“A topical selection approach to improve long-term gene expression”

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With ex vivo approaches for skin gene therapy, we experienced a decrease of in vivo gene expression over time.

Potential explanations include:
1. inefficient gene delivery to keratinocyte stem cells
2. partial silencing or inactivation of genes delivered by viral vectors
l) Topical Selection of MDR-expressing Keratinocytes

Principal advantages:

- Does not require direct KSC identification because cells capable of long term MDR expression are being selected.
- MDR confers resistance to a broad range of distinct selective agents
- Limited skin toxicity of mitotic inhibitors avoids widespread necrosis and inflammation
- MDR is an endogenous protein and will not elicit an immune response
RV VECTOR

MDR1

LTR

LTR

HUMAN KC & FB

RAFT CULTURE

TOPICAL SELECTION

Colchicine

MDR Transduced KC

MDR Transduced KC
Grafts of Human Skin Equivalents
MDR Expression in MDR+KC Grafts - Week 7

No Colchicine

+Colchicine Tx
FACS Analysis Of MDR+KC% During Topical Colchicine Treatment (Week 7)

- Vehicle control: 23%
- Colchicine (100 µg/gm): 36%
- Colchicine (200 µg/gm): 68%
Topical Colchicine Treatment Increases MDR+KC% In A Dose Dependent Manner At Week 7

\[ p = 0.008 \]
Topical Colchicine Results in Sustained Increased Percentages of MDR+KC

Colchicine Treatment
DELAYED SELECTION RESTORES %MDR+KC

The graph shows the percentage of %MDR+KC over weeks. There are three lines indicating different selection conditions:

- **No Selection**: A line indicating no selection with a peak around 12 weeks.
- **Selection at 4WKS**: A line starting from a peak at 4 weeks and decreasing over time.
- **Selection at 12 WKs**: A line starting from a peak at 12 weeks and decreasing over time.

The y-axis represents %MDR+KC, ranging from 0 to 100, while the x-axis represents weeks, ranging from 0 to 24.
Topical colchicine increases the level of MDR expression in MDR+KC at week 15

Isotype control

Vehicle control

Colchicine (200 µg/gm)
Why does topical colchicine treatment maintain MDR expression?

To distinguish between these mechanisms, we have to determine if cells that are not expressing the MDR protein still contain the MDR gene.
Colchicine Tx Increases MDR Gene Copy Number in Grafts (Week 15)

Relative copy number

48% MDR+  7% MDR+  45% MDR+

p=0.008
Summary

Topical colchicine treatment can increase both the percentage and the duration of in vivo $MDR1$ gene expression in keratinocytes.

Topical colchicine treatment is associated with an increased level of $MDR1$ expression in individual keratinocytes.

Topical colchicine treatment is able to select for keratinocyte progenitor cells that both contain and express the $MDR1$ gene.
Potential disadvantages of this approach:

1. Producing high titer bicistronic vectors that contain the MDR selectable marker gene has been difficult, historically.

2. With retroviral or lentiviral bicistronic vectors, the size of the MDR selectable marker gene (4.5 kb) will limit the size of a desired therapeutic gene that can be inserted.

3. Need to determine why the percentage of MDR+KC reached a plateau of 50% during topical colchicine selection.
Increasing the topical colchicine dose to human skin grafts

Then the cream is applied using a syringe and the hole is covered using a tape that is easily removable for subsequent cream application.
Atrial Natriuretic Peptide (ANP) Pathway

Heart

Corin

Pro-ANP

nucleus

Cardiomyocyte

ANP

NPR-C

clearance

Kidney

NPR-A

cGMP

NEP

degradation

↓ systemic vascular resistance

↓ central venous pressure

↓ inhibition Renin-Angiotensin-Aldosterone pathway

↓ cardiac output

↓ Blood Volume

↓ Arterial Pressure

↓ Blood Volume

Natriuresis and Diuresis
# Bicistronic Retroviral Vectors

## XIX-MDR

<table>
<thead>
<tr>
<th>5’ LTR</th>
<th>Ψ</th>
<th>CMV</th>
<th>EMPTY</th>
<th>IRES</th>
<th>MDR1</th>
<th>LTR 3’</th>
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## XIX-ANP-MDR

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![Image of a signal peptide and its variants](image-url)
Systemic Delivery of ANP:

In vitro feasibility studies with raft cultures have demonstrated that:
1. Both keratinocytes and fibroblasts secrete biologically active ANP at levels anticipated to achieve a biological effect in vivo
2. Keratinocytes that express ANP are able to differentiate and stratify normally

Human skin equivalents containing different combinations of ANP expressing keratinocytes and/or fibroblasts are being grafted to immunocompromised mice

Systemic ANP effects will be assessed by monitoring blood pressure and by measuring serum levels of human ANP.
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