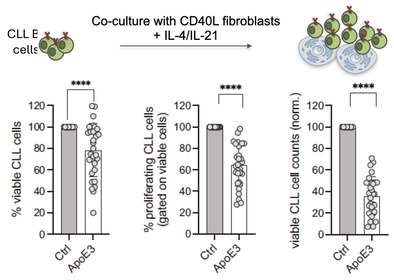
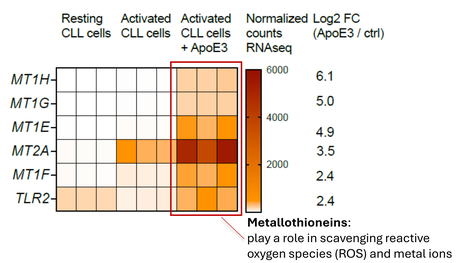
P #1006: ApoliproteinE induces ferroptosis in chronic lymphocytic leukaemia cells

Nardi F[1], Del Prete R[1], Drago R[1,2], Di Rita A[1,2], Vallone FE[3], Ciofini S[2], Malchiodi M[2], Pezzella L[1], Tinti L[1], Cicaloni V[1], Salvini L[1], Licastro D[4], Pezacki AT[5,6], Chang CJ[5,6], Marotta G[2], Naldini A[2], Deaglio S[3], Vaisitti T[3], Gozzetti A[2], Bocchia M[2], Kabanova A[1]
[1]Fondazione Toscana Life Sciences, Siena. [2]University of Siena.[3]University of Turin. [4]AREA Science Park, Padriciano, Trieste. [5]Princeton University. [6]University of California, Berkeley.

1. ApoE affects CLL cell proliferation through oxidative stress and metal disbalance

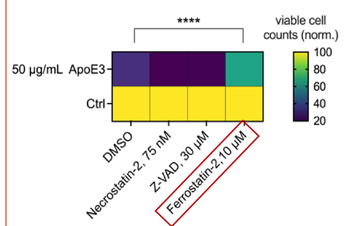


Recombinant **ApoE inhibits viability and proliferation of CD40L-activated CLL cells**

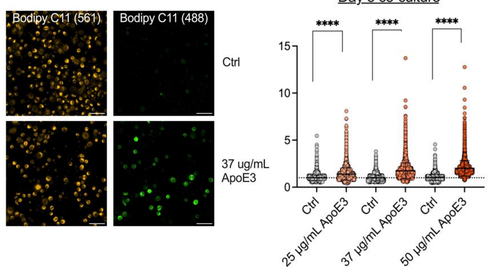


RNA siquensing analysis of ApoE-treated and untreated cells revealed a signature of **oxidative stress** and **cellular response to metal toxicity**

2. ApoE induces lipid peroxidation and ferroptosis in proliferating CLL cells

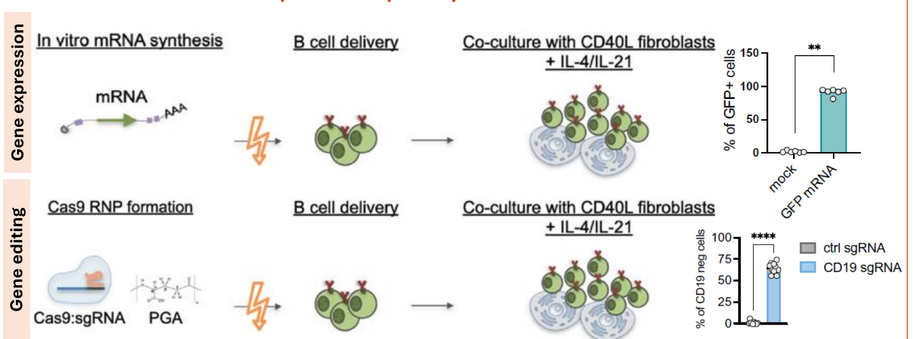


Inhibitors screen pointed out ApoE toxicity is counteracted by the **ferroptosis inhibitor ferrostatin-2**



ApoE-induced lipid peroxidation evaluated using the fluorescent fatty acid analogue C11-BODIPY(581/591)

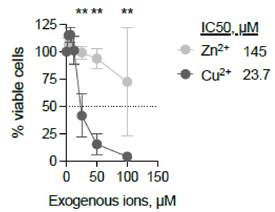
3. Robust methods for manipulation of primary B cells



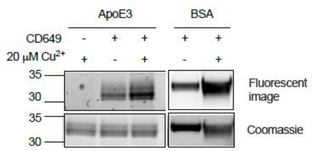
INTRODUCTION

Chronic lymphocytic leukaemia (CLL) shows a vastly heterogeneous clinical course, ranging from patients with favourable prognosis to those with rapidly progressive disease which ultimately may give rise to a highly aggressive lymphoma, known as Richter syndrome (RS) [1][2]. Identification of intrinsic vulnerabilities in CLL represents a key approach to better understand molecular basis of its heterogeneity and identify targets to optimize current treatment schedules. We previously described that CLL cells are characterized by an ectopic expression of the immunomodulatory receptor ILT3/LILRB4 [3]. Here, we describe a natural suppression mechanism mediated by its ligand, the apolipoprotein E (ApoE), which is an abundant serum protein regulating metabolic homeostasis.

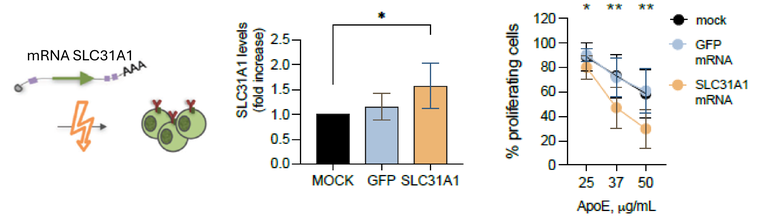
4. ApoE toxicity against CLL cells is regulated by copper



CLL cells are resistant to Zn^{2+} toxicity, while being **sensitive to Cu^{2+}**

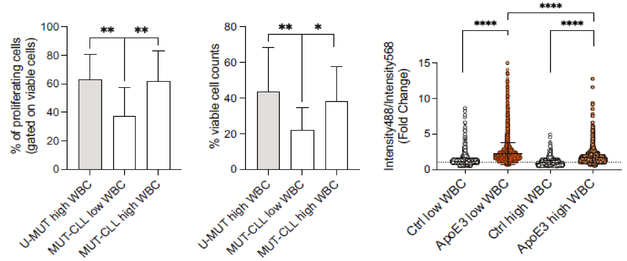


ApoE is a copper binding protein

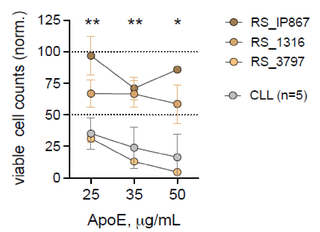


Overexpression of the copper importer SLC31A1 raises CLL cell sensitivity to ApoE

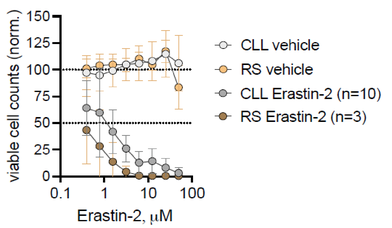
5. Aggressive CLL cells could become resistant to ApoE toxicity but not ferroptosis



CLL cells from patients with low white blood cell counts are more sensitivity to ApoE inhibition



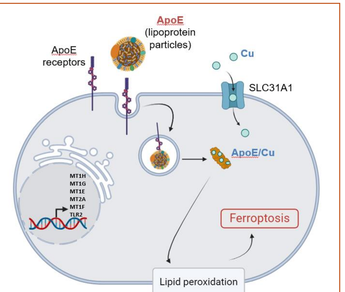
Richter syndrome xenografts are **resistant to ApoE**



Both CLL cells and **Richter syndrome** xenografts are **sensitive** to **drug-induced ferroptosis**

CONCLUSION(S)

Our findings suggest a natural ApoE-mediated suppression axis in CLL. We show that **ApoE affects CLL cell viability and proliferation through the induction of ferroptosis**, an iron-dependent form of cell-death driven by massive lipid peroxidation and membrane damage[4]. Moreover, metal binding properties of ApoE may further exacerbate its toxic effect by inducing **copper accumulation** within cells. We further show that escape from ApoE-mediated inhibition is possible in aggressive CLL cells and Richter syndrome xenograft. Nevertheless, **aggressive CLL and Richter syndrome xenografts retain their sensitivity to drug-induced ferroptosis**, suggesting ferroptosis as a new promising therapeutic target in aggressive CLL.



REFERENCES

1: Kipps TJ et al, *Nat Rev Dis Primers* 2017; 3:17008.

2: Wang Y, Ding W, *Clin Adv Hematol Oncol* 2020; 18(6):348-357.

3: Zurli V et al, *Blood* 2017; 130(18):2006-2017.

4: Stockwell BR, Jiang X, Cell Chem Biol. 2020; 27(4):365-375.

This work has been done with the support from the Italian Foundation on Cancer Research (My First AIRC grant 21495) and the European Union - NextGenerationEU through the Italian Ministry of University and Research under PNRR - M4C2-I1.3 Project PE_00000019 "HEAL ITALIA", CUP B63D22000680006.