OLD AND NEW DRUGS IN THE ERA OF TARGET THERAPY AND BIOMOLECULAR PREDICTORS (FROM HORMONAL MANIPULATION TO…)

The radiation oncologist's point of view

DOTT LORENZO LIVI

RADIOTERAPIA

UNIVERSITA' DI FIRENZE

The radiation oncologist's point of view

- BIOLOGICAL RESEARCH
- PRE-CLINICAL RESEARCH
- ROUTINE AND CLINICAL RESEARCH

BIOLOGICAL RESEARCH

Tumor heterogeneity represents one potential limiting factor for the antitumor activity of inhibitors targeting a single-cellular pathway.

The complex interactions between tumor-specific signaling and radiation response provide a rationale for targeting multiple-signaling pathways.

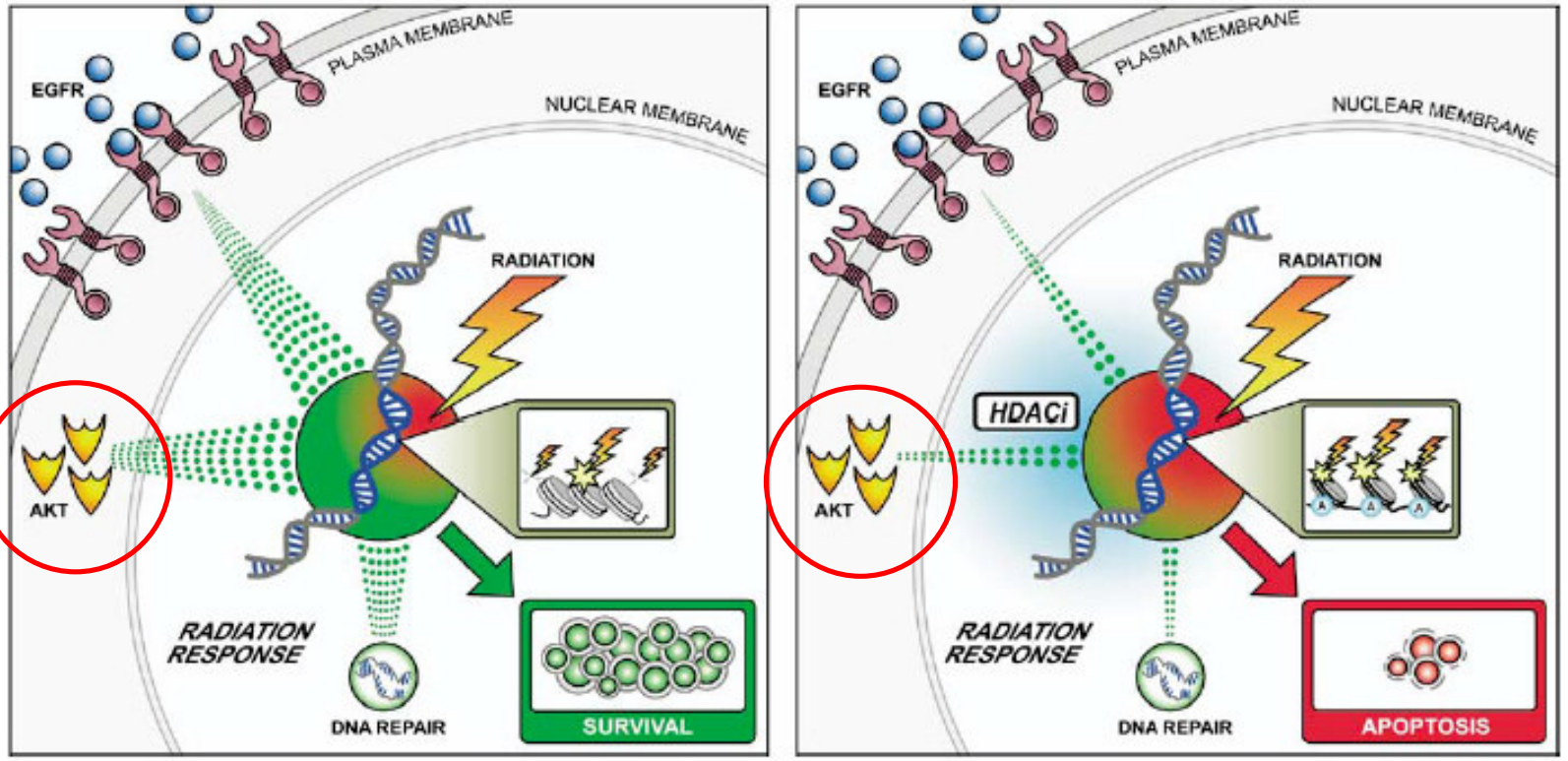
This may be achieved by agents that have the capacity to target multiple oncoproteins or through the combination of multiple single-target agents.

BIOLOGICAL RESEARCH

The epidermal growth factor receptor (EGFR) family consists of four transmembrane receptor tyrosine kinases: EGRF (HER1), HER2 (ErbB2, neu), HER3 (ErbB3), and HER4 (ErbB4), whose function is to transmit extracellular cues to intracellular signal transduction pathways that regulate proliferation, survival, and differentiation responses. At least two members of the family, EGFR and HER2, are frequently dysregulated. Both EGFR and HER2 overexpression have been associated with resistance of tumor cells to chemotherapy and radiotherapy.

Yarden Y., *Eur JCancer 2001;37(Suppl. 4):S3-S8*
Arteaga CL *J Clin Oncol 2001;19:32S-40S*

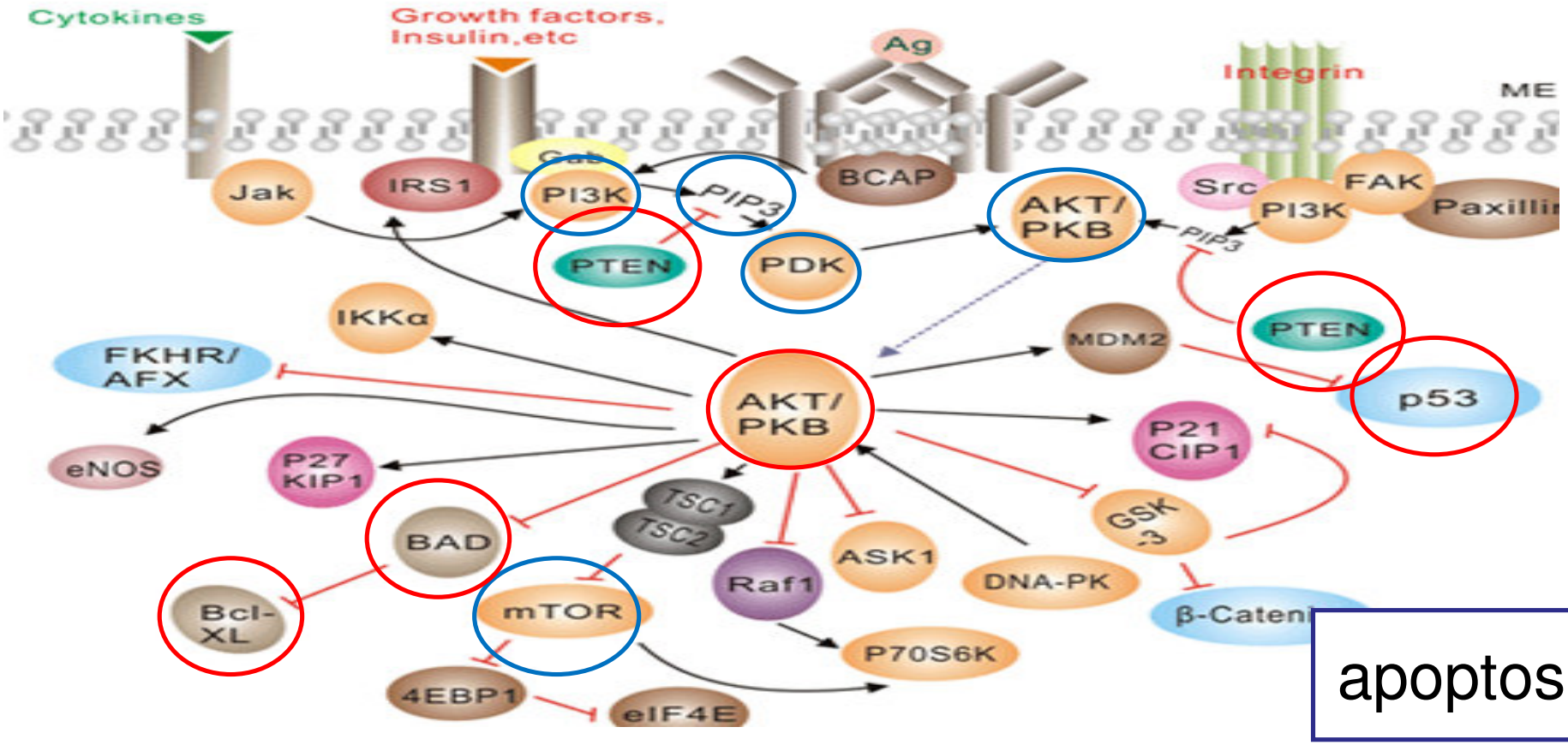
BIOLOGICAL RESEARCH



Histone deacetylases (HDAC): enzymes that remove acetyl groups from an e-N-acetyl lysine amino acid on a histine HDAC inhibitors may act as potent XRT sensitizers by abrogating promitogenic/survival and DNA repair–signaling pathways. The capacity of HDAC inhibitors to downmodulate the expression of EGFR and ErbB2, therefore representing another possible mechanism for HDAC inhibitors to enhance the antitumor activity of radiation.

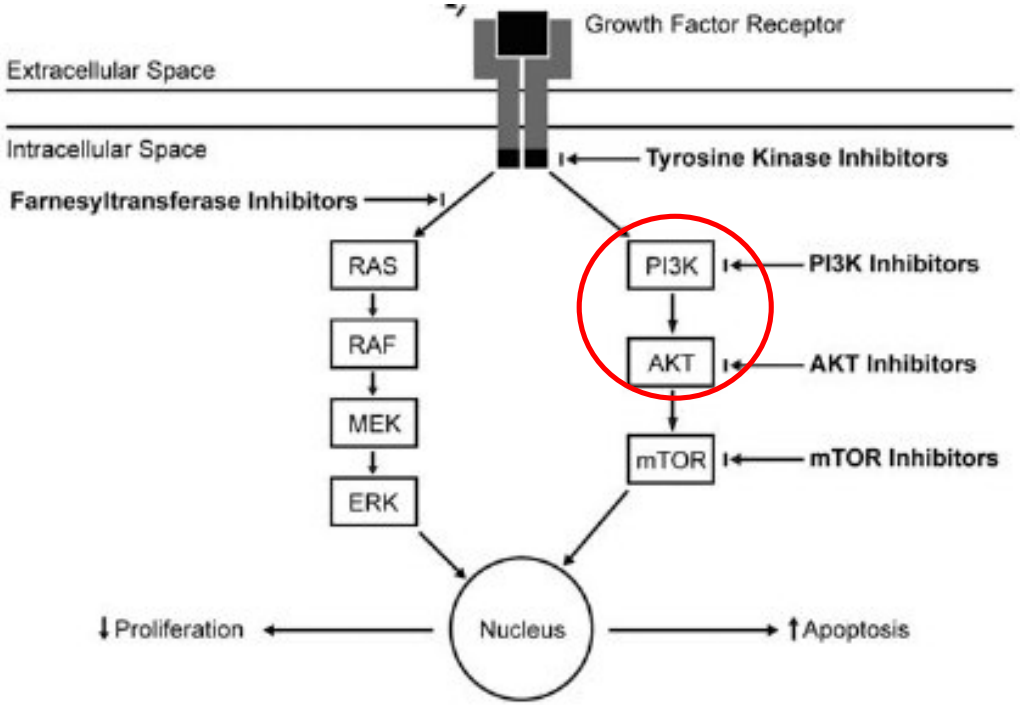
Johnstone RW, Nat Rev Drug Discov 1:287-299, 2002
Yu X, J Natl Cancer Inst 94:504-513, 2002
Chinnaiyan P Int J Radiat Oncol Biol Phys 62:223-229, 2005

BIOLOGICAL RESEARCH



Akt activation can be regulated through the tumor suppressor PTEN. PTEN protein acts as a phosphatase to dephosphorylate phosphatidylinositol (3,4,5)-trisphosphate This dephosphorylation is important because it results in inhibition of the AKT signaling pathway. it acts as part of a chemical pathway that signals cells to stop dividing and causes cells to undergo programmed cell death

BIOLOGICAL RESEARCH



Constitutive activation of AKT is known to contribute to radioresistance and is therefore a significant target for increasing radiosensitivity. Breast cancer cells are rendered more resistant to radiation-induced apoptosis by AKT overexpression.

Chinnaiyan P Semin Radiat Oncol 16:59-64, 2006

BIOLOGICAL RESEARCH

Heat-shock protein 90 (HSP90) has a crucial role in both the stabilisation and regulation of various proteins, including those related to radioresistance. Inhibition of Hsp90 may therefore provide a strategy for enhancing the radiosensitivity of tumour cells.

Inhibition involves the selective degradation of several key proteins attributed to radiation resistance, including EGFR, ErbB2, Raf-1, and AKT IGF-1R signaling blockade may increase cellular radiosensitivity by blocking AKT activation through the inhibition of both PI3K and ATM.

McKenna WG. Oncogene 22:5866-5875, 2003
Stingl L. *British Journal of Cancer* 102: 1578-1591, 2010

PRE-CLINICAL RESEARCH

ICTR 2003 **Translational Research and Pre-Clinical Strategy Study**

EFFECTS OF THE EGFR/HER2 KINASE INHIBITOR GW572016 ON EGFR- AND HER2-OVEREXPRESSING BREAST CANCER CELL LINE PROLIFERATION, RADIOSENSITIZATION, AND RESISTANCE

Methods and Materials: Primary human breast cancer cell lines that endogenously overexpress EGFR or HER2 and luminal mammary epithelial H16N2 cells stably transfected with HER2 were evaluated for the effect of GW572016 on inhibition of ligand-induced or constitutive receptor phosphorylation, proliferation, radiosensitization, and inhibition of downstream signaling.

Results: GW572016 inhibited constitutive and/or ligand-induced EGFR or HER2 tyrosine phosphorylation of all five cell lines, which correlated with the antiproliferative response in all but one cell line. GW572016 radiosensitized EGFR-overexpressing cell lines, but HER2-overexpressing cells were unable to form colonies after brief exposure to GW572016 even in the absence of radiation, and thus could not be evaluated for radiosensitization. One cell line was resistant to the antiproliferative and radiosensitizing effects of GW572016, despite receptor inhibition. Exploration of potential mechanisms of resistance in SUM185 cells revealed failure of GW572016 to inhibit downstream ERK and Akt activation, despite inhibition of HER2 phosphorylation. In contrast, sensitive HER2-overexpressing cell lines demonstrated inhibition of both ERK and Akt phosphorylation.

Conclusion: GW572016 potently inhibits receptor phosphorylation in either EGFR- or HER2-overexpressing cell lines and has both antiproliferative and radiosensitizing effects. Resistance to GW572016 was not due to a lack of receptor inhibition, but rather with a lack of inhibition of ERK and Akt, suggesting that measurement of inhibition of crucial signaling pathways may better predict response than inhibition of receptor phosphorylation. The SUM185 cell line provides a valuable model for studying mechanisms of resistance of EGFR/HER2 inhibitor therapy.

Zoug H. Int. J. Radiation Oncology Biol. Phys., Vol. 58, No. 2, pp. 344–352, 2004

PRE-CLINICAL RESEARCH

LAPATINIB IN COMBINATION WITH RADIATION DIMINISHES TUMOR REGROWTH IN HER2+ AND BASAL-LIKE/EGFR+ BREAST TUMOR XENOGRAFTS

Purpose: To determine whether lapatinib, a dual epidermal growth factor receptor (EGFR)/HER2 kinase inhibitor, can radiosensitize EGFR+ or HER2+ breast cancer xenografts.

Methods and Materials: Mice bearing xenografts of basal-like/EGFR+ SUM149 and HER2+ SUM225 breast cancer cells were treated with lapatinib and fractionated radiotherapy and tumor growth inhibition correlated with alterations in ERK1 and AKT activation by immunohistochemistry.

Results: Basal-like/EGFR+ SUM149 breast cancer tumors were completely resistant to treatment with lapatinib alone but highly growth impaired with lapatinib plus radiotherapy, exhibiting an enhancement ratio average of 2.75 and a fractional tumor product ratio average of 2.20 during the study period. In contrast, HER2+ SUM225 breast cancer tumors were highly responsive to treatment with lapatinib alone and yielded a relatively lower enhancement ratio average of 1.25 during the study period with lapatinib plus radiotherapy. Durable tumor control in the HER2+ SUM225 model was more effective with the combination treatment than either lapatinib or radiotherapy alone. Immunohistochemical analyses demonstrated that radiosensitization by lapatinib correlated with ERK1/2 inhibition in the EGFR+ SUM149 model and with AKT inhibition in the HER2+ SUM225 model.

Conclusion: Our data suggest that lapatinib combined with fractionated radiotherapy may be useful against EGFR+ and HER2+ breast cancers and that inhibition of downstream signaling to ERK1/2 and AKT correlates with sensitization in EGFR+ and HER2+ cells, respectively.

Sambade MJ. Int. J. Radiation Oncology Biol. Phys., Vol. 77, No. 2, pp. 575–581, 2010

PRE-CLINICAL RESEARCH

Radiation biology

Mechanism of lapatinib-mediated radiosensitization of breast cancer cells is primarily by inhibition of the Raf > MEK > ERK mitogen-activated protein kinase cascade and radiosensitization of lapatinib-resistant cells restored by direct inhibition of MEK

Materials and methods: Response of EGFR downstream signaling pathways was assessed by Western blot and clonogenic cell survival assays in breast tumor cells after irradiation (5 Gy), lapatinib, CI-1040, or combined treatment.

Results: In SUM102 cells, an EGFR+ basal breast cancer cell line, exposure to ionizing radiation elicited strong activation of ERK1/2 and JNK, which was blocked by lapatinib, and weak/no activation of p38, AKT or STAT3. Direct inhibition of MEK1 with CI-1040 resulted in 95% inhibition of surviving colonies when combined with radiation while inhibition of JNK with SP600125 had no effect. Lapatinib-mediated radiosensitization of SUM102 cells was completely abrogated with expression of constitutively active Raf. Treatment of lapatinib-resistant SUM185 cells with CI-1040 restored radiosensitization with 45% fewer surviving colonies when combined with radiation.

Conclusions: These data suggest that radiosensitization by lapatinib is mediated largely through inhibition of MEK/ERK and that direct inhibition of this pathway may provide an additional avenue of radiosensitization in EGFR+ or HER2+ breast cancers.

Sambade MJ,Radiotherapy and Oncology 93 (2009) 639–644

PRE-CLINICAL RESEARCH

Everolimus (RAD-001), marketed by Novartis under the tradenames Zortress (USA) and Certican (Europe and other countries) in transplantation medicine, and Afinitor in oncology is the 42-O-(2-hydroxyethyl) derivative of sirolimus and works similarly to sirolimus as an mTOR (mammalian target of rapamycin) inhibitor. It is currently used as an immunosuppressant to prevent rejection of organ transplants

Concomitant Radiotherapy is not recommended.
Novartis suggests to wait at least 30 days!!!!!
Nothing from the literature

INIBITORI DEL RECETTORE HER-2 E RADIOTERAPIA

- BIOLOGICAL RESEARCH
- PRE-CLINICAL RESEARCH
- ROUTINE AND CLINICAL RESEARCH

ROUTINE AND CLINICAL RESEARCH

TAM+RT: in vitro studies

Reference	Cell line	Estrogen receptor	Incubation time (h) prior to irradiation	Hormone	Interaction
Wazer *et al.* (1989)	MCF-7	+	2	17β-estradiol	+
				Tamoxifen	–
Wazer *et al.* (1993)	MDA-MB-231	–	2	Tamoxifen	No interaction
Böhning *et al.* (1996)	MCF-7	+	1–4	17β-estradiol	+
				Tamoxifen	–
Villalobos *et al.* (1995)	MCF-7 BUS	+	3*	Estradiol	+
Villalobos *et al.* (1996)	MCF-7 BUS	+	3*	Estradiol	+
	T47D B8	+		Estradiol	No interaction
	EVSA-T	–		Estradiol	No interaction
Paulsen *et al.* (1996)	MCF-7	+	2	Tamoxifen	–
				Estradiol	No interaction
	MDA-MB-231	–		Tamoxifen	No interaction
				Estradiol	+
Sarkaria *et al.* (1994)	MCF-7	+	5*	4OH-TAM	No interaction
Newton *et al.* (1998)	MCF-7	+	1	Tamoxifen	+
				ZM 182780	+

Schmidberger H, Endocr Relat Cancer 2003

ROUTINE AND CLINICAL RESEARCH

TAM+RT: clinical studies

Author	N° pts	10-y OS	10-y DFS	10-y LR
Pierce 2005	107 TAM seq, 202 conc.	88%vs90% p=0.65	83%vs83% p=0.76	5%vs7% p=0.54
Ahn 2005	254 TAM seq, 241 conc.	82%vs84% p=0.45	10-y DMFR 78%vs82% p=0.12	10-y LRFR 86%vs90% p=0.86
Harris 2005	278 TAM seq, 278 conc.	81%vs86% p=0.64	85%vs76% p=0.35	3%vs7% p=0. 64

ROUTINE AND CLINICAL RESEARCH

TAM+RT: clinical studies

Author	N° pts	10-y OS	10-y DFS	10-y LR
Pierce 2005	**No difference in OS, DFS, LR**			
Ahn 2005				
Harris 2005				

ROUTINE AND CLINICAL RESEARCH

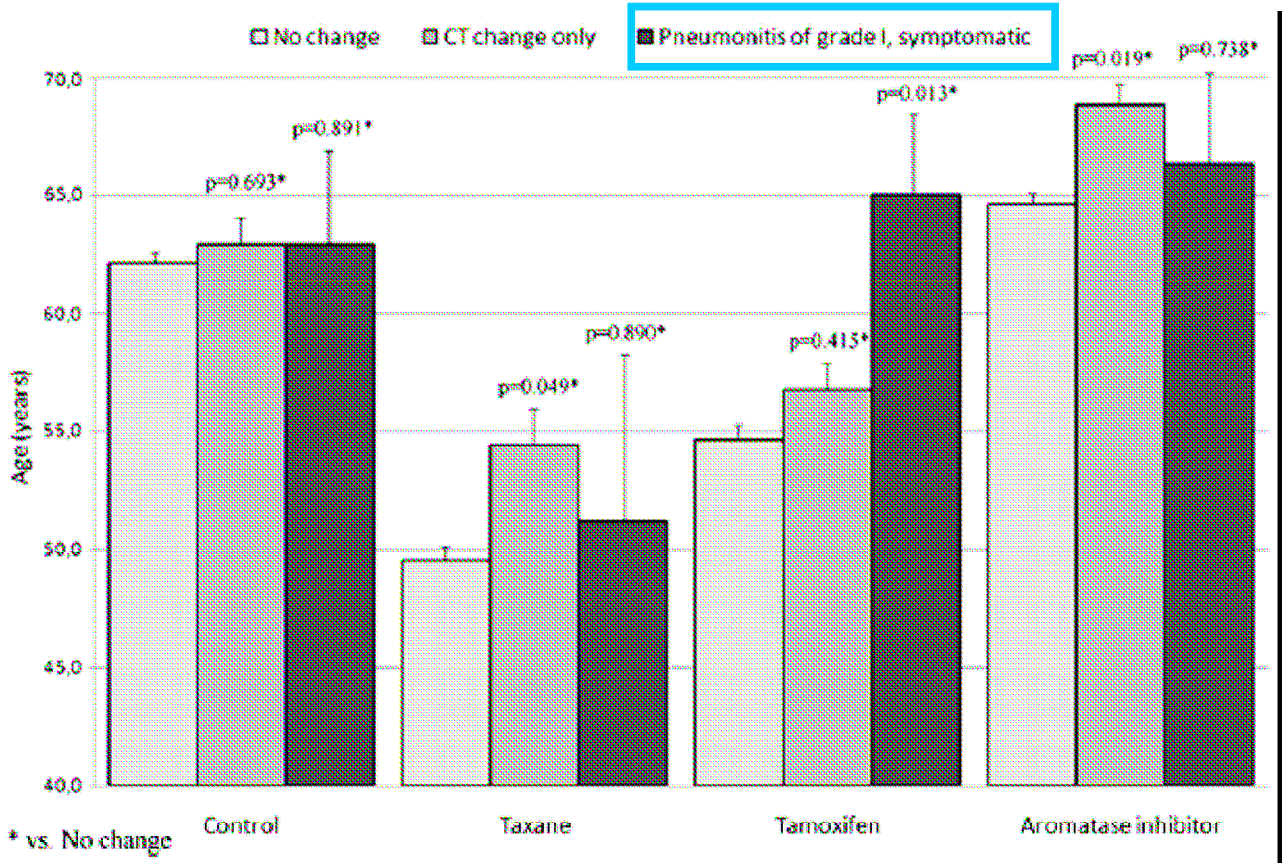
AI+RT: clinical studies

Author	N° pts	RT technique	3-y OS	3-y LR	Toxicity
Ishitobi 2009 **Retrospective**	113 AI+RT conc., 151 seq	Electron beam portal to the chest wall+ photon field to the axilla, IM and SPCL	100%vs100%	100%vs98% p=0.68	**No association** with AI (pneumonitis)
Varga 2010 **Prospective**	82 AI+RT conc., 77 TAM+RT conc.	Photon fields to the breast ± SPCL and axilla	NR	NR	**No association** with AI (pneumonitis G1 and fibrosis G1)

ROUTINE AND CLINICAL RESEARCH

Table 5. Multivariate analysis of the effects of age, MLD, and systemic therapy on early and late radiogenic lung sequelae

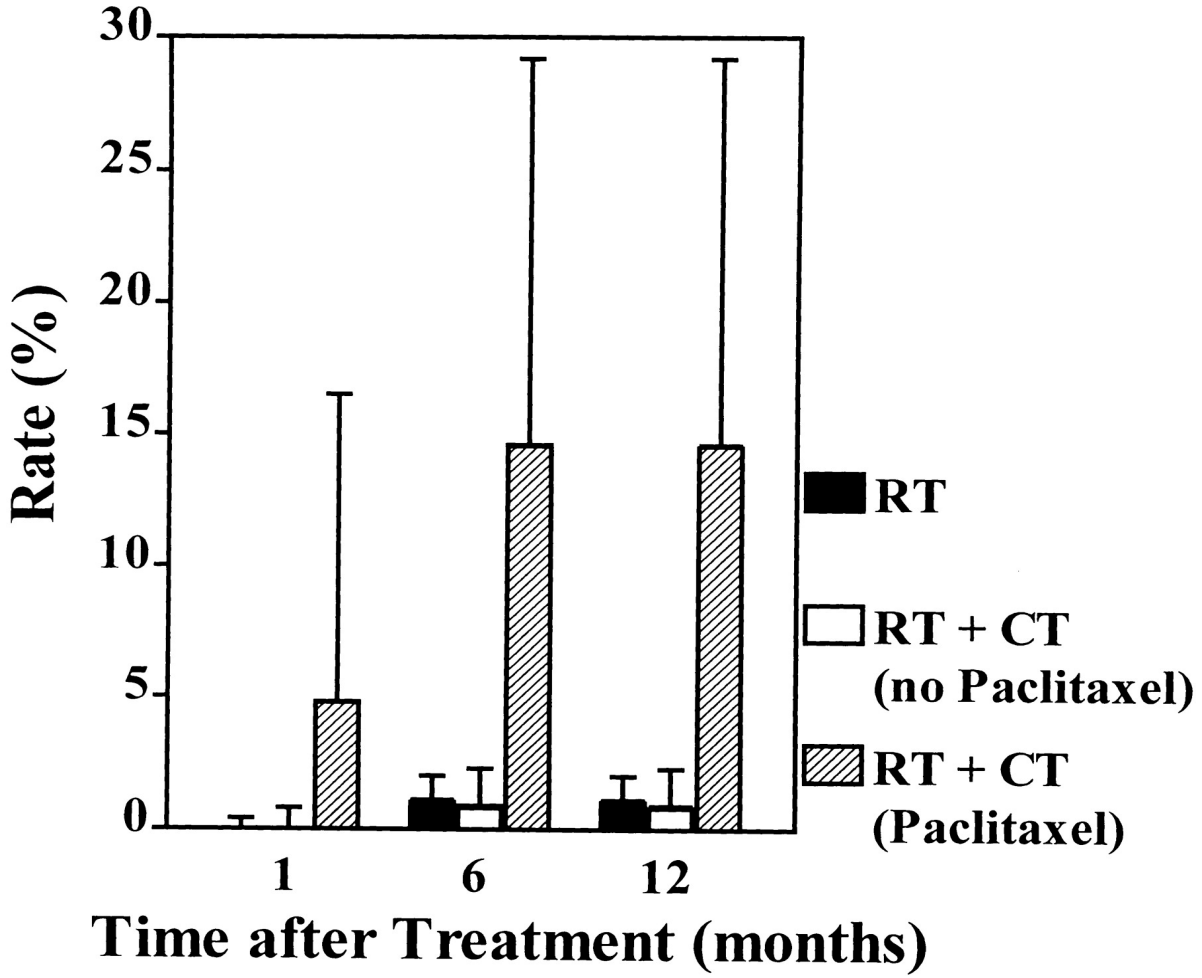
	Grade 1 pneumonitis, symptomatic			Grade 1 pneumonitis, any			Grade 1 fibrosis		
Factor	OR	95% CI	*p*	OR	95% CI	*p*	OR	95% CI	*p*
Age	1.041	0.991–1.094	0.106	1.035	1.011–1.061	0.005	1.074	1.042–1.107	0.001
MLD	1.126	1.009–1.256	0.033	1.113	1.049–1.181	0.001	1.207	1.124–1.295	0.001
Systemic treatment			0.064			0.080			0.010
Taxane	0.465	0.066–3.268	0.442	0.674	0.309–1.470	0.322	0.750	0.294–1.915	0.548
Tamoxifen	2.775	0.746–10.323	0.128	1.679	0.863–3.266	0.127	2.442	1.120–5.326	0.025
Aromatase inhibitor	0.804	0.188–3.435	0.768	0.955	0.504–1.806	0.887	0.765	0.359–1.632	0.488



Varga Z, IJROBP 2010

ROUTINE AND CLINICAL RESEARCH

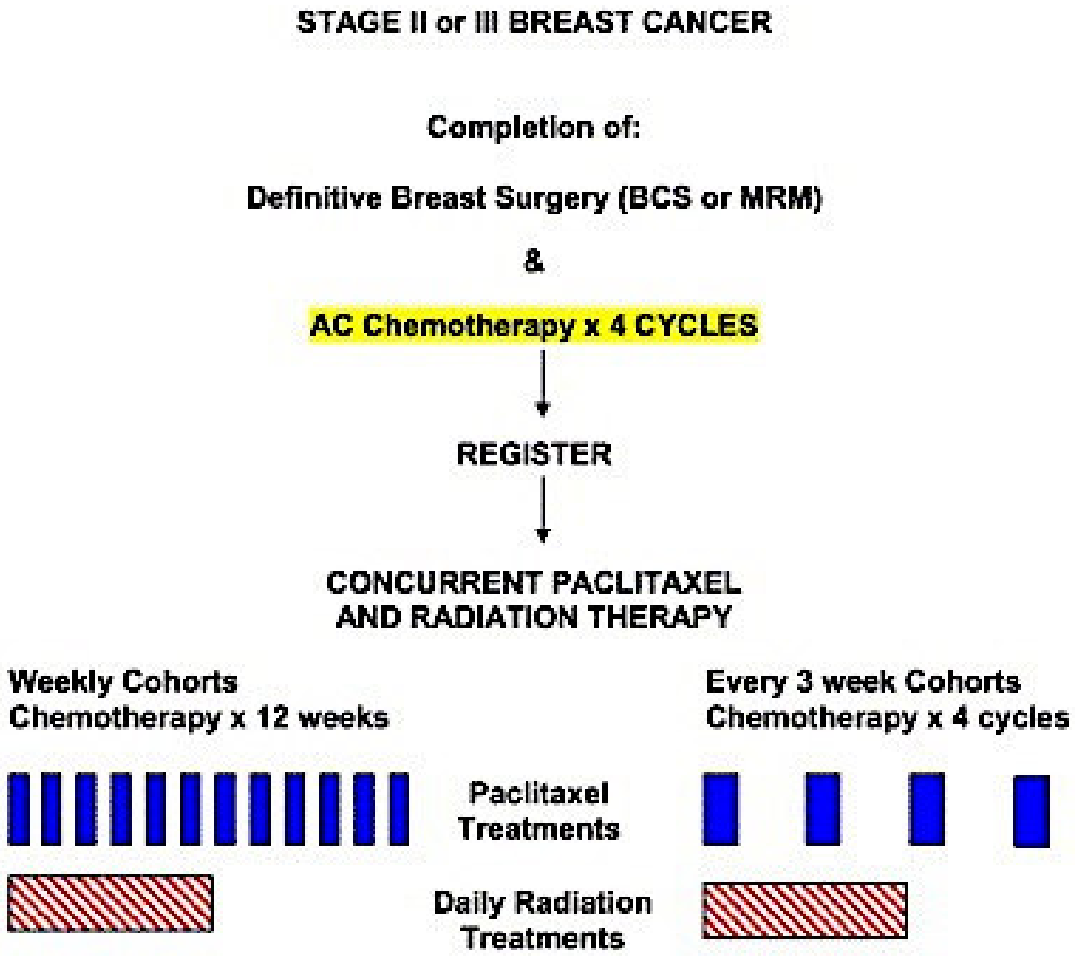
Lung toxicity and old drugs: RT and taxanes



Taghian A G et al. JNCI J Natl Cancer Inst 2001;93:1806-1811

JNCI

ROUTINE AND CLINICAL RESEARCH
concomitan treatment



ROUTINE AND CLINICAL RESEARCH

Table 3. Toxicity related to concurrent therapy

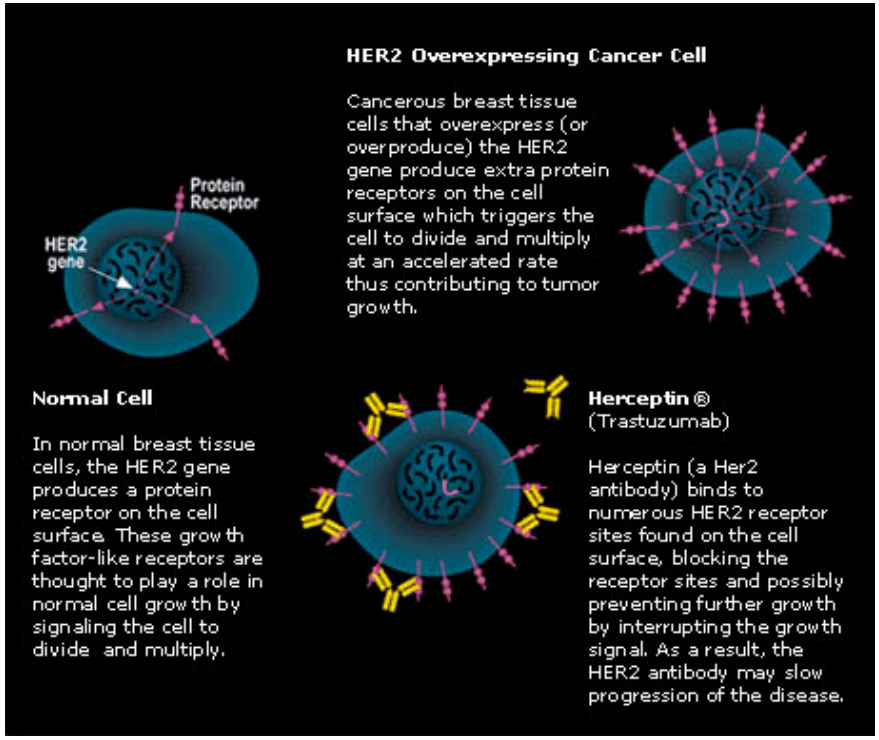
	Cohort				
	1	2	3	4	Total
Paclitaxel schedule		Weekly	Every 3 weeks		
Paclitaxel dose	60 × 12	60 (mod)* × 12	135 × 2, 175 × 2	175 × 4	
No. of patients	8	8	8	16	40
Dose-limiting toxicity	2	2	0	0	4
Dose-limiting toxicity description	Grade 3 radiation pneumonitis (n = 2)	Grade 2 radiation pneumonitis requiring steroids (n = 1); liver function test abnormal with 3-week delay (n = 1)			
Radiation pneumonitis					
Grade 1	0	0	0	2	2
Grade 2	0	1†	1	1	3
Grade 3	2†	0	0	0	2
Radiation dermatitis					
Grade 1	4	3	6	13	26
Grade 2	2	5	0	1	8
Grade 3/4	0	0	0	0	0

* (mod) denotes modifications to treatment schedule (See text).
† Denotes patients treated with steroids for pneumonitis.

Concomitant RT + taxanes: unaccetable lung toxicity

ROUTINE AND CLINICAL RESEARCH

Trastuzumab (herceptin) blocks her2-activated cell signalling reducing cell proliferation restoring ability to undergo apoptosis by inhibiting the phosphatidylinositol 3 kinase/Akt pathway increases cellular sensitivity to chemotherapy and radiotherapy



ROUTINE AND CLINICAL RESEARCH

HERA trial **2009** **4 ys FU**	No T → 72.2% T → 78.6% Increase DFS→ 6.2%	No T →87.7% T →89.3% Increase OS→ 1,6%
NSABP-B31 **NCCTG-9831** **2007** **4 ys FU**	No T → 71,3% T → 85.9% Increase DFS→ 11.8%	No T→89.4% T →92.6% Increase → OS 2.6%
FinHer study **2009** **5 ys FU**	No T→ 73,0% T → 83,3% Increase DFS 10.3%	No T→82,3% T →91,3% Increase → OS 9%

ROUTINE AND CLINICAL RESEARCH

Trastuzumab and Cardiac Toxicity:

- the incidence of cardiac dysfunction in the trastuzumab arm (HERA Trial) at a median follow-up time of 1 year was 0.6% for severe CHF and 7.0% for left ventricular (LV) dysfunction
- approximately 10 % had a substantial decrease in the left ventricular ejection fraction (LVEF).
- the risk of cardiac dysfunction with trastuzumab treatment increases with the use of anthracyclines.

ROUTINE AND CLINICAL RESEARCH

The mechanism of cardiac dysfunction associated with trastuzumab is not clearly understood**.** The ErbB2 receptor is expressed on cardiomyocytes, in addition to tumor tissue, where it exerts a protective effect on cardiac function (maintenance of normal cardiac contractility and dependence on HER2 for myocyte survival); thus, interference with ErbB2-signaling (Trastuzumab) may block this protective effect

Procter M, JCO 2009
Perez EA, Clin Breast Cancer 2008

ROUTINE AND CLINICAL RESEARCH

It is possible that radiation-associated cardiac damage occurs by both microvascular (fibrotic) and macrovascular (coronary atherosclerosis) damage occurring after a longer latency period.
Modern irradiation techniques seem to be associated with a limited risk of heart complication.
The use of anthracycline, other cardiotoxic chemotherapies and targeted therapies should incite for great caution by performing a careful treatment planning and optimisation

Doyen J. *Cancer Radiother.* 2010 Jul;14(4-5):319-26

ROUTINE AND CLINICAL RESEARCH

Radiotherapy and Oncology 90 (2009) 122–126

Contents lists available at ScienceDirect

Radiotherapy and Oncology

journal homepage: www.thegreenjournal.com

Cardiac toxicity

Acute cardiotoxicity with concurrent trastuzumab and radiotherapy including internal mammary chain nodes: A retrospective single-institution study

Richard Shaffer[a], Scott Tyldesley[a,*], Martin Rolles[c], Stephen Chia[a], Islam Mohamed[b]

[a] British Columbia Cancer Agency, Vancouver, Canada
[b] British Columbia Cancer Agency, Kelowna, Canada
[c] Singleton Hospital, Swansea NHS Trust, Wales, UK

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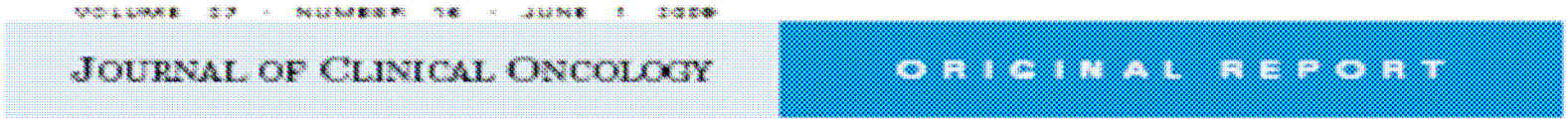
ABSTRACT

Purpose: To examine the acute cardiotoxicity of internal mammary chain (IMC) irradiation with concurrent trastuzumab.
Materials and Methods: Clinical and cardiac function data were collected on 59 patients with early breast cancer who were treated with adjuvant trastuzumab and chemotherapy with or without radiotherapy (often including IMC) at BC Cancer Agency in 2005.
Results: Forty-four of fifty-nine patients received adjuvant radiotherapy (RT). Thirteen had left-sided IMC RT. For left-sided RT, IMC inclusion increased the mean percentage dose to 5% of the heart, but the mean doses to 50% and 90% of the heart were similar. Median baseline left ventricular ejection fraction (LVEF) was 62% and similar in all groups. Median absolute decrease in LVEF after RT was 4%, which was not significantly different according to side or inclusion of IMCs. Trastuzumab was stopped in 11 of 59 patients (18.6%) due to decrease in LVEF. After median follow up of 15 months, three patients developed clinical congestive heart failure, none of whom received left-sided IMC RT.
Conclusions: There was no excess acute cardiotoxicity observed with the combination of left-sided IMC irradiation and concurrent trastuzumab.

ROUTINE AND CLINICAL RESEARCH



Radiotherapy and Adjuvant Trastuzumab in Operable Breast Cancer: Tolerability and Adverse Event Data From the NCCTG Phase III Trial N9831

No significant differences among arms were found in incidence of acute skin reaction, pneumonitis, dyspnea, cough, dysphagia, or neutropenia.

ROUTINE AND CLINICAL RESEARCH

ELSEVIER

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

EJC

The acute skin and heart toxicity of a concurrent association of trastuzumab and locoregional breast radiotherapy including internal mammary chain: A single-institution study

Results: Median age was 52 years (25–76). Chemotherapy with anthracycline was administered in 92% of patients. All patients received trastuzumab every three weeks (8 mg/kg followed by 6 mg/kg) for a median duration of 12 months (3-40). The IMC was irradiated in 83% of patients. There were: 87 grade 1, 14 grade 2 and 2 grade 3 skin reactions. There were 13 oesophagitis: 9 grade 1; 3 grade 2, and 1 grade 3. Out of 101 patients with assessments after 6 months, late telangiectasia grade 1 occurred in 5 patients, local pain grade 1 in 19 patients and grade 2 in 3 patients, fibrosis grade 1 in 16 patients. A reversible grade $\geqslant$2 left ventricular systolic dysfunction occurred in 6 patients.

Conclusion: In this prospective study of breast cancer patients treated with trastuzumab-radiotherapy with, in most cases, anthracycline-based chemotherapy and IMC irradiation, both the rate of abnormal LVEF after concurrent trastuzumab-radiotherapy and the skin toxicity were deemed acceptable. Further follow-up is needed.

Caussa L EJC 2010 in press

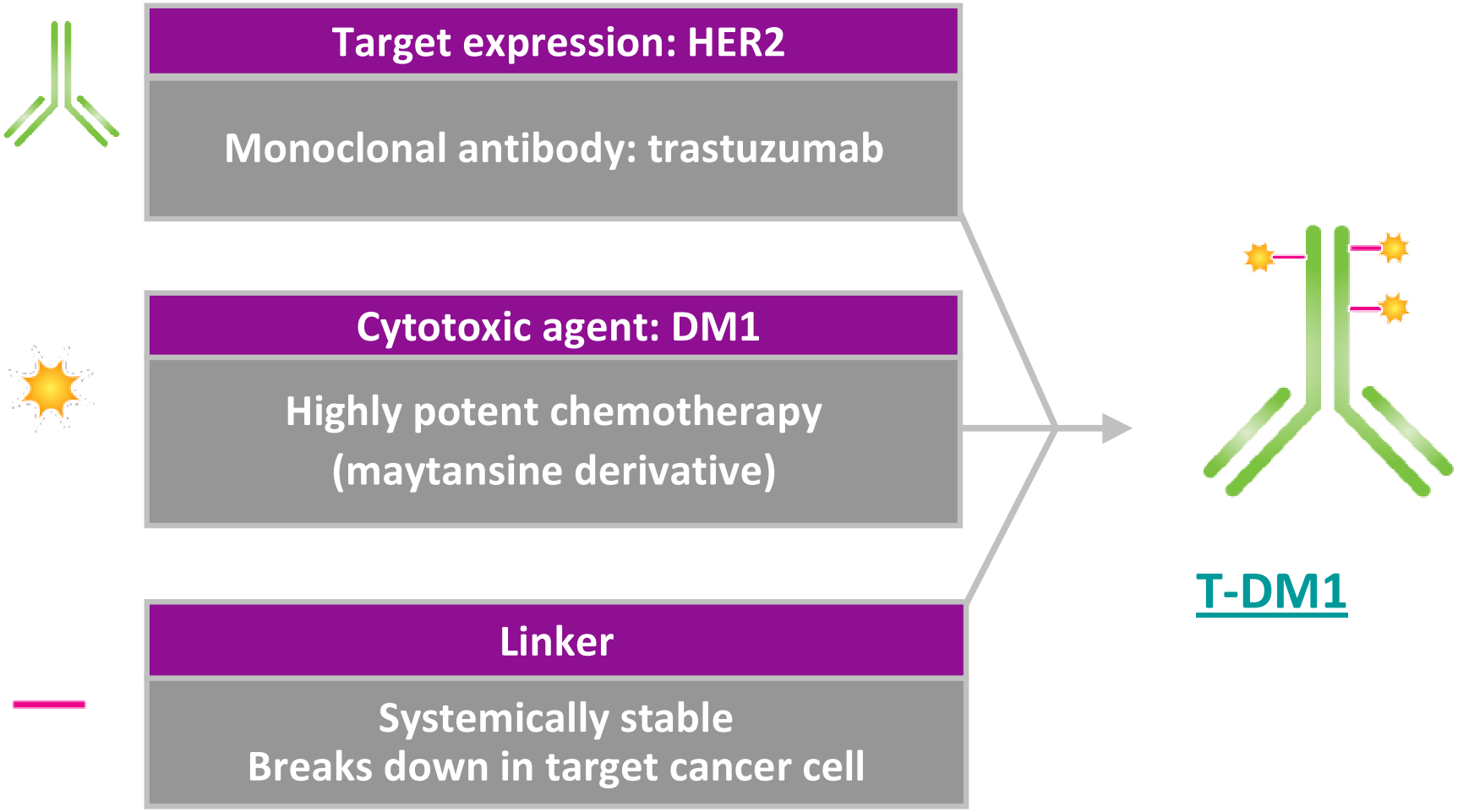
ROUTINE AND CLINICAL RESEARCH

Adjuvant trastuzumab in breast cancer: experience from the University of Florence.

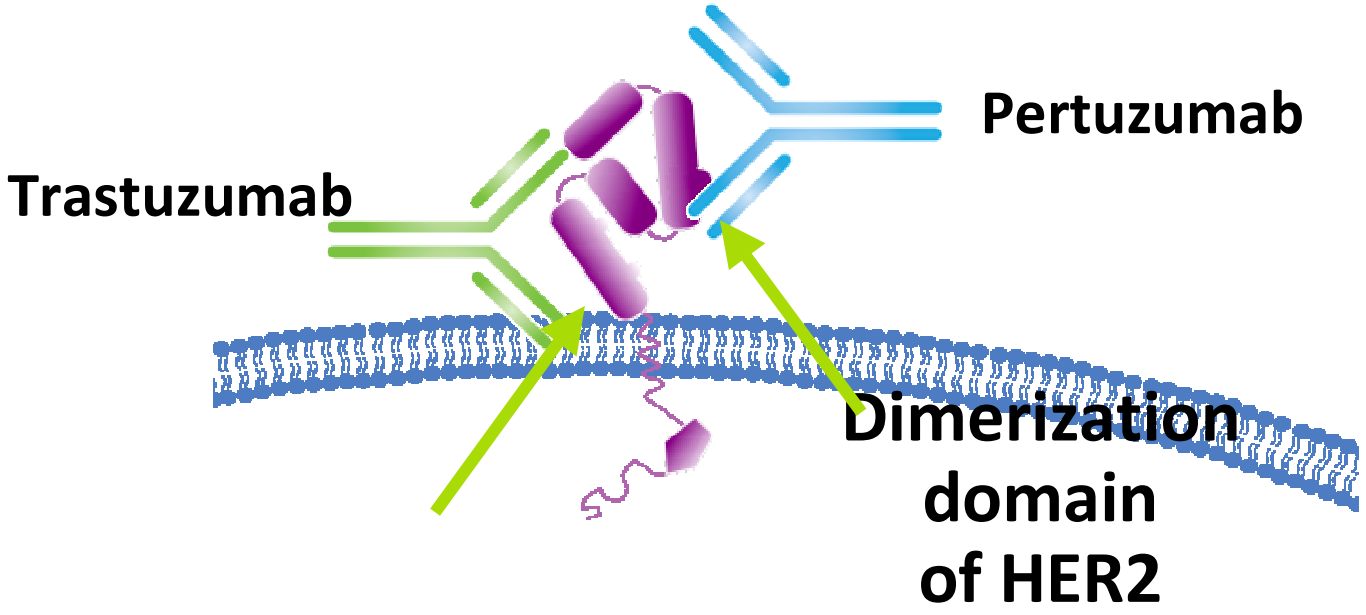
…in our experience trastuzumab given postoperatively with adjuvant chemotherapy was well tolerated and produced optimal clinical results in terms of disease-free survival.

Livi L. J Chemother. 2010 Apr;22(2):115-8.

Trastuzumab emtansine (T-DM1): the first-in-class HER2-targeted antibody-drug conjugate



Pertuzumab, the first HER2 Dimerization Inhibitor, demonstrates synergistic activity with trastuzumab



- **Preferentially inhibits ligand-independent HER2 signaling**
- **Prevents shedding of HER2 ECD**
- **Flags cells for destruction by the immune system**

- **Inhibits formation of HER2 dimer pairs**
- **Suppresses multiple HER signalling pathways, leading to a more comprehensive blockade of HER2-driven signalling**
- **Flags cells for destruction by the immune system**

Junttila et al. Cancer Cell 2009

Avastin Overview

- Monoclonal antibody specific for VEGF ligand
- Validated antiangiogenesis in cancer therapy
  - > 200,000 patients treated worldwide with Avastin
- Clinical validation in numerous settings
  - Avastin approved
    - Colorectal cancer and non-small cell lung cancer (worldwide)
      - Progression-free and overall survival
    - Metastatic breast cancer (ex-US)
      - Progression-free survival (PFS)

Indication Statement

Avastin®, in combination with paclitaxel, for the treatment of patients who have not received chemotherapy for their locally recurrent or metastatic HER2-negative breast cancer.

Radiotherapy
Safety and efficacy of association it not known

CONCLUSIONS

- BIOLOGICAL RESEARCH
  The most straightforward approach is to target a specific molecule involved in tumor-cell survival, including EGFR, IGF-1R, Ras, PI3K, and AKT.

- PRE-CLINICAL RESEARCH
  Promising new preclinical data show potential therapeutic benefit for combining molecularly targeted agents with radiation

CONCLUSIONS

3. ROUTINE AND CLINICAL RESEARCH

a) the data on combining targeted therapies with radiation are still scarce and do not allow for meaningful conclusions.

b) the long-term outcome of trastuzumab-related heart failure is unknown so it is important to spare the heart volume during RT, but trastuzumab given postoperatively is well tolerated and produced optimal clinical results