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

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Chemotherapy-induced alopecia mediated by 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**
- **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**

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No protection of p53-mutant cancer cells

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

ALRN-6924: Structure & key design properties

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

<table>
<thead>
<tr>
<th>Binding Affinity</th>
<th>Kd, nM</th>
<th>MDM2</th>
<th>MDMX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native p53</td>
<td>770</td>
<td>480</td>
<td></td>
</tr>
<tr>
<td>ALRN-6924</td>
<td>13.7</td>
<td>8.9</td>
<td></td>
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</tbody>
</table>
ALRN-6924 is a decoy that mimics p53 and selectively binds to MDMX + MDM2, releasing p53 to induce cell cycle arrest.

Low-dose ALRN-6924
Low-level p53 release → Cell cycle Arrest\(^1\)

High-dose ALRN-6924
High-level p53 release → Apoptosis\(^1\)

\(^1\) Chen et al.; Cold Spring Harb Perspect Med. 2016
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**

**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 induced 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

Hair follicle isolation

ALRN-6924 treatment

Paclitaxel treatment

End of the organ culture

0 h

24 h

42 h

66 h

Anagen VI HFs

serum-free anagen scalp HF organ culture from healthy donors


Do Not Post
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.
**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)
**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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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

Number of K15+Ki-67+ cells in the bulge

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 DNA damage.

Number of K15⁺γH2AX⁺ cells in the bulge

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)

Number of K15⁺Vimentin⁺ cells in the bulge

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

Paclitaxel

Dystrophic anagen pathway (Less alopecia, retarded hair regrowth)

Apoposis EMT DNA damage

Mitotic-catastrophe Cytotoxicity

Minimize HF damage & acute alopecia?

Fully recovered Hair follicle

Paclitaxel

Bulge (Stem cells)

Bulb (HM KCs)

Hair shaft Shedding

Dystrophic catagen pathway (Massive alopecia, accelerated hair regrowth)
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.
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