



**HAL**  
open science

# DIFFERENTIAL EFFECTS OF PROTON THERAPY AND PHOTON THERAPY ON HEAD AND NECK SQUAMOUS CELL CARCINOMA (HNSCC) POST-TREATMENT AGGRESSIVENESS

Marilena Lupu-Plesu, Audrey Claren, Sonia Martial, Papa-Diogop N'diaye,  
Kevin Lebrigand, Damien Ambrosetti, Isabelle Peyrottes, Julien Feuillade,  
Joël Hérault, Maeva Dufies, et al.

► **To cite this version:**

Marilena Lupu-Plesu, Audrey Claren, Sonia Martial, Papa-Diogop N'diaye, Kevin Lebrigand, et al..  
DIFFERENTIAL EFFECTS OF PROTON THERAPY AND PHOTON THERAPY ON HEAD AND  
NECK SQUAMOUS CELL CARCINOMA (HNSCC) POST-TREATMENT AGGRESSIVENESS.  
Séminaire du Cancéropôle Provence-Alpes-Côte d'Azur, Jun 2017, SAINT-RAPHAEL, France. hal-  
02382956

**HAL Id: hal-02382956**

**<https://hal.science/hal-02382956v1>**

Submitted on 4 Dec 2019

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



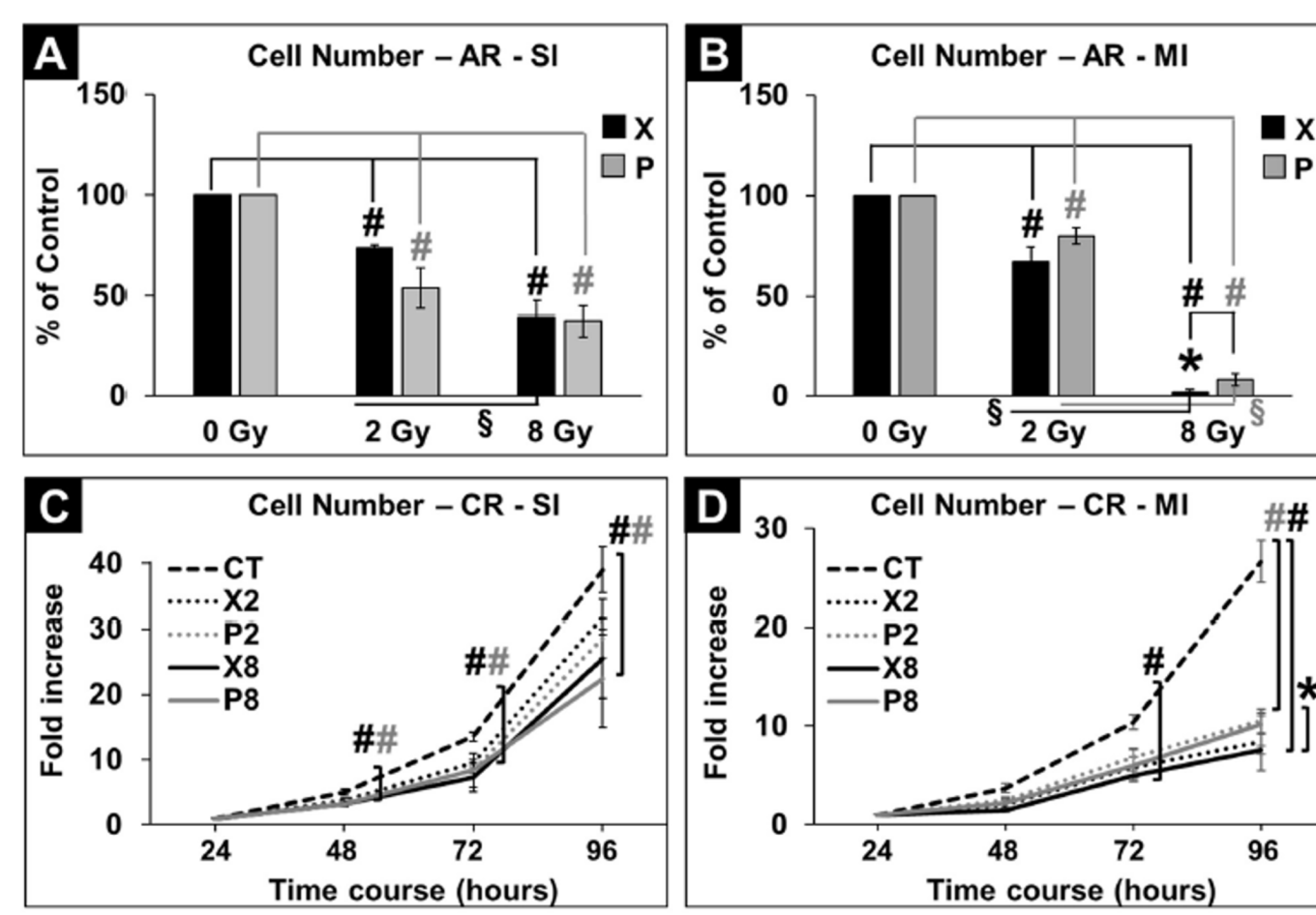
# DIFFERENTIAL EFFECTS OF PROTON THERAPY AND PHOTON THERAPY ON HEAD AND NECK SQUAMOUS CELL CARCINOMA (HNSCC) POST-TREATMENT AGGRESSIVENESS

Marilena Lupu-Plesu, Audrey Claren, Sonia Martial, Papa-Diogop N'Diaye, Kevin Lebrigand, Damien Ambrosetti, Isabelle Peyrottes, Julien Feuillade, Joël Héroult, Maeva Dufies, Jérôme Doyen, and Gilles Pagès

Institute for Research on Cancer and Aging (IRCAN), Nice, CNRS UMR 7284, INSERM U1081, 28 Avenue de Valombrose, 06107 Nice, France ; Centre Antoine Lacassagne, 33 Avenue de Valombrose, 06100 Nice, France ; University of the Côte d'Azur, CNRS, Institute of Molecular and Cellular Pharmacology, F06560 Sophia Antipolis, France ; Nice University Hospital, 30 Avenue de la Voie Romaine, 06000 Nice, France

The treatment of squamous cell carcinoma of the aerodigestive tract (SCC) by standard radiotherapy is challenging because of the proximity of organs at risk. Due to its higher precision in tumor targeting, protontherapy could become the treatment of choice for SCC. Additionally, recent studies have shown that proton irradiation suppresses angiogenic genes and impairs tumor cell invasion/growth.

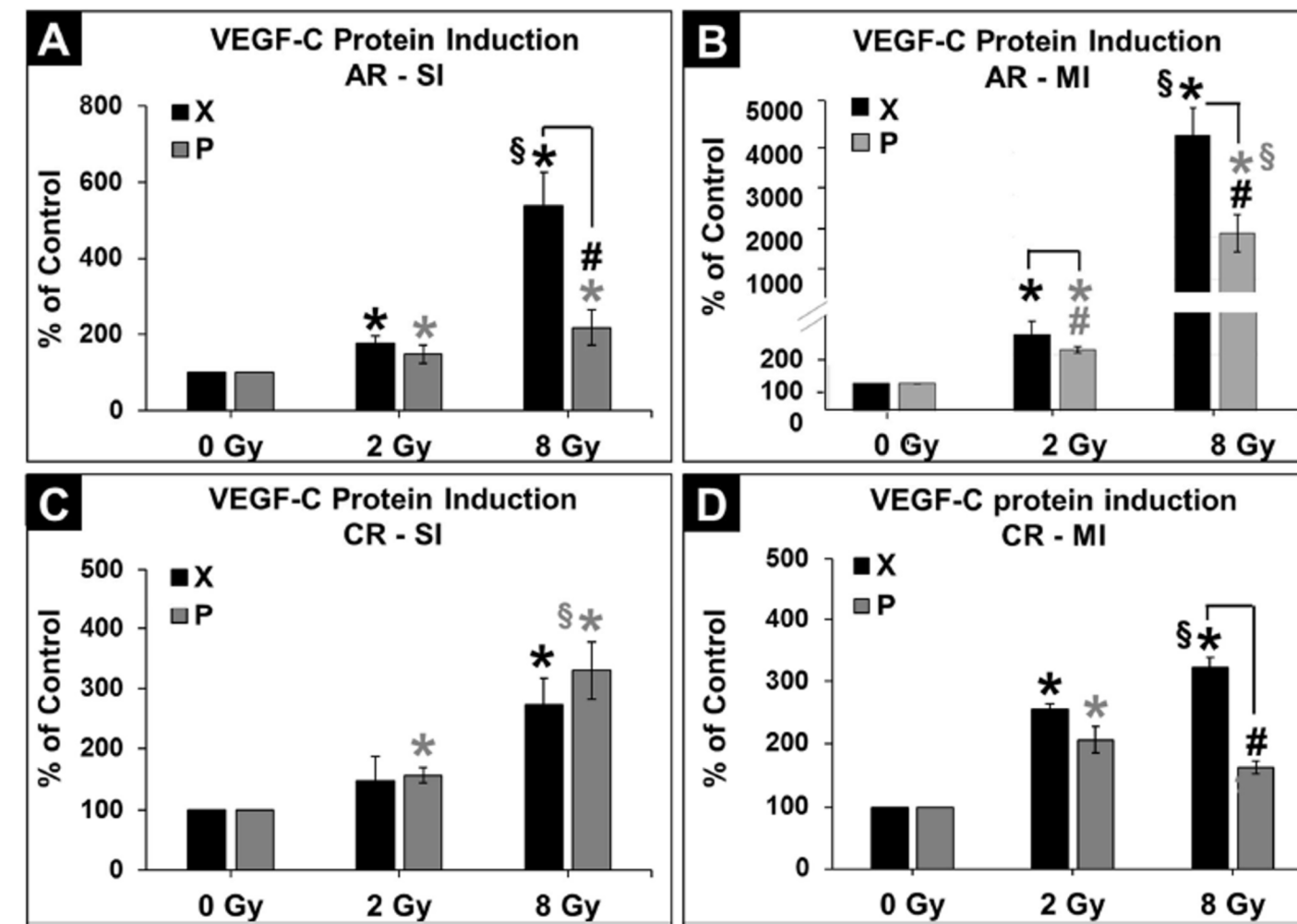
We investigated the effectiveness of single and multiple proton (P) versus photon (X) irradiations in SCC cells on their proliferation potential and on the expression of genes / proteins involved in proliferation, (lymph)angiogenesis, metastasis and anti-tumor immunity.



## CELL PROLIFERATION

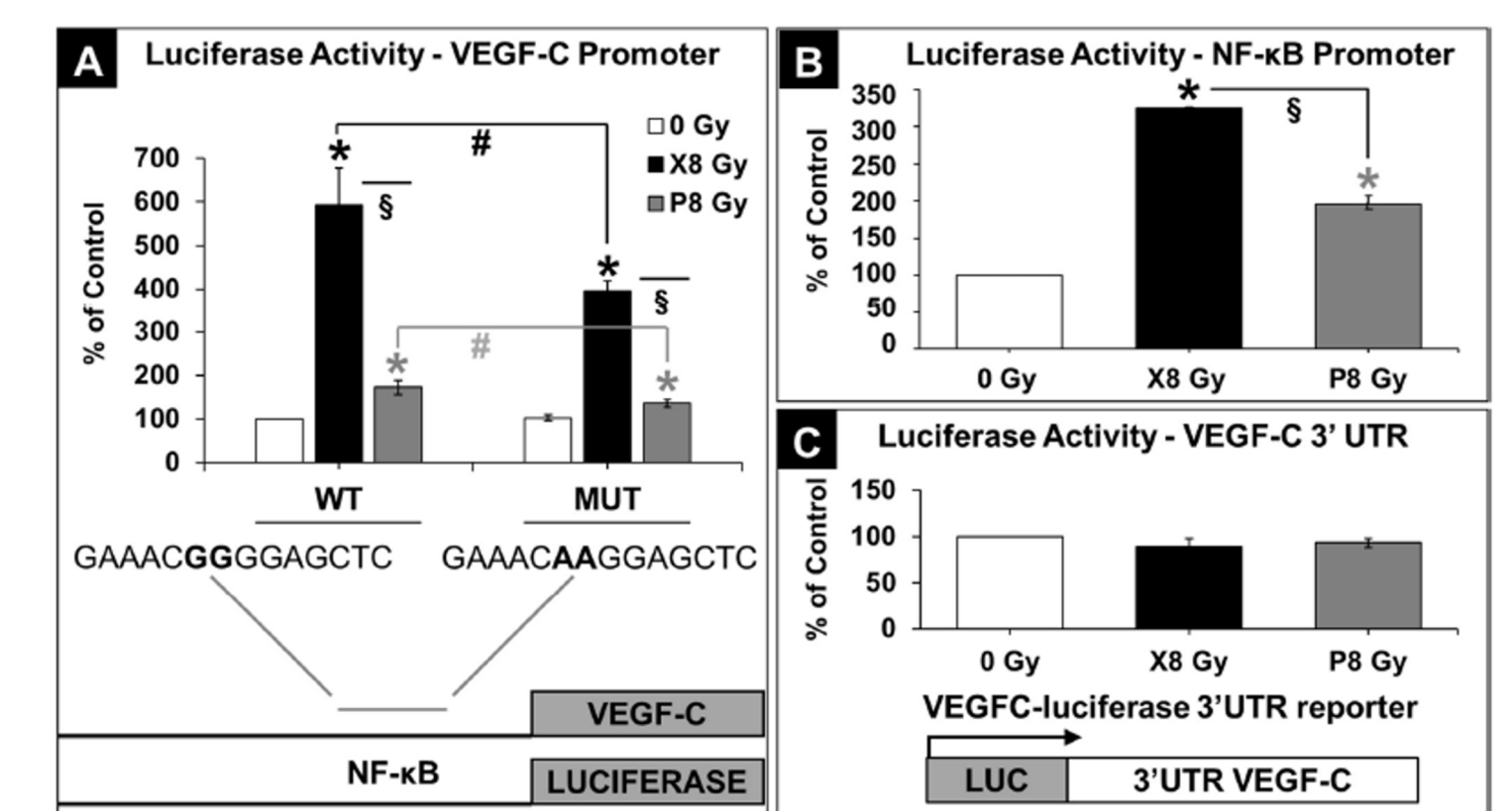
Cell proliferation after a single or multiple proton (P) or photon (X) irradiations at 2 Gy or 8 Gy.

Both P and X irradiations acutely and chronically affected cell growth and cell viability as measured by cell counting of viable cells 48, 72, 96 hours after cell seeding.  $p < 0.05$



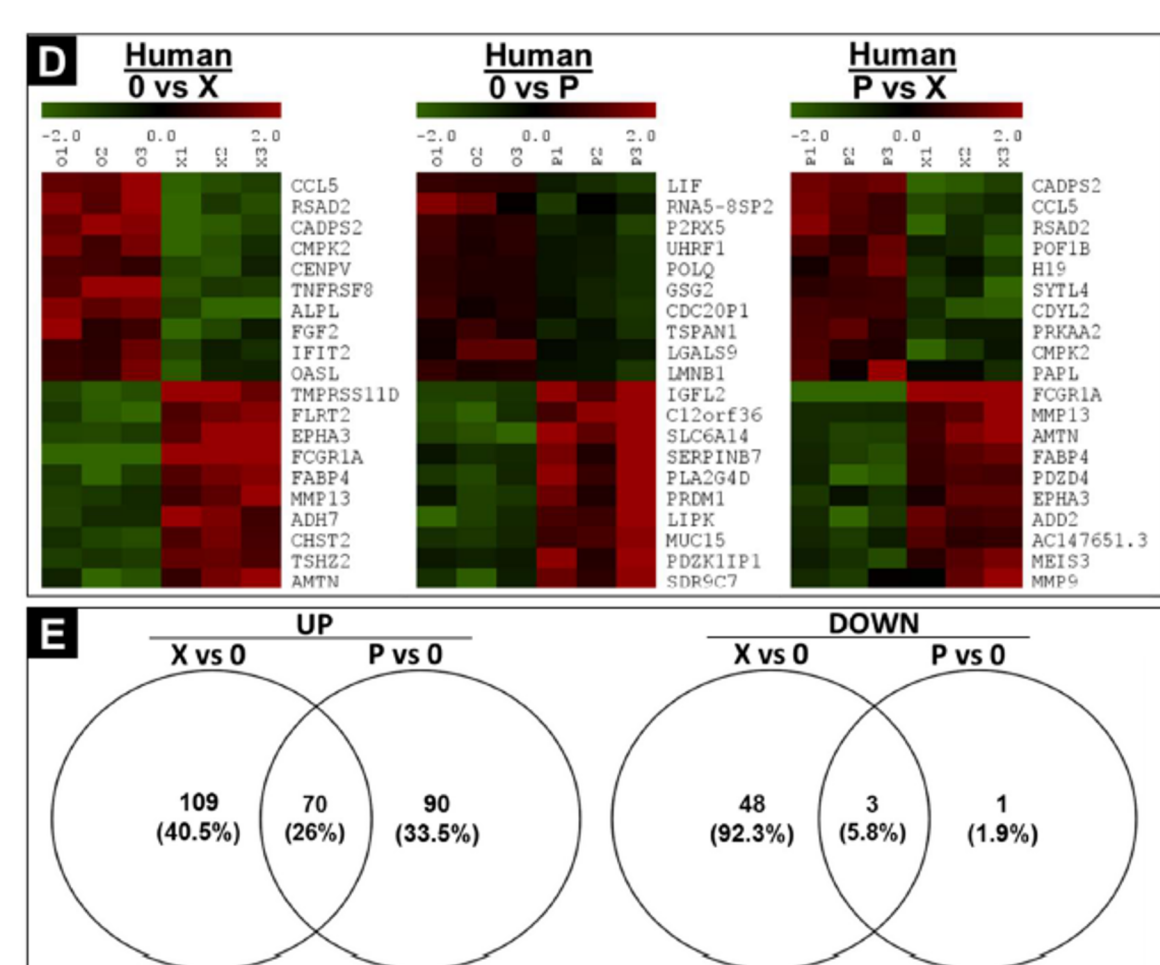
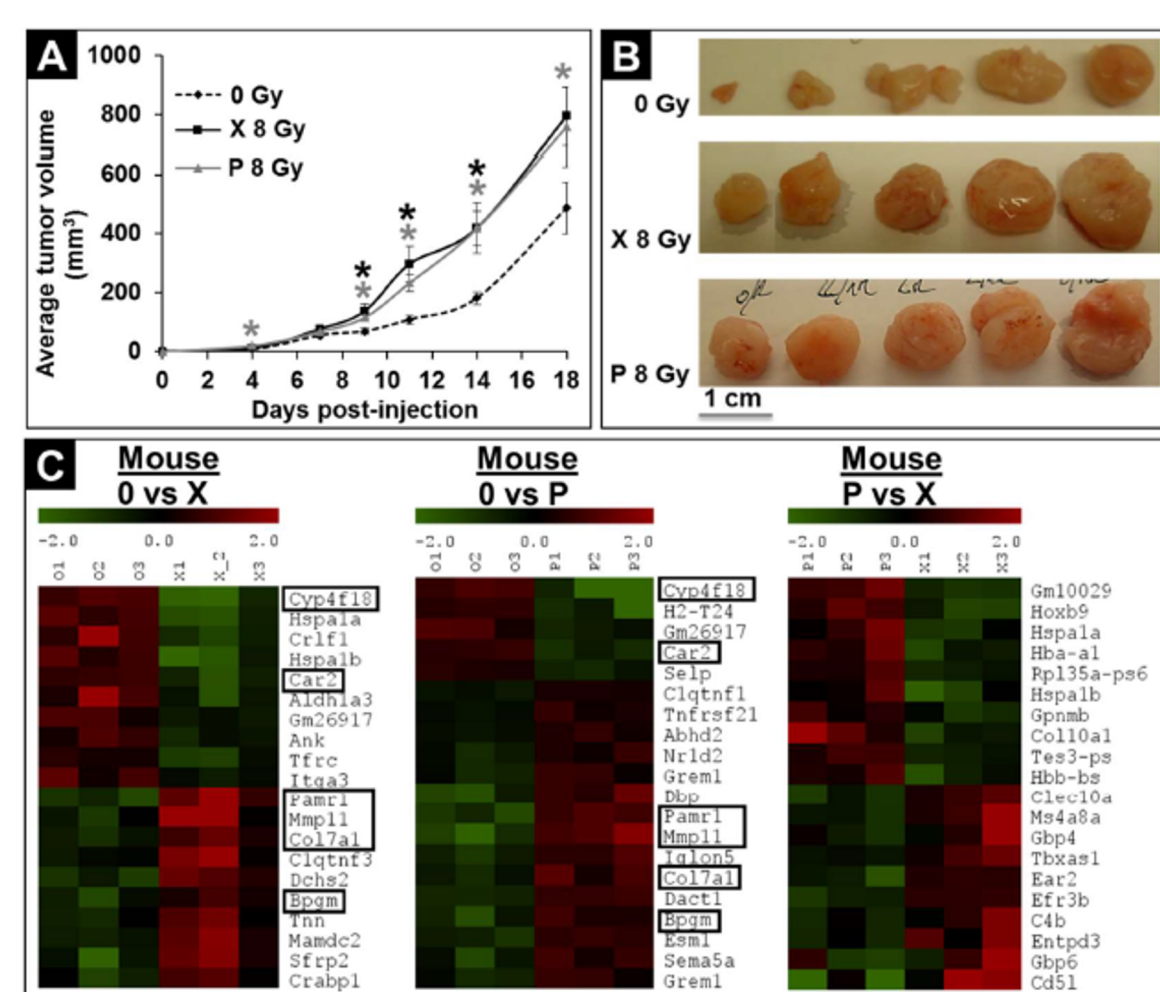
## VEGF-C PROTEIN INDUCTION

In CAL33 cells, VEGF-C protein levels increased in a dose-dependent manner following both P and X irradiations. Furthermore, they were significantly lower after P irradiation, except for the CR-SI setting where the two types of radiations.



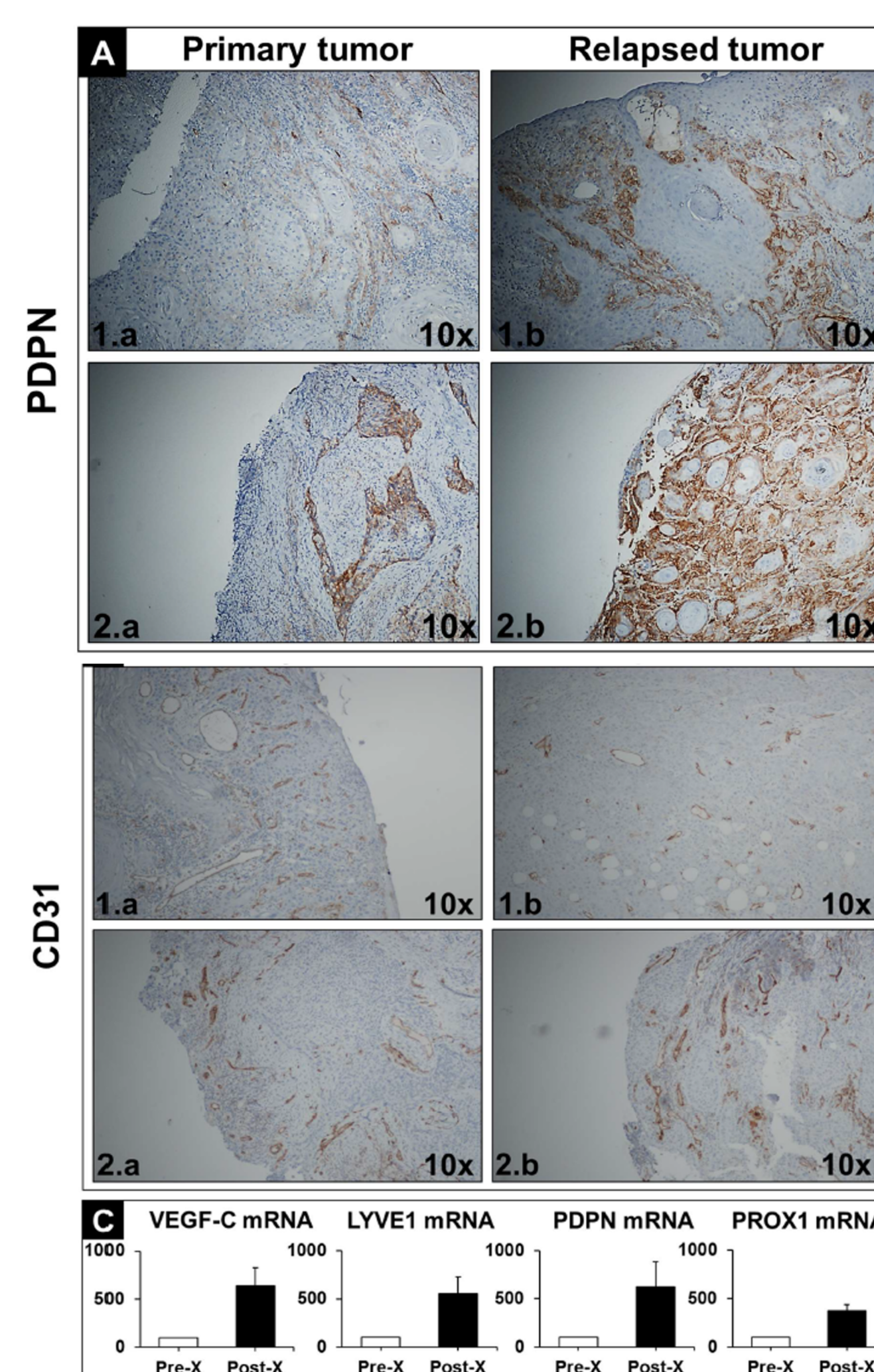
## X AND P IRRADIATIONS STIMULATE VEGF-C PROMOTER ACTIVITY

Irradiation by either X or P stimulated the activity of the VEGF-C promoter especially in CAL33 cells surviving to multiple X irradiations (6- and 18-fold increase, respectively,  $p < 0.001$ ). Mutation of the NF-kB binding site (MUT) had no effect on the basal VEGF-C promoter activity in non-irradiated cells. However, in cells surviving to MI by P and X, the activity of the MUT, as compared to WT, promoter was significantly decreased (by 33%,  $p = 0.004$  and by 30%,  $p = 0.027$ , respectively).



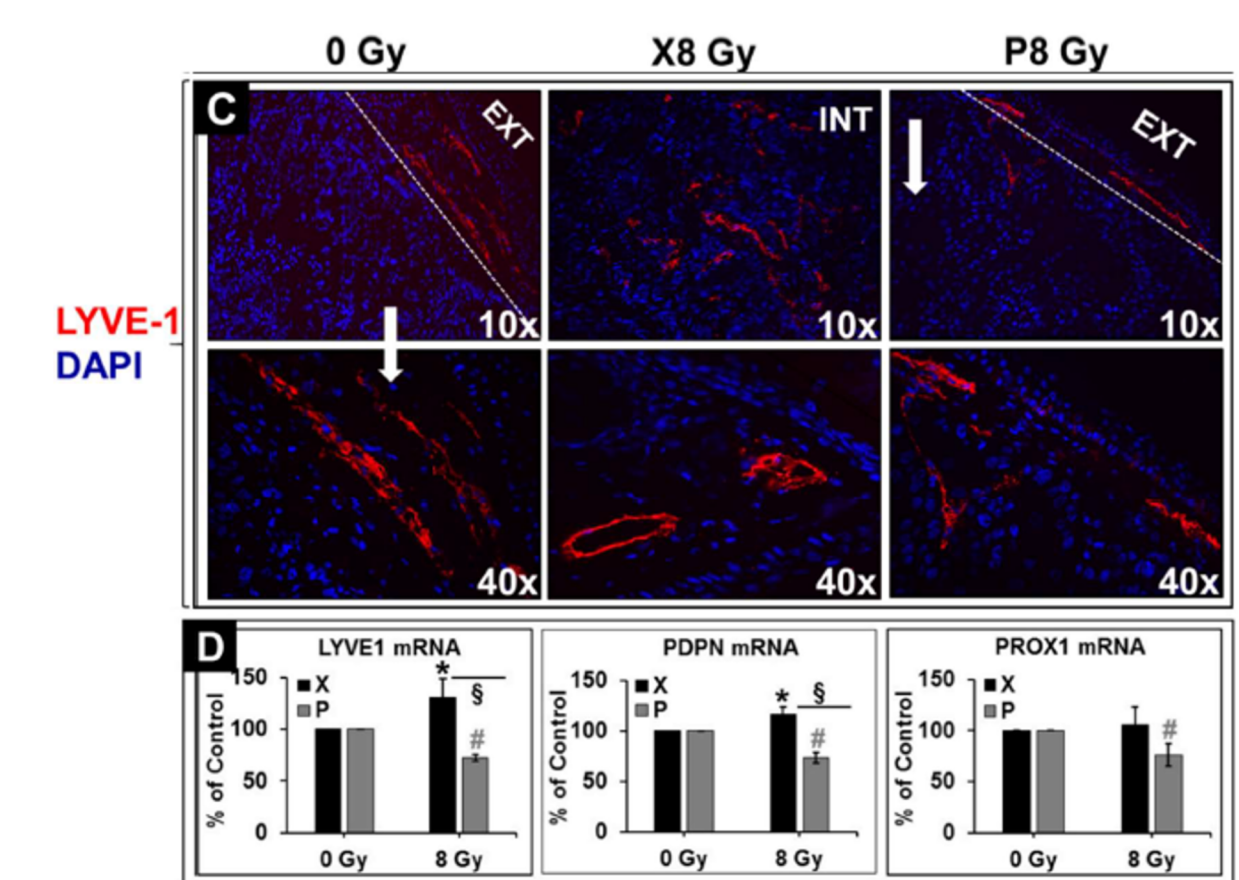
## ANALYSIS OF TUMORS GENERATED BY SUBCUTANEOUS XENOGRAFTS

(A) Average tumor volume (mm<sup>3</sup>); (B) Representative images of tumor xenografts; (C) Heatmap of ten most up- and down-regulated mouse genes in tumors generated by non-irradiated cells vs P or X tumors, and in P vs X tumors; (D) Heatmap of ten most up- and down-regulated human genes in tumors generated by non-irradiated cells vs P or X tumors, and in P vs X tumors; (E) Venn diagrams showing common up-regulated and down-regulated human genes between P and X tumors. Framed genes are commonly expressed in P and X tumors. Selection is adjusted  $p$  value  $< 0.05$  and  $\text{loFC} > 1$ .



## EVALUATION OF LYMPHANGIOGENESIS IN BIOPSIES FROM HNSCC PATIENTS

Representative images of immunohistochemistry for (A) PDPN and (B) CD31 expression: (1) oral and (2) hypopharyngeal localization; Left panels (1.a, 2.a) – primary tumor; Right panels (1.b, 2.b) – relapsed tumor in the same patient after surgery and chemo-X radiotherapy (brown, PDPN/CD31; blue, hematoxylin - nuclei); (C) quantification of PDPN, VEGF-C, LYVE1 and PROX1 mRNA expression.



## REPRESENTATIVE FLUORESCENCE IMAGES IN MURINE XENOGRAFTS

LYVE1 (lymphatic endothelial cells, red) / Hoechst (nuclei, blue), showing different patterns of lymphatic vessels development in X (both periphery and interior of the tumor), P and CT (periphery of the tumor) groups; dashed white lines delimit the tumor edge; CT, control (tumors generated by non-irradiated cells); (D) Murine LYVE1, PDPN and PROX1 mRNA levels in xenografts.

| A mRNA levels (% of Control) - AR - SI |        |      |           | B mRNA levels (% of Control) - AR - MI |            |                     |           |      |     |     |     |
|--|--------|------|-----------|--|------------|---------------------|-----------|------|-----|-----|-----|
| Gene                                   | 2 Gy   | 8 Gy | 2 Gy 8 Gy | Gene                                   | 2 Gy       | 8 Gy                | 2 Gy 8 Gy |      |     |     |     |
| Lymphangiogenesis and metastasis       | VEGF-A | 122  | 159       | 143                                    | 160        | VEGF-A              | 127       | 155  | 145 | 159 |     |
|  | VEGF-C | 130  | 227       | 159                                    | 209        | VEGF-C              | 227       | 1206 | 146 | 155 |     |
|  | VEGF-D | 103  | 92        | 155                                    | 81         | VEGF-D              | 124       | 259  | 124 | 156 |     |
| Inflammation                           | IL6    | 129  | 135       | 119                                    | 72         | IL6                 | 136       | 200  | 82  | 172 |     |
|  | IL8    | 147  | 416       | 162                                    | 460        | IL8                 | 133       | 242  | 138 | 309 |     |
| Proliferation                          | CCL2   | 104  | 123       | 171                                    | 134        | CCL2                | 117       | 112  | 109 | 149 |     |
|  | TRP-2  | 89   | 112       | 102                                    | 102        | TRP-2               | 121       | 78   | 105 | 144 |     |
| Anti-tumor immunity                    | Pd-L1  | 85   | 108       | 88                                     | 93         | Pd-L1               | 173       | 51   | 121 | 111 |     |
|  | PD-L1  | 108  | 103       | 91                                     | 112        | Anti-tumor immunity | PD-L1     | 138  | 142 | 122 | 204 |
| Gene score                             | 4      | 6    | 8         | 3                                      | Gene score | 4                   | 6         | 8    | 3   |     |     |

## QUANTITATIVE GENE EXPRESSION, AS PERCENTAGE OF CONTROL (0 GY), IN EITHER P OR X IRRADIATED CAL33 CELLS BELONGING TO (A) AR-SI, (B) AR-MI, (C) CR-SI AND (D) CR-MI GROUPS

Highlighted values – significantly different ( $p < 0.05$ ) expression levels, as compared to control, for genes associated to favorable (dark grey) and non-favorable (black) outcomes; \*, significantly different expression levels after low, as compared to high dose(s) of either P or X irradiation; #, significantly different expression levels after either low or high dose(s) of P, as compared to X irradiation.

## CONCLUSIONS:

### In vitro results:

- Protontherapy leads to lower expression of factors involved in (lymph)angiogenesis, inflammation and immune tolerance.
- Acquisition of less aggressive phenotype after protontherapy

### In vivo results:

- VEGF-C mRNA levels increase in a dose-dependent manner and with the irradiation number, except in the cells surviving after three proton irradiations.
- Subcutaneous xenograft of proton-irradiated cells generate tumors with less lymphatic vessels than the tumors obtained by photon-irradiated cell xenograft

### Patient studies:

- Relapsed tumors (after photontherapy) present a marked expression of genes involved in lymphangiogenesis/metastasis.

These observations suggest that photontherapy would lead to less pronounced lymphangiogenesis/metastasis, as compared to protontherapy.