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)

- 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
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

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†

Questions addressed

Does ALRN-6924:

- prevent general HF toxicity induced by paclitaxel (Taxol) or 4-hydroperoxycyclophosphamide (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

- 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

ALRN-6924 significantly enhances p21 expression in the hair matrix bulb of human anagen scalp HF's 

ex vivo

This shows the expected p53 activation

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


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**Number of Melanin clumps**

- **Vehicle**
- **PTX**
- **ALRN 6924**
- **ALRN 6924 + PTX**

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

**Number of apoptotic K15\(^+\) cells in the bulge**

- Vehicle
- PTX
- ALRN 6924
- ALRN 6924 + PTX

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

**Image:**
- **Vehicle:** K15/Cas3
- **PTX:** K15/Cas3
- **ALRN:** K15/Cas3
- **ALRN + PTX:** K15/Cas3
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.
ALRN-6924 protects from 4-HC-induced catagen

Hair cycle staging

% of HF s in each hair cycle stage

Vehicle  4-HC  ALRN 6924  ALRN 6924 + 4-HC

Anagen  Early Catagen

Hair cycle score

Arbitrary unit (hair cycle score)

Vehicle  4-HC  ALRN 6924  ALRN 6924 + 4-HC

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

- **Vehicle**
- **4-HC**
- **ALRN 6924**
- **ALRN 6924 + 4-HC**

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

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