1. **Title**: Radio-sensitization of Keap-1 mutant human lung cancer cells by FDA approved anti-psoriatic drug
2. **Introduction:**

Radiotherapy (RT) combined with other treatment modalities is the predominant strategy for management of lung cancer. Despite significant advancements in administration and efficacy of RT, radio-resistance limits therapeutic outcome leading to tumor relapse and recurrence. Normally, cells are able to combat oxidative stress induced by radiation through a resolute antioxidant and cytoprotective system primarily regulated by nuclear factor erythroid 2-related factor 2 (Nrf-2 or NFE2L2) and it is commonly known as the master regulator of oxidative stress.

Under normal conditions, the Kelch-like ECH-associated protein 1 (Keap-1)negativelyregulates Nrf-2 levels by sequestering Nrf-2 in the cytoplasm and aiding its degradation through ubiquitination. Oxidative stress or exposure to xenobiotics leads to oxidation of specific cysteine residues (Cys273 and Cys288) on Keap-1, causing conformational changes that dissociate it from Nrf-2 facilitating its translocation to nucleus. Nrf-2 heterodimerizes with small Maf protein (sMaf) in the nucleus, and subsequently binds to regulatory regions in DNA known as Antioxidant Response Elements (ARE). This interaction results in the upregulation of transcription genes related to antioxidant defense, cytoprotection and DNA repair. Among its target genes are NAD(P)H quinone oxidoreductase 1, Glutamate-cysteine ligase (both catalytic and modifier subunits), Sulfiredoxin 1 (SRXN1), Thioredoxin reductase 1 (TXNRD1), Heme oxygenase-1, Glutathione S-transferase, and multidrug resistance-associated proteins.Nrf-2 also modulates the synthesis and utilization of critical molecules like glutathione, thioredoxin, and NADPH. Upregulation of these genes and redox factors restore the homeostasis.This leads to activation of many detoxification and cytoprotective genes that help in combating the oxidative stress.

The cytoprotective function of Nrf-2 acts as a bane when cancer cells are exposed to radio-therapy as it provides survival advantage and thus contributing to relapse. Unregulated Nrf-2 expression that may arise due to mutations in Keap-1 and hyper-methylation of Keap-1 promoter fosters radio-resistance by promoting protective mechanisms in cancer cells, hampering the efficacy of radiation-induced damage. The Cancer Genome Atlas (TCGA) data suggests that 20-25% of lung cancers and ~20% of ovarian cancers have altered Nrf-2 expression. The identification of “Nrf-2 addicted” cancers and the resultant survival advantage has shifted the focus towards identification of Nrf-2 inhibitors for better therapeutic outcome.

The rationale behind the project was to identify a clinically relevant Nrf-2 inhibitorsthat can be used to sensitize radio-resistant lung cancers. Keap-1 mutant radio-resistant A549 human lung cancer cells were chosen as the model due to constitutive expression of Nrf-2. Clobetasol propionate (CP), a synthetic glucocorticoid, is known to inhibit Nrf-2 via activation of β-Trcp mediated proteosomal degradation. US-FDA has approved CP for treatment of psoriasis. Our studies have demonstrated sensitization of human lung cancer cells to radiation induced killing when combined with CP. Further, combination of CP with radiation led to enhanced iron release, oxidative stress, reduced DNA damage repair and subsequent cell death through ferroptosis in radio-resistant human lung cancer cells.

3. Objectives:

1. Identification of Nrf-2 inhibitor that can sensitize radio-resistant Nrf-2 overexpressing cancers (in-vitro and in-vivo).
2. Understanding the implications of Nrf-2 inhibitor by studying gene expression changes through mechanism trancriptomics.
3. Elucidation and validation of mechanism of radio-sensitization by Nrf-2 inhibitor in-vitro.
4. Identification of a druggable Nrf-2 mediated pathway for efficient radiotherapy.

4. Materials and methods:

4.1 Reagents: Dulbecco's Modified Eagles Medium (DMEM),Fetal bovine serum (FBS) and trypsin-EDTA were obtained from Himedia (India).Clobetasol propionate, JC-1, propidium iodide (PI), liproxstatin-1 (LIP-1) and deferoxamine (DFO) were purchased from Sigma Chemical Co. (MO, USA).DAPI with antifade, MitoSOX Red, 2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDA) dye, MitoTracker Red, Hoechst 33342 and opti MEM were purchased from ThermoFisher Scientific (MA, USA). Cell based lipid peroxidation kit, Nrf-2 transcription factor activity assay kit and anti-γH2AX antibody was procured from Abcam (USA). Nrf2 and phosho-Nrf2 antibody was purchased from Boster Biological Technology (CA, USA).GPX4 antibody was purchased from Santacruz biotechnology (California, USA).Nrf-2 plasmid was procured from OriGene (Rockville, USA). RNA isolation kit, cDNA synthesis kit, SYBR green PCR mix and X-treme Gene 9 DNA transfection reagentwere procured from Roche Diagnostics (GmBh, Germany).

4.2 Cell culture, treatment and irradiation

A549 (Human lung adenocarcinoma) cells were procured from Health Protection Agency Culture Collections, UK. The cells were maintained as monolayers in DMEM containing 10% FBS, streptomycin (0.1 mg/ml) and penicillin (100 U/ml) in a humidified incubator maintained at 37°C and 5% CO_2. The cells seeded overnight were treated with vehicle or CP (100 nM, 4 h), followed by exposure to γ-ray irradiation (4Gy) using ^{60}Co Teletherapy machine Bhabhatron-II (Panacea Medical Technologies, Bangalore, India) at a dose rate of 1 Gy/min.

Cell death and proliferation:
Radio-sensitization of A549 cells was studied using MTT assay, flow cytometry based propidium iodide PI assay, monitoring cell growth kinetics using Incucyte live cell imaging system and clonogenic assay. A549 spheroids were grown on ultra-low attachment plates and subsequently stained with PI to study extent of cell death.
Animal studies: A549 xenografts were implanted in 6-8 weeks old female NOD/SCID BALB/c mice (6 mice per group). When tumor size reached 100 mm^3, mice were treated with intraperitoneal administration of CP (1 mg/kg) or vehicle (DMSO) three times in a week or exposed to radiation alone to the tumor (3x2 Gy) or administered CP followed by local radiation to the tumor. The tumor volume was recorded for 21 days followed by animal sacrifice and measurement of tumor weight.

Nrf-2 levels: Real-time PCR, immunoblotting and immunofluorescence were used to study the levels of Nrf-2 in cells treated with CP, radiation and combination of CP+radiation. Nrf-2 transcription factor activity was studied in nuclear extracts by colorimetric method.

Quantification of cancer stem cells: Cells (5000 per group) seeded in black 96-well plate were treated as mentioned in 4.2, and cultured for 48 h, washed, incubated in DMEM without phenol red containing Hoechst 33342 (10 μg/ml) for 90 min at 37°C and acquired using High Throughput cell analyser (Acumen Cellista, SPT Labtech, UK).

Transcriptomics study: Total RNA was extracted from the control and three treatment groups (n=2) i.e. cells treated with CP, 4 Gy radiation and combination of CP+ 4 Gy. Library preparation was performed by following manufacturer instructions for Illumina sequencing on Novaseq 6000 system and the differential gene expression analysis was carried out using R packages.

Transfection: A549 cells were transfected with 1μg Nrf-2 over-expression plasmid using X-tremeGENE 9 DNA Transfection reagent as per manufacturer's protocol and cells were seeded and cultured for respective experiments.

ROS Measurement: Cellular ROS was studied by staining cells withH_2DCFDA (Ex. 485 nm / Em. 535 nm), and mitochondrial ROS was studied usingMitoSOX Red (Ex. 510nm/ Em. 580nm) using a multimode microplate reader (Synergy Hybrid Biotek, VT, USA) or a flow cytometer or imaging under Leica fluorescence microscope (Leica Dmi8, Wetzlar, Germany).Mitochondrial membrane potential (MMP) was studied using JC-1 staining

Ferroptosis studies: Morphological features of ferroptosis were studied using Transmission Electron Microscope. Iron levels were studied BODIPY based dye developed by Pachpatilet. al. Lipid peroxidation was studied using Liperfluo. Flow cytometric analysis of intracellular antibody staining was used to study protein markers of ferroptosis.

Results:

1. **Clobetasol propionate sensitized human lung cancer cells to radiation induced killing**

CP induced radio-sensitization in A549 lung cancer cells as seen in Figure 1. The viability of cells treated with the combination of CP and radiation was significantly less as compared to radiation or CP alone (Fig. 1A). Flow cytometry based PI assay was used as a means to study cell death and it can was observed that the percentage of SubG1 cells was >40% in cells treated with CP+radiation compared to CP or radiation alone (Fig. 1B). Live cell imaging indicated that number of floating and rounded cells were higher and the growth kinetics measured as confluence was significantly slower in cells treated with CP+radiation (Fig. 1C and 1D). Clonogenic survival fraction is the gold standard method to study anti-proliferative and anti-cancer activities. It was observed that ~40% cells survived and formed colonies even when cells were exposed to 4 Gy radiation. However, combining CP with radiation completely suppressed the clonogenic growth and survival fraction of A549 cells (Fig. 1E and 1F). Interestingly, side population assay CP alone as well as in combination with radiation decreased the percentage of cancer stem cells which often contribute to radio-resistance (Fig. 1G). The radio-sensitization effects of CP were further observed in A549 spheroids as the size, integrity and compactness of CP+radiation treated spheroids was very compromised. The PI uptake was relatively higher in spheroids treated with CP+radiation indicating higher degree of cell

death (Fig. 1H and 1I). Finally, radio-sensitization was validated in-vivo using mice bearing A549 tumor xenografts. The tumor size and weight was significantly lower in mice that received combined treatment of CP and radiation (Fig. 1J, 1K and 1L). These results strongly suggested that using an Nrf-2 inhibitor can help the Nrf-2 overexpressing cells overcome radio-resistance and CP could be used as an efficient radio-sensitizer.

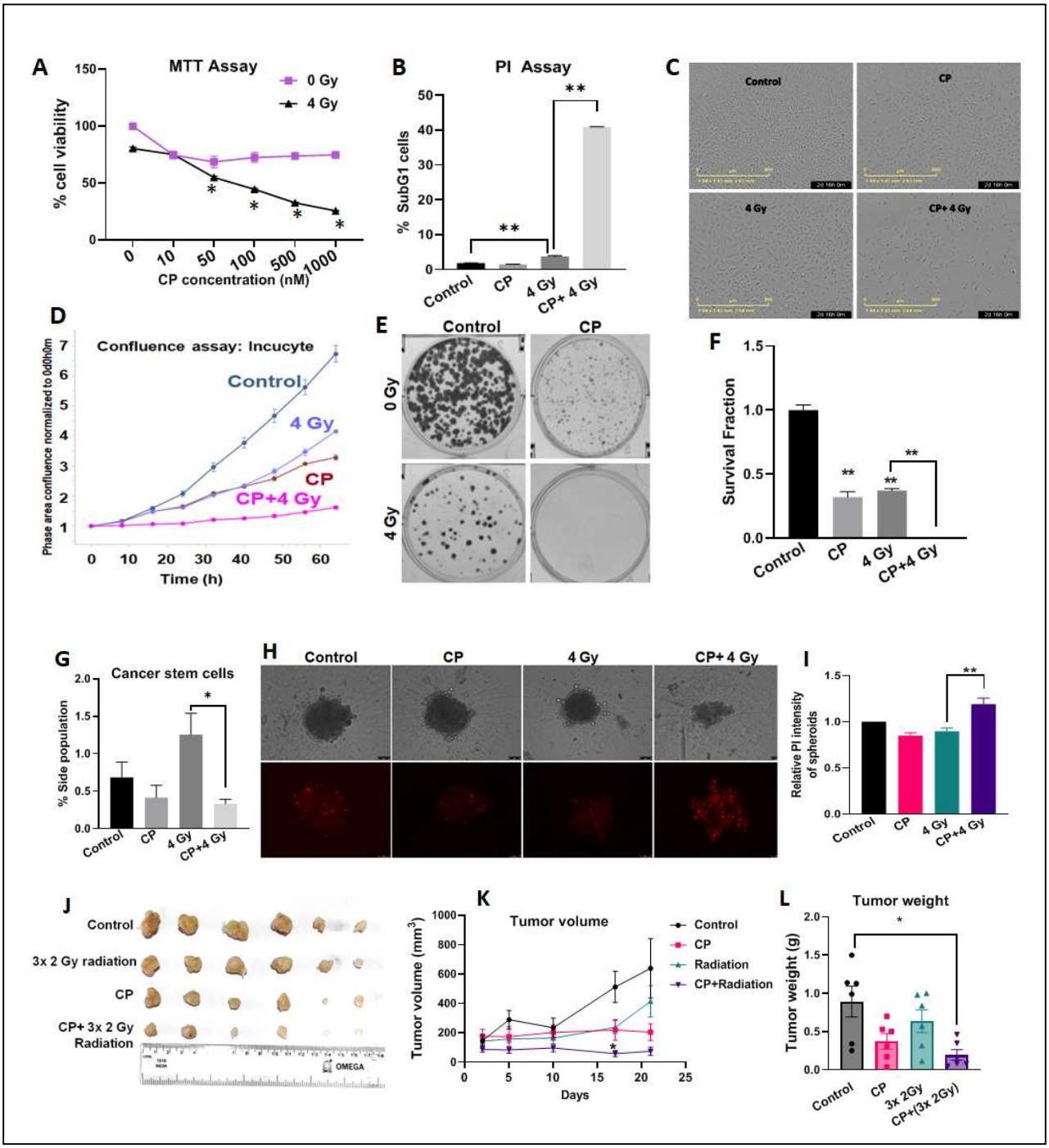

Figure 1: Radio-sensitization of A549 human lung cancer cells, spheroids and xenografts by CP

2. CP inhibited Nrf-2 in A549 human lung cancer cells

Immuno-fluorescence images in Fig. 2A indicated that treatment of cells with radiation resulted in increased expression as well as nuclear translocation of Nrf-2 whereas CP treatment reduced both constitutive expression as well as radiation induced nuclear translocation of Nrf-2. The same was confirmed by western blotting (Fig. 2B). The Nrf-2 transcription factor activity was higher when cells were exposed to radiation which was reduced below control levels in cells treated with CP and combination of CP and radiation (Fig. 2C).

Transcriptomics based analysis revealed that the expression of anti-oxidants was significantly downregulated and DCFDA based study indicated elevated ROS levels in cells treated with CP+4 Gy (Fig. 2D and 2E). RT-PCR based gene expression analysis suggested that CP treatment resulted in downregulation of Nrf-2 and its dependent genes (Fig. 2F). Further, DNA damage was studied by γ-H2AX foci and it was observed that cells treated with CP+4 Gy had more number of foci per cell indicating severe DNA damage and delayed repair (Fig. 2G).

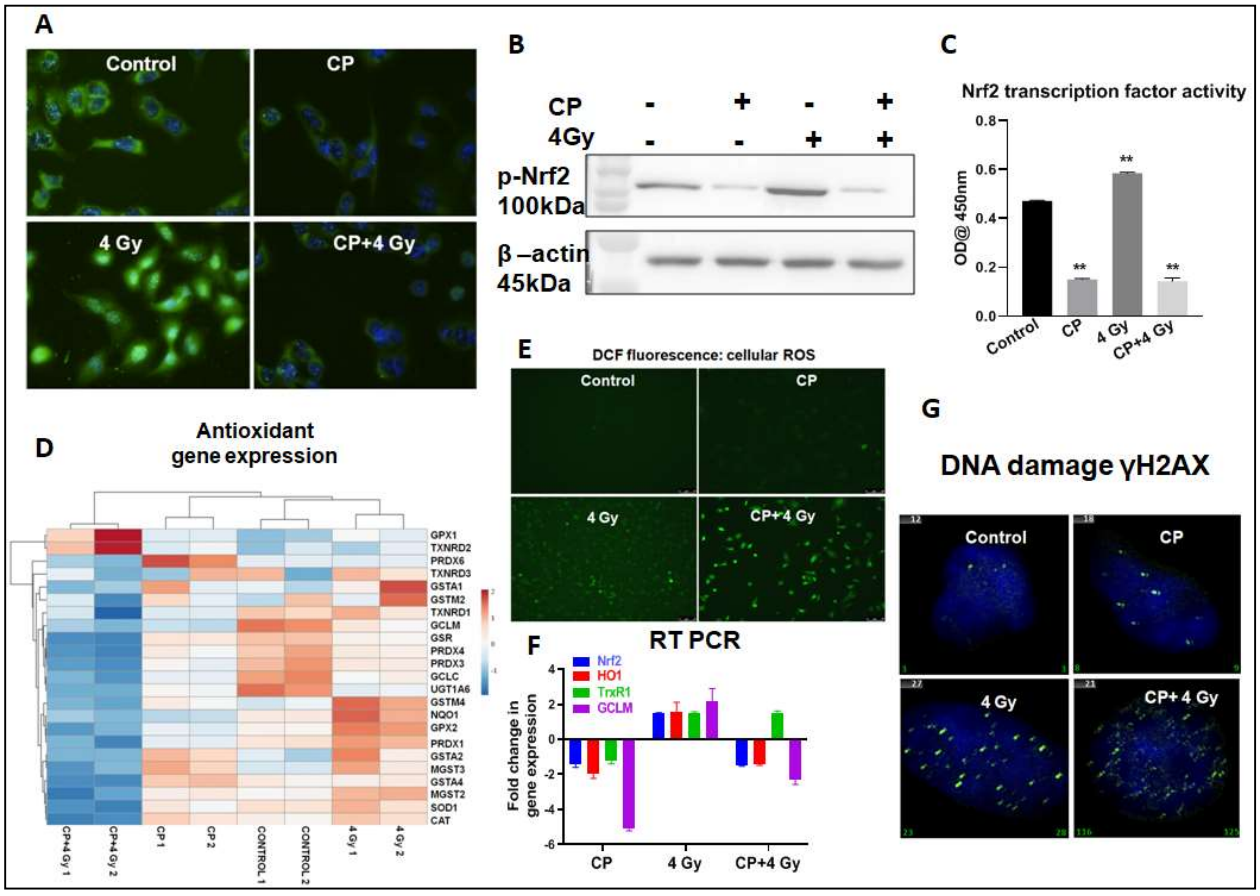

Figure 2: Inhibition of Nrf-2 and its dependent genes by CP

3. Identification of ferroptosis as the mechanism of CP-mediated radio-sensitization

RNA Seq data analysis of the KEGG pathways in CP+4 Gy treated cells showed enrichment of pathways involved in fatty acid synthesis and metabolism, glutathione metabolism and ferroptosis (Fig. 3A). Ferroptosis is an iron mediated form of programmed cell death that is characterized by the accumulation of lipid peroxides and the resulting damage to cell membranes. Nrf-2 is known to be one of the most central players in mitigating ferroptotic damage. Morphologically, TEM based ultrastructure study indicated severe damage to mitochondrial cristae and membrane rupture along with mitochondrial swelling and increased vacuolization in cells treated with CP+4 Gy (Fig. 3B, 3C and 3D). Intracellular free iron levels were found to be increased in cells treated with combination of CP+4 Gy (Fig. 3E) along with increased lipid peroxidation (Fig. 3F). GPX4 is an Nrf-2 dependent mitigator of lipid peroxidation and its expression was decreased in cells treated with CP+4 Gy (Fig. 3G). CP mediated radiosensitization was abrogated in the presence of ferroptosis inhibitor liproxstatin-1 (LIP-1) and iron chelator deferoxamine (DFO), further validating the role of ferroptosis in radio-sensitization (Fig. 3H, 3I and 3J).

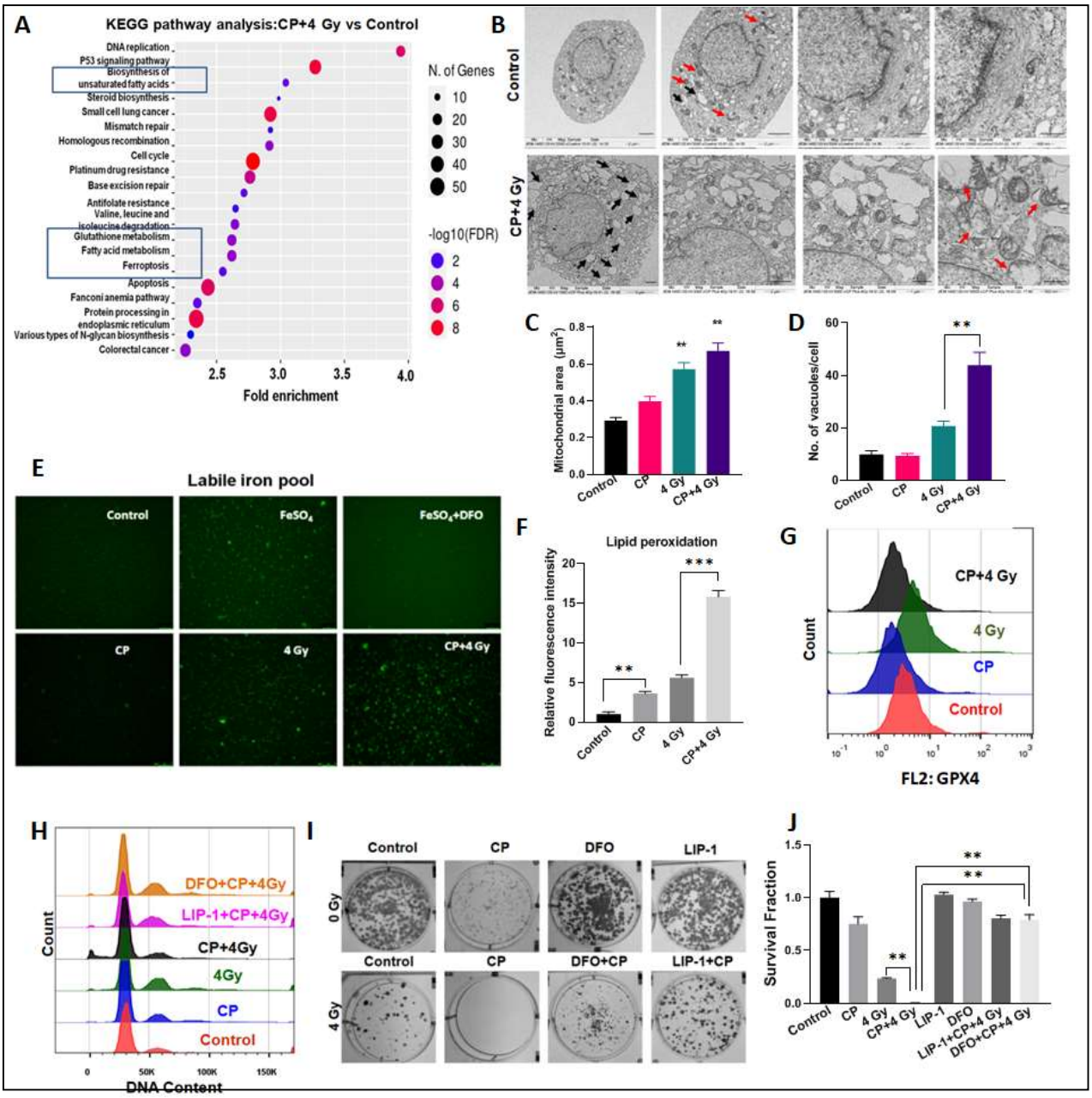

Figure 3: CP mediated radio-sensitization is driven by ferroptosis

4. Role of mitochondrial ROS and Nrf-2 in ferroptosis driven radio-sensitization

Loss of mitochondrial membrane potential (MMP) was observed in cells treated with CP and CP+4 Gy (Fig. 4A). CP+4 Gy treated cells had higher levels of mitochondrial ROS compared to 4 Gy radiation alone, which was scavenged by mitochondrial targeted anti-oxidant mitoTEMPO (Fig. 4B). Subsequently, it was observed that the CP+4 Gy induced increase in intracellular iron levels as well as lipid peroxidation was abolished in the presence of mitoTEMPO (Fig. 4C and 4D). Radio-sensitization by CP was also inhibited by mitoTEMPO suggesting that increase in mt ROS served as a trigger for iron dependent lipid peroxidation and subsequent radio-sensitization (Fig. 4E and 4F). In addition, overexpression of Nrf-2 also abrogated the increase in mt ROS as observed in Fig. 4G. Overexpression of Nrf-2 also inhibited the CP+radiation induced increase in lipid peroxidation and iron levels (Fig. 4H and I). Subsequently, role of Nrf-2 was validated by observing inhibition of CP mediated radio-sensitization in Nrf-2 overexpressing cells (Fig. 4J and 4K). This suggested that as the master regulator of oxidative stress, Nrf-2 played a crucial role in mt ROS mediated ferroptosis and radiation response in A549 cells.

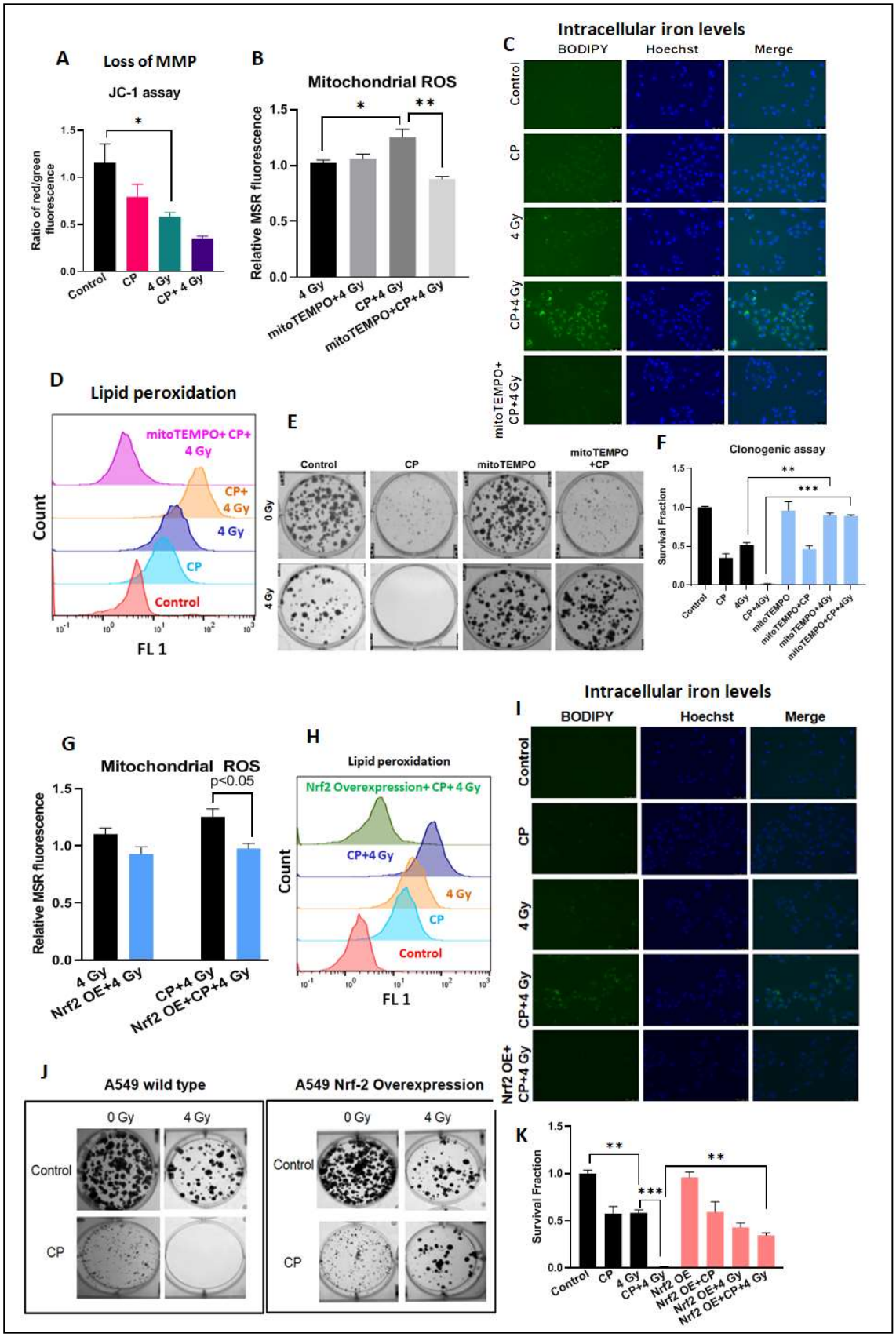

Figure 4: Role of mt ROS and Nrf-2 in ferroptosis driven radio-sensitization by CP

Statistical analysis: Three independent experiments were carried out for all the studies. Graphpad Prism 9.0 software was used to carry out the statistical analysis using ordinary one-way or two-way ANOVA followed by Sidak's or Tukey's multiple comparison test. Data are presented as mean ± SEM. The difference was considered significant when $p<0.05$, and p values are indicated in the graph or shown by * such that * indicates $p<0.05$ and ** indicates $p<0.01$.

Discussion:

The project was aimed at identifying, investigating and validating a potent and clinically relevant radio-sensitizer for Nrf-2 overexpressing lung cancer cells. Human lung adenocarcinoma A549 cells harbour mutation in Keap-1 resulting in unregulated and constitutive expression of Nrf-2 which provides survival benefit to cancer cells against radio-therapy. The hypothesis guiding the project was that inhibition of the constitutively high levels of Nrf-2 can weaken the defence mechanism of cells against oxidative stress and thus the cells can be more sensitive towards radiation induced killing. Clobetasol propionate (CP) is a reported Nrf-2 inhibitor and is approved by US-FDA for psoriasis treatment. Cells treated with CP prior to radiation treatment were studied for multiple end points. It was observed by MTT assay, PI assay, live cell imaging and clonogenic assay that treatment of A549 cells with a combination of CP and radiation resulted in efficient and significant radio-sensitization. Combination treatment of CP along with radiation resulted in complete loss of colony forming ability of cells. CP in combination with radiation exhibited sensitization enhancement ratio of 2.6 indicating potent radio-sensitization. Cancer stem cells (CSCs) often determine intrinsic radio-resistance and side population assay indicated that CP alone as well as in combination with radiation depleted the CSCs. Combination treatment led to loss of integrity due to dispersal of cells and enhanced PI uptake in A549 spheroids. Further, the radio-sensitization effect of CP was validated in A549 xenograft mice model it was observed that size as well as weight of tumors in mice treated with combination of CP and radiation was lesser as compared to radiation alone.

Immunofluorescence, immunoblotting and transcription factor activity assays suggested that CP inhibited both constitutive as well as inducible expression, nuclear localisation and activity of Nrf-2. Subsequently, transcriptomics based analysis was carried out to study gene expression changes and among many other parameters, the expression of anti-oxidants was found to be significantly downregulated in CP+4 Gy treated cells. Owing to inhibition of Nrf-2 by CP and subsequent downregulation of the dependent anti-oxidant genes, cellular ROS levels were very high in CP+4 Gy treated cells. Our lab had previously demonstrated the role of Nrf-2 in ROS mediated DNA damage response and it was observed that inhibition of Nrf-2 by CP followed by exposure to radiation resulted in greater DNA damage in the cells as compared to radiation alone.

Analysis of transcriptomics data indicated enrichment of pathways involved in fatty acid and glutathione metabolism, synthesis of unsaturated fatty acids and ferroptosis. Ferroptosis is an iron mediated mode of death marked by increased lipid peroxidation and mitochondrial damage. CP+radiation induced ferroptosis was studied by morphological features, iron levels, lipid peroxidation and protein markers like GPX4 and ACSL4. Inhibitors of ferroptosis abrogated CP mediated radio-sensitization. Nrf-2 activation can lead to the upregulation of various antioxidant enzymes like GPX4 that may help counteract lipid peroxide accumulation that is characteristic of ferroptosis. Many genes involved in lipid metabolism (FABP1, LIPH,

ACLY, SCD1 etc) and iron metabolism (HMOX1, FECH1, FTL etc) are regulated by Nrf-2. Transcriptomics data analysis revealed that treatment of cells with CP+radiation resulted in downregulation of many ferroptosis inhibitor genes (GCLC, GCLM, SLC7A11, FTH1 etc) and upregulation of ferroptosis activators (TP53, HMOX1, SLC3A2 etc). Many recent studies have suggested the cells resistant to ferroptosis are also radio-resistant and induction of ferroptosis is one of the recently identified ways to improve radiation sensitivity of cells. Hence, induction of ferroptosis when cells receive a combined treatment of CP with radiation is an effective strategy to enhance radiation sensitivity of cancer cells.

To further elucidate the mechanism of ferroptosis driven radio-sensitization, mitochondrial ROS (mt ROS) and mitochondrial membrane potential (MMP) were studied. It was observed that combination of CP+radiation led to significant increase in mt ROS compared to radiation alone along with decline in MMP. Scavenging mt ROS using mitoTEMPO resulted in decline in the intracellular iron level as well as levels of lipid peroxidation. Subsequently, scavenging of mt ROS resulted in completed abrogation of CP mediated radio-sensitization. These results strongly indicated that mt ROS acts as trigger for release of intracellular iron which participates in Fenton type reaction leading to generation of mt ROS which drives lipid peroxidation, ultimately causing ferroptotic cell death. It was also observed that Nrf-2 overexpression resulted in decrease of mt ROS, intracellular iron levels and lipid peroxidation which subsequently abrogated CP mediated radio-sensitization. This suggests that as the master regulator of oxidative stress, Nrf-2 plays a central role in modulating mitochondrial oxidative stress which in turn drives ferroptosis.

In summary, this research has discovered that CP, an FDA-approved substance, has a newfound role as a novel radio-sensitizer. It functions by triggering ferroptosis when combined with radiation exposure. The investigation and confirmation of the Nrf-2 dependent pathway of ferroptosis, driven by mitochondrial ROS, were carried out. These findings offer a comprehensive understanding of the potential to enhance radiation sensitivity by targeting ferroptosis through established and approved clinical agents.

Impact of the research in the advancement of knowledge or benefit to mankind:

Lung cancer is one of the most prevalent and aggressive malignancies and already a significant challenge due to its high incidence and mortality rates. Based on The National Cancer Registry Programme Report 2020, 103,371 lung cancer cases in India were estimated in 2022. However, the burden is further compounded by the persistent issue of relapse. The recurrence of lung cancer not only inflicts immense financial and emotional suffering on patients and their families but also places an overwhelming strain on our healthcare resources. In a country where accessibility to quality medical care can be a challenge for many, the recurrence of lung cancer underscores the urgent need for comprehensive and innovative approaches to treatment, surveillance, and support.One of the major causes of relapse and recurrence of lung cancer is radio-resistance. There is no approved and clinically available radio-sensitizer for lung cancer patients. Dysregulation resulting in overexpression of Nrf-2 is a major factor contributing to radio-resistance and approximately 20-25% lung cancers have altered Nrf-2 expression.In such patients, inhibition of Nrf-2 prior to radiotherapy can substantially improve the efficacy of radio-therapy and prevent recurrence and relapse.

In terms of knowledge, the research has contributed significantly in identifying novel triggers of ferroptosis. Ferroptosis is a relatively new phenomenon and has been linked to radio-resistance in few studies. This research has elaborated the role of Nrf-2 in ferroptosis resistance and its feasibility in becoming a druggable

target of achieving radiation sensitization. This has opened the horizon of further research in iron metabolism and lipid metabolism in radiation responses. The project outcome has also highlighted the direct role of mitochondrial ROS as a trigger for iron release in cells that drives ferroptotic death.

The outcome of this research is identification of Clobetasol propionate (CP) as a novel radio-sensitizer for Keap-1 mutant Nrf-2 overexpressing lung cancer cells. CP has been approved by US-FDA as an anti-psoriatic agent which increases its translational potential. Patients with high Nrf-2 levels are at high risk of tumor relapse post radiotherapy. Screening such lung cancer patients having high expression of Nrf-2 and combining CP with radiotherapy can help to improve the prognosis.

Literature references

1. Uzel, E.K., M. Figen, and Ö. Uzel, *Radiotherapy in lung cancer: current and future role.* Sisli Etfal Hastan Tip Bul, 2019. **53**(4): p. 353-60.
2. Aravindan, N., et al., *Acquired tumor cell radiation resistance at the treatment site is mediated through radiation-orchestrated intercellular communication.* International Journal of Radiation Oncology Biology Physics, 2014. **88**(3): p. 677-685.
3. Choi, E., et al., *A clinical drug library screen identifies clobetasol propionate as an NRF2 inhibitor with potential therapeutic efficacy in KEAP1 mutant lung cancer.* Oncogene, 2017. **36**(37): p. 5285-5295.
4. Sánchez-Ortega, M., A.C. Carrera, and A. Garrido, *Role of NRF2 in lung cancer.* Cells, 2021. **10**(8): p. 1879.
5. Singh, A., et al., *Gain of Nrf2 function in non-small-cell lung cancer cells confers radioresistance.* Antioxidants & redox signaling, 2010. **13**(11): p. 1627-1637.
6. Qin, S., et al., *Nrf2 inhibition sensitizes breast cancer stem cells to ionizing radiation via suppressing DNA repair.* Free Radical Biology and Medicine, 2021. **169**: p. 238-247..
7. Dixon, S.J., et al., *Ferroptosis: an iron-dependent form of nonapoptotic cell death.* cell, 2012. **149**(5): p. 1060-1072.
8. Stockwell, B.R., *Ferroptosis turns 10: Emerging mechanisms, physiological functions, and therapeutic applications.* Cell, 2022. **185**(14): p. 2401-2421.
9. Lei, G., et al., *The role of ferroptosis in ionizing radiation-induced cell death and tumor suppression.* Cell research, 2020. **30**(2): p. 146-162.
10. Ye, L.F., et al., *Radiation-induced lipid peroxidation triggers ferroptosis and synergizes with ferroptosis inducers.* ACS chemical biology, 2020. **15**(2): p. 469-484.
11. Anandhan, A., et al., *NRF2 controls iron homeostasis and ferroptosis through HERC2 and VAMP8.* Science Advances, 2023. **9**(5): p. eade9585.
12. Jayakumar, S., D. Pal, and S.K. Sandur, *Nrf2 facilitates repair of radiation induced DNA damage through homologous recombination repair pathway in a ROS independent manner in cancer cells.* Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 2015. **779**: p. 33-45.
13. Sanchez-Vega, F., et al., *Oncogenic Signaling Pathways in The Cancer Genome Atlas.* Cell, 2018. **173**(2): p. 321-337 e10.
14. Feng, L., et al., *SLC7A11 regulated by NRF2 modulates esophageal squamous cell carcinoma radiosensitivity by inhibiting ferroptosis.* Journal of Translational Medicine, 2021. **19**: p. 1-16.

15. Sun, M., et al., *Coroglaucigenin enhances the radiosensitivity of human lung cancer cells through Nrf2/ROS pathway.* Oncotarget, 2017. **8**(20): p. 32807.
16. Seiler, A., et al., *Glutathione peroxidase 4 senses and translates oxidative stress into 12/15-lipoxygenase dependent-and AIF-mediated cell death.* Cell metabolism, 2008. **8**(3): p. 237-248.
17. Liu, C.-Y., et al., *Glutathione peroxidase 4 expression predicts poor overall survival in patients with resected lung adenocarcinoma.* Scientific Reports, 2022. **12**(1): p. 20462.
18. Doll, S., et al., *ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition.* Nature chemical biology, 2017. **13**(1): p. 91-98.