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AIRO2022

Radioterapia di precisione per un'oncologia innovativa e sostenibile

BOLOGNA, 25-27 NOVEMBRE
PALAZZO DEI CONGRESSI

Associazione Italiana Radioterapia e Oncologia clinica

Società Italiana di Radiobiologia

Neurotrophin-induced effects on mucosal melanoma cells after exposure to low and high-LET radiations

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Associazione Italiana Radioterapia e Oncologia clinica

Società Italiana di Radiobiologia

DICHIARAZIONE

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Come da nuova regolamentazione della Commissione Nazionale per la Formazione Continua del Ministero della Salute, è richiesta la trasparenza delle fonti di finanziamento e dei rapporti con soggetti portatori di interessi commerciali in campo sanitario.

- Posizione di dipendente in aziende con interessi commerciali in campo sanitario **(NIENTE DA DICHIARARE / CNAO)**
- Consulenza ad aziende con interessi commerciali in campo sanitario **(NIENTE DA DICHIARARE / CNAO)**
- Fondi per la ricerca da aziende con interessi commerciali in campo sanitario **(NIENTE DA DICHIARARE / CNAO)**
- Partecipazione ad Advisory Board **(NIENTE DA DICHIARARE / CNAO)**
- Titolarità di brevetti in compartecipazione ad aziende con interessi commerciali in campo sanitario **(NIENTE DA DICHIARARE / CNAO)**
- Partecipazioni azionarie in aziende con interessi commerciali in campo sanitario **(NIENTE DA DICHIARARE / CNAO)**
- Altro

Gyneacological melanoma - What do we know?

Therapeutic algorithm for melanomas of the lower genital tract.

Therapeutic strategy of Gynecological melanoma

Surgery

Vulvar melanoma (AJCC stage)

Stage IA–IIC

WLE with a clinical tumor-free 1-cm margin circumferentially for a melanoma with a Breslow thickness up to 2 mm, and 2 cm for thicker tumors. Bilateral SLN biopsy sampling. If SLN negative, WLE is adequate. If SLN positive → managed as stage III. Excision of suspicious groin lymph nodes if clinically palpable or suspicious on imaging (ultrasonography, CT, MRI, PET) → consider groin lymphadenectomy as in stage III.

Stage III

WLE with tumor-free margins as recommended for stage IA–IIC; consider radical vulvectomy. If SLN positive in ipsilateral groin → unilateral groin lymphadenectomy. If SLN positive in both groins → bilateral groin lymphadenectomy

Stage IV

WLE with tumor-free margins as recommended for stage IA–IIC; consider radical vulvectomy. Nodal management as in stage III. Consider resection of metastatic lesions

Vaginal melanoma (AJCC stage)

Stage IA–IIC

WLE with a clinical 1-cm margin circumferentially for a melanoma with a Breslow thickness up to 2 mm, and 2 cm for thicker tumors. Due to the location → consider more radical procedures (vaginectomy, pelvic exenteration). SLN biopsy is challenging → excision of suspicious lymph nodes/lymphadenectomy if clinically palpable or suspicious on imaging (ultrasonography, CT, MRI, PET).

Stage III

Loco-regional surgery as in stage IA–IIC. According to the location of the disease → unilateral groin lymphadenectomy versus bilateral groin lymphadenectomy

Stage IV

Loco-regional surgery as in stage IA–IIC. Nodal management as in stage III. Consider resection of metastatic lesions

Cervical melanoma (FIGO stage)

Stage IA–IIA

Radical hysterectomy with upper vaginectomy and pelvic lymphadenectomy → to guide adjuvant treatment

Stage IIB–IVA

The indication and the extent of the surgical procedures (pelvic exenteration) should be tailored according to patient's conditions/tumor localization. Consider radiotherapy as alternative approach

Stage IVB

Radical surgery (from radical hysterectomy/upper vaginectomy to pelvic exenterative procedures) with negative margins may improve the local control of the disease.

Adjuvant treatments

Use of adjuvant radiotherapy, chemotherapy, immunotherapy in early stages is not supported by the literature.

Positive margins (without possible re-resection)/histologically positive nodes → Radiation therapy may be offered (Carbon ion therapy as alternative)

Immune-checkpoint inhibitors (nivolumab, ipilimumab) and targeted therapy (imatinib, vemurafenib, dabrafenib) constitute a promising option.

Systemic treatments (metastatic/recurrent disease)

Radiation therapy

In patients with unresectable tumors → consider radiotherapy combined with chemotherapy and immunotherapy.

Vulvar melanoma: treatment radiotherapy plan adopted from cutaneous melanomas Vaginal/Cervical melanomas: combine with brachytherapy

Chemotherapy

Dacarbazine, temozolomide, nitrosourea, and paclitaxel with or without cisplatin or carboplatin have shown disappointed results, but they should be considered in this setting

Targeted therapy

Immune-checkpoint inhibitors

Nivolumab and ipilimumab have been shown the most promising results in cutaneous melanoma. These should be specifically investigated in melanoma of the female genital tract.

c-KIT inhibitor (imatinib)

BRAF inhibitors (vemurafenib, dabrafenib)

MEK inhibitors (trametinib, cobimetinib)

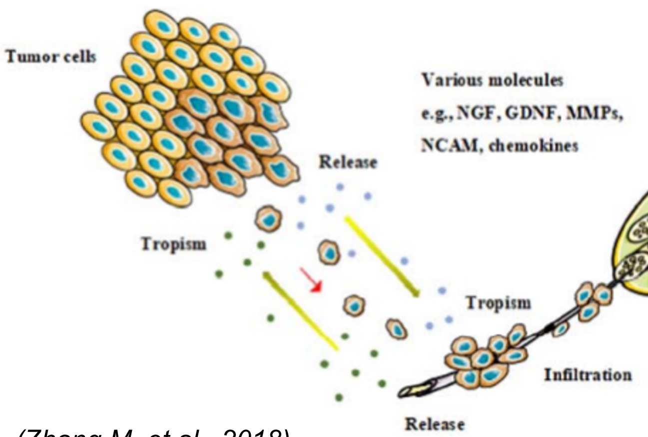
Patients with metastatic/recurrent disease should be encouraged to participate in clinical trials

WLE: wide local excision; SLN: sentinel lymph node.

(Gadducci A. et al., 2018)

- Melanomas of the lower genital tract are rare and aggressive malignancies
- Metastatic tumours and highly neurotropic
- Chemo- and radioresistant
- Poor clinical outcome of patients → 5-year OS of 35-70%for vulvar, 13-32% for vaginal and 10% for cervical melanoma

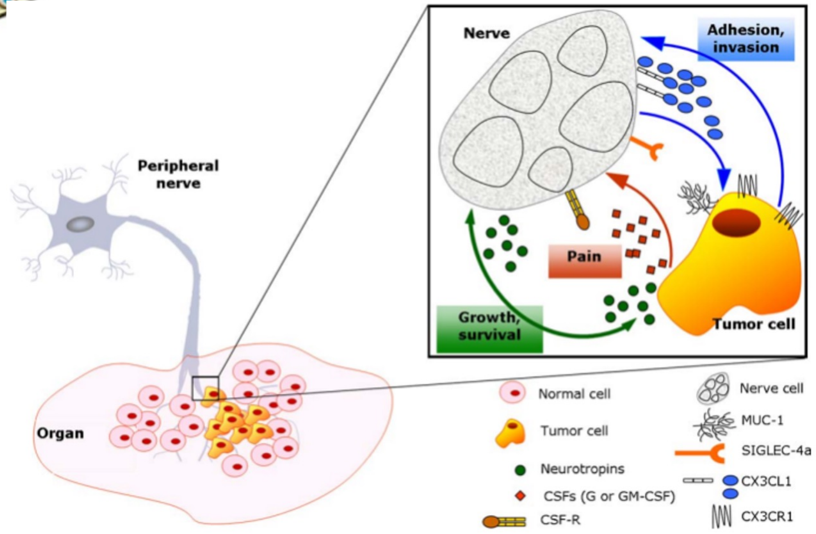
Perineural Invasion (PNI)



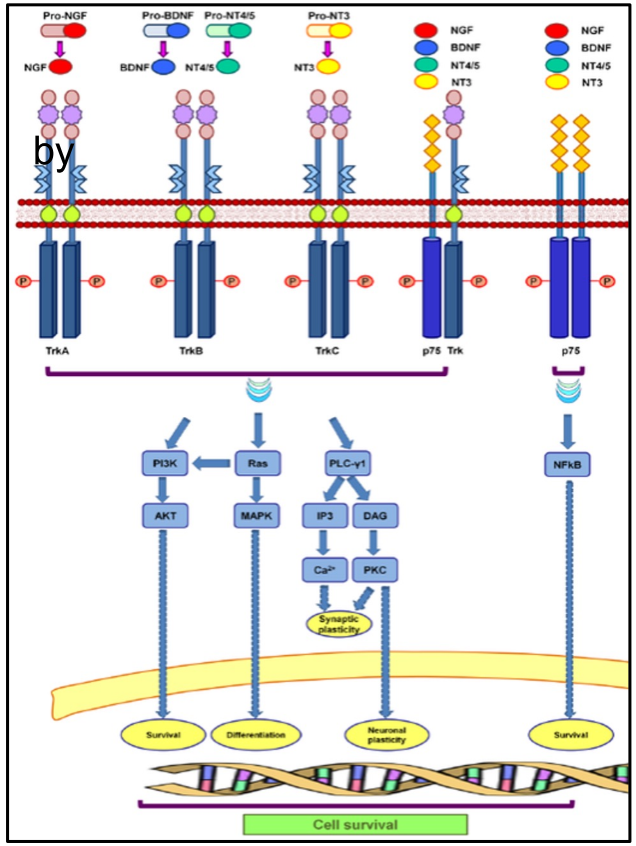
(Zhang M. et al., 2018)

Various molecules, including NGF, GDNF, NCAM and chemokines, secreted by tumor cells and nerve cells in the tumor microenvironment, promote tumor cell migration toward nerves and invasion of the nerves.

Signal transduction pathways activated by neurotrophins.

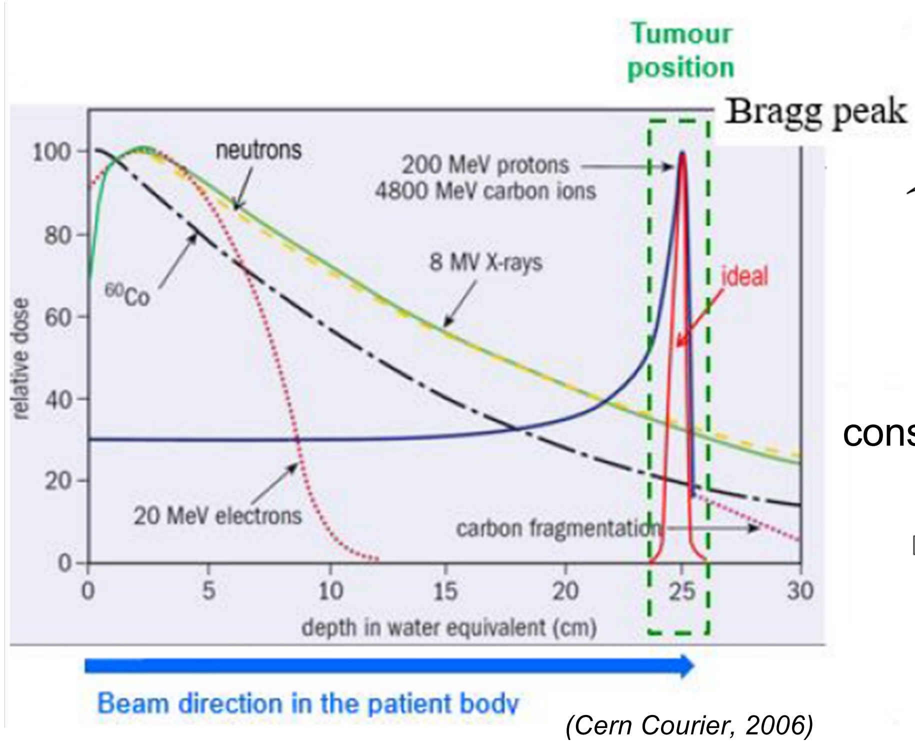


(Marchesi F. et al., 2010)



(Bucci C. et al., 2014)

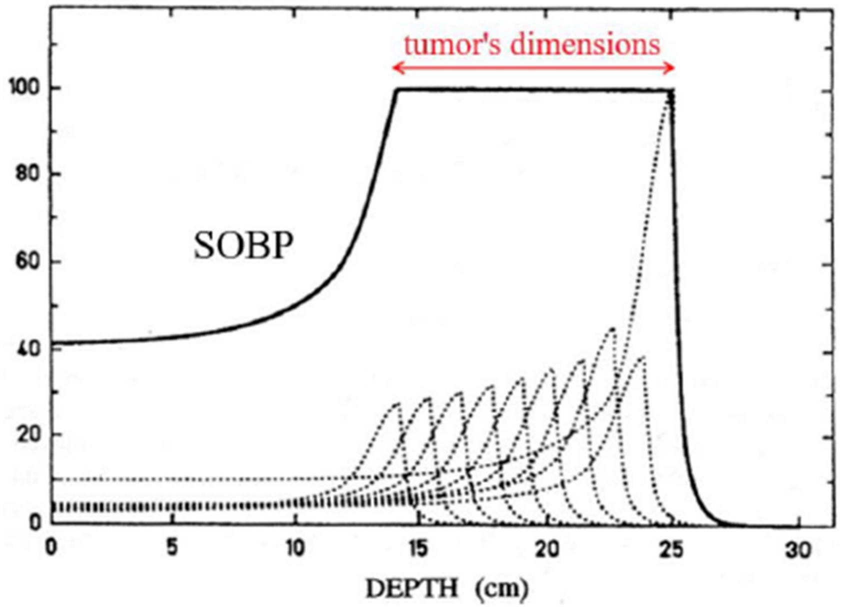
Conventional Radiotherapy VS Hadrontherapy



(Cern Courier, 2006)

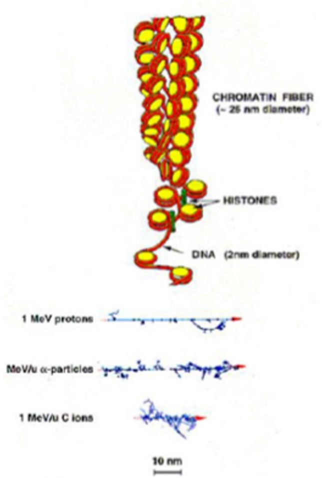
the dose distribution of charged particles is favourable

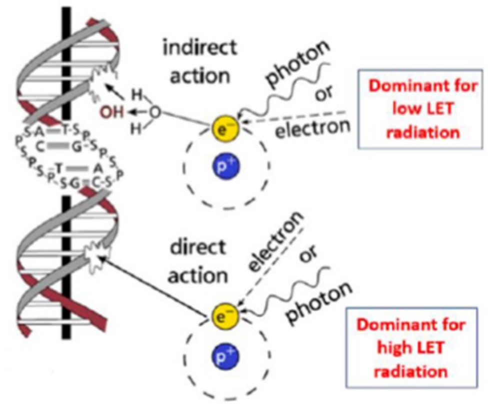
considering the biological advantages…



(Davino D. et al., 2016)

Low and high-LET radiations induce diverse changes in migration and invasion of different cancer cell lines





(Müssig D., 2013.)

- *low LET* irradiation induces ***SSB*** (single strand breaks)
- ***high LET*** irradiation induces ***complex clustered DSB*** (double strand breaks) → ***more difficult to repair*** → ***irreversible***

Migration and invasiveness of human tumor cell lines after irradiation.

Organ	Cell line	Radiation Dose (Gy) (LET)	Migration	Invasion	Key molecules
CNS	U87	γ-ray: 2, 10	+ at 2 Gy	N.D.	αvβ3, αvβ5
		C-ion: 0.5, 3 (91.5 ± 1.5 keV/μm)	– at both doses	N.D.	
	U87	X-ray: 1, 3, 10	N.C. at all doses	N.D.	β3 and β1 integrin
		C-ion: 1, 3, 10	– at 3, 10 Gy	N.D.	(partial correlation)
	U87 EGFR++	X-ray: 2, 6	+ at 2 Gy, – at 6 Gy	N.D.	EGFR/AKT/ERK1/2
		C-ion: 2, 6 (100 keV/mm)[a]	– at both doses	N.D.	
	LN229 EGFR++	X-ray: 2, 6	– at both doses	N.D.	EGFR/AKT/ERK1/2
		C-ion: 2, 6 (100 keV/μm)[a]	– at both doses	N.D.	
	SF126	X-ray: 4	N.D.	+	–
		C-ion: 2 (80 keV/μm)[b]	N.D.	+	NOS/PI3K/AKT2/RHOA
Colon	HCT116	X-ray: 1, 3, 10	– at 10 Gy	N.D.	β1 integrin (partial
		C-ion: 1, 3, 10	– at all doses	N.D.	correlation)
	HCT116 p21wt	X-ray: 1, 3, 10	– at all doses	N.D.	p21 was not
		C-ion: 1, 3, 10	– at all doses	N.D.	affected
	HCT116 p21-/-	X-ray: 1, 3, 10	– at all doses	N.D.	
		C-ion: 1, 3, 10	– at all doses	N.D.	
Lung	A549	X-ray: 0.5, 2, 10	– at 10 Gy	– at 10 Gy	PI3K/AKT
		C-ion: 0.25, 1, 5 (50 keV/μm)[c]	– at 1, 5 Gy	– at 1, 5 Gy	
	A549	X-ray: 0.5, 2, 10	– at 2, 10 Gy	– at 10 Gy	ANLN
		C-ion: 0.25, 1, 5	– at all doses	– at 1, 5 Gy	
	A549	X-ray: 2, 8	+ at both doses	N.D.	RHO
		C-ion: 2, 8(108 keV/μm)[b]	+ at both doses	N.D.	
	EBC-1	X-ray: 0.5, 2, 8	N.C. at all doses	N.C. at all doses	N.D.
		C-io: 0.25, 1, 4	– at 4 Gy	– at 1, 4 Gy	
Pancreas	MIAPaCa-2	X-ray: 2, 4, 8	+ at 2 Gy, - at 8 Gy	+ at 2, 4 Gy	RHOA/RAC1,
		C-ion: 2 (80 keV/μm)[b]	–	–	MMP-2
		C-ion: 0.5, 1, 2, 4 (80 keV/mm)[b]	– at 1, 2, 4 Gy	– at 1, 2, 4 Gy	
	AsPC-1	C-ion: 2 (80 keV/μm)[b]	–	N.C.	–
	BxPC-3	C-ion: 2 (80 keV/μm)[b]	–	N.C.	–
	Panc-1	X-ray: 2, 4, 8	N.C. at all doses	+ at 2, 4 Gy	RHOA/RAC1, uPA/plasmin
		C-ion: 0.5, 1, 2, 4(80 keV/μm)[b]	– at 4 Gy	+ at 1, 2, 4 Gy	NOS/PI3K/AKT2/RHOA/RAC1 uPA/plasmin
Sarcoma	HT1080	X-ray: 0.5, 2, 8	+ at 0.5 Gy	+ at 0.5, 2 Gy, - at 8 Gy	aVb3
		C-ion: 0.2, 1, 4	– at all doses	– at all doses	MMP-2
		Proton: 0.5, 2, 8	– at all doses	– at all doses	MMP-2

+, enhanced; –, reduced; N.C., No statistically significant change was observed; N.D., not determined.
[a] Dose-averaged LET.
[b] Mono-energic beam with a narrow Bragg Peak.
[c] Middle of the spread-out Bragg peak.

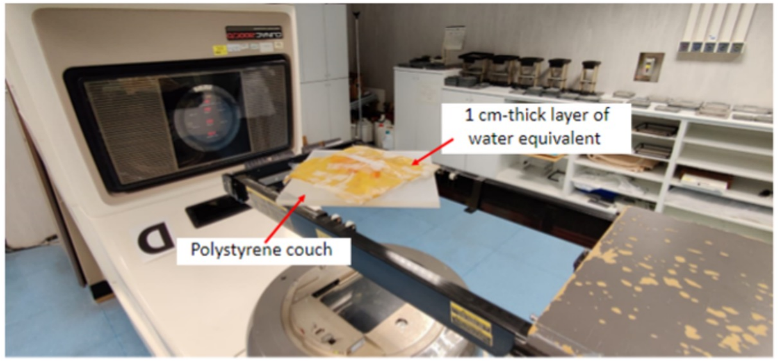
(Fujita M. et al., 2015)

Materials and methods

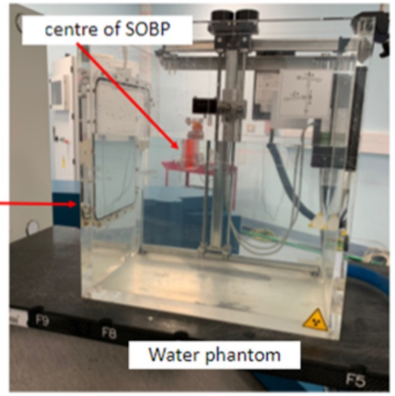
Emanuele Frittitta

Experimental setups

X-rays



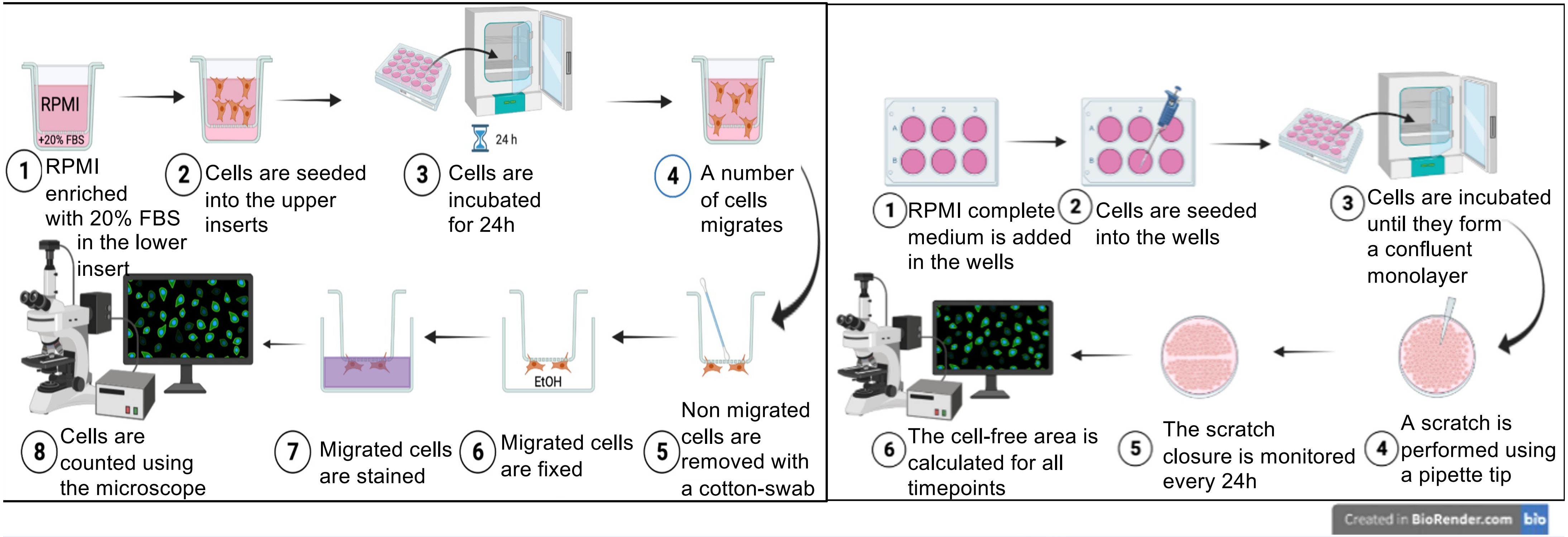
C-ions



Cell migration

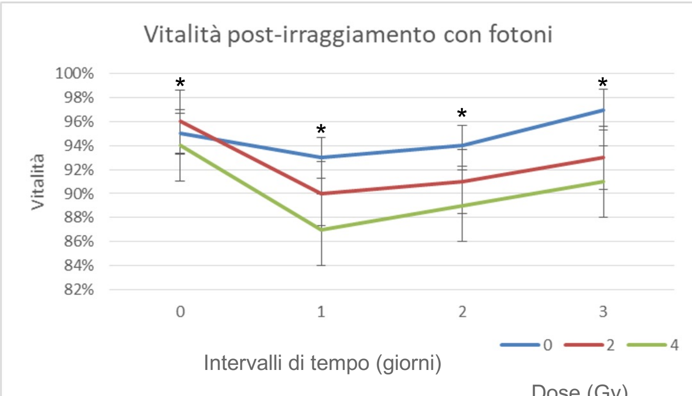
Transwell migration assay

Scratch migration assay

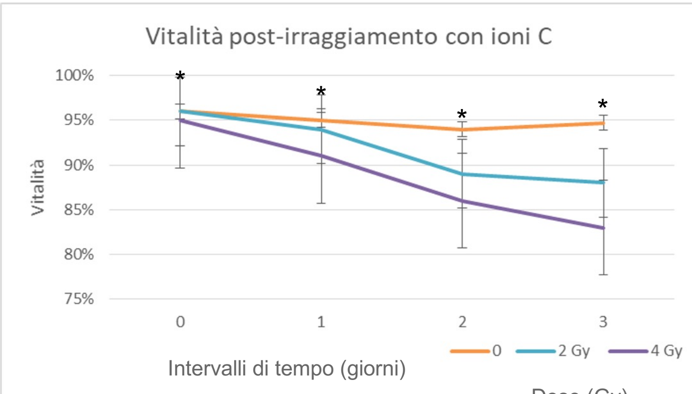


Results

Cell vitality after exposure to radiations

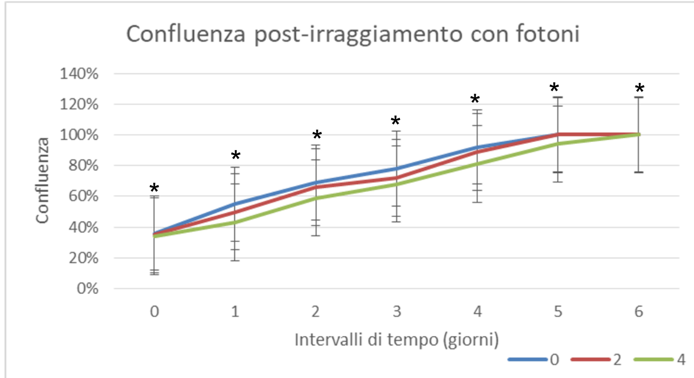


* P<0.001 statistically significant difference

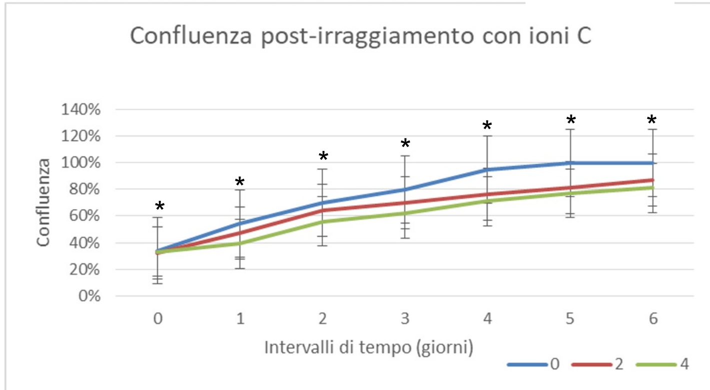


* P<0.001 statistically significant difference

Cell proliferation after exposure to radiations



* P<0.001 statistically significant difference



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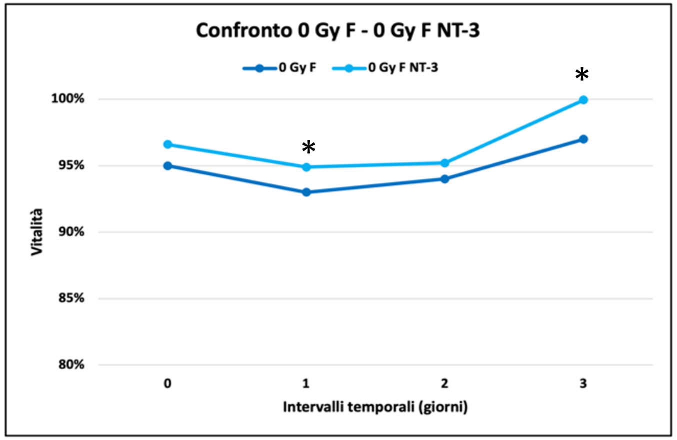
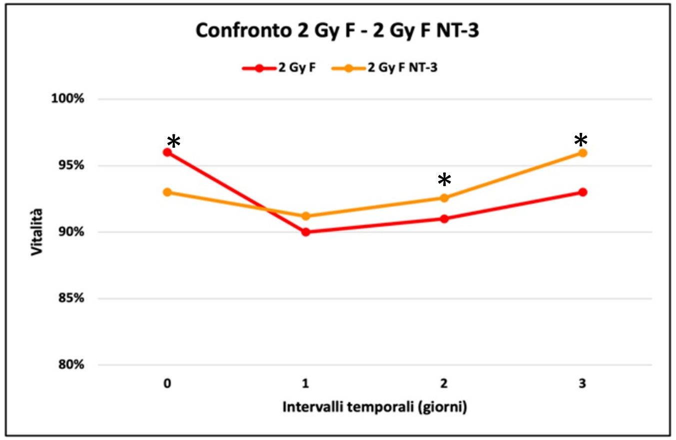
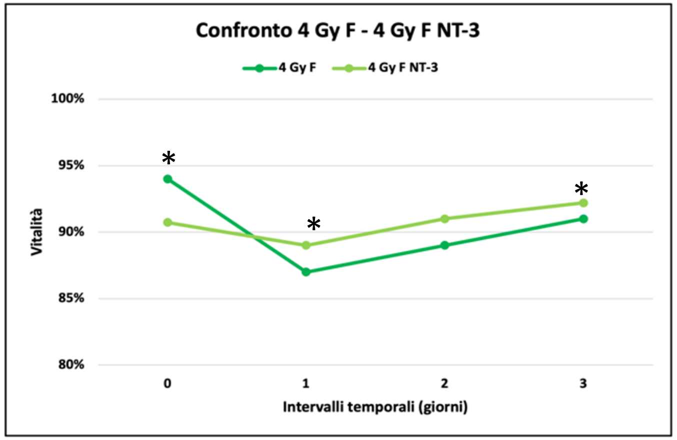
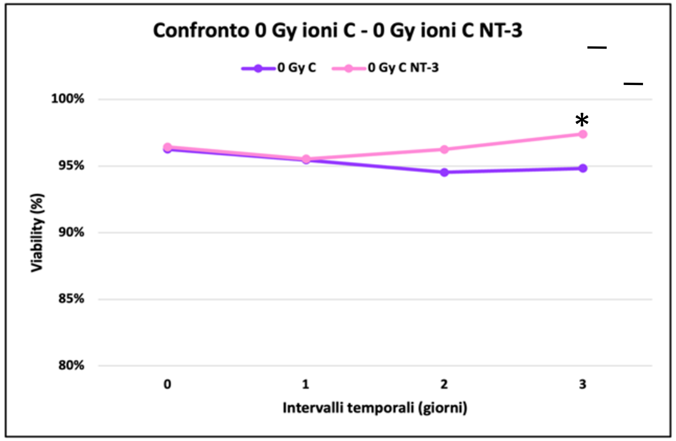
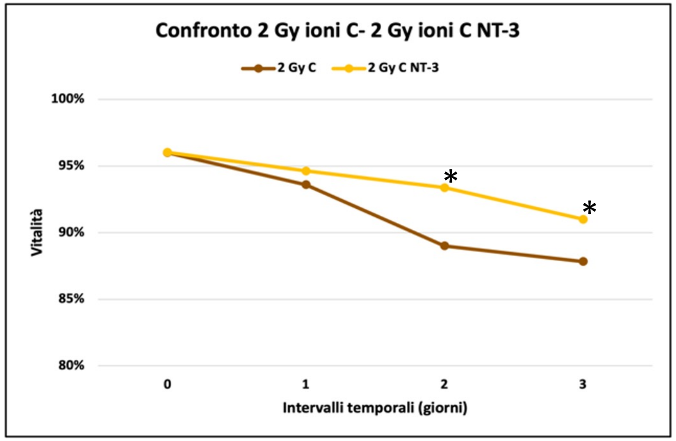
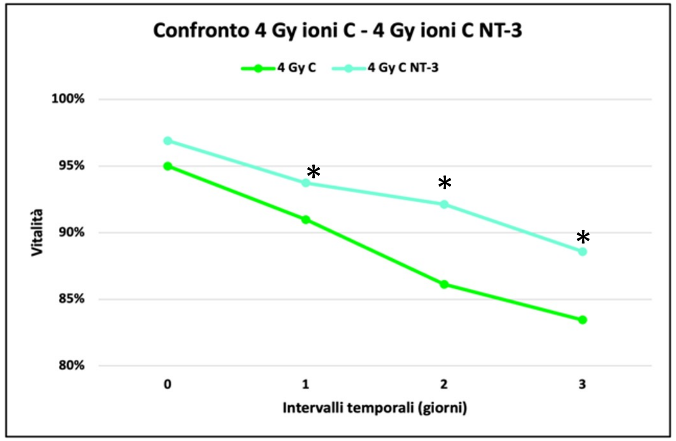




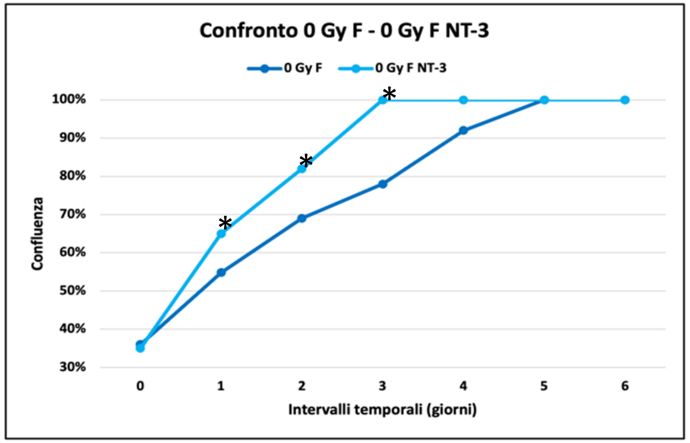


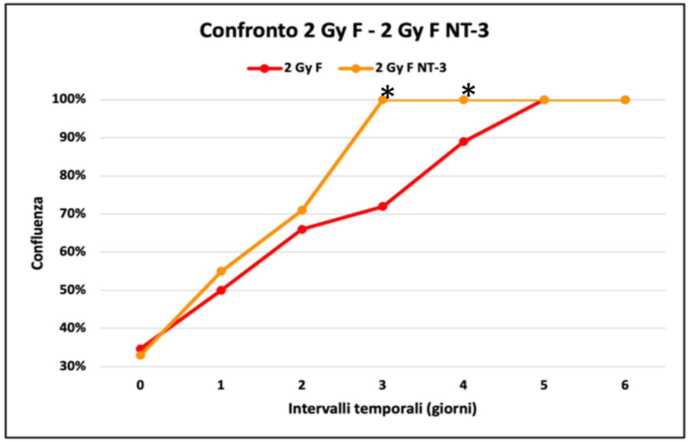


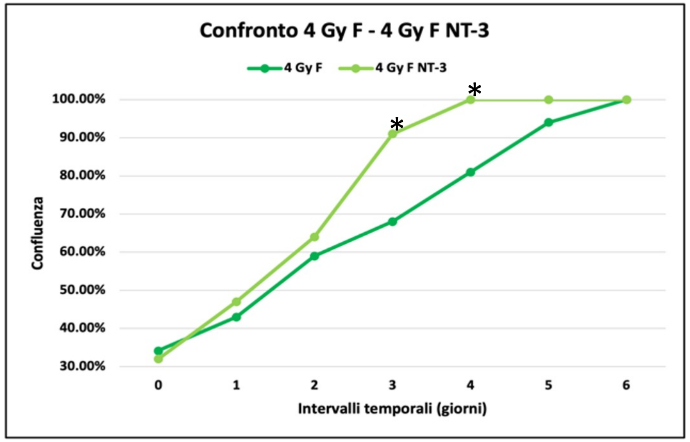


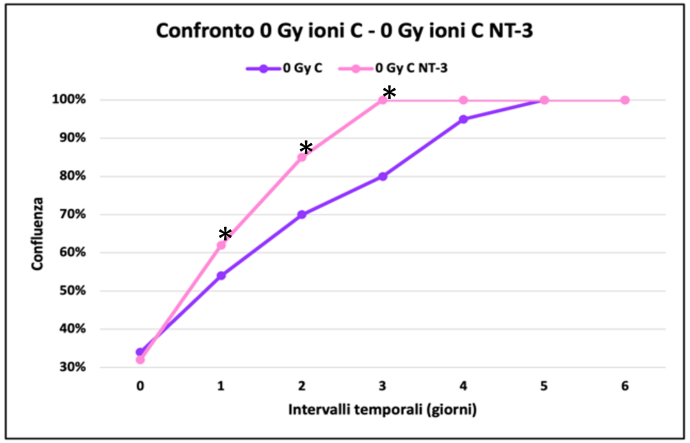


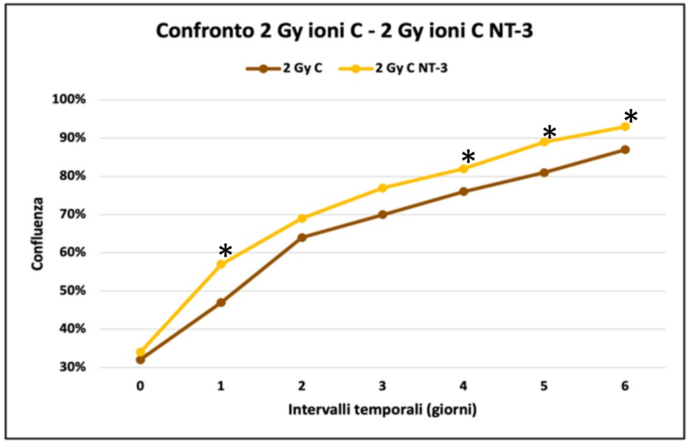
* P<0.001 statistically significant difference

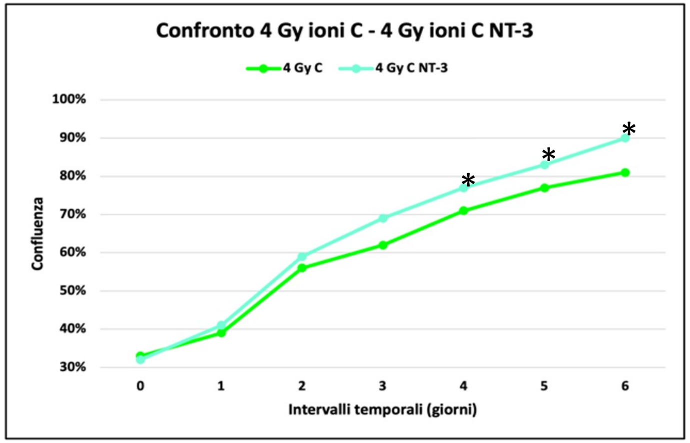






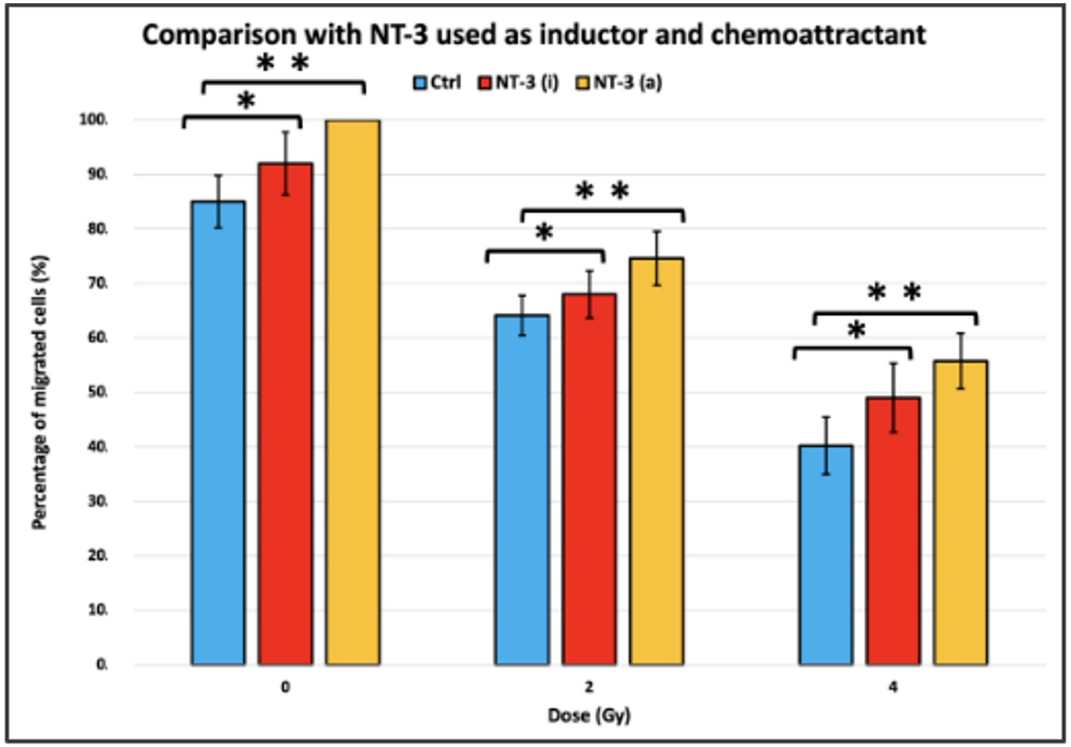






* P<0.001 statistically significant difference

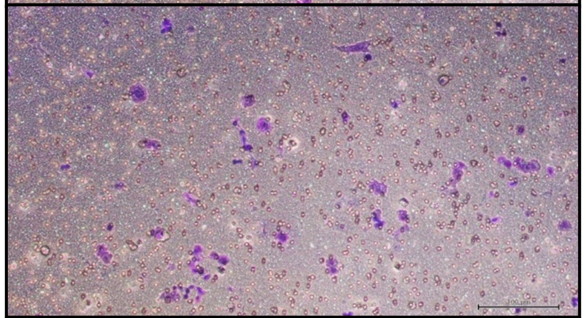
Cell migration - Transwell assay



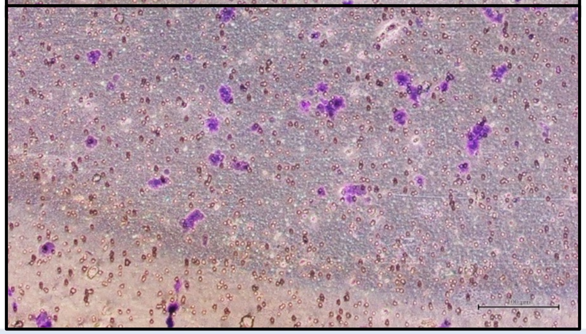
* P<0.05 and ** P <0.01 statistically significant difference

4 Gy C-ion irradiated cells

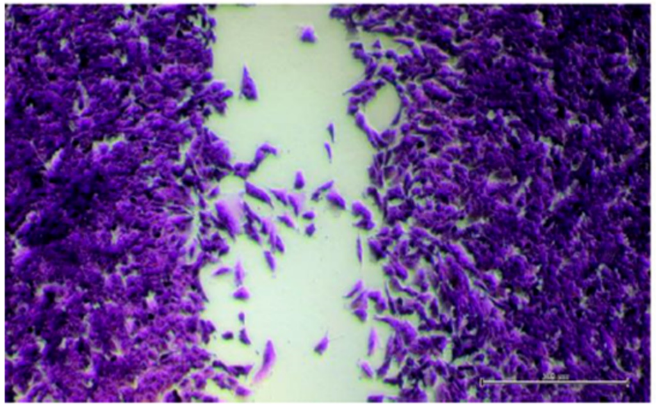
4 Gy C-ion irradiated cells + NT-3 as inductor

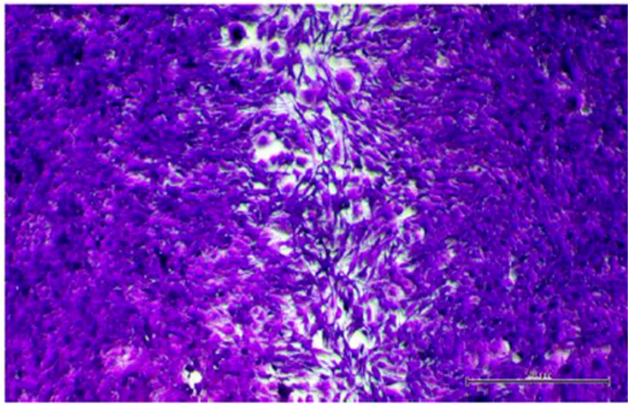


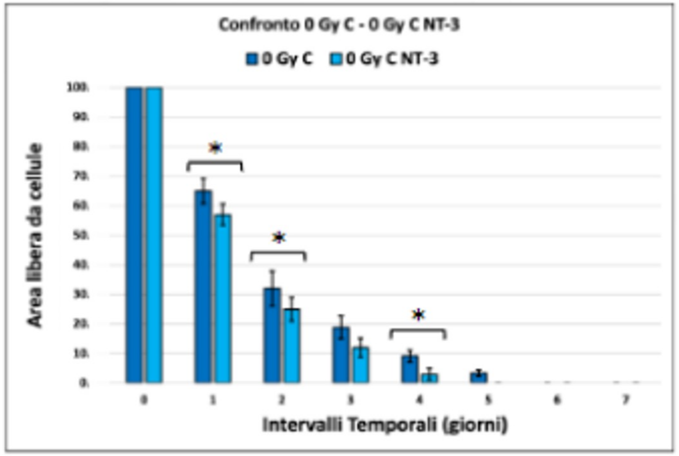
4 Gy C-ion irradiated cells + NT-3 as chemoattractant

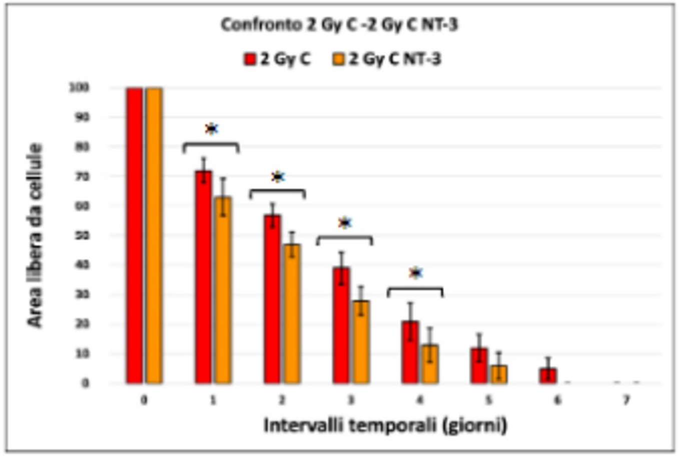


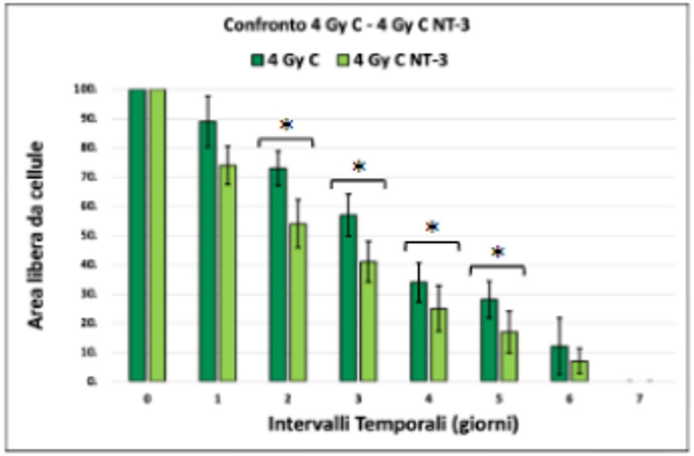
Cell migration - Scratch assay











* P<0.001 statistically significant difference

Conclusions

- ➢ C-ions significantly reduce cell viability, proliferation and migration of mucosal melanoma cells in a dose-dependent way when compared to photons.
- ➢ The addition of NT-3 increases cell viability, proliferation and migration of mucosal melanoma cells even after the exposure to radiation.This increase is more significant after photon irradiation.
- ➢ NT-3 exhibits a more significant effect when acting as a chemoattractant than acting as an inductor.

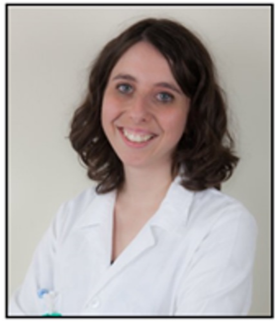
What's next?

- ➢ Use of other cell lines corresponding to different
- ➢ Co-culture tumour cells with neural cells
- ➢ Use of 3D *in vitro* models (scaffolds, organoids…)
- ➢ Use of *in vivo* models (xenograft mice models, zebrafish…)
- ➢ *Ex vivo* experiments using samples from patients' biopsies

Grazie per la vostra attenzione !

Our Radiobiology team...

Federica Carnevale



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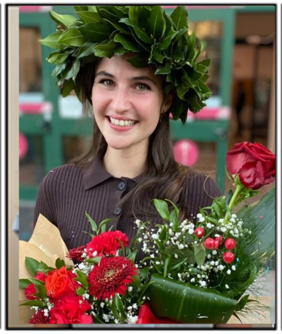

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