclitaxel (100 nM) treatment

End of the organ culture

0 h

24 h

42 h

66 h

Bodo et al. AJP 2007, Langan et al. EXD 2015, Purba et al. EMBO Mol Med 2019, Piccini et al. JEADV 2021.

Previously…

Isolated
Anagen VI HFs

Dystrophic anagen pathway
(Less alopecia, retarded hair regrowth)

Paclitaxel or 4-HC

Bulge (Stem cells)

Bulb (HM KCs)

ALRN-6924

Paclitaxel

Apoptosis
EMT
DNA damage

Mitotic-catastrophe
Cytotoxicity

Minimize HF damage & acute alopecia?

Fully recovered Hair follicle

Hair shaft Shedding

Shortened dystrophic telogen

?

Dystrophic catagen pathway
(Massive alopecia, accelerated hair regrowth)

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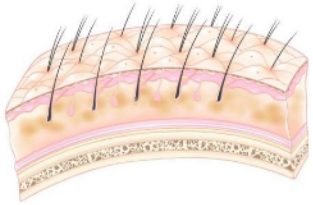
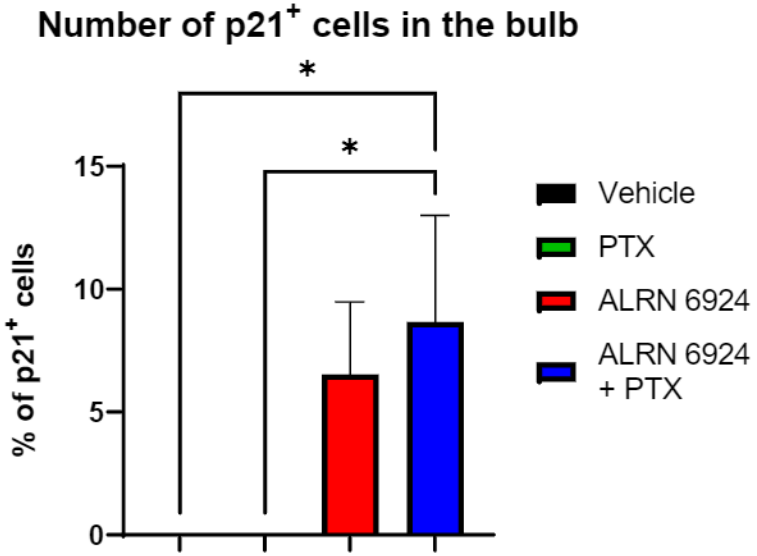
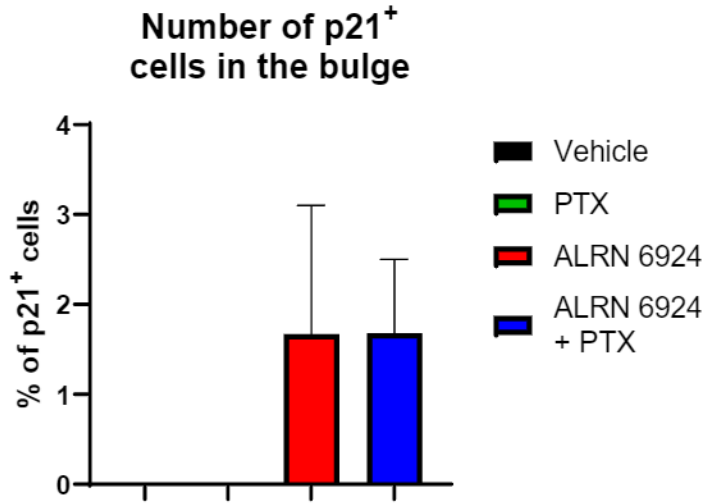
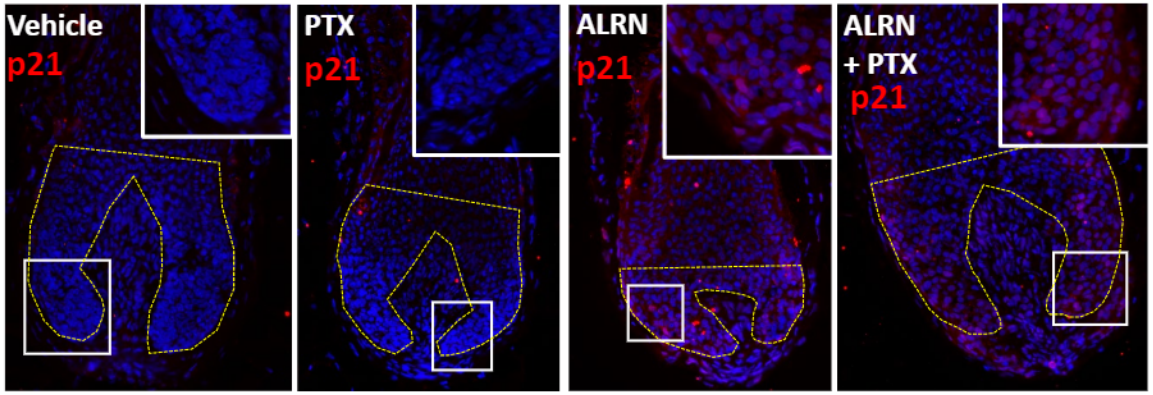
ALRN-6924 significantly enhances p21 expression in the hair matrix bulb of human anagen scalp HFs *ex vivo*

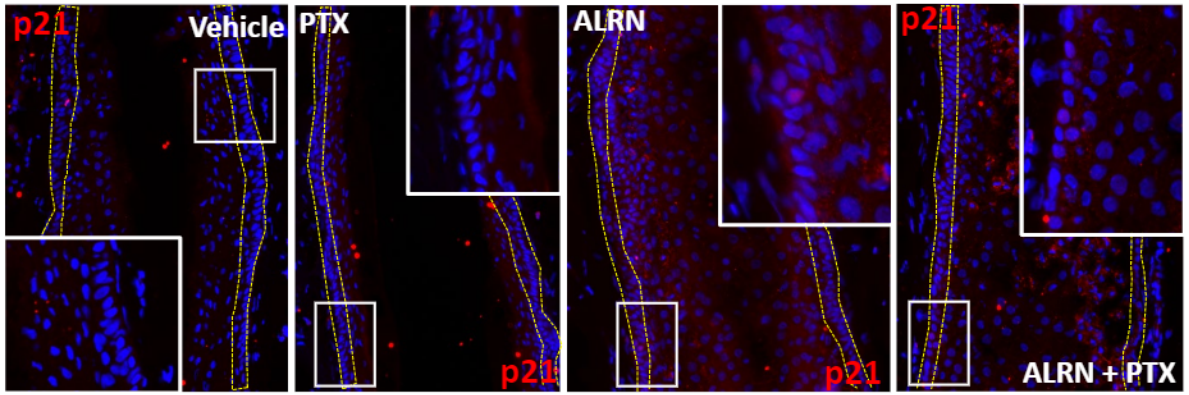




This shows the expected p53 activation







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Mean +/- SEM; n=5-7-15 HFs from **1 donor**; Mann Whitney test; *p<0.05. Yellow dotted areas: p21 evaluation area

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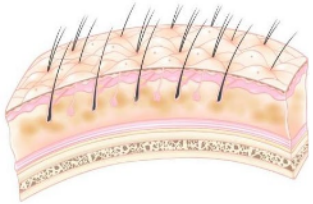
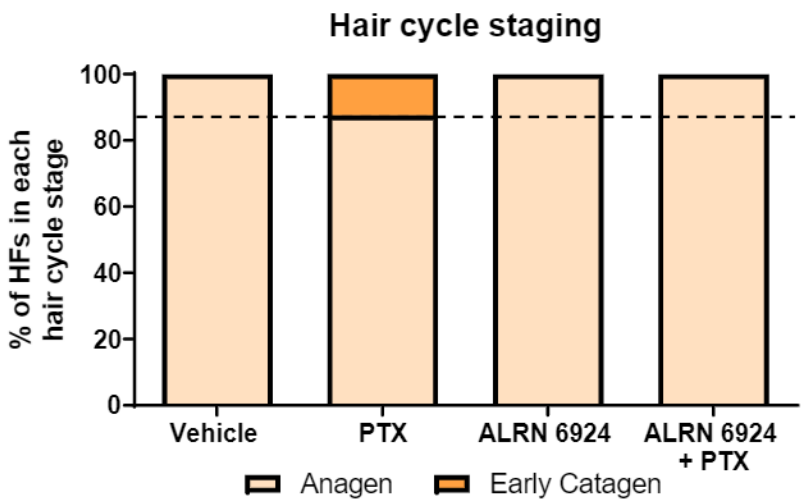
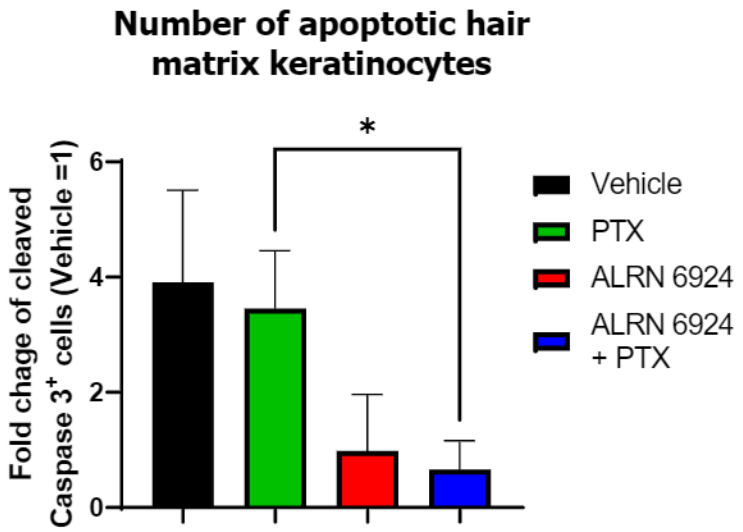
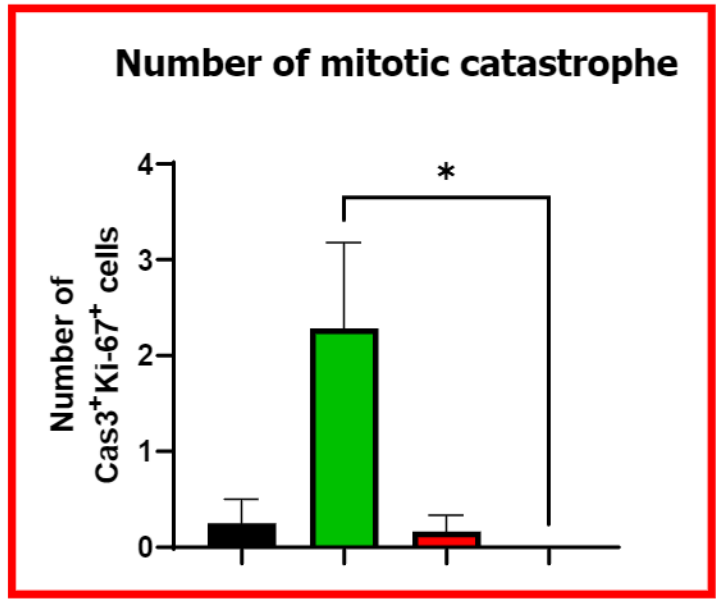
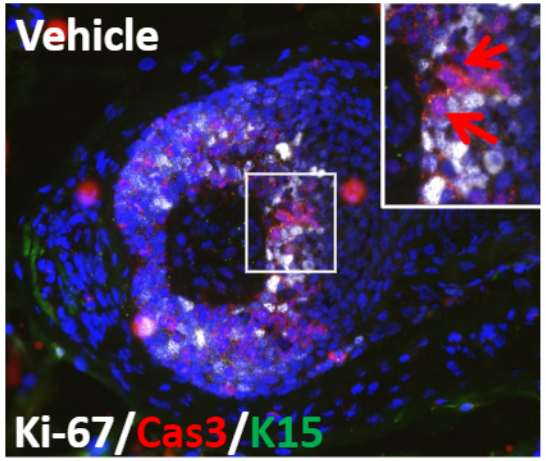
ALRN-6924 protects from PTX-induced apoptosis in the hair matrix, and prevents PTX-induced mitotic catastrophe, - without inducing catagen!

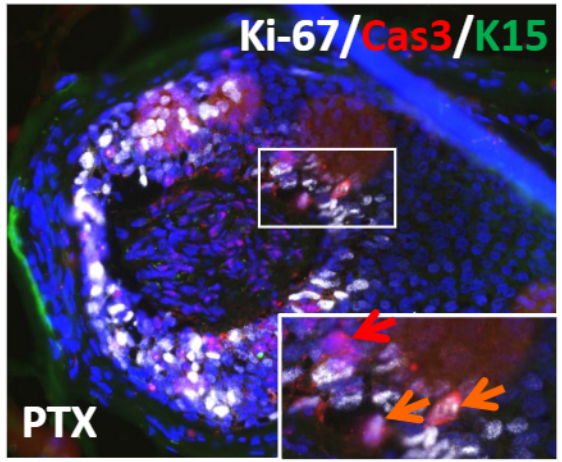


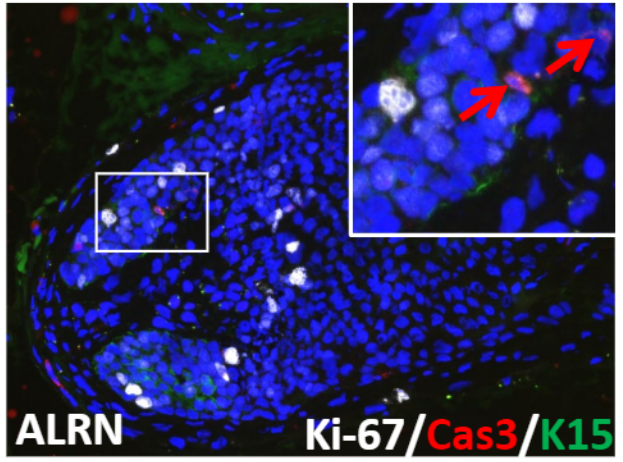


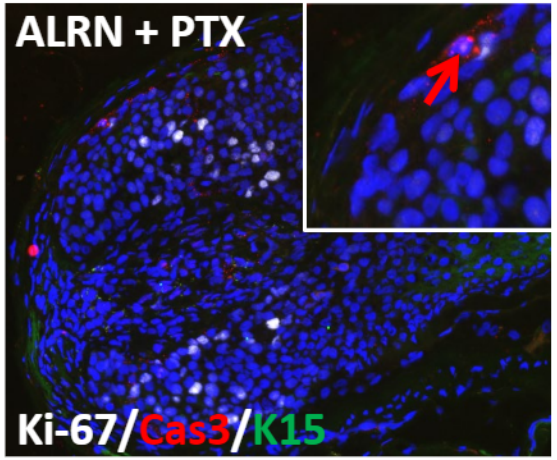












Mean +/- SEM; n=4-8 HFs from **1 donor**; Mann–Whitney test, *p<0.05. Red arrows: cleaved-caspase-3 positive cells; orange arrows: Mitotic catastrophe (Ki-67/Caspase-3 double positive cells)

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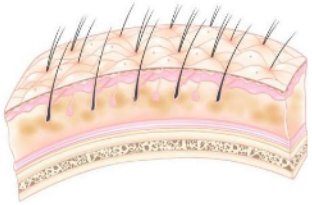
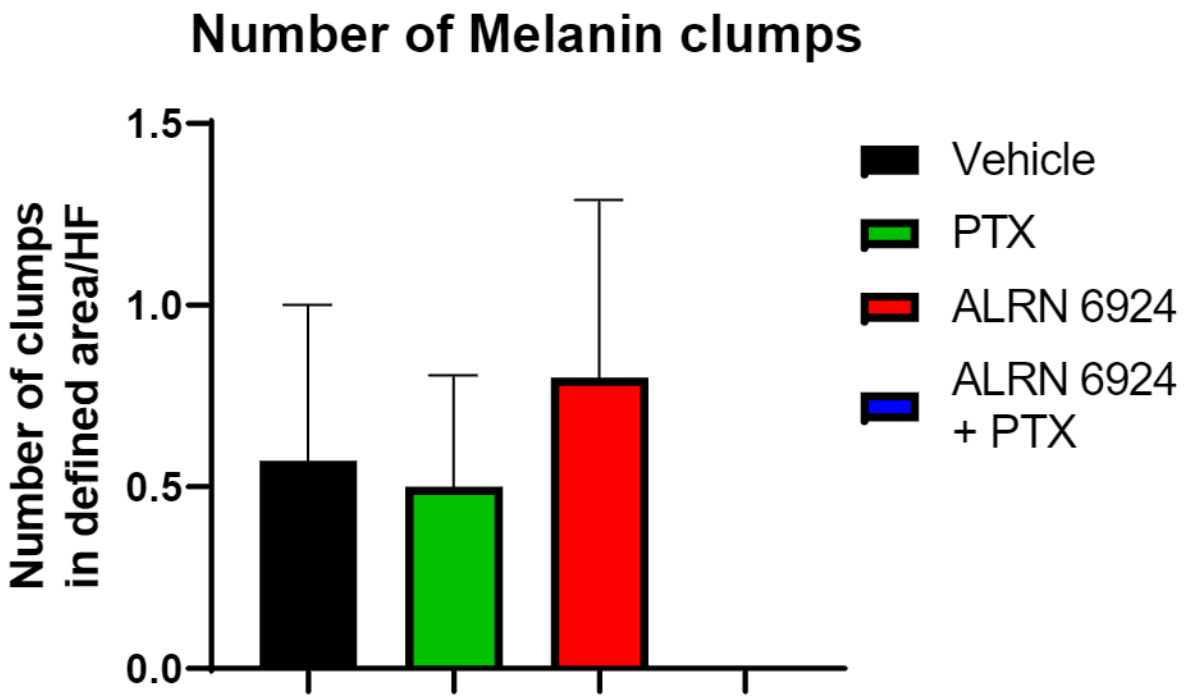
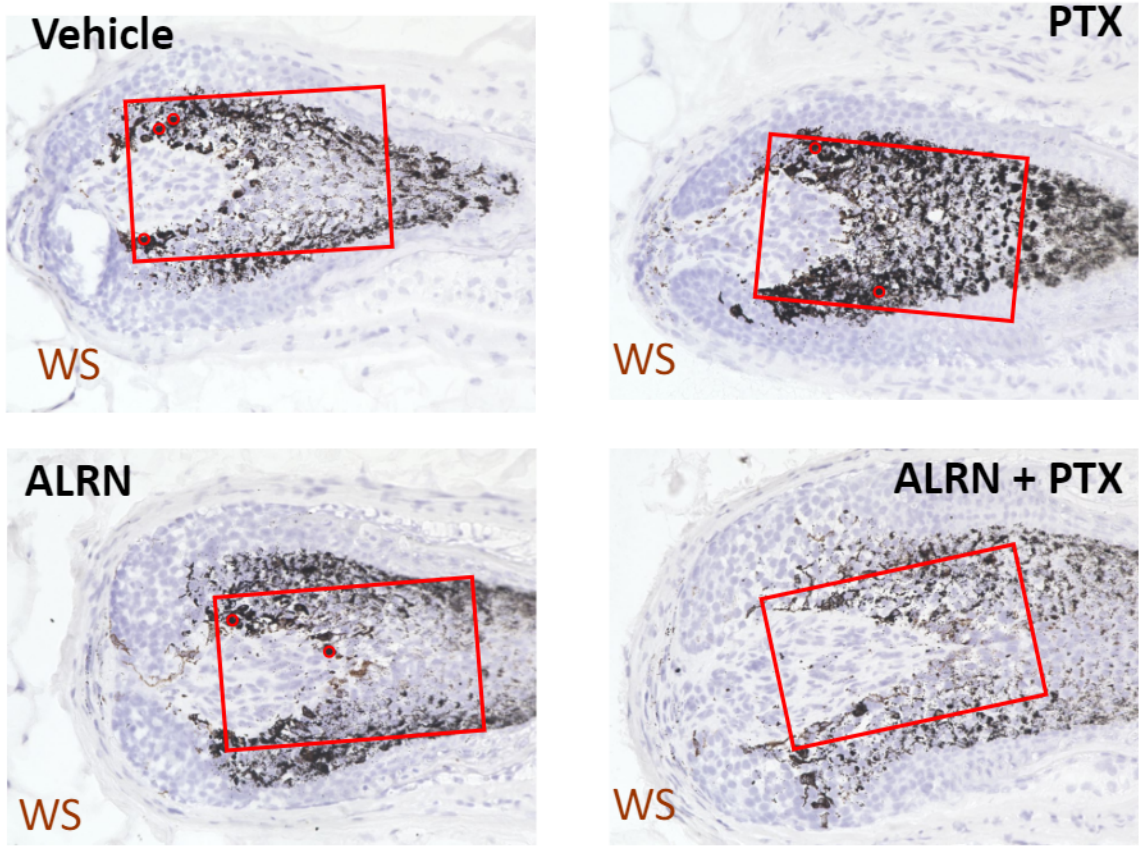
ALRN-6924 itself does not promote melanin clumping, but prevents PTX-induced melanin clumping



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

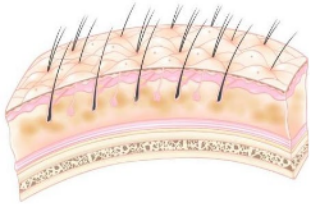




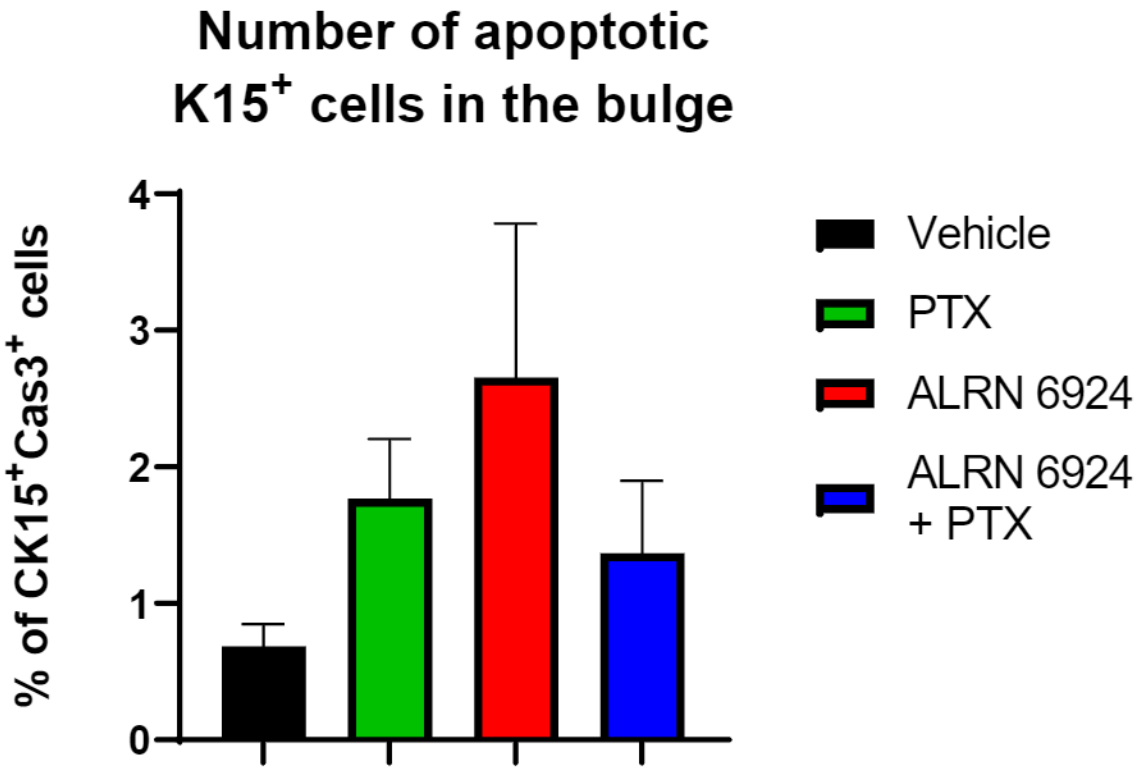
⬭ : Melanin clump

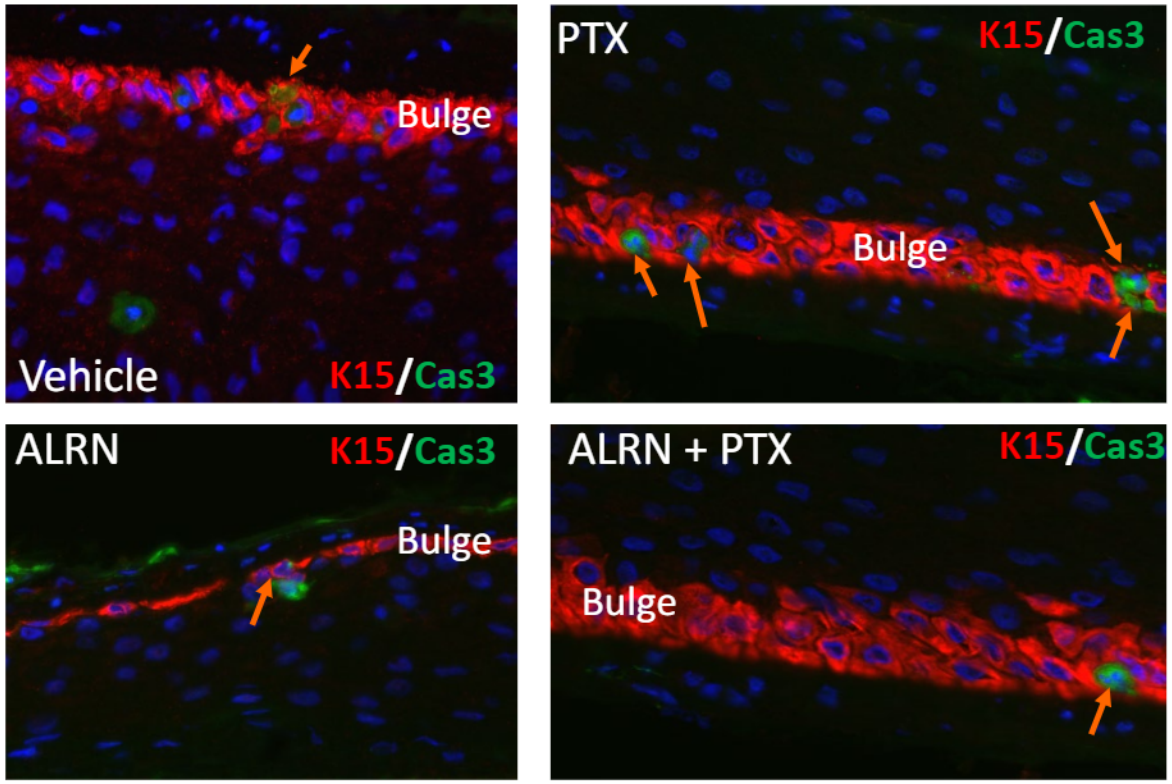
Mean +/- SEM; n=5-10 HFs from **1 donor**; Mann Whitney test, not significant.

ALRN-6924 itself does not promote apoptosis of K15+ eHFSCs, but may prevents apoptosis induction by PTX



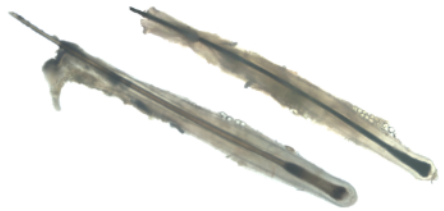
Keratin 15 (K15) HF stem cells marker





Mean +/- SEM; n=9-10 HFs from **1 donor**; Mann–Whitney test, ns.

Experimental Design 4-HC



Isolated
Anagen VI HFs

ALRN-6924 (1 µM) treatment

4-HC (30 µM) treatment

End of the organ culture

0 h

24 h

42 h

66 h

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Bodo et al. AJP 2007, Langan et al. EXD 2015, Purba et al. EMBO Mol Med 2019, Piccini et al. JEADV 2021.

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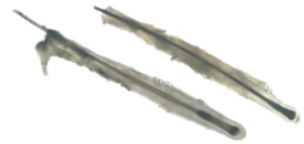
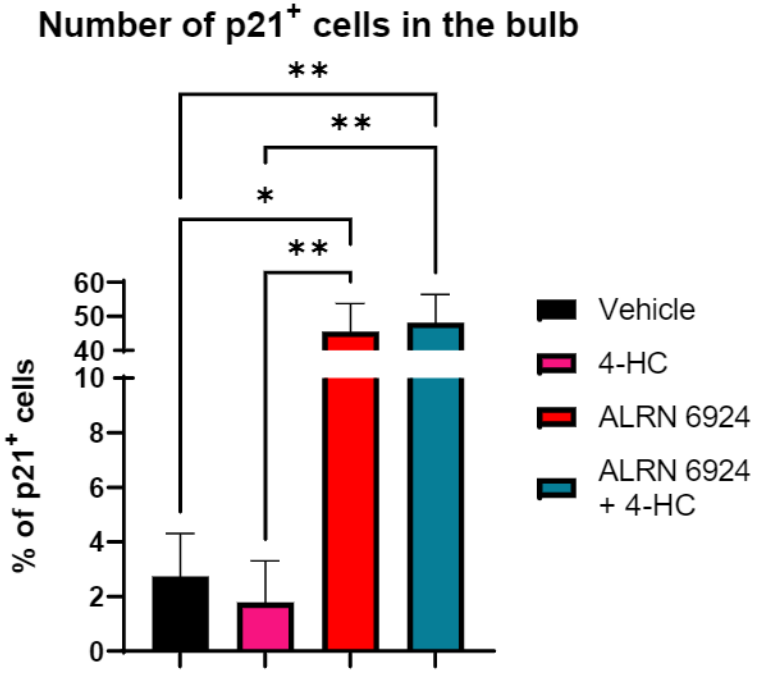
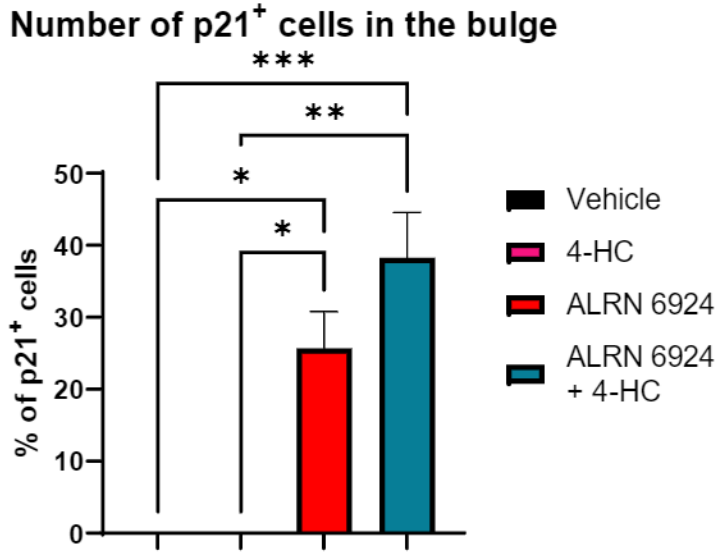
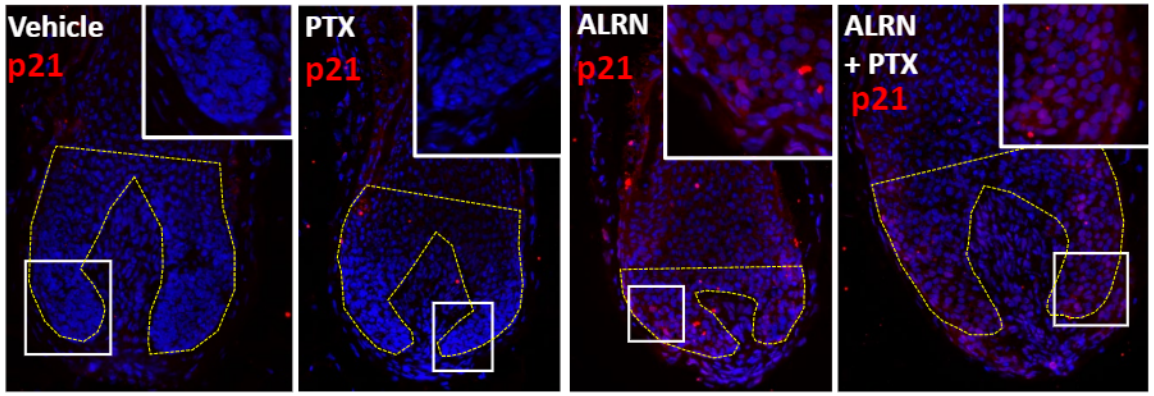
ALRN-6924 significantly enhances p21 expression in the anagen hair matrix bulb of human scalp HFs *ex vivo*

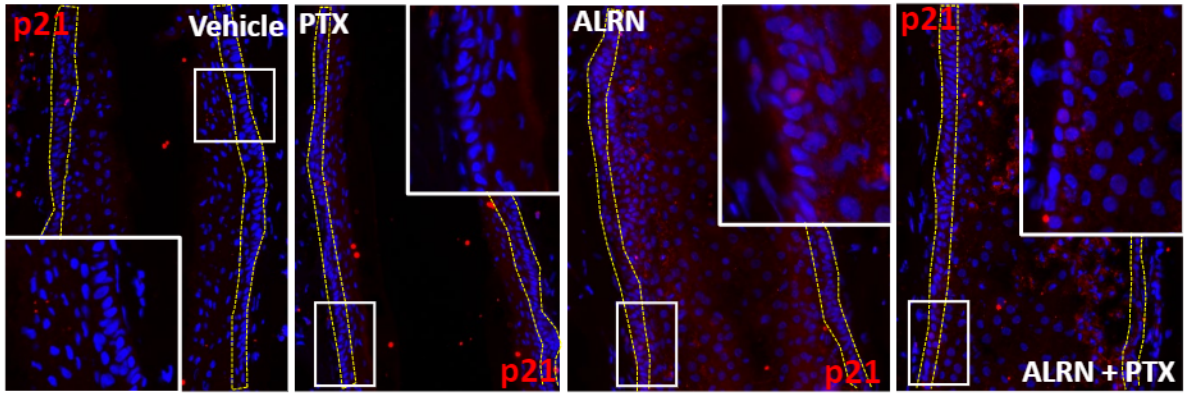




This shows the expected p53 activation







Mean +/- SEM; n=6-7 HFs from **1 donor**; Mann Whitney test; *p<0.05, **p<0.01; ***p<0.001. Yellow dotted areas: p21 evaluation area

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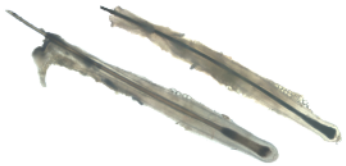
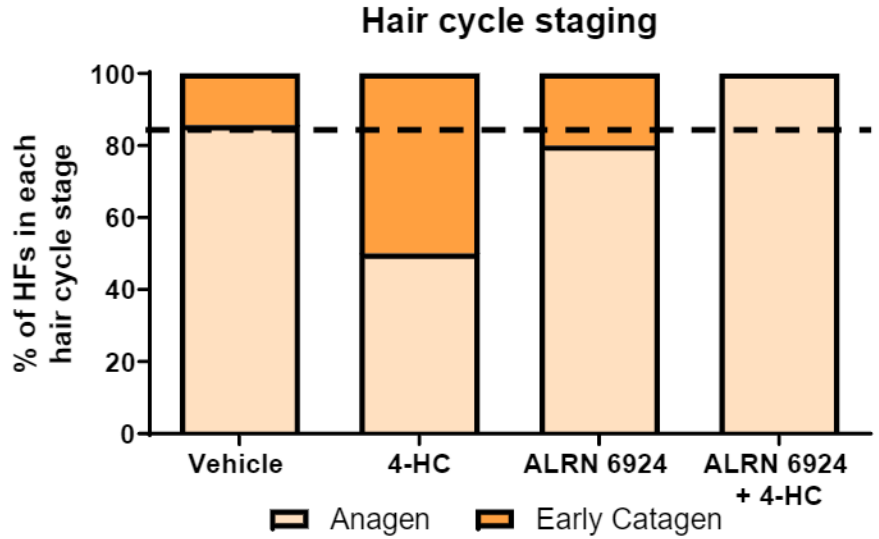
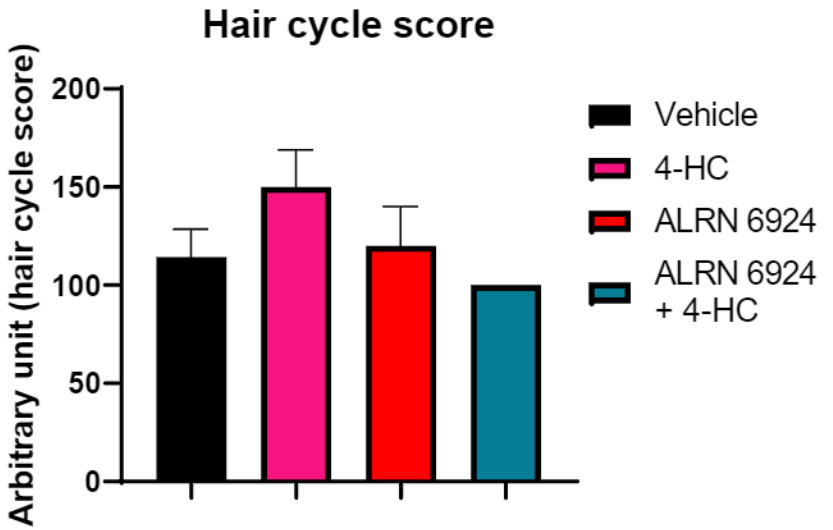
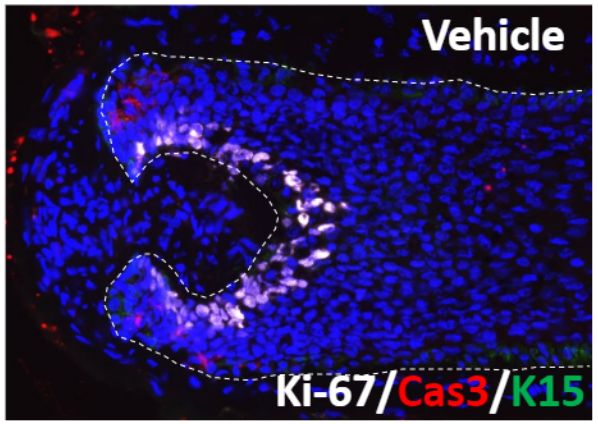
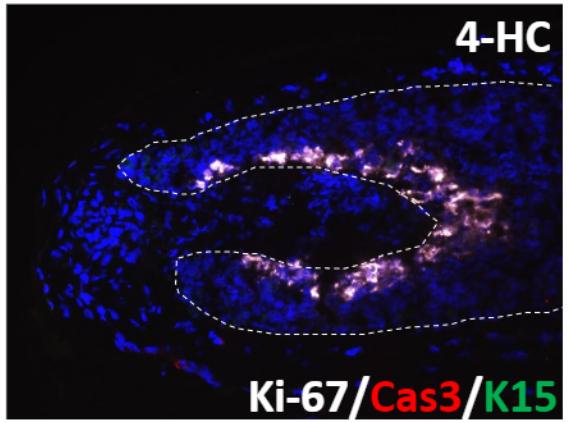
ALRN-6924 protects from 4-HC-induced catagen

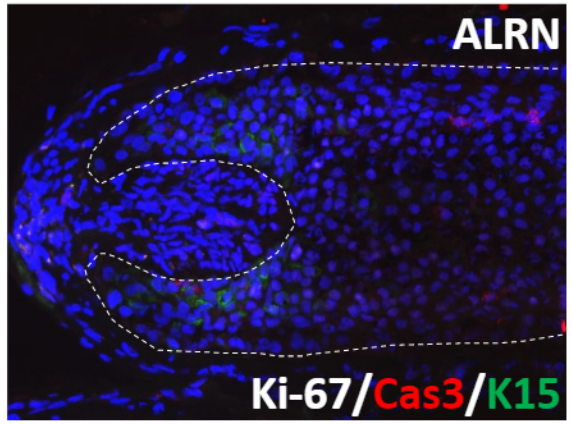


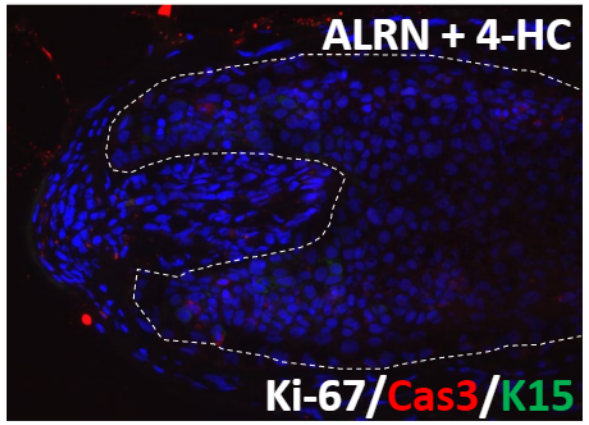












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Mean +/- SEM; n=5-8 HFs from **1 donor**; Mann–Whitney test.

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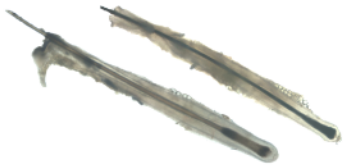
ALRN-6924 itself does not promote melanin clumping, but prevents 4-HC-induced melanin clumping



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

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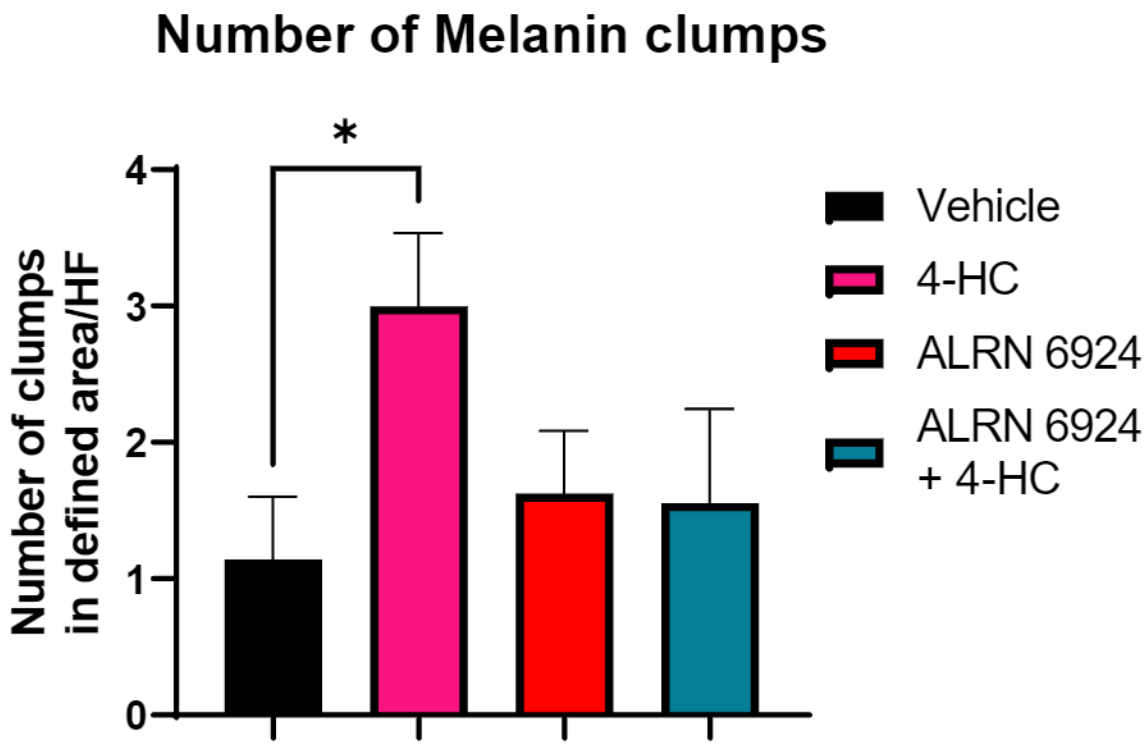

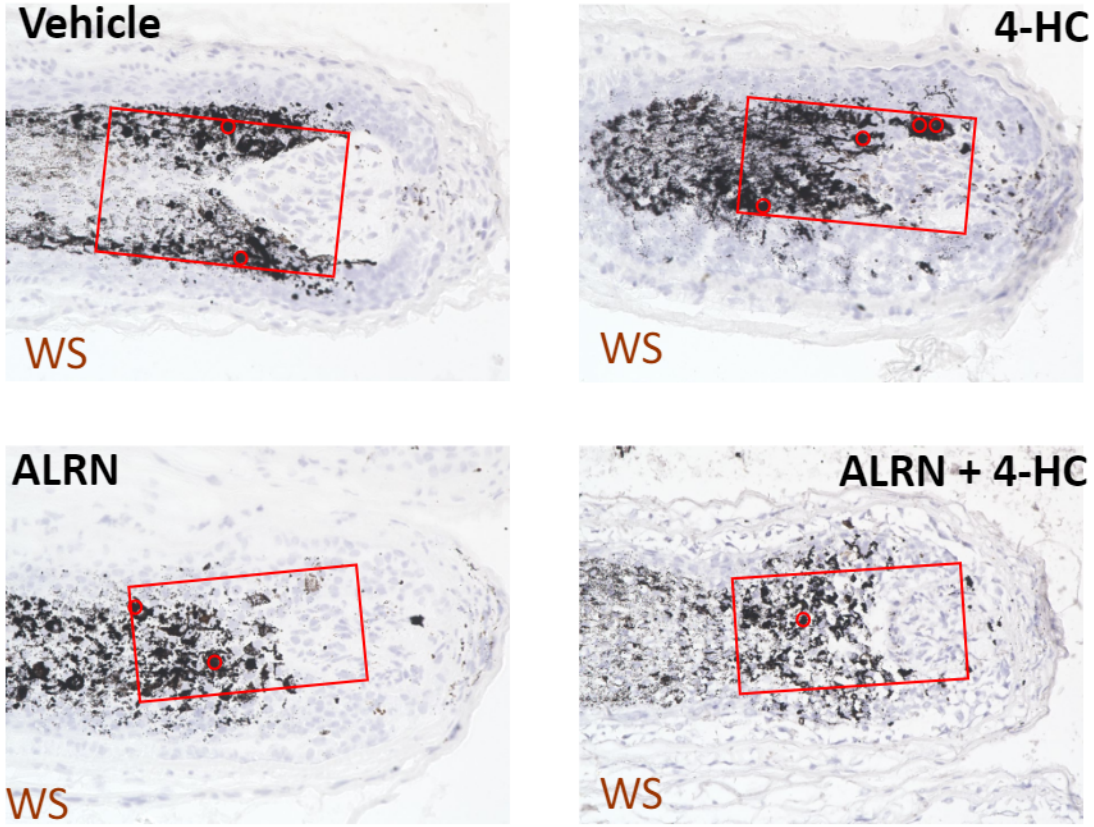

⬭ : Melanin clump

Mean +/- SEM; n=7-9 HFs from **1 donor**; Mann Whitney test, *p<0.05.

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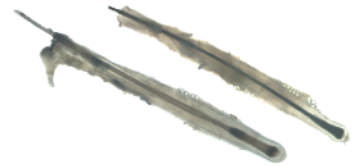
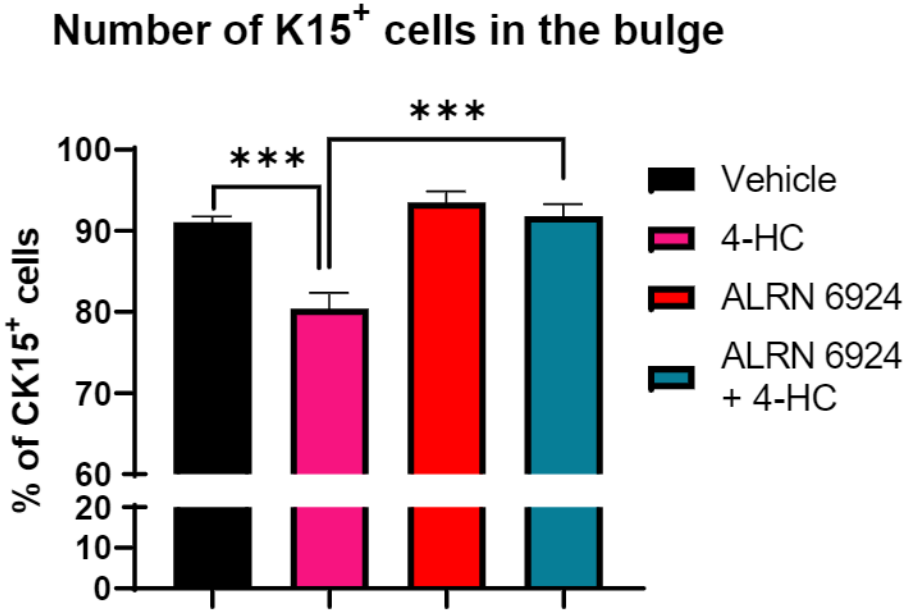
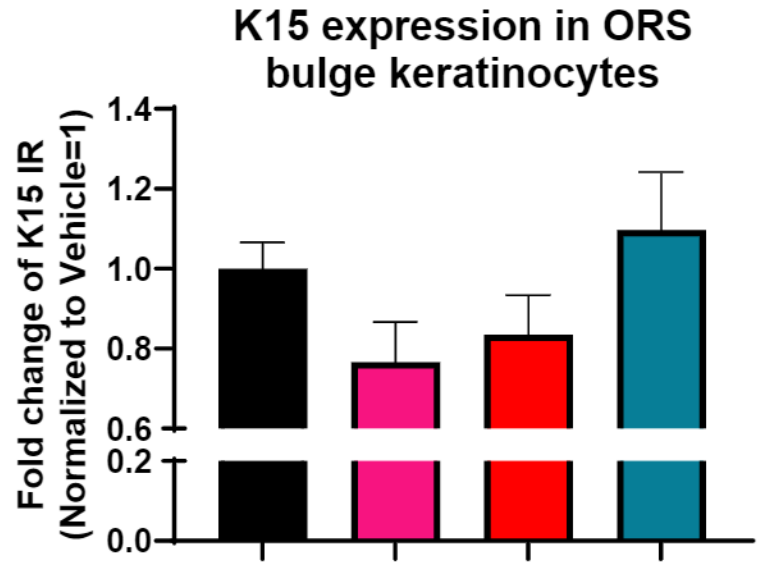
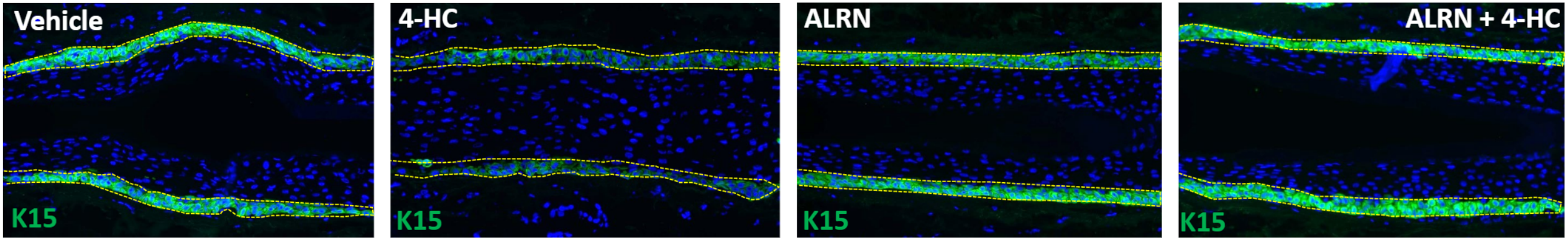
In short-term HF culture, ALRN-6924 does not affect keratin 15 expression and the number of K15$^+$ bulge keratinocytes, but **significantly prevents the decrease of K15$^+$ cell number from 4-HC action.**









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Mean +/- SEM; n=9-11 HFs from **1 donor**; Student's t test, ***p<0.001. Yellow dotted areas: K15 evaluation area

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Conclusions & Perspectives

- **ALRN-6924 does not induce premature catagen** *ex vivo* and is thus unlikely to cause telogen effluvium
- **ALRN-6924** inhibits **PTX-induced catagen and** mitotic catastrophe in the hair matrix, indicating that it may **favor a mild form of dystrophic anagen.**
- **ALRN-6924 inhibits 4-HC-induced catagen** and **protects human scalp HFs** from the **4-HC** mediated **cytotoxicity.**
- **Most importantly, ALRN-6924 reduces PTX-induced HF stem cells apoptosis,** and **significantly prevents the decrease of $K15^+$ cell number from 4-HC action** *ex vivo* and thus promises to **reduce the incidence and degree of permanent alopecia after taxane and 4-HC therapy.**

These *ex vivo* data support our working hypothesis that ALRN-6924 can SELECTIVELY protect healthy HFs and their stem cells against permanent taxane (and 4-HC?)-induced alopecia.

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Takahiro Suzuki, PhD
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D. Allen Annis, PhD
Manuel Aivado, MD, PhD

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