TRPV3 antagonist treatment reduces pro-inflammatory pathways in Olmsted syndrome keratinocytes

Michael McGrath[1], **Lucile Marchal[1]**, Ephraim Brener[2], Liora Braiman[2,] and Alain Hovnanian[1, 3, 4]

[1] INSERM UMR 1163, Laboratory of genetic skin diseases, Imagine Institute, 75015 Paris, France
[2] Kamari Pharma Ltd, Sapir 3 street, 1st floor, Ness Ziona Science Park, 7403626 Israel
[3] University of Paris City, 75006 Paris, France.
[4] Department of Genetics, Necker hospital for sick children, Assistance Publique des Hopitaux de Paris (AP-HP), 75015, France.
- Corresponding author: Alain Hovnanian, alain.Hovnanian@inserm.fr

Introduction:

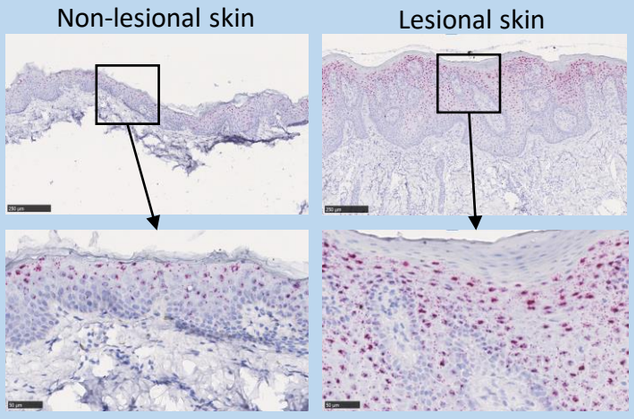
Olmsted syndrome (OS) is a rare, very painful, and severe form of palmoplantar keratoderma. It is most often caused by dominant variants in the transient receptor potential vanilloid 3 (TRPV3) gene encoding a thermosensitive calcium channel which forms a functional complex with EGFR. TRPV3 variants are mainly gain of function leading to abnormal keratinocyte activation through amplified calcium influx causing increased release of pro-inflammatory factors, angiogenic factors and EGFR/mTOR signalling activation. Currently there are no TRPV3 targeted therapies in clinic. Here we show that highly specific TRPV3 antagonist KM-001 reduces calcium influx, disease associated marker levels (pRPS6, NF-kB p65, pSTAT3) and pro-inflammatory mediators secretion (TNF-α and IL-8) in OS keratinocytes. KM-001 could therefore become a viable therapeutic option for OS.

1. Expression of TRPV3 is observed in the upper layer of the epidermis and is increased in lesional skin of OS patient with dominant mutation

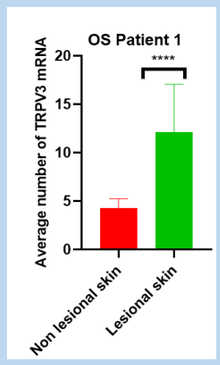
Using mRNA *in-situ* hybridization on Olmsted patient non-lesional and lesional skin sections. Each red spot represents one TRPV3 mRNA. Spots were quantified using QuPath software and the average number of TRPV3 mRNA per epidermal cell per area was calculated.

Data for patient 1 (dominant TRPV3 mutation) is presented below :

Non-lesional skin | Lesional skin



Scale bars are respectively 250 µM and 50 µm.



Unpaired t-test was performed p-value < 0.0001.

As shown in OS patient 1 skin sections above, TRPV3 is principally expressed in the upper layers of the skin, including spinous and granular layers.

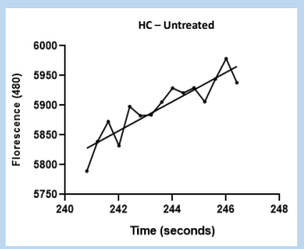
TRPV3 expression is variable between healthy skin controls, OS patients skin and seems to also depend on biopsy zone (data not shown).

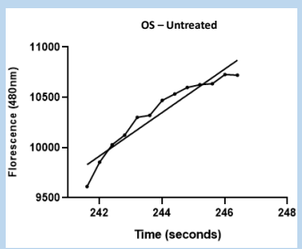
Differences in expression have been observed between lesional and non-lesional skin but are not significant for all patients tested.

Cells from patient 1 and other TRPV3 dominant mutated patients were used for the following experiments

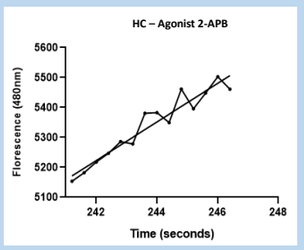
2. Increased Calcium influx into OS keratinocytes and reduction following treatment with TRPV3 antagonist KM-001

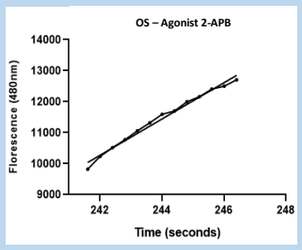
Using FDSS/µCELL high throughput calcium imaging technology





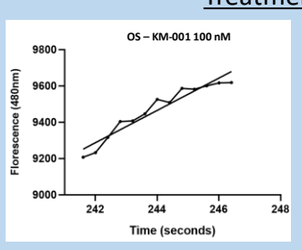
HC : Healthy control keratinocytes
OS : Olmsted syndrome keratinocytes

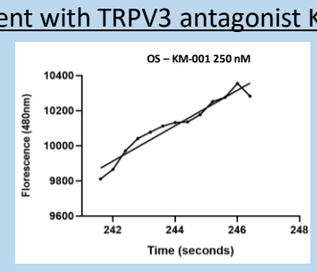




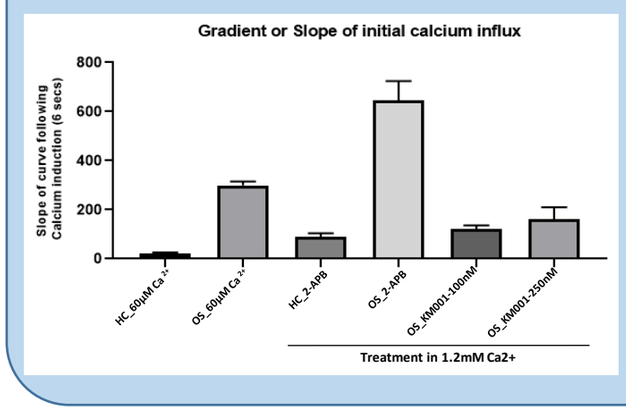
Keratinocytes were screened with varying concentrations of KM-001 and TRPV3 agonist 2-APB

Treatment with TRPV3 antagonist KM001





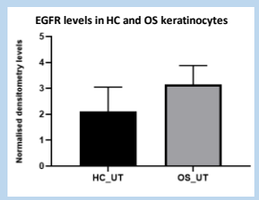
Agonist causes an increase calcium influx into both HC and OS keratinocytes.

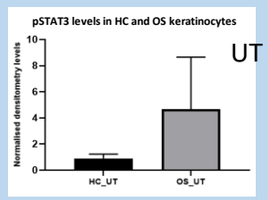


Treatment with TRPV3 antagonist KM-001 decreases the calcium influx into the cell compared to untreated control

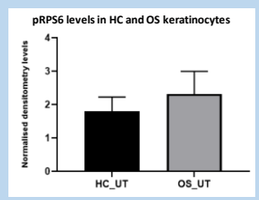
3. OS signalling pathway characterisation

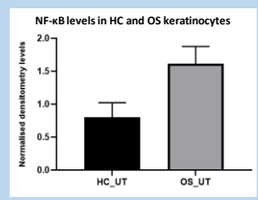
Western blot analysis was used to characterise the signalling pathways involved in OS keratinocytes

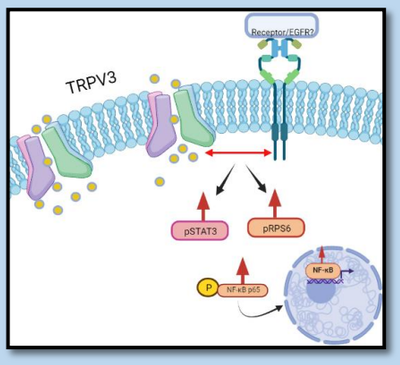




UT : Untreated

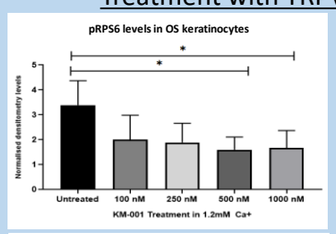


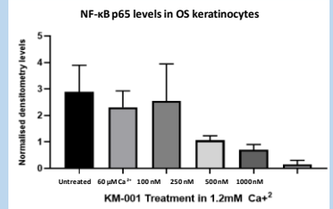




OS keratinocytes in high calcium conditions leads to an increase in **pRPS6**, **pSTAT3, EGFR** and **NF-kB p65**.
Suggesting a crosstalk between TRPV3-EGFR signalling

Treatment with TRPV3 antagonist KM001

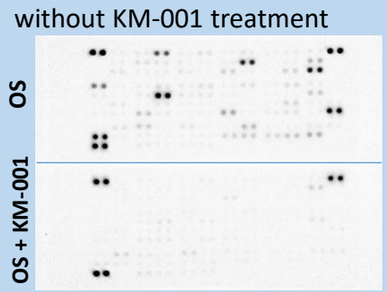




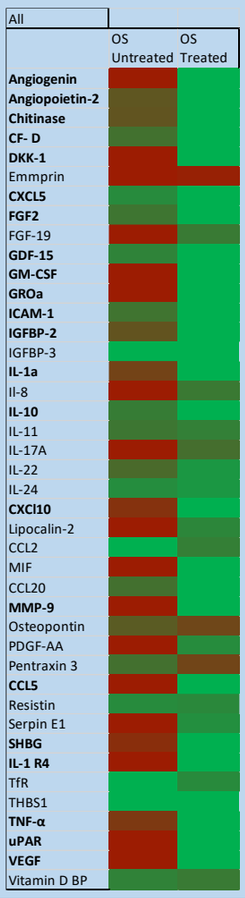
Treating OS cells with TRPV3 antagonist KM-001 causes a decrease in the disease-associated markers pRPS6 and NF-kB p65. Providing strong evidence for its use in treating OS. Paired students t-test p-value <0.01.

4. Reduction of secreted key pro-inflammatory mediators in OS keratinocytes following treatment with TRPV3 antagonist KM-001

Using a cytokine/chemokine proteome profiler we determined the immunological secretory signature of OS keratinocytes with and without KM-001 treatment

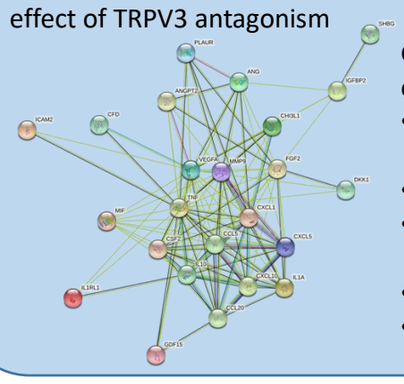


Factors
Green - **decrease** in relative density



KM-001 treatment has a drastic impact on OS secretory profile. Reducing the release of many key pro-inflammatory mediators (**TNFα**, **IL-8, CXCL5**) implicated in OS pathology.

Using STRING software and GO analysis we were able to determine the signalling and immunological pathways implicated in OS and effect of TRPV3 antagonism



GO analysis, increased frequency of terms relating to:

- **Chronic inflammatory response**
- **Epithelial apoptosis**
- **Immune cell activation/differentiation**
- **IL-6 response**
- **Calcium signalling**

Conclusion and Perspectives:

- TRPV3 expression is variable among OS patients and healthy controls skin
- TRPV3 expression is increased in some lesional skin sections obtained from OS patient including patient 1 (dominant TRPV3 mutation).
- OS patient derived keratinocytes maintain a disease-like phenotype in culture.
- Variant TRPV3 increases cytosolic calcium in OS keratinocytes.
- There is a connection with TRPV3-EGFR/mTOR/NF-kB signaling leading to a pro-inflammatory phenotype of OS cells in culture.

Treatment of OS cells with KM-001 a TRPV3 antagonist

- Reduces calcium influx, pRPS6 and NF-kB p65 levels.
- Reduces pro-inflammatory factors including: **TNFα**, **IL-8 and CXCL5**
- Reduces angiogenic factors including: **VEGF**, **angiogenin** and **angiopoietin-2**

Thus, this preliminary data shows that treatment with TRPV3 antagonist KM-001 has positive effects on OS keratinocytes in culture and could become a viable treatment for Olmsted patients.