Report Erasmus project +KA171 University of Camerino - School of Biosciences and Veterinary Medicine and University of Cincinnati – College of Medicine

University of Camerino – School of Biosciences and Veterinary Medicine
University of Cincinnati – Department of Internal Medicine
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Spending time at the University of Cincinnati as part of the Erasmus+ KA171 exchange was a truly formative experience for me, both scientifically and personally. My project focused on investigating **ferroptosis**, a specific form of programmed cell death that is characterized by iron accumulation, excessive lipid peroxidation, and reactive oxygen species (ROS)[1]. In the lab of Dr. Cristina Andreani at the Vontz Center for Molecular Studies, I had the opportunity to work at the cutting edge of cancer research—focusing specifically on **non-small cell lung cancer (NSCLC)**, and how different genetic mutations, particularly in the **KRAS gene**, affect a tumor's vulnerability to ferroptosis.

From the start, I was immersed in a collaborative and intellectually stimulating environment. Dr. Andreani and I planned a series of experiments to explore how ferroptosis could be leveraged as a therapeutic strategy. Our hypothesis stemmed from previous work published by the group[2,3], which showed that **KRAS-mutant** and **KRAS wild-type** lung cancers exploit distinct mechanisms to avoid ferroptosis. My goal was to understand how two key players—**Thioredoxin Reductase 1 (TrxR1)** and **Heme Oxygenase 1 (HO-1)**—influence ferroptosis sensitivity in these cancer cells.

To probe this, we used **two gold-based compounds**, CS47 (a novel compound developed at the University of Camerino[4]) and **Auranofin**, an FDA-approved drug already known to inhibit TrxR1. Interestingly, we observed that **KRAS wild-type cells** were much more sensitive to TrxR1 inhibition than their **mutant counterparts**, which appeared to be protected.

We began to suspect that this protection might come from **higher levels of glutathione (GSH)**—a major cellular antioxidant—in KRAS-mutant cells. To test this, I treated the cells with **Buthionine Sulfoximine (BSO)**, a known inhibitor of GSH synthesis, alongside CS47 and Auranofin. The result was striking: **KRAS-mutant cells became sensitized to TrxR1 inhibition**, supporting our hypothesis that GSH plays a compensatory role in redox regulation when TrxR1 is blocked.

Curious about whether we could "rescue" cells from ferroptosis, I then tested if adding back key antioxidant molecules—**L-Cysteine** and **N-Acetyl-L-Cysteine (NAC)**—could reverse the effects of TrxR1 inhibition. These molecules are known to support both the glutathione and thioredoxin systems. After 48 hours of treatment, both compounds were able to **completely reverse cell death** caused by CS47 and Auranofin in KRAS wild-type cells, underscoring the critical role of **cysteine availability** in ferroptosis regulation.

We then shifted focus to **selenium biology**, given that TrxR1 is a **selenoprotein** containing the rare amino acid **selenocysteine**. I tested whether supplementing with selenium-containing compounds—**Selenite** and **Selenocystine**—could overcome the loss of TrxR1 activity. However, these treatments did not significantly rescue the cells, suggesting that **TrxR1 is required for converting these oxidized selenium sources into their active counterparts**, and that simply providing selenium is not sufficient to restore redox balance.

At this point, another player emerged in our story: **HO-1**, an enzyme encoded by the **HMOX1** gene. RNA-seq data from Dr. Andreani's lab showed that TrxR1 inhibitors trigger a sharp increase in HMOX1 expression. This was intriguing because **HO-1 breaks down heme into biliverdin, carbon monoxide (CO), and free iron**, and has been linked to ferroptosis via iron overload[5]. To test HO-1's role, I treated cells with **Hemin**, a compound that induces HO-1. Unexpectedly, Hemin **slightly protected** the cells instead of exacerbating death. This led us to consider that Hemin's other antioxidant effects might be masking HO-1's true contribution.

To get clearer answers, I worked with Dr. Andreani to **overexpress a GFP-tagged version of HO-1** in KRAS wild-type cells. After sorting the cells based on GFP intensity and confirming expression by Western blot, I exposed them to CS47 and Auranofin. The results were striking: **cells with high HO-1 expression were significantly more sensitive to treatment**, demonstrating that **HO-1 enhances TrxR1-induced ferroptosis** in a dose-dependent manner.

Throughout my stay, I had the chance to contribute to this exciting body of work, which is now part of a manuscript in preparation for submission. Being listed as a contributing author is deeply rewarding, and I am proud to have helped generate insights that will guide future projects—possibly exploring how **dietary interventions or iron modulation** might influence TrxR1-targeting therapies.

Beyond the Bench

My experience extended well beyond the lab. I had the rare opportunity to **shadow Dr. Alex Evans**, a clinical pharmacist at the UC Blood Healing Center. Observing her interactions with patients gave me a new appreciation for **personalized medicine**—the careful balancing act of optimizing therapies based on individual responses and tolerances.

I also attended several **seminars and research presentations** held at the Vontz Center (please refer to the table 1 below). These talks covered cutting-edge topics in redox signaling, metabolism, and cancer biology, and sparked ideas for how interdisciplinary thinking can drive innovation.

Conclusion

Participating in the Erasmus+ KA171 program has been an incredible opportunity to deepen my scientific training, work on an exciting translational project, and experience firsthand the dynamic research culture of an international academic center. I return home not only with new data and technical skills, but also with a deeper curiosity for redox biology and a stronger motivation to pursue a career in cancer research.

Table1. List of attended workshops and seminars

03/06	Moderators: Issac Choi & Jacob Kurek **Wen-Xing Ding, PhD, FAASLD** Host: Dr. Chunying Du, UC Department of Cancer Biology William Warner Abercrombie Professor, Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center ***"Autophagy in liver diseases: too much is as bad as too little"***
03/13	Moderators: Shreya Shyamsunder & Robby Beal **Szu-Aun Long, MD,** Dr. Andrew Waters Lab, UC Department of Surgery-Oncology ***"Evaluating direct KRASQ61H inhibition in pancreatic cancer models"*** **Banzhan Ruan, PhD,** Dr. Jun-Lin Guan Lab, UC Department of Cancer Biology ***"Conditional knockout of Tsc1 and RASA1 in endothelial cells leads to capillary barrier dysfunction in mice"***
O3/18	10x Genomics Symposium In Collaboration with the Core at UC By Jacob Gordon
03/24	LI-COR Biosciences - Overview of your Odyssey or Pearl imaging system and advanced applications- Image acquisition - Data analysis with Empiria Studio Software - Assay best practices and troubleshooting
03/25	Dr. Fujimoto Diet intervention and AI-based methods for Lung Cancer
03/27	Moderators: Joe Ungvary & Emily Wachter **Jacob Kurek, CCB PhD Student,** Dr. Andrew Volk Lab, CCHMC ***"Chromatin assembly in terminal erythropoieses"*** **Devyani Sharma, CCB PhD Student,** Dr. Marie-Dominique Filippi Lab, CCHMC ***"Cardiolipin and the regulation of hematopoietic stem cells"***
04/03	Moderators: Sam Zumwalde & Sreelakshmi Sanam **Kate Von Handorf, CCB PhD Student,** Dr. David Plas Lab, UC Department of Cancer Biology ***"Tumor microenvironment-conscious kinase targeting in glioblastoma"*** **Dina Secic, CCB PhD Student,** Dr. Maria Czyzyk-Krzeska Lab, UC Department of Cancer Biology ***"Determination of the allocation of copper to cytochrome c oxidase using a non-radioactive Cu tracer and size-exclusion coupled with ICP-MS"***
04/10	Moderators: Evan Peters & Julie Fisher **Sara Alharbi, CCB PhD Student,** Dr. Tim Le Cras Lab, CCHMC ***"MEK inhibition restores dysregulated genes in human endothelial cells expressing the NRASQ61R mutation***

lymphangiomatosis"
Bibek Karki, CCB PhD Student, Dr. Tom Cunningham Lab, UC Department of Cancer Biology
"Evolutionary origins and innovations sculpting the mammalian PRPS enzyme complex"

04/1 Moderators: Charlie Nims & Grace Goodhart

7 **Lindsay Bischoff, CCB PhD Student,** Dr. Elisa Boscolo Lab, CCHMC
CARDELL FELLOW
"In-vivo investigation of the mechanisms driving venous malformation downstream of mutant TIE2"

04/2 Moderators: Josh Jones & Angelle Jones

4 **Antonio Barrientos, PhD** Host: Dr. Maria Czyzyk-Krzeska, UC Department of Cancer Biology
Professor of Neurology and Biochemistry & Molecular Biology, University of Miami, Miller School of Medicine, Miami FL
"Regulation of Mitochondrial Translation"

References

1 Dixon, S. J. & Stockwell, B. R. The Hallmarks of Ferroptosis. *Annual Review of Cancer Biology* **3**, 35-54 (2019). https://doi.org/10.1146/annurev-cancerbio-030518-055844

2 Bartolacci, C. *et al.* Targeting de novo lipogenesis and the Lands cycle induces ferroptosis in KRAS-mutant lung cancer. *Nature Communications* **13**, 4327-4327 (2022). https://doi.org/10.1038/s41467-022-31963-4

3 Galassi, R. *et al.* Anticancer Activity of Imidazolyl Gold (I/III) Compounds in Non-Small Cell Lung Cancer Cell Lines. *Pharmaceuticals* **17**, 1133-1133 (2024).

4 Galassi, R. *et al.* Synthesis and characterization of azolate gold(i) phosphane complexes as thioredoxin reductase inhibiting antitumor agents. *Dalton Trans.* **41**, 5307-5318 (2012). https://doi.org/10.1039/C2DT11781A

5 Hassannia, B., Vandenabeele, P. & Vanden Berghe, T. in *Cancer Cell* (2019).