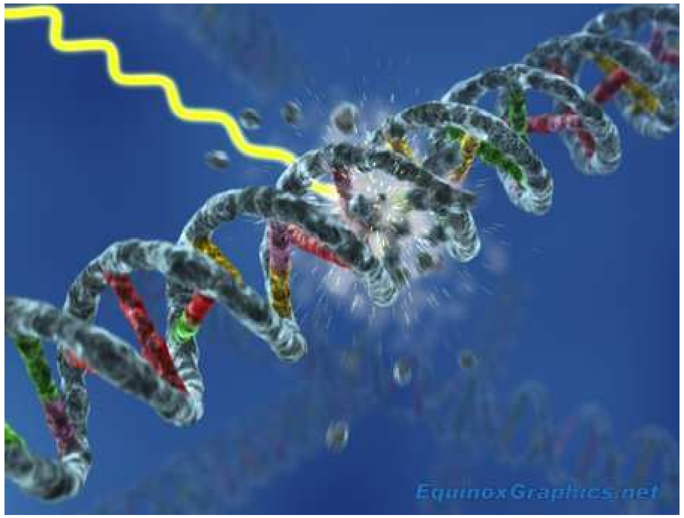
Consiglio Nazionale delle Ricerche Istituto di Bioimmagini e Fisiologia Molecolare (IBFM)

A radiobiological experiment on breast cancer cell line using laser driven electron accelerators

Minafra L[1], Cammarata FP[1], Bravatà V[1], Forte GI[1], Lamia D[1], Gizzi L[2], Labate L[2], Baffigi F[2], Fulgentini L[2], Koester P[2], Gilardi MC[1] and Russo G[1]

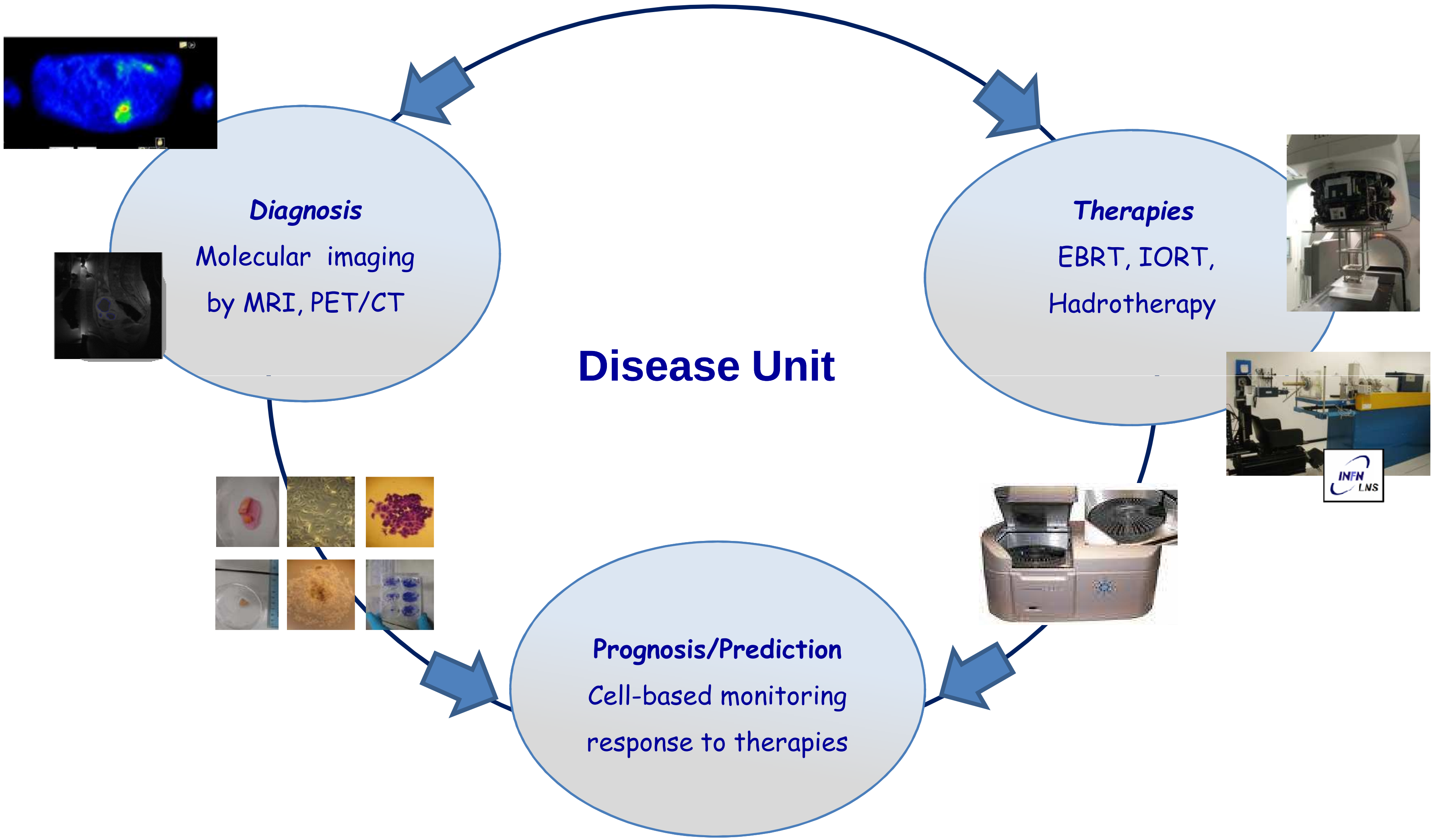
1.Institute of Bioimaging and Molecular Physiology (IBFM) – CNR, SS Cefalù (PA)
2. National Optic Institute (INO) – CNR, Pisa



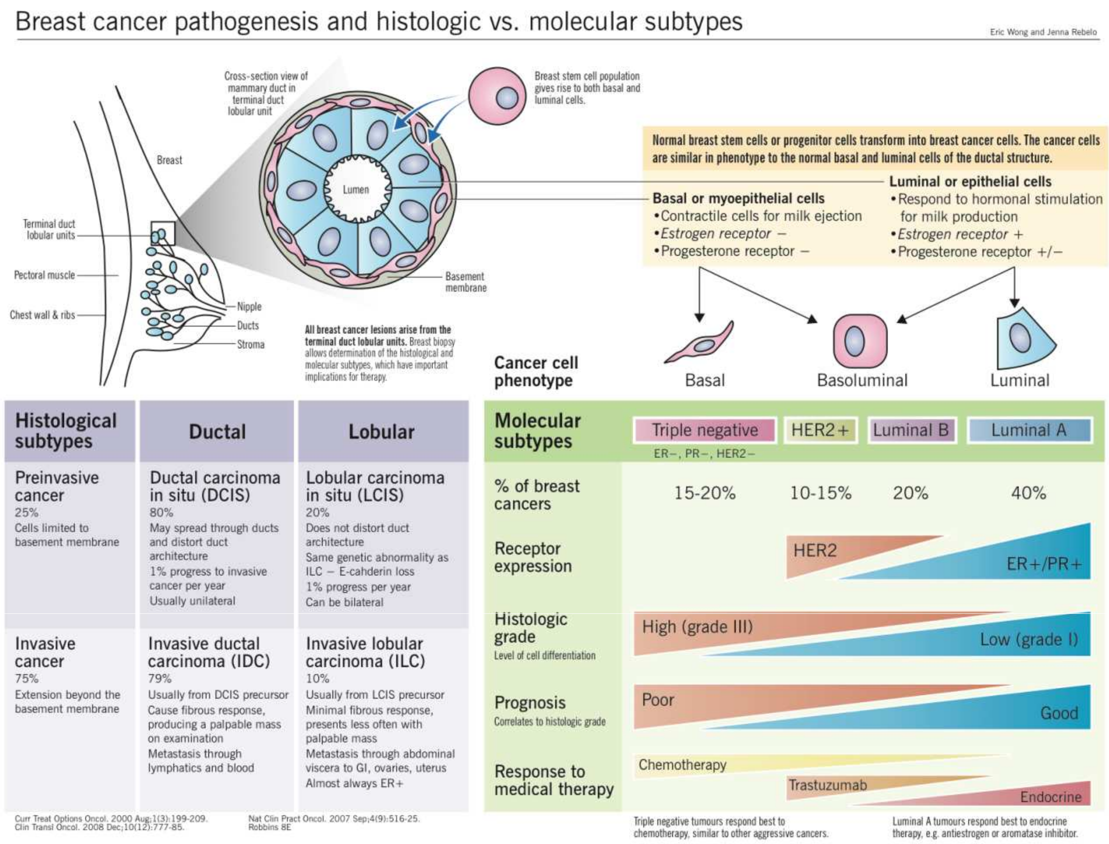
Catania, 8 September 2016

Luigi Minafra (PhD)
IBFM-CNR
luigi.minafra@ibfm.cnr.it

THE IBFM TRANSLATIONAL RESEARCH MODEL



BREAST CANCER



- Breast cancer (BC) is a very complex, multifactorial disease, highly heterogeneous at a molecular and clinical level and presents distinct subtypes (>30) associated with different clinical outcomes.

- BC affects 1 out of 8 women during their lifetime and represents 29 % of all cancers affecting women.

- To treat BC radiation therapy (RT) plays an important role, often used in combination with surgery and chemotherapy.

ARTICLE

A collection of breast cancer cell lines for the study of functionally distinct cancer subtypes

Richard M. Neve,[1,2,9,*] Koei Chin,[2,9] Jane Fridlyand,[2,6] Jennifer Yeh,[2] Frederick L. Baehner,[2] Tea Fevr,[2] Laura Clark,[1] Nora Bayani,[1] Jean-Philippe Coppe,[1] Frances Tong,[3] Terry Speed,[3] Paul T. Spellman,[1] Sandy DeVries,[2] Anna Lapuk,[1] Nick J. Wang,[1] Wen-Lin Kuo,[1] Jackie L. Stilwell,[1] Daniel Pinkel,[2] Donna G. Albertson,[2] Frederic M. Waldman,[2] Frank McCormick,[2] Robert B. Dickson,[7] Michael D. Johnson,[7] Marc Lippman,[8] Stephen Ethier,[4] Adi Gazdar,[5] and Joe W. Gray[1,2]

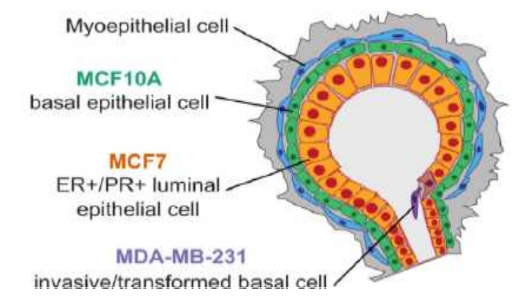


Table 1. Source, clinical, and pathological features of tumors used to derive breast cancer cell lines used in this study

	Cell line	Gene cluster	ER	PR	HER2	TP53	Source	Tumor type	Age (years)	Ethnicity	Culture media	Culture conditions
1	600MPE	Lu	+	[−]		−		IDC			DMEM, 10% FBS	37°C, 5% CO_2
2	AU565[a]	Lu	−	[−]	+	$+^{WT}$	PE	AC	43	W	RPMI, 10% FBS	37°C, 5% CO_2
3	BT20	BaA	−	[−]		$++^{WT}$	P.Br	IDC	74	W	DMEM, 10% FBS	37°C, 5% CO_2
4	BT474	Lu	+	[+]	+	+	P.Br	IDC	60	W	RPMI, 10% FBS	37°C, 5% CO_2
5	BT483	Lu	+	[+]		−	P.Br	IDC, pap	23	W	RPMI, 10% FBS	37°C, 5% CO_2
6	BT549	BaB	−	[−]		$++^{M}$	P.Br	IDC, pap	72	W	RPMI, 10% FBS	37°C, 5% CO_2
7	CAMA1	Lu	+	[−]		+	PE	AC	51	W	DMEM, 10% FBS	37°C, 5% CO_2
8	HBL100	BaB	−	[−]		++	P.Br	N	27		DMEM, 10% FBS	37°C, 5% CO_2
9	HCC1007[d]	Lu	+	[−]		[+/−]	P.Br	Duc.Ca	67	B	RPMI, 10% FBS	37°C, 5% CO_2
10	HCC1143[d]	BaA	−	[−]		$++^{M}$	P.Br	Duc.Ca	52	W	RPMI, 10% FBS	37°C, 5% CO_2
11	HCC1187[d]	BaA	−	[−]		$++^{M}$	P.Br	Duc.Ca	41	W	RPMI, 10% FBS	37°C, 5% CO_2
12	HCC1428[d]	Lu	+	[+]		[+]	PE	AC	49	W	RPMI, 10% FBS	37°C, 5% CO_2
13	HCC1500[d]	BaB	−	[−]		−	P.Br	Duc.Ca	32	B	RPMI, 10% FBS	37°C, 5% CO_2
14	HCC1569[d]	BaA	−	[−]	+	$-^{M}$	P.Br	MC	70	B	RPMI, 10% FBS	37°C, 5% CO_2
15	HCC1937[d]	BaA	−	[−]		[−]	P.Br	Duc.Ca	24	W	RPMI, 10% FBS	37°C, 5% CO_2
16	HCC1954[d]	BaA	−	[−]	+	[+/−]	P.Br	Duc.Ca	61	EI	RPMI, 10% FBS	37°C, 5% CO_2
17	HCC202[d]	Lu	−	[−]	+	[−]	P.Br	Duc.Ca	82	W	RPMI, 10% FBS	37°C, 5% CO_2
18	HCC2157[d]	BaA	−	[−]		[+]	P.Br	Duc.Ca	48	B	RPMI, 10% FBS	37°C, 5% CO_2
19	HCC2185[d]	Lu	−	[−]		[+]	PE	MLCa	49	WH	RPMI, 10% FBS	37°C, 5% CO_2
20	HCC3153[d]	BaA	−	[−]		[−]					RPMI, 10% FBS	37°C, 5% CO_2
21	HCC38[d]	BaB	−	[−]		$++^{M}$	P.Br	Duc.Ca	50	W	RPMI, 10% FBS	37°C, 5% CO_2
22	HCC70[d]	BaA	−	[−]		$++^{M}$	P.Br	Duc.Ca	49	B	RPMI, 10% FBS	37°C, 5% CO_2
23	HS578T	BaB	−	[−]		$+^{M}$	P.Br	IDC	74	W	DMEM, 10% FBS	37°C, 5% CO_2
24	LY2	Lu	+	[−]		+/−	PE	IDC	69	W	DMEM, 10% FBS	37°C, 5% CO_2
25	MCF10A[b]	BaB	−	[−]		$+/-^{WT}$	P.Br	F	36	W	DMEM/F12*	37°C, 5% CO_2
26	MCF12A[b]	BaB	−	[−]		+	P.Br	F	60	W	DMEM/F12*	37°C, 5% CO_2
27	MCF7	Lu	+	[+]		$+/-^{WT}$	PE	IDC	69	W	DMEM, 10% FBS	37°C, 5% CO_2
28	MDAMB134VI	Lu	+	[−]		$+/-^{WT}$	PE	IDC	47	W	DMEM, 10% FBS	37°C, 5% CO_2
29	MDAMB157	BaB	−	[−]		−	PE	MC	44	B	DMEM, 10% FBS	37°C, 5% CO_2
30	MDAMB175VII	Lu	+	[−]		$+/-^{WT}$	PE	IDC	56	B	DMEM, 10% FBS	37°C, 5% CO_2
31	MDAMB231	BaB	−	[−]		$++^{M}$	PE	AC	51	W	DMEM, 10% FBS	37°C, 5% CO_2
32	MDAMB361	Lu	+	[−]	+	$-^{WT}$	P.Br	AC	40	W	DMEM, 10% FBS	37°C, 5% CO_2
33	MDAMB415	Lu	+	[−]		+	PE	AC	38	W	DMEM, 10% FBS	37°C, 5% CO_2
34	MDAMB435	BaB	−	[−]		$+^{M}$	PE	IDC	31	W	DMEM, 10% FBS	37°C, 5% CO_2
35	MDAMB436	BaB	[−]	[−]		[−]	PE	IDC	43	W	L15, 10% FBS	37°C, no CO_2
36	MDAMB453	Lu	−	[−]		$-^{WT}$	PF	AC	48	W	DMEM, 10% FBS	37°C, 5% CO_2
37	MDAMB468	BaA	[−]	[−]		[+]	PE	AC	51	B	L15, 10% FBS	37°C, no CO_2
38	SKBR3[a]	Lu	−	[−]	+	+	PE	AC	43	W	McCoys 5A, 10% FBS	37°C, 5% CO_2
39	SUM1315MO2[c]	BaB	−	[−]		[+]	Sk	IDC			Ham's F12, 5%-IE	37°C, 5% CO_2
40	SUM149PT[c]	BaB	[−]	[−]		[+]	P.Br	Inf Duc.Ca			Ham's F12, 5%-IH	37°C, 5% CO_2
41	SUM159PT[c]	BaB	[−]	[−]		[−]	P.Br	AnCar			Ham's F12, 5%-IH	37°C, 5% CO_2
42	SUM185PE[c]	Lu	[−]	[−]		[−]	PE	Duc.Ca			Ham's F12, 5%-IH	37°C, 5% CO_2
43	SUM190PT[c]	BaA	−	[−]	+	[+/−]	P.Br	Inf			Ham's F12, SF-IH**	37°C, 5% CO_2
44	SUM225CWN[c]	BaA	−	[−]	+	++	CWN	IDC			Ham's F12, 5%-IH	37°C, 5% CO_2
45	SUM44PE[c]	Lu	[+]	[−]		[−]	PE	Ca			Ham's F12, SF-IH**	37°C, 5% CO_2
46	SUM52PE[c]	Lu	[+]	[−]		[−]	PE	Ca			Ham's F12, 5%-IH***	37°C, 5% CO_2
47	T47D	Lu	+	[+]		$++^{M}$	PE	IDC	54		RPMI, 10% FBS	37°C, 5% CO_2
48	UACC812	Lu	+	[−]	+	$-^{WT}$	P.Br	IDC	43		DMEM, 10% FBS	37°C, 5% CO_2
49	ZR751	Lu	+	[−]		−	AF	IDC	63	W	RPMI, 10% FBS	37°C, 5% CO_2
50	ZR7530	Lu	+	[−]	+	$-^{WT}$	AF	IDC	47	B	RPMI, 10% FBS	37°C, 5% CO_2
51	ZR75B	Lu	+	[−]		+/−					RPMI, 10% FBS	37°C, 5% CO_2

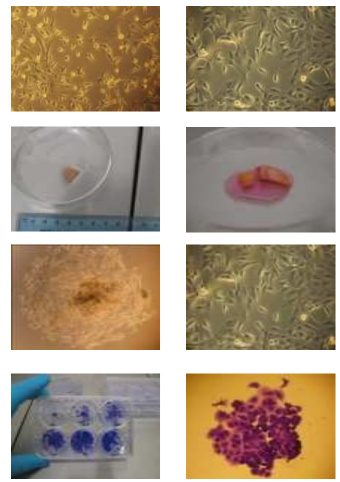
There are a large number of immortalized cell-lines available as study models for BC. *Neve RM et al. Cancer Cell , 2006*

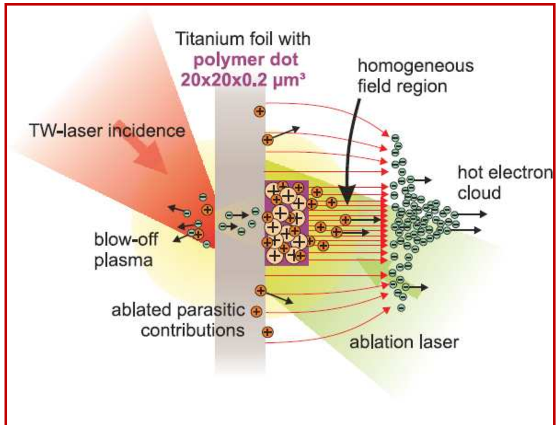
RADIOBIOLOGY KNOWHOW

Biological effects induced by IR have been studied by ***in-vitro, ex-vivo*** and ***in-vivo*** approaches and by the integration of **multidisciplinary skills** of biologists, physicists, engineers and physicians of our group.

The principal *in-vitro* activities have been carried out on **breast cancer cell lines,** immortalized and primary cells from patient tumour biopsies (*ex-vivo*) with different beams:

- **electrons,**
- **protons and**
- **electrons produced by laser plasma interaction**

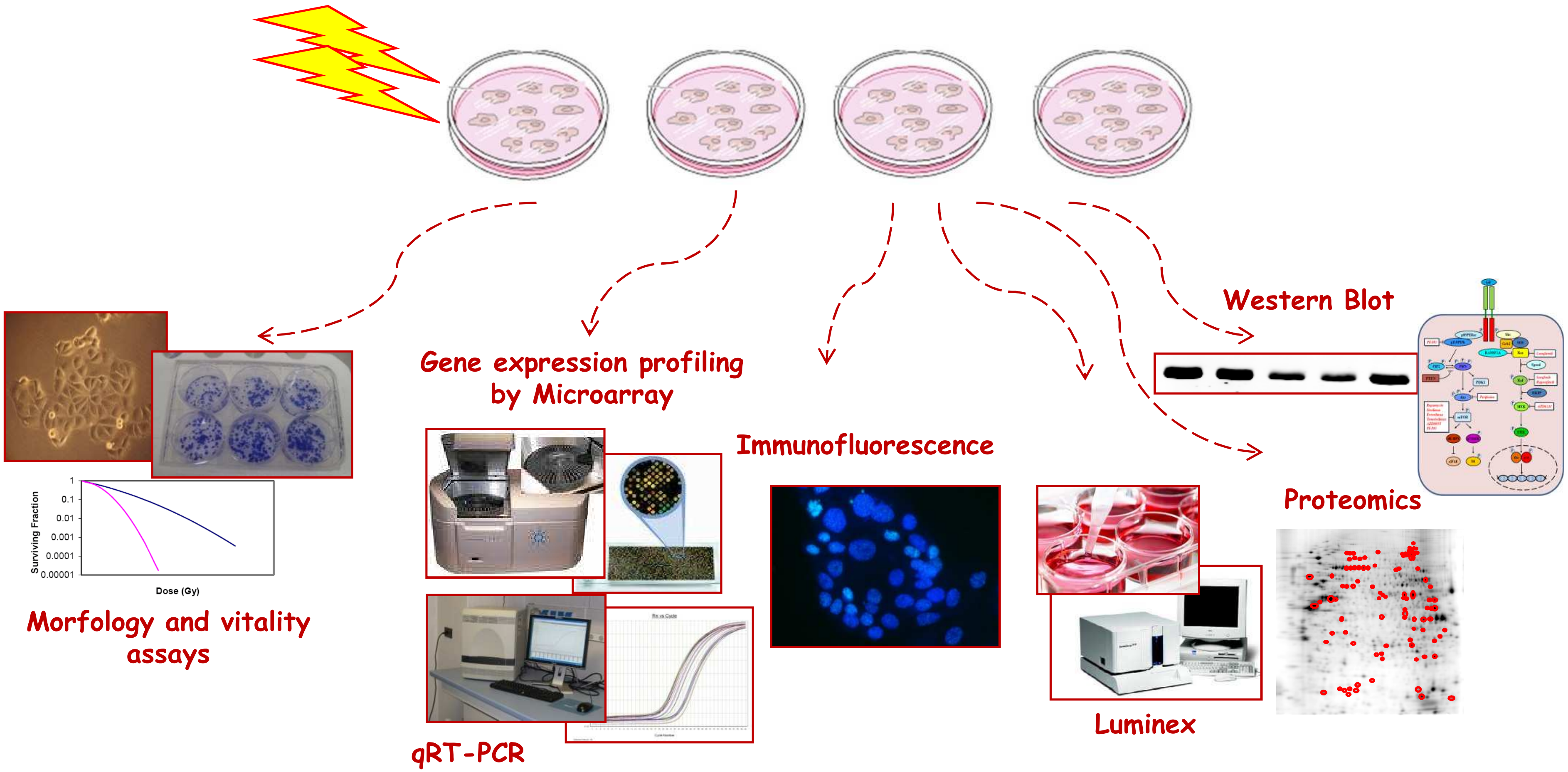




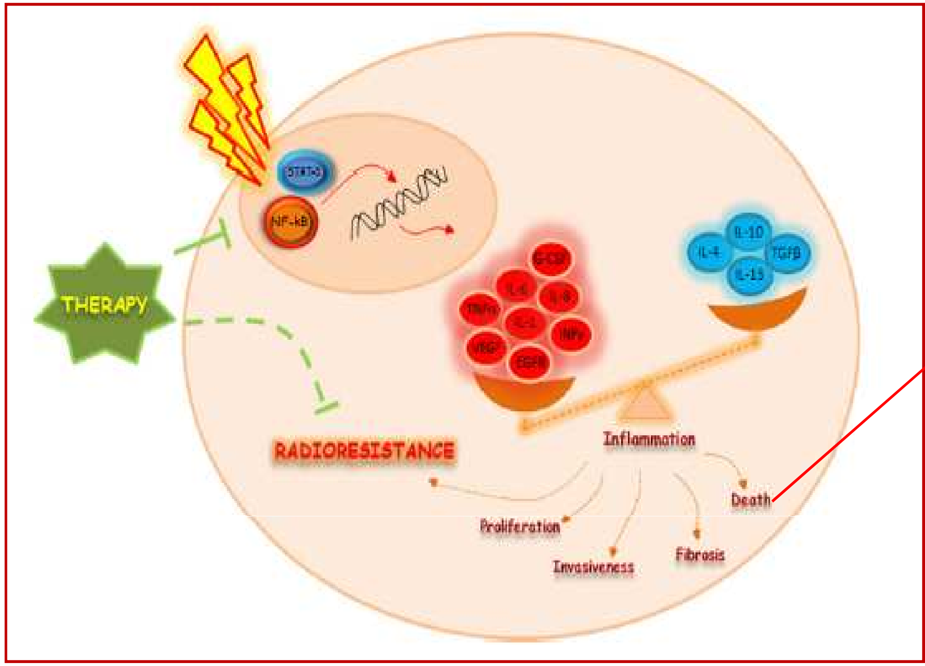
LNS-INFN

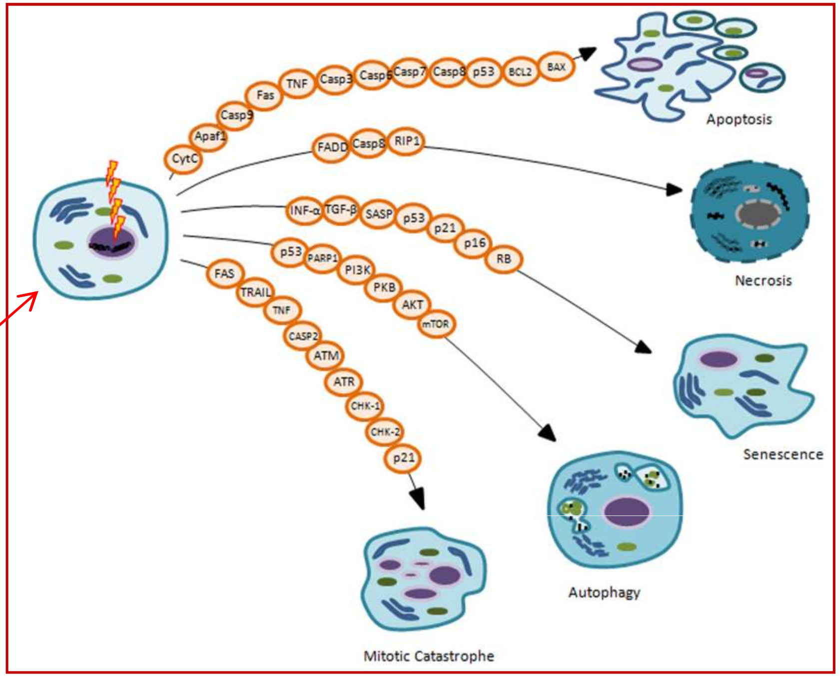
IN VITRO/EX VIVO CELL-BASED MODELS

Main goal is to highlight molecular mechanisms, by OMIC approach, involved in the response to radiation treatment with different types of beam, in order to identify radiosensitivity/radioresistance biomarkers and personalize treatments



CELL RESPONSE TO IONIZING RADIATION





Immunological response

Di Maggio FM, Minafra L et al. Journal of Inflammation 2015; 12:14

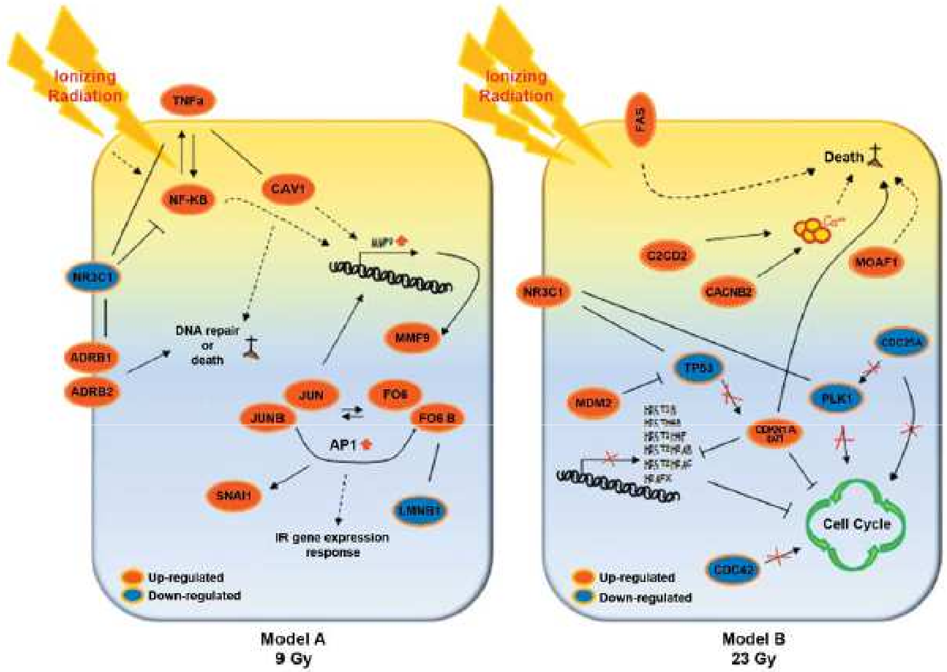
Different cell death mechanisms

Minafra L and Bravatà V. Transl Cancer Res 2014; 3 (1):32-47

Cell and molecular response to IR is highly complex, dependent on: LET, dose rate, dose fractionation, radiation dose and type of the irradiated cells or tissues (*Hellweg CE, Spitta LF et al. Front Oncol 2016 ;6:61*).

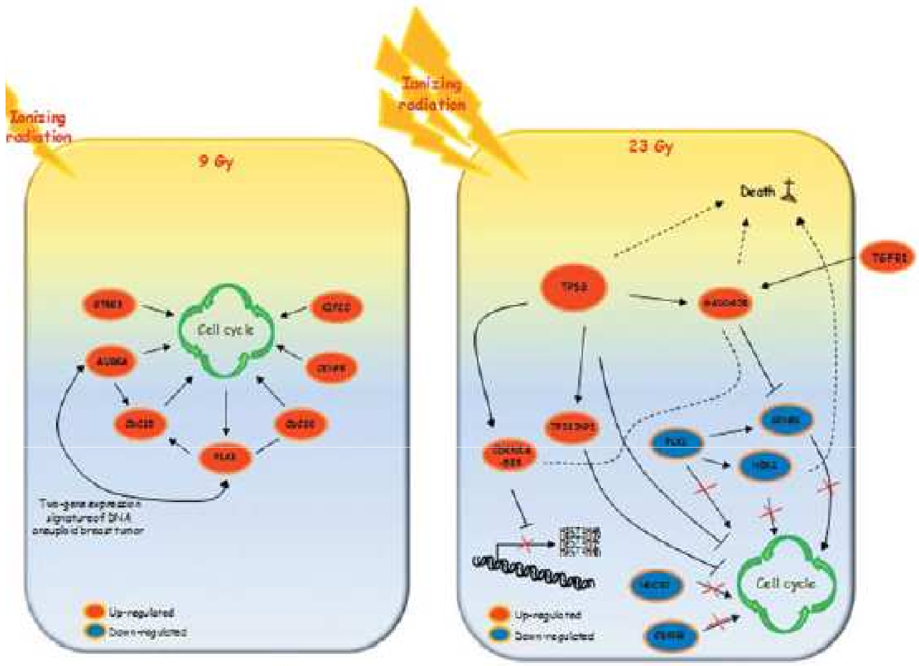
Molecular models of response to high dose electron RT

MCF7 breast cancer cell line



Bravatà V, Minafra L et al.
Anticancer Res 2015;35(5):2577-91

MCF10A normal breast cell line



Minafra L , Bravatà V et al.
Anticancer Res. 2015; 35(6):3223-34

In the cellular response to radiation, the activation of several signal transduction pathways by IR, results in an altered expression of a series of target genes, defining in cell death or survival.

RADIOBIOLOGICAL LASER DRIVEN EXPERIMENT

CNR - PISA - Italy - INTENSE LASER IRRADIATION LABORATORY - Istituto Nazionale di Ottica

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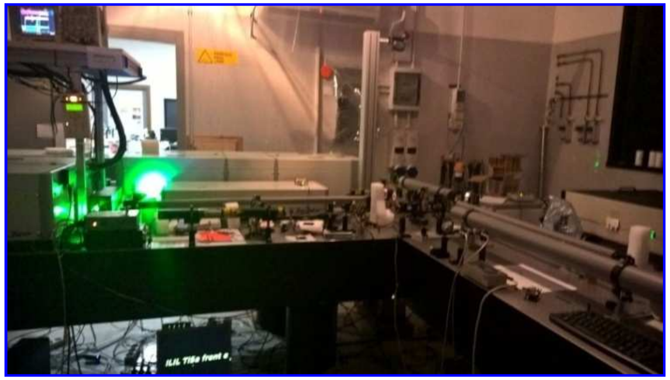
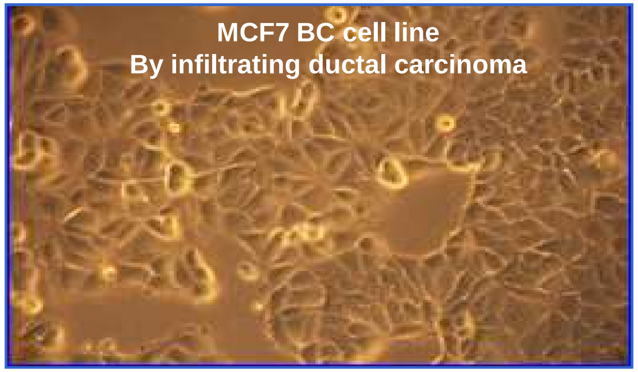
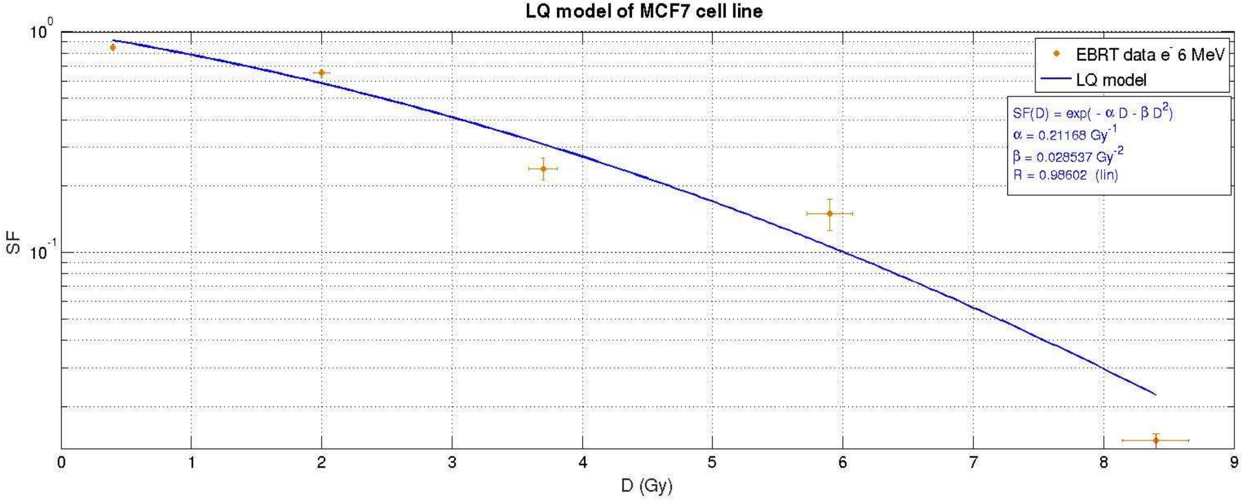


RADIOBIOLOGICAL LASER DRIVEN EXPERIMENT



Electron beam	**LDA (ILIL)**
D/pulse	0.07 Gy/pulse
Dose rate	4 Gy/min
t_{pulse}	10^{-12} s
Peak dose rate	7×10^{10} Gy/s
Energy	up to 20 MeV
Frequency	0.5 Hz

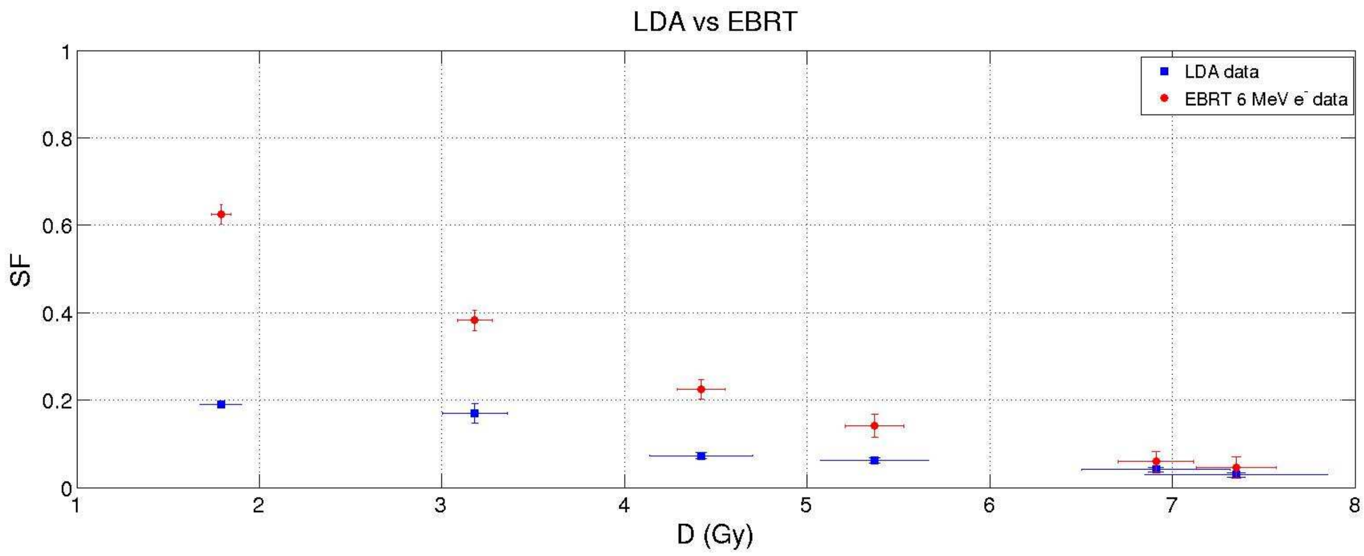
EBRT ON MCF7 BC CELL LINE



D (Gy) (± SD)	EBRT SF (± SD)
0.40 ± 0.01	0.85 ± 0.03
2.0 ± 0.1	0.65 ± 0.02
3.7 ± 0.1	0.24 ±0.03
5.9 ± 0.2	0.15 ± 0.02
8.4 ± 0.3	0.014 ± 0.001

Dose rate: 2 Gy/min
T_{pulse}: 10^{-6} s
Energy: 6 MeV

LDA VS EBRT ON MCF7 BC CELLS

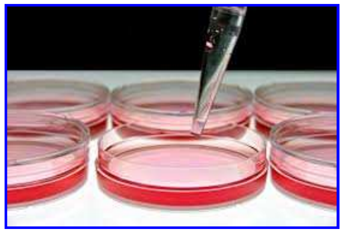


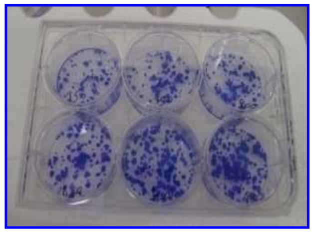
D (Gy) (± SD)	**EBRT SF** (± SD)	**LDA SF** (± SD)
1.8 ± 0.1	0.63 ± 0.02	0.19 ± 0.01
3.2 ± 0.2	0.38 ± 0.04	0.17 ± 0.02
4.4 ± 0.3	0.22 ± 0.03	0.07 ± 0.01
5.4 ± 0.3	0.14 ± 0.02	0.06 ± 0.01
6.9 ± 0.4	0.06 ± 0.01	0.04 ± 0.01
7.4 ± 0.5	0.05 ± 0.01	0.03 ± 0.01

CONCLUSION

- **Our radiobiological experiment with laser electron beam represents the first one performed on MCF7 BC cell line.**

- **Further studies and experiments are needed to improve laser technology and to better understand the biological effects regarding both ultra-short duration of particle bunches and the extremely high dose rate released.**





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