



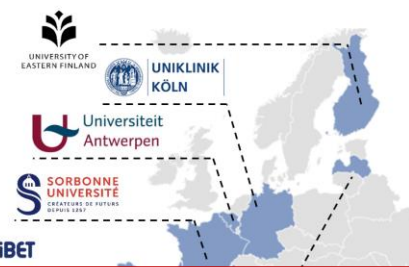
IT-DED³

Drug discovery: design, synthesis and biological evaluation of novel ferroptosis and necroptosis inhibitors

Camilla Scarpellini (ESR2)

Joint meeting OcuTher and IT-DED³
Nice, October 16, 2019

Work Package 1: general overview



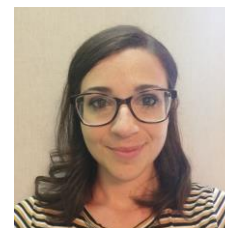
**Design, Synthesis and
Biochemical evaluation
of novel Ferroptosis
and RIPK1 inhibitors**



• ESR2 Camilla Scarpellini



• ESR1 Alba Ramos Llorca



• ESR3 Agnese Compagnone



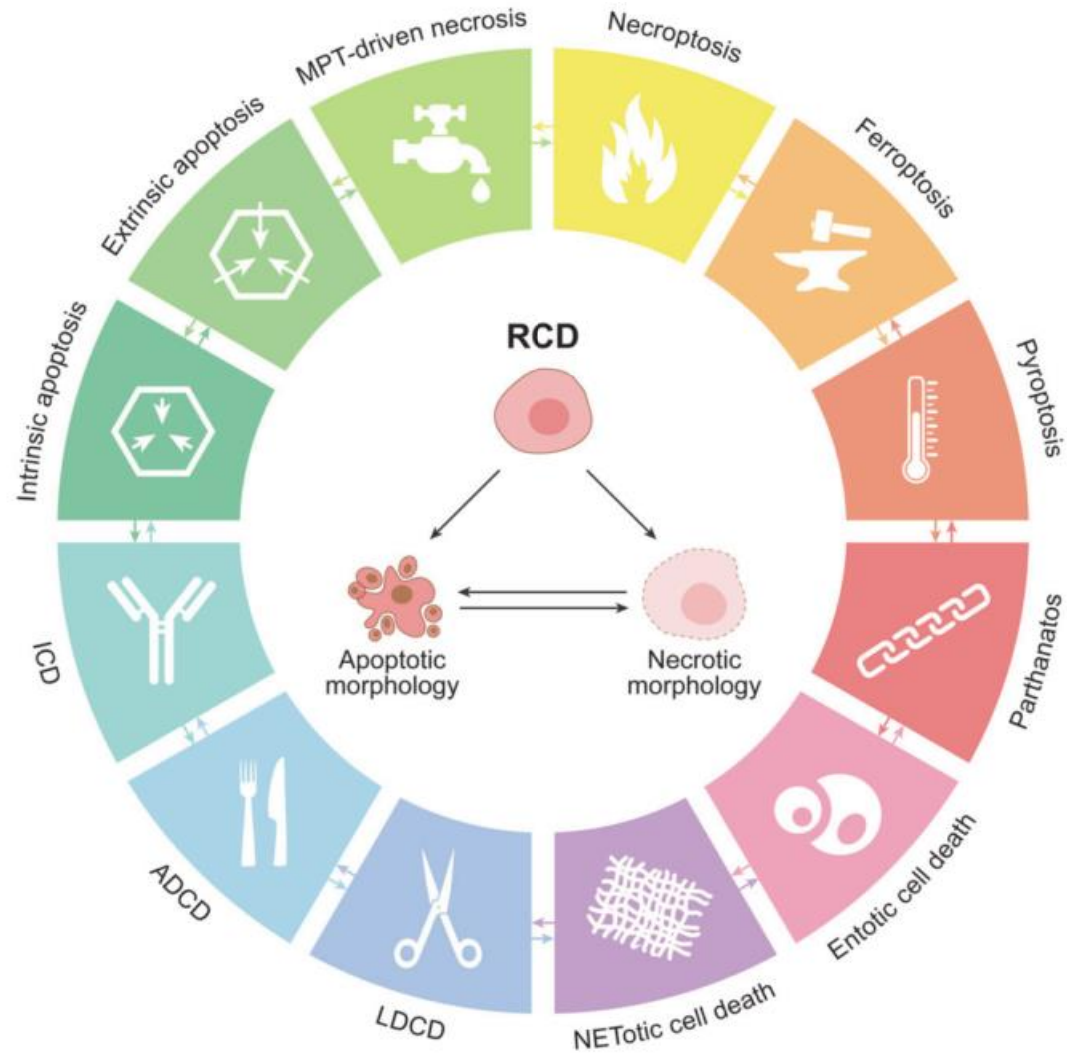
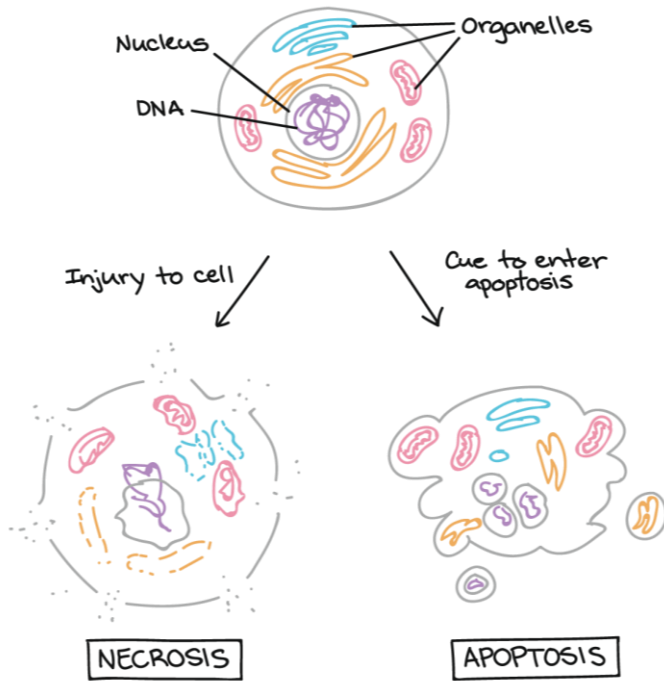
• ESR12 Maha Abdallah



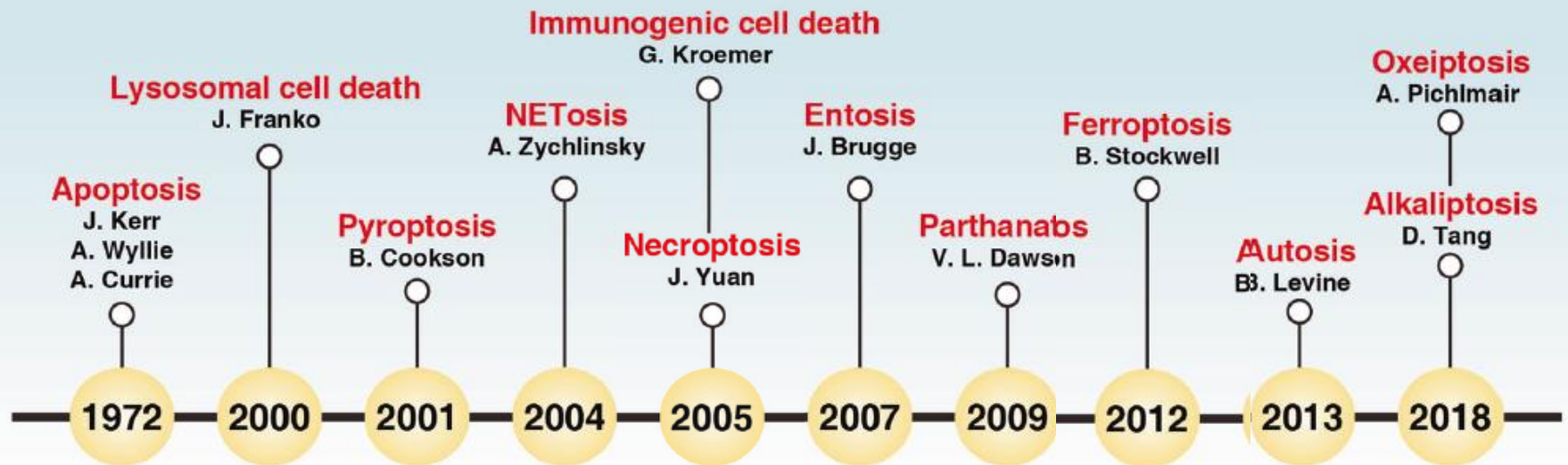
• ESR4 Nikolaos Katsinas



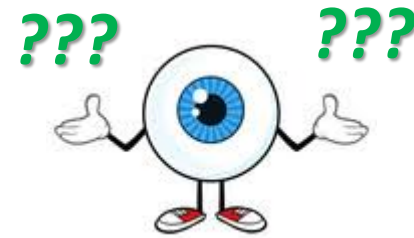
Cell death overview



Cell death as dynamic field

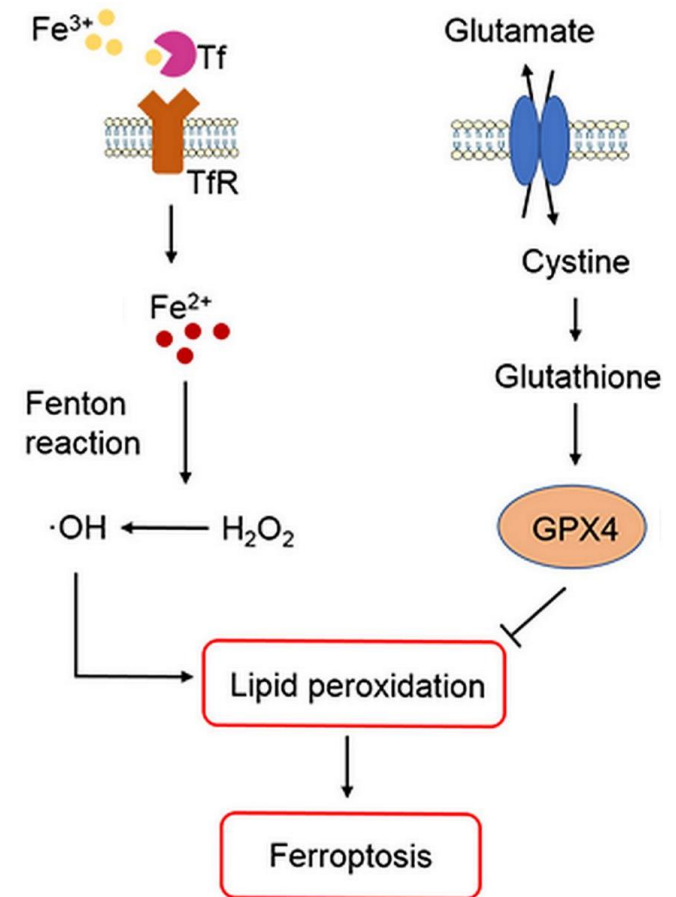


What is rroptosis?



“Ferroptosis is an iron-catalyzed, nonapoptotic form of regulated necrosis that results in oxidative lipid damage in cell membranes”

- Accumulation of **lipid hydroperoxides**
- Disruption of membrane integrity
- **GPX4** reduction of LOOH to LOH



Wan J, et al. *Stroke and Vascular Neurology*, **2018**, 000205

Dixon S. J., et al., *Cell*, **2012**, 149, 1060–1072,

Ferroptosis and dry eye diseases



Why we hypothesized the connection between **ferroptosis** and **dry eye**?

- The **accumulation of lipid hydroperoxide byproducts** is associated with conjunctival diseases such as allergic conjunctivitis and **dry eye**;
- **GPx4 might be a new therapeutic target for conjunctival disorders such as dry eye and keratoconjunctivitis.**

Sakai O et al. *Invest Ophthalmol Vis Sci.* **2015**; 56:538–543

- **Oxidative stress induce cell death in Retinal Pigment (RPE) through ferroptosis in addition to apoptosis and necroptosis**

Totsuka, K., et al., *Experimental Eye Research*, **2019**, 181:316-324

Cornea

The Role of Oxidative Stress and Inflammation in Conjunctivochalasis

Samantha K. Ward^{1,2}, Tate Uttomsi Wabumateu^{1,2,3}, Mitsuo Doi^{1,2,3}

Contents lists available at [ScienceDirect](#)

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Clinical and Epidemiologic Research

The Impact of Conjunctivochalasis on Dry Eye Symptoms and Signs

nder,¹ Allison L. McClellan,³ Katherine T. McManus,³

Sophia Seen¹ and Louis Tong^{1,2,3,4}

oxidative stress

ARTICLE

Oxidative stress induces ferroptotic cell death

Kiyohito Totsuka^{a,1}, Takashi Ueta^{a,b,1}, Takatoshi Suguru Nakagawa^a, Demetrios G. Vavvas^d, Megu

Cornea

Role of Glutathione Peroxidase 4 in Conjunctival Epithelial Cells

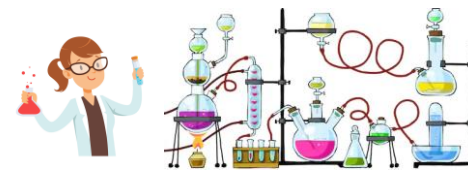
Osamu Sakai,^{1,2} Takatoshi Uchida,^{1,2} Hirofumi Imai,³ Takashi Ueta,¹ and Shiro Amano^{4,5}

Dry Eye Disease: Oxidative Stress Decline in the Lacrimal Gland

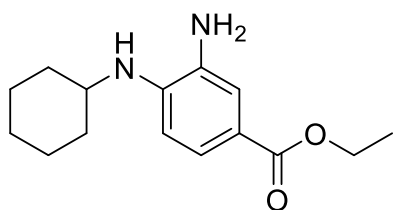
ta, MD,* Takamasa Ishii, PhD,† Naoaki Ishii, PhD,†
 Ōzuo Tsubota, MD*



Ferroptosis inhibitors



Develop new analogues of **UAMC-3203** and **UAMC-3206**

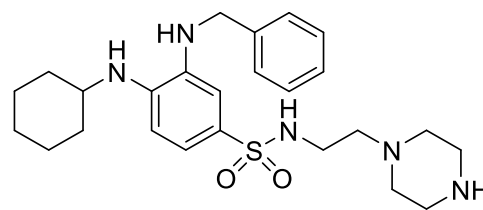


Fer-1

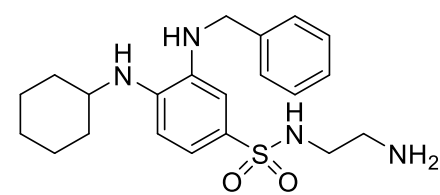
Novel Fer-1 analogues with improved *potency, stability* and *solubility*



Stability issue

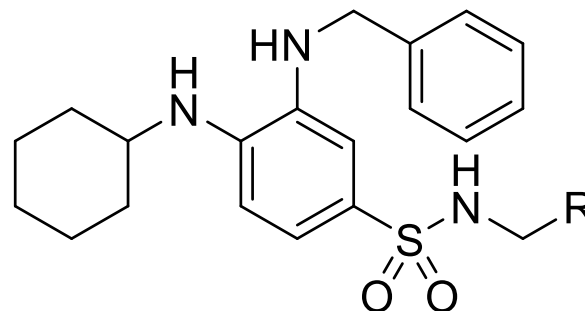


8.39
UAMC-3203

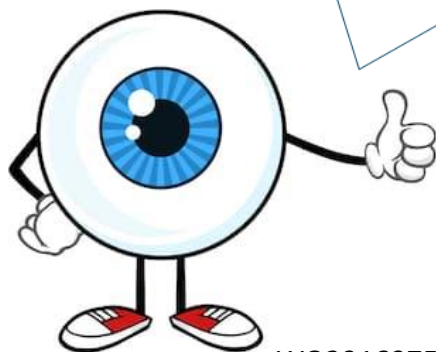


8.38
UAMC-3206

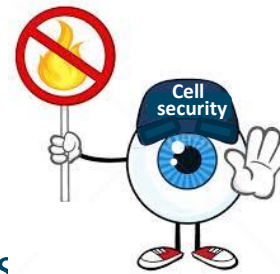
Improve the ADME profile of the analogues



Synthesis of novel molecules for dry eye application



... and Necroptosis?



“Necroptosis is a form of regulated necrosis depends on RIPK1 and/or RIPK3 activity, responsible of inflammation”

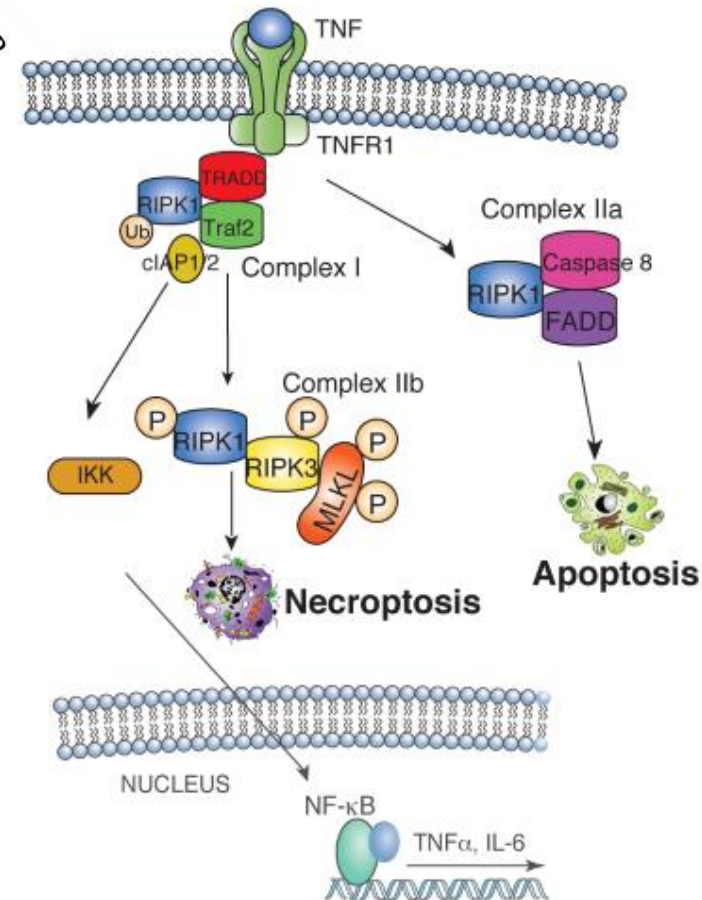
➤ **RIPK1** has a pivotal role

➤ Inducible by **TNF**

TNF-stimulation can yield multiple outcomes:

1. *Cell survival* (**NF- κ B**)
2. *Apoptosis* (**Caspases**)
3. *Necroptosis* (**RIPKs**)

RIPK1-RIPK3 complex (**NECROSOME**)



Necroptosis and dry eye diseases



Why we hypothesized the connection between **necroptosis** and **dry eye**?

- Recent studies suggest **necroptosis** as a major **mechanism of (retinal pigment epithelium) RPE cell death** in response to oxidative stress in Age-related macular degeneration (AMD).
- RIPK1** plays a key role in necroptosis, which is a type of programmed necrosis that is **involved in ocular diseases**

Janga K. et al., *Experimental Cell Research*, **2017**, 359, 30–38

- In the pathogenesis of DED **inflammation is the principal mechanism**;
- Cytokines and **IL- β and TNF- α detected in human conjunctiva**;

Wei Y. et al., *Eye Contact Lens*. **2014**, 40(4): 248–256

(12) **United States Patent**
Gamache et al.

(10) Patent No.: **US 6**
(45) Date of Patent:

(54) USE OF NF- κ B INHIBITORS TO TREAT DRY

6,071,904 A 6/2000 Ali et al. ..

(7) Cell Death and Differentiation (2010) 17, 482–487
© 2010 Macmillan Publishers Limited All rights reserved 1350-9047/10 \$32.00
www.nature.com/cdd

Signaling Pathways

Stress-activated Protein Kinase Signaling Pathway:

Dry I

STEPHEN C. PELUGFELD
MIC

ELSEVIER

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

HHS Public Access
Author manuscript
Ageing Res Rev. Author manuscript; available in PMC 2016 November 01.
Published in final edited form as:
Ageing Res Rev. 2015 November ; 24(0 0): 286–298. doi:10.1016/j.arr.2015.09.002.

EXPERIMENTAL EYE RESEARCH

RIPK1 is not essential for TNFR1-induced activation of NF- κ B

WW-L Wong¹, IE Gentle¹, U Nachbur¹, H Andertor

Clinical Ophthalmology

Open Access Full Text Article

Dry eye syndrome: developments and lifitegrast in perspective

B Wang¹ and PN Moynagh^{1,2}

Protective effect of RIPK1-inhibitory compound in *in vivo* models for retinal degenerative disease

Ki-Hong Jang^a, Yun-Ju Do^{a,1}, Tae-Sung Koo^b, Jun-Sub Choi^{c,2}, Eun Ju Song^d, Yeseong Hwang^a, Hyun Ju Bae^e, Ju-hee Lee^e, Eunhee Kim^{a,*}



ss and in AMD

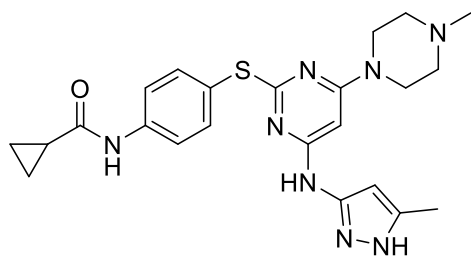
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s in cell death

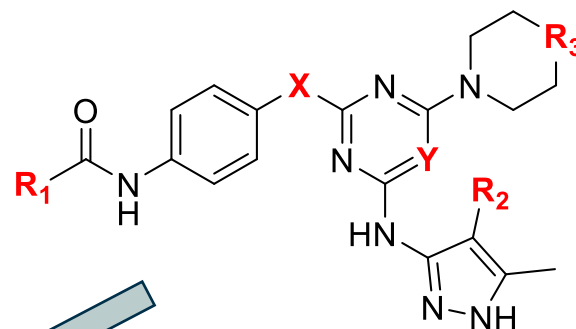


Necroptosis inhibitors → RIPK1 inhibitors

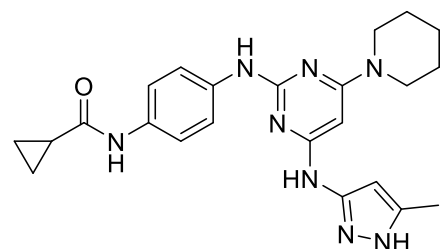


Tozasertib (VX-680)

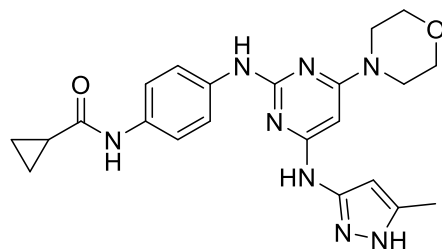
- **Type I** Aurora Kinase (AurK) inhibitor
- Disruption of cell growth
- Low dissociation constant towards RIPK1



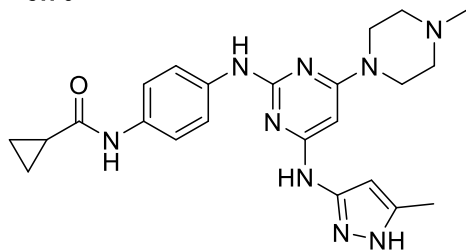
Tozasertib-analogues as
Type I RIPK1-inhibitors



5.70



5.71



5.72

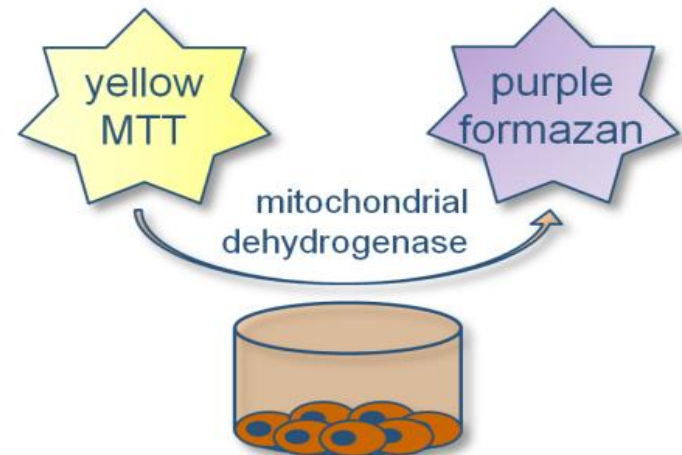
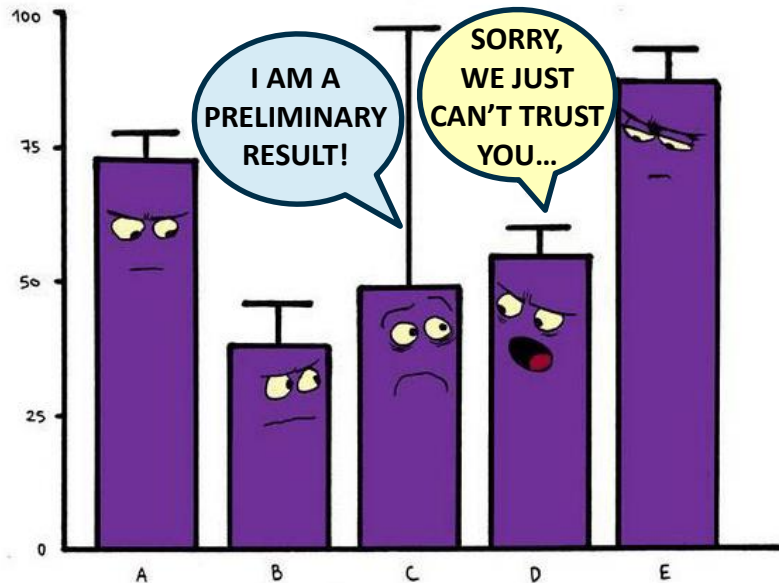
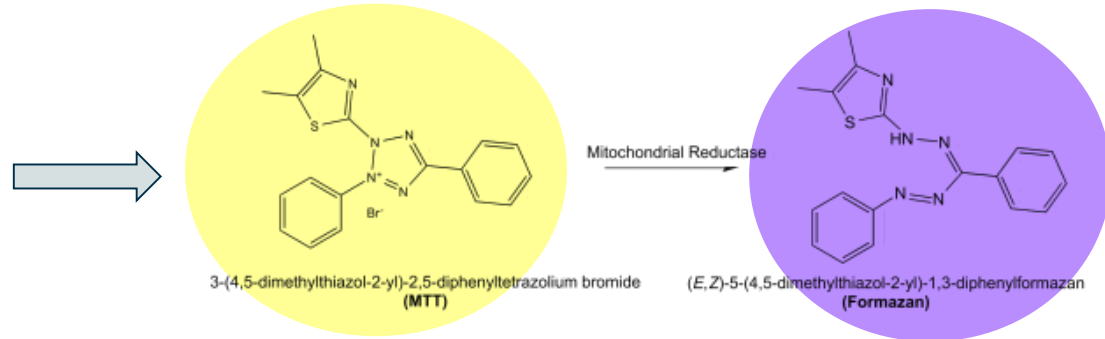
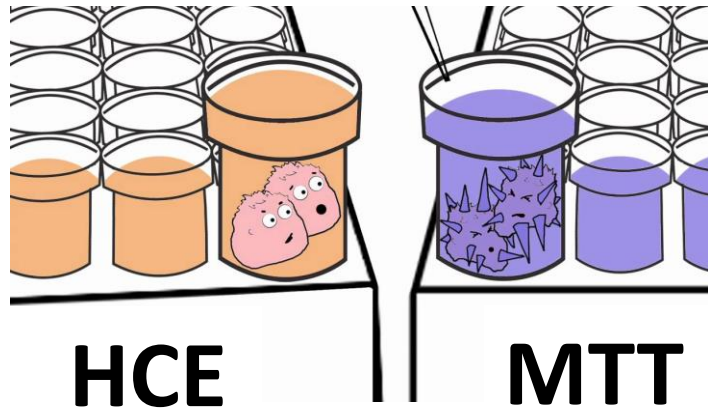
**Novel RIPK1 inhibitors
with improved activity
for dry eye application**



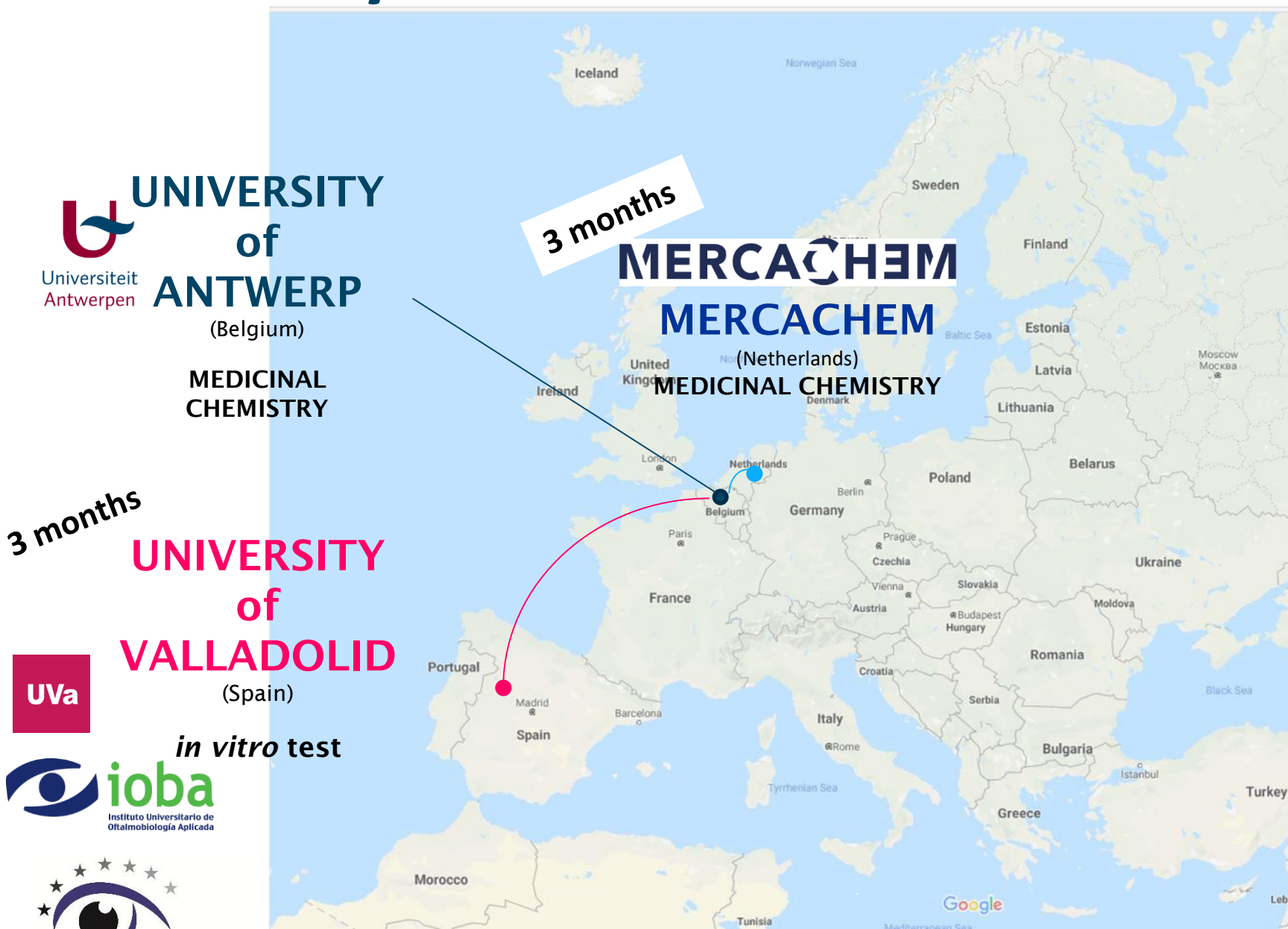
Ferroptosis: *in vitro* experiment

Verify ferroptosis in human cornea cells (HCE)

SECONDMENT



ESR2 – My secondment



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Prof. Koen Augustyns
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Dr. Amalia Enríquez de Salamanca
Prof. Yolanda Diebold



All members of the consortium
All project partners

and you for your kind attention!

