# *Review Article* Radiation-induced tumor neoantigens: imaging and therapeutic implications

Christopher D. Corso, Arif N. Ali, Roberto Diaz

Department of Radiation Oncology, Emory University School of Medicine; Winship Cancer Institute of Emory University, Atlanta, GA 30322, USA

Received January 11, 2011; accepted January 23, 2011; Epub January 25, 2011; Published February 15, 2011

**Abstract:** Exposure of tumor cells to ionizing radiation (IR) is widely known to induce a number of cellular changes. One way that IR can affect tumor cells is through the development of neoantigens which are new molecules that tumor cells express at the cell membrane following some insult or change to the cell. There have been numerous reports in the literature of changes in both tumor and tumor vasculature cell surface molecule expression following treatment with IR. The usefulness of neoantigens for imaging and therapeutic applications lies in the fact that they are differentially expressed on the surface of irradiated tumor cells to a greater extent than on normal tissues. This differential expression provides a mechanism by which tumor cells can be "marked" by radiation for further targeting. Drug delivery vehicles or imaging agents conjugated to ligands that recognize and interact with the neoantigens can help to improve tumor-specific targeting and reduce systemic toxicity with cancer drugs. This article provides a review of the molecules that have been reported to be expressed on the surface of tumor cells in response to IR either *in vivo* or *in vitro*. Additionally, we provide a discussion of some of the methods used in the identification of these antigens and applications for their use in drug delivery and imaging.

Keywords: Radiation-induced neoantigens, targeted therapy, nanoparticles, ionizing radiation

#### Introduction

Ionizing radiation (IR) is a commonly employed treatment modality for many types of human cancers. In clinical practice, radiation is often used in combination with other types of treatments including chemotherapy and surgery [1]. For example, concomitant chemotherapy plus radiation is the standard of care for treating patients with head and neck cancer, lung cancer, rectal cancer, cervical cancer, glioblastomas, and anal cancer [2-6]. It is estimated that nearly 60% of all cancer patients receive radiation as a component of their treatment [7]. This widespread use and the need for new, targeted therapies to avoid systemic toxicity associated with standard chemotherapy has prompted researchers to explore new ways to utilize radiation for cancer therapy [8-14]. One of the unique aspects of using IR for treating tumors is that it can be delivered to a focused tissue volume using either external beams or internal sources such as brachytherapy seeds. This allows for the deposition of high cumulative radiation doses at the tumor site while sparing the normal surrounding tissues. IR can achieve cell killing through interaction with water molecules and subsequent formation of hydroxyl radicals that lead to DNA strand breaks [15]. These DNA strand breaks are cytotoxic primarily in rapidly dividing cells such as cancerous neoplasms. IR has been shown to elicit phenotypic changes in both normal and cancerous cells that survive an insult [16, 17].

Over the past 15 years, there has been increasing interest in molecules that are expressed on the surface of tumor cells following irradiation. The usefulness of these neoantigens for imaging and therapeutic applications lies in the fact that they are differentially expressed on the surface of irradiated tumor cells to a greater extent than on normal tissues. Drug delivery vehicles or imaging agents conjugated to ligands that recognize and interact with the differentially expressed neoantigens can help to improve specific tumor targeting and reduce systemic toxicity with cancer drugs. Conceptually, the IR can be described as "marking" the tumor for further destruction or imaging. This is a role that suits radiation well because of the tissue penetration and precise delivery to the perceived tumor volume that can be achieved.

Tumors are heterogeneous masses consisting of networks of microvessels and cancer cells. Both endothelial cells and cancer cells comprising tumors have been shown to respond to IR by increasing the surface expression of various molecules [18-22]. However, most of the reports to date have focused on targeting tumor endothelial neoantigens [8, 9, 12, 23]. An advantage of targeting endothelial antigens lies in improved penetration into tumors, improved drug bioavailability, and direct access from the intravascular space [24]. In addition, studies have shown that some molecules like  $\alpha_{2b}\beta_3$  integrin are expressed following exposure to IR on many types of tumor vasculature [23]. This suggests that therapeutic or imaging schemes targeting such endothelial molecules would be widely applicable to most, if not all, solid tumors treated with radiation. Figure 1 illustrates a proposed model that explains how irradation of tumor cells leads to the overexpression of neoantigens in tumor endothelial cells which can then be targeted for drug delivery. The incident IR induces cellular signaling pathways which lead to the upregulation of antigen production and expression on the cell surface. Nanoparticles encapsulating chemotherapeutic drugs conjugated with antigen-specific ligands can then be delivered to the tumor intravascularly.

While antigens that are upregulated at the surface of tumor endothelial cells offer excellent prospects for tumor therapy, other studies have explored the surface expression of molecules directly on cancer cells following irradiation [21, 25-28]. In addition to inducing surface expression of antigens, IR may further improve the delivery of agents - especially bulky ones like liposomes or nanoparticles - to tumor cells because it transiently increases the permeability of the vasculature [29]. There are currently a number of approaches being studied to improve delivery of nanotherapeutics directly to tumors and facilitate tumor penetration through normalization of the vasculature and the interstitial matrix [30-33].

A wide variety of methods that exploit radiation induced neoantigens for imaging or cancer therapeutics have been reported in the literature. Site-directed drug delivery is one of the more commonly investigated applications. Immunoliposomes encapsulating combretastatin were used to target E-selectin on irradiated mouse mammary tumors and melanomas [13, 14]. Others have used drug-encapsulated nanoparticles conjugated to antigen-specific ligands for targeting irradiated tumors [12, 23, 34]. The effect of IR on tumor cell immunogenicity has also been a topic of interest. IR has been shown to increase surface expression of molecular targets which promotes tumor cell killing by endogenous cytotoxic cells [16, 18, 35]. In mouse models it has been shown that irradiation of cutaneous melanoma prior to resection resulted in a dramatic reduction in lung metastasis after systemic challenge with untreated melanoma cells [36]. This evidence indicates that IR may play an important role in enhancing the immunogenicity of cellular tumor vaccines [26, 37]. More recently, work on the identification of a molecule TIP-1 that translocates to the surface prior to cell death has led to an interest in developing post-irradiation tumor imaging modalities and may be useful in predicting tumor responsiveness to radiation [38].

The goal of this article is to provide a review of the molecules that have been reported to be expressed on the surface of tumor cells in response to IR either *in vivo* or *in vitro* (**Table 1**). Additionally, we provide a discussion of some of the methods used in the identification of these antigens and applications for their use in drug delivery and imaging. New strategies for targeting cancers by way of radiation induced antigens have the potential to be very useful in the fields of drug delivery, immunotherapy, and imaging.

# Cell adhesion molecules

Cell adhesion molecules mediate cell-cell and cell-matrix interactions and are expressed widely in human tissue. They possess many biologically important roles and are involved in tumor development and invasion, inflammatory responses, migration and differentiation, embryogenesis, cell growth, coagulation, wound repair and tissue integrity [39-42]. There are a number of different families of adhesion molecules including integrins, selectins, cadherins,



Figure 1. Ionizing radiation leads to the upregulation of surface molecules in tumor endothelial cells that can be targeted for drug delivery. A schematic illustrating a generalized mechanism by which irradiation can be used to deliver tumor targeted therapies. 1) lonizing radiation is incident upon the tumor from an external source. 2) Radiation induces the upregulation of the neoantigen and its subsequent translocation to the cell surface. 3) Intravascular nanoparticles conjugated with a targeting moiety bind to the irradiated tumor endothelial cell surface. 4) The nanoparticle is endocytosed and the drug contents are released to the surrounding tumor cells.

Table 1. Table of radiation-induced molecules in endothelial or tumor cell lines

[20]
[20]
[20, 44]
[20]
[45, 46]
[43]
[52]
[51]
[48, 50]
[14]
[27, 53]
[16]
[16]
[16]
[53]
[54]
[11, 47, 50,
52]
[17, 48, 50]
[50]
[51]

# Radiation induced neoantigens

	ARP-1, ARK-RS multiple myeloma cell line	In vitro	[56]
	Cells from isolated human primary multiple myeloma tumors	In vitro	[57]
			[57]
NCAM (CD56)	ET-1, RM82 Ewing tumor cell line	In vitro and in vivo: murine	[56]
V-CAM	HDMEC cell line	In vitro	[48]
	Endothelium of tumors:		
	B16/OVA melanoma cell lines	In vivo: murine	[34]
PECAM-1 (CD31)	HDMEC	In vitro	[17]
$\alpha_{2b}\beta_3$ Integrin	Endothelium of tumors:	In vivo: murine	[]
02003	GI 261 murine glioma		[23]
	Lewis lung carcinoma cell line		[23]
	B16F0 and B16F10 murine melanoma cell lines		[9 13]
	B16 melanoma cell line	In vitro	[58]
avB₂ Integrin	187MG 1 N-18 1 N-229 human malignant glioma cell lines	In vitro	[60]
B <sub>1</sub> Integrin	A549. SKMFS1 lung carcinoma cell lines	In vitro	[19]
D1 mogm	COL 0-320 colon carcinoma cell line	In vitro	[25]
F-cadherin (CD324)	4549 lung carcinoma cell lines	In vitro	[20]
	T-SCC squamous cell carcinoma lines	In vitro and in vivo: murine	[64]
MHC Class I	HCT 116 WiDr HT-29 SW480 SW620 MC38 colon	In vitro and in vivo: murine	[16 67]
	carcinoma cell lines		[10, 01]
	A5/19 NCLH23 NCLH6/17 lung carcinoma cell line	In vitro	[16]
	MKN/15 gastric adeposarcinoma cell line	In vitro	[10]
	UCI101 UCI107 SKOV 3 T 222 ovarian carcinoma cell lines	In vitro	[21] [27 53]
	Cells from freshly isolated human ovarian tumors	In vivo: human	[27, 33]
	SiHa HT 2 CaSki carvical carcinoma call line	In vitro	[33] [54 55]
	APD 1 APK PS multiple myolema cell lines		[54, 55]
	MolluSo, B16 E10 molonomo colluino		
	Celle from isolated human primary multiple myoleme tumore		[00, 07]
	CLOSE diama coll line		[37]
CD80 (P7 1)	B215 mastantama cell line		[00]
CD80 (B7.1)	mbAT2f mbAT2 hangtama call ling		[20]
	NULTER fibrablest cell line		[20]
	NIH313 IIDroblast cell line		[20]
0000	AK7 mesourenoma cen ine		[20]
CD20	INI9, INI9/ BCI2, Ramos neoplastic B-cell lines		[[/]]
	GMISIOB lymphoblastic cell line		[[1]]
NKCOD Liganda (MICA	Raji, Daudi Burkitti iymphoma cell lines		[78]
NKG2D Ligands (MICA,	HeLa cervical carcinoma cell line		[35]
MICB, ULBP1,2,3)			(00)
Fas (CD95/Apo-1)	MCF-7, ZR-75.1, U2-US breast carcinoma cell line	In vitro	[28]
	U87 glioma cell line	In vitro	[81]
	Cells from isolated numan gliomas	In vitro	[81]
	Biopsy tissue from numan nead and neck lymphoma	In vivo: numan	[82]
	MC38 murine colon adenocarcinoma	In vitro	[18]
FasL	C15 nasopharyngeal carcinoma cell line	In vivo: murine	[83]
Irail-R1 & Irail-R2	SaUS2, HUS osteosarcoma cell lines	In vitro	[86]
(DR4 & DR5)	MCF7 breast carcinoma cell line (TRAIL-R2 only)	In vitro	[85]
	Hep-2R numan larynx squamous cell carcinoma line	In vitro	[90]
GRP78 (BIP)	HUVECS grown in coculture with GL261 glioma cell line	In vitro	[12]
	Endothelium of tumors:		
	GL261 murine glioma	In vivo: murine	[12]
	MDA-MB-231 breast carcinoma cell line		[12]
Calreticulin	CT26 colon carcinoma cell line	In vitro	[103]
	B16F0 melanoma cell líne	In vitro	[36]
ERp57 (grp58/PDIA3)	CT26 colon carcinoma cell line	In vitro	[105]
CEA	MKN45 gastric adenocarcinoma cell line	In vitro	[16, 21]
	Caco-2, HCT 116, WiDr, HT-29, LS 174T, SW1463, SW480,	In vitro	[16]
	T84, LoVo, COLO 205 colon carcinoma cell lines		
	A549, SK-LU-1, SW900, NCI-H23, GLL-1 lung carcinoma cell	In vitro	[16, 109]
	lines	In vitro	[16]
	22Rv1, LNCaP prostate carcinoma cell line		
TIP-1 (TAX1BP3)	H460, LLC lung carcinoma cell line	In vitro and In vivo: murine	[38, 113]

the immunoglobulin superfamily, and cell surface proteoglycans. The effects of IR on the expression of these molecules varies depending on the individual adhesion molecule and the irradiated cell line/tissue [39].

#### P-Selectin

P-selectin is a molecule that is involved in the adhesion and activation of circulating inflammatory cells and platelet aggregation [22]. Hallahan et al. studied P-selectin expression in irradiated tumor blood vessels and found that prior to irradiation P-selectin is located within the endothelium. After irradiation however, Pselectin was translocated to the lumen of the blood vessels. Its expression on the endothelial cell surface was transient, and platelet aggregation studies showed resolution by 48 hours after irradiation [43]. The surface expression of Pselectin following irradiation was observed in all neoplasms studied and in both rat and mouse species [20, 23]. The effect was only observed in irradiated tissues, suggesting preferential expression after IR treatment. Other studies have shown increased expression of P-selectin on human umbilical vascular endothelial cells (HUVECs) in vitro [43]. These observations have led to the use of single chain variable fragment (ScFv) sequences for targeted visualization of radiation induced P-selectin in lung tumors [44]. P-selectin has also been used as a targeting molecule for the delivery of drug-filled nanocapsules to tumors following irradiation leading to prolonged survival and reduction of metastasis in mouse models [45, 46].

# E-Selectin

E-selectin is another molecule which is upregulated at both the mRNA and the protein level in endothelial cells in response to IR [47-50]. Eselectin expression in HUVECs begins to increase at 2 hours following irradiation and peaks at 4-6 hours [47]. The elevated levels of surface expression of E-selectin persist on endothelial cells for several days in treated murine lung tissue [49]. In human dermal microvascular endothelial cells (HDMECs), E-selectin was not shown to be present in untreated controls. but expression levels were increased after treatment with both 5 and 10 Gy [48]. In the oral mucosa of head and neck cancer patients treated with total doses of 30-60 Gy, immunohistochemical staining showed a significant increase in expression of E-selectin and may be involved in the development of oral mucositis following irradiation [51]. Recently, Patillo *et al.* targeted irradiated mouse mammary tumors with anti-E-selectin conjugated immunoliposomes containing the antivascular drug combretastatin disodium phosphate [14]. They found that unirradiated MCa-4 tumor endothelial cells expressed a basal level of E-selectin, while irradiated tumors showed significantly increased Eselectin expression [14]. Tumor treatment in xenografted mice with the targeted liposomes plus fractionated radiation led to a significant delay in tumor growth compared to fractionated radiation or targeted liposomes alone [14].

# ICAM-1

Another molecule of interest is ICAM-1 which is a member of the lg superfamily. It is constitutively expressed on the surface of various cells, including macrophages, leukocytes and endothelial cells and is involved in leukocyte trafficking, vascular integrity, and inflammation [22, 52]. Increased surface expression of ICAM-1 was found on tumor cells from freshly isolated human ovarian tumors and ovarian cancer cell lines following high dose irradiation [27, 53]. Cervical cancer cell lines were also found to exhibit upregulated surface ICAM-1 after treatment with IR [54]. Santin et al. later showed that combining retinoic acid treatment with high dose gamma irradiation induces an additive effect and leads to increased surface expression of ICAM-1 over either treatment alone [55]. The results indicate that the combination of these two treatments may provide a powerful antiproliferative effect. A study of human colon, lung and prostate cancer cell lines showed upregulation of ICAM-1 at the surface and indicated that it may be involved in enhanced T-cell binding to tumor targets and enhanced costimulatory signaling with T-cells [16]. Both ICAM-1 and NCAM expression were increased in an Ewing tumor cell line following irradiation [56]. Additionally, ICAM-1 upregulation was seen in multiple myeloma cell lines as well as myeloma primary tumors after irradiation [57]. The upregulation persisted in multiple myeloma cells for 6 days. This indicates that ICAM-1 may be a useful target for tumor therapeutics or imaging applications and may be involved in increasing the immunogenicity of the cells [57].

Endothelial cell expression of ICAM-1 has been

another topic of interest. It has been shown that ICAM-1 is increased on the surface of HDMECs. HUVECs, and human microvascular endothelial cells (HMEC) after treatment with IR which suggests that it participates in the radiationinduced inflammatory reaction of endothelium [17, 47, 48, 50, 52]. There have been no reports of attempts for radiation-guided targeting of ICAM-1 for therapeutic purposes to date. However, Kiani et al. studied the upregulation of ICAM-1 by quantifying the delivery of microspheres coated with anti-ICAM-1 antibodies to tumor cells both in vivo and in vitro [11]. They found enhanced microsphere adhesion to HU-VECs under in vitro flow conditions. In an in vivo rat cranial window model in which they irradiated cerebral tissue, they observed a 13-fold increase in adhesion of the anti-ICAM microspheres compared to control indicating that ICAM-1 may be a suitable target for drug delivery [11].

#### V-CAM

V-CAM is a molecule that has been shown in multiple reports to exhibit no increase in surface expression following IR in HUVECs, HDMECs, and oral mucosa [17, 47, 51]. In contrast, a study by Heckman et al. found that V-CAM upregulation was increased on an mRNA and protein expression level in HDMECs [48]. The elevated protein expression persisted up to 24 hours after irradiation. Lugade et al. further showed V-CAM upregulation in tumor vasculature of B16/OVA melanomas xenografted into mice thighs after treatment with either 3 or 15 Gy. They related this upregulation following irradiation to the creation of a tumor microenvironment that selects for increased infiltration and retention of T cells [34]. To our knowledge, V-CAM has never been targeted in vivo for drug delivery or imaging applications.

# Integrins

Integrins are heterodimeric transmembrane molecules that are comprised of an  $\alpha$  and  $\beta$  subunit and are subdivided into subfamilies based upon the  $\beta$  subunit [39]. Each subunit contributes to ligand specificity and contains ligand binding sites. Integrins serve as receptors for many proteins including Ig superfamily adhesion molecules and extracellular matrix proteins like the collagen, fibronectin, vitronectin, and fibrinogen receptors [9]. The conserved peptide

sequence arginine-glycine-aspartic acid (RGD) is a major recognition site for several integrins and is commonly found in ligands that bind to integrins including  $\alpha_{2b}\beta_3$ . Onoda et al. found a transient increase in the expression of  $\alpha_{2b}\beta_3$  in B16 melanoma cells after low-dose gamma irradiation. This increased expression resulted in enhanced adhesion to fibronectin in vitro and increased induction of metastases in vivo suggesting the association between upregulation of integrins in cells surviving radiation and increased malignant potential [58]. To assess the targeting of  $\alpha_{2b}\beta_3$  in irradiated tumors, B16F0 tumor bearing mice were treated with integrinspecific peptides. Increased binding was demonstrated within tumor blood vessels after irradiation as compared to controls [9]. In a seminal paper from 2003, Hallahan et al. reported the first demonstration of radiation-guided drug delivery to tumor blood vessels. They showed a radiation-induced accumulation of  $\alpha_{2b}\beta_3$  within the lumen of the microvasculature of tumors isolated 6 hours after irradiation with no increase in untreated controls [23]. Irradiated tumors were then targeted with radiopharmaceuticals by use of  $\alpha_{2b}\beta_3$  ligands conjugated to nanoparticles and liposomes. A significant growth delay was observed in irradiated tumors [23]. More recently, RGD conjugated liposomes incorporating the anti-angiogenic drug combretastatin were used to target  $\alpha_{2b}\beta_3$  in irradiated mouse melanomas. A single dose of 5 Gy combined with immunoliposome treatment lead to a 5.1-day tumor growth delay compared to untreated controls [13].

Other studies have shown a radiation-induced upregulation of various  $\alpha$  and  $\beta_1$  integrins in lung cancer cell lines and colon cancer cell lines *in vitro* which may have implications for the induction of malignant phenotypes following IR [19, 25].  $\beta_1$  integrin and its associated signaling pathways have been explored as a potential therapeutic target for breast cancer [59].  $\alpha_v\beta_3$  integrin mRNA expression was shown to be upregulated in response to IR in 3 glioma cell lines which lead to increased migration and invasiveness [60]. These data suggest that improved therapeutic efficacy of radiotherapy in malignant gliomas may be aided by inhibition of migration and invasion.

#### Cadherins

The cadherins are molecules involved in cell-cell

adhesion and maintenance of epithelial integrity. They are thought to play an important role in the invasiveness and the metastatic potential of cancer cells [61]. E-cadherin is a calciumdependent cell adhesion molecule. Its reduced expression by tumor cells may be related to the invasive potential of some cancers [62, 63]. Akimoto et al. demonstrated increased expression of E-cadherin and  $\alpha$ -catenin after fractionated irradiation of the A549 human lung cancer cell line which led to a reduction in invasive capacity in the irradiated cells [61]. A study by Ebara et al. on the effects of IR on the surface expression of E-cadherin found it to be increased in thyroid gland squamous cell carcinoma cells both in vivo and in vitro [64]. The authors suggest that this upregulation may decrease the metastatic propensity through inhibition of detachment of the tumor cells from the primary site [64].

#### Immunoregulatory molecules

The discovery that immunomodulatory molecules are upregulated post-IR on the surface of certain tumor cells has led to research involving tumor cell vaccines and potentiation of cytotoxic T lymphocyte (CTL) mediated tumor killing [37]. Studies have demonstrated that an enhancement in the immune system's response to tumor antigens can be achieved using various cancer vaccines targeting tumor associated antigens [65].

#### MHC Class I

MHC Class I molecules are important for presentation of intracellular antigens to class Irestricted T cells. These molecules have been shown to be upregulated on tumor cells following irradiation in a variety of cell lines including multiple myeloma, melanoma, colon, lung, ovarian, and gastric tumor cell lines [16, 21, 27, 57, 66, 67]. In tumor cells, MHC Class I molecules play a role in antigen presentation of tumor molecule epitopes to circulating T cells, and their upregulation has been shown to enhance the effectiveness of immunotherapy in high grade gliomas treated with IR [68]. Conversely, through the downregulation of MHC molecules. tumor cells may evade immune recognition and elimination [69]. Reits et al. showed that immunotherapy was successful in eradicating murine colon adenocarcinoma through CTL mediated killing only when preceded by radiotherapy of the tumor tissue [67].

Three schemes through which IR leads to the upregulation of MHC class I molecules were described: early effect, repair response, and late effect [67]. The early effect increase of MHC Class I occurs in the first 0-4 hours after irradiation and involves damage and degradation of the existing cellular proteins with subsequent presentation of the degradation products via MHC class I. The repair response effect occurs when irradiation forms free radicals through water radiolysis which leads to oxidation of protein amino acids. This modification targets affected proteins for degradation and subsequent upregulation of MHC Class I. Finally, the late effect involves mTOR pathway activation, increased protein synthesis, and an increase of the intercellular peptide pool for subsequent presentation [67]. These findings may help to explain the abscopal effect which is a phenomenon in which IR can inhibit distant tumors outside of the region where local IR is absorbed [70].

# CD80

CD80 is a co-stimulatory molecule that is essential for activating T lymphocytes. Recognition of MHC class I peptide presentation without CD80 co-stimulation leads to T cell anergy. Typically, CD80 is found on activated antigen presenting cells like B-cells and macrophages. However, Morel et al. showed that IR can induce CD80 neoexpression in various murine tumor cells including hepatomas and mastocytomas, which prior to irradiation constitutively exhibited no CD80 expression [26]. These findings may further explain the irradiation-enhanced immunogenicity of many tumor cells, which may present the tumor antigen and then directly provide the costimulatory signal to the lymphocyte, provided that MHC Class I molecules and CD80 molecules are expressed on the same cell. Galiximab is a monoclonal antibody to CD80 which has been demonstrated to have antitumor activity against various B-cell lymphoma models in preclinical trials [71]. However, the role of radiation in enhancing the effects of anti-CD80 therapy has yet to be fully explored.

# CD20

A majority of B-cell lymphomas express the CD20 surface antigen which has been a mo-

lecular target for immunotherapies like rituximab, zevalin, ofatumumab and others [72-74]. CD20 is a nonglycosylated phosphoprotein that is thought to be involved in the regulation of cell cycle progression of B-lymphocytes [75]. Despite the advances made with antibody therapies like rituximab, approximately two-thirds of lymphoma patients eventually develop disease recurrence and die of their disease [76]. Therefore, novel treatments are needed to cure these patients. Kunala et al. showed that CD20 surface expression was enhanced following treatment with low doses of IR in vitro [77]. This effect was found to be dose dependent up to 10 Gy and the greatest levels of expression were found at 24 hrs following irradiation. In a more recent study by Gupta et al. low dose irradiation of Burkitt lymphoma cells led to enhanced cellsurface CD20 expression peaking within 24 hours of treatment [78]. However, treatment with antioxidants abrogated the CD20 induction suggesting a mechanism involving redox regulation. These results suggest that pretreatment with radiation prior to subsequent immunotherapy could improve tumor targeting and increase the efficacy of the treatment. It may also explain increased benefits of radioimmunotherapies in which localized irradiation is an induction agent responsible for the upregulation of CD20 leading to greater intrinsic immune responses and tumor cell killing.

# NKG2D

Other molecules involved in the immunogenicity of tumor cells have been explored for radiationinduction at the cell surface. NKG2D ligand surface expression was explored by Kim *et al.* in HeLa cervical carcinoma cell lines after treatment with IR [35]. It was discovered that the upregulation of the NKG2D ligands led to enhanced susceptibility to natural killer (NK) cellmediated cytolysis. This observation implies a role of NK cells in the immune response to cancer cells after treatment with radiation and may signify a new family of molecules to be further explored for tumor-targeted therapies.

#### Death pathway and apoptosis-associated molecules

# Fas

lonizing radiation leads to cell death through a sequence of events that involves many signaling pathways which vary depending on whether the cell undergoes early or delayed cell death [79]. One molecule that is implicated in cellular apoptosis following irradiation is Fas (also called APO-1 or CD95). Fas is a cell-surface death receptor expressed in a variety of tissues and interaction with its ligand, FasL plays a role in the control of the immune system as well as in triggering apoptosis [80]. Chakraborty et al. showed directly that upregulation of Fas on murine colon adenocarcinoma cells after irradiation lead to enhanced cytotoxic T lymphocyte activity. These results hold implications for further work exploring a combination of immunotherapy and radiation therapy [18]. Sheard et al. showed that after irradiation, Fas was upregulated at the surface in p53-wild type breast carcinoma cells in vitro in a dose-dependent manner [28]. This effect was observed at doses at and below 10 Gy. However, p53 null and mutant cell lines failed to up-regulate Fas levels and halt their cell cycles in the G<sub>1</sub> phase indicating that p53 status is an important determinant of radiation induction of Fas. Another study by Ishikawa et al. showed that Fas expression levels increased in a dose dependent manner following irradiation of human malignant glioma cells and the U87 glioma cell line [81]. This observation was accompanied by enhanced cellmediated cytotoxicity of the glioma cells in vitro which suggests that local irradiation combined with immunotherapy may be effective in the management of malignant gliomas. In another study, Ogawa et al. demonstrated that tumor tissue from patients with malignant lymphomas showed differentially increased Fas expression before and after IR treatment [82]. The Faspositive lymphomas showed high radiosensitivity. However, radioresistant squamous cell tumors showed negative or only slight elevations in Fas levels which suggests that Fas expression may be useful as a marker of radiation efficacy in some tumor types [82].

# FasL

FasL is another molecule that has been studied in tumor cells following treatment with IR. It is known to be the ligand to the Fas receptor, and is an essential determinant of apoptosis. Abdulkarim *et al.* demonstrated strong surface coexpression of FasL and Fas on nasopharyngeal carcinoma cells treated with radiation *in vivo* in which a large percentage of cells underwent apoptosis [83]. These results led them to conclude that these molecules can play a major role in the anti-tumor effect of IR at therapeutic doses [83]. The death receptor pathways that are involved during radiation-induced early and delayed cell death were studied by Luce *et al.* in a number of cell lines [79]. Following radiation, they observed early upregulation of Fas, TRAIL-R2 (DR5), and TNF-R2 which was associated with sensitization of the cells to apoptosis. However, late expression of FasL, TRAIL, and TNF- $\alpha$ was associated with mitotic catastrophe and delayed cell death.

#### TRAIL

The TRAIL receptors TRAIL-R1 (DR4) and TRAIL-R2 possess an intercellular death domain which is capable of activating the extrinsic pathway for apoptosis after TRAIL-induced receptor trimerization [84]. TRAIL is non-toxic when delivered intravascularly in vivo and preferentially kills neoplastically transformed cells over normal cells [85]. A study by Chinnaiyan et al. showed that TRAIL-R2 could be upregulated in the MCF7 breast carcinoma cell line with radiation and that the addition of TRAIL to treatment regimens led to large increases in apoptotic cell death [85]. Tumors xenografted into the hindlimbs of nude mice showed growth regression when treated with combined IR and TRAIL. More recently, these receptors were studied in human osteosarcoma cells after treatment with IR. It was found that both TRAIL receptors (DR4 and DR5) were upregulated at the cell surface [86]. Combined treatment with IR and TRAIL synergistically enhanced apoptosis and decreased cell viability in osteosarcoma cell lines. These two studies provide support for combining IR with TRAIL to improve tumor responsiveness and eradication.

Another therapeutic scheme that has gained interest recently involves the use of monoclonal antibodies targeted to TRAIL-R2 [87, 88]. A number of studies have shown the beneficial effects of combining radiation with anti-TRAIL-R2 treatment both *in vitro* and *in vivo* [89, 90]. Nagane *et al.* demonstrated significantly suppressed growth of subcutaneous glioma xenografts leading to complete regression in tumorburden mice following treatment with the direct agonist anti-TRAIL-R2 mAb KMTR2 [87].

#### Phosphatidylserine

One of the most widely exploited molecules of apoptosis is the cell membrane phospholipid

phosphatidylserine (PS). While it is normally confined to the cytosolic side of the membrane, induction of apoptosis leads to rapid externalization of PS. This occurs as a result of disruption of the enzymes responsible for maintaining cell membrane phospholipid asymmetry [91]. This 'eat me' signal helps macrophages recognize and eliminate dying cells. PS exposure is now understood to be a ubiquitous phenomenon of apoptosis that is independent of cell type and cell death inducing trigger [92]. It is no surprise then, that radiation-induced apoptosis of tumor cells leads to the expression of PS. Molecules that bind to extracellular PS with high affinity, such as Annexin V, are commonly used to target PS as an indicator of cells undergoing apoptosis [91]. PS has previously been evaluated clinically in follicular lymphoma, head and neck squamous cell carcinoma, breast cancer, and lung cancer as a marker of apoptosis and has been correlated to ultimate prognosis and treatment response [93-97]. A more in depth discussion of PS and its applications is provided below in the tumor imaging section.

# Miscellaneous molecules

# GRP78

There are a variety of molecules that have been shown to be upregulated in response to IR that do not fit neatly into the previously discussed categories. One of them is glucose regulated protein-78 or GRP78 (also known as BiP) which is an endoplasmic reticulum (ER) chaperone that is involved in suppression of stress-induced apoptosis [98]. On the cell surface, it can function in the transduction of extracellular stimuli to intracellular signals in the promotion of tumorigenesis [99, 100]. From a therapeutic viewpoint, GRP78 is a practical target because it is expressed at elevated levels in tumors as compared to normal tissues. Recently, Passarella et al. showed that GRP78 expression is increased in tumor endothelium after treatment with IR [12]. In co-culture experiments in which tumor cells are grown in two-tiered dishes containing HUVECs, they found that the expression of GRP78 only occurred when HUVECs and tumor cells were irradiated in co-culture together. This implies that some signaling is taking place between tumor and endothelial cells that leads to the upregulation of GRP78 in response to IR. Attempts to target GRP78 for cancer therapy have generally shown encouraging results [12, 101, 102]. Passarella *et al.* used peptideconjugated nanoparticles to deliver paclitaxel to breast carcinoma tumors grown in nude mice and observed a significant delay in tumor tripling time for tumors treated with combination therapy of IR and the targeted nanoparticle [12]. Similarly, Katanasaka *et al.* implemented peptide-modified liposomes targeting GRP78 for the delivery of doxorubicin to colon carcinoma tumor-bearing mice and demonstrated suppressed tumor growth and prolonged survival [102].

# Calreticulin

Calreticulin is another molecule that is primarily found in the ER, but is translocated to the cell surface following ER stress. It serves as a signal that allows dying cells to be recognized and ingested by dendritic cells and its expression therefore leads to increased cancer cell immunogenic apoptosis [103, 104]. Perez et al. showed that translocation of calreticulin to the cell surface is increased in response to irradiation of B16F0 melanoma cells in vitro [36]. Irradiation of subcutaneous melanomas in mice prior to resection resulted in a large reduction in systemic metastases when challenged with unirradiated melanoma cells. The results confirm that calreticulin is involved in increasing the immunogenicity of melanoma cells which are targeted for removal by dendritic cells. Calreticulin translocation is reported to be controlled by another ER stress protein ERp57/ GRP58 and they are translocated to the surface together in response to IR and a number of other cytotoxic insults [105]. For therapeutic applications, induction of calreticulin by proapoptotic peptides has been shown to enhance the immunogenicity of tumor cells and ultimately the antitumor efficacy of these peptides [106].

# CEA

Carcinoembryonic antigen (CEA) is a marker that is often used to assay for liver metastasis from colon cancers and is also used to track the response of primary colon cancer to treatment. It is one of the most widely used tumor markers worldwide and is a glycoprotein that is actually classified in the Ig superfamily [107]. This classification implies a role in cell adhesion, but its localization on the apical surface of mature enterocytes in healthy colon is difficult to reconcile [107]. While CEA is reported to be prevalent in a variety of human malignancies, a number of studies have shown the induction of CEA in tumor cells following irradiation [108]. Hareyama et al. were the first to show that the expression of CEA was increased on the surface membrane of irradiated gastric adenocarcinoma cells and that this expression was regulated at the gene expression level [21]. Matsumoto et al. showed upregulated CEA expression on the surface of GLL-1 human lung cancer cells [109]. The peak expression was observed 4 days after irradiation and combination treatment with interferonv showed additional effect. Garnett et al. demonstrated surface increases of CEA in colon, lung and prostate tumor cell lines and that this increased expression led to improved killing by CEA-specific CTLs [16]. CEA is a target that has been shown to improve responsiveness to immune-mediated killing, and may be a useful marker for other targeted therapeutic modalities including radioimmunotherapy and drug encapsulated nanoparticles [110, 111].

# TIP-1

PDZ domain proteins play roles in acting as molecular scaffolds and in maintenance of cell polarity and cell signaling [112]. Recently, TIP-1 which is an atypical PDZ domain protein in that it functions as a negative regulator of PDZbased scaffolding was found to be translocated to the cell surface following IR in a dose dependent manner [38, 112]. Targeting TIP-1 was found to be a useful assessment of tumor response to IR due to the discovery that TIP-1 is expressed by dying tumor cells prior to apoptosis. TIP-1 was also recently shown to be an effective target for drug delivery. Peptideconjugated paclitaxel encapsulated, doxorubicin encapsulated, and platinum nanoparticles were found to aggregate at the site of irradiated tumors leading to improved bioavailability of the drug and significant tumor growth delay when compared to controls [113-115].

# Strategies for identifying and targeting inducible antigens

Surface protein analysis is commonly performed by flow cytometry using primary and fluorescently-labeled secondary antibodies [9, 21, 68, 116]. The use of propidium iodide is typically employed as a second stain to identify dead cells that have a ruptured membrane. This is

because propidium iodide is a membrane impermeant DNA stain that will provide a fluorescent signal in non-viable cells. This is particularly important when the protein under investigation is expressed in large quantities in internal cellular compartments where a ruptured membrane would result in false positive staining. Other useful techniques for identifying and quantifying surface proteins include membrane fraction processing followed by western blotting, and plasma membrane biotinylation in which neutravidin coated beads or gel are used to trap surface proteins conjugated with biotin. The proteins are then removed from the beads or column and are electrophoresed. Staining methods are also widely used for surface antigen detection including immunohistochemical and immunocytochemical techniques. To characterize the nature of overexpression of the antigen, mRNA levels are often detected by northern blotting. Increased mRNA expression indicates the upregulation is, at least in part, through transcriptional mechanisms. Unchanged mRNA levels from pre- to post-treatment, given an increase in protein surface expression, may indicate either translocation of the protein from one cellular compartment to the surface, or a change in translational regulation.

Typically, a radiation-induced antigen is identified through one of the previously mentioned methods before it is targeted for drug delivery, often through the use of monoclonal antibodies. However, the use of specific antibodies for antigen discovery requires at least some a priori knowledge of what to look for before it can be found. A different option involves using phage display to determine ligands that bind to the tumor cells [117]. This can be performed without prior knowledge of any particular molecular target. The usefulness of this technique lies in the ability to screen a massive number of phages (up to 1010 variants) each which encode a different peptide sequence. The enormous variety is achieved by the introduction of a DNA library into the phages. The recombinant DNA encodes the peptide sequences which are subsequently produced and expressed on the protein capsid. The phages which express a variety of peptide sequences can be delivered intravenously, often in a mouse, for in vivo targeting studies [118]. Phages that bind to normal tissues are discarded, while phages that bind only to the tumor are harvested and the DNA can be amplified within bacteria infected with the phage. Serial rounds of "biopanning" can be performed which serve to enrich the phages homing specifically to the tumor. Phage DNA can then be sequenced to determine the amino acid sequence of peptides on the capsid which can then be reproduced for use as targeting moieties.

There have been a number of studies employing tumor targeting peptides for imaging, drug delivery, or as potential drugs themselves [117, 119]. Phage display has also proven to be useful for identifying peptides that target radiationinduced antigens [120]. Hallahan et al. utilized a phage-displayed peptide RGDGSSV to target  $\alpha_{2b}\beta_3$  integrin in irradiated tumors [23]. Later, phage display was used to discover a set of peptides that were capable of targeting irradiated gliomas [12]. One of the most promising peptides. GIRLRG. was found to bind to GRP-78. Subsequent studies showed that GIRLRG could be conjugated to paclitaxel encapsulated nanoparticles. Breast tumor (MDA-MB-231) and glioma (GL261) cell line xenografts in nude mice were irradiated and then treated with the peptide-nanoparticle conjugate and showed a significant increase in tumor tripling time as illustrated in Figure 2 [12]. In 2008, Han et al. published an article in which they discovered a peptide (HVGGSSV) that displayed potential as an in vivo molecular imaging probe [118]. In a subsequent paper, they identified the target for HVGGSSV as TIP-1 through a separate phage display experiment where a T7 phage-displayed human lung tumor cDNA library was screened against beads coated with the HVGGSSV peptide [38]. After 5 rounds of screening, they isolated the phages that bound to the beads, and subsequently sequenced the phage clones which encoded the amino acid sequence of TIP-1 [38]. The peptide was then shown to be capable of discerning between responding and nonresponding tumors because it binds to TIP-1 which is expressed prior to cell death [38]. One of the most recent examples in the literature of peptides being employed for targeting irradiated tumors is by Hariri et al. where they conjugated the HVGGSSV peptide to a nanoparticle containing paclitaxel and studied the tumor targeting abilities [113]. They found improvement in tumor growth delay in vivo with a combination of IR and targeted nanoparticle. These results indicate that short peptide sequences can be used in a similar fashion to monoclonal antibodies for targeting radiation-induced antigens and that



**Figure 2.** GIRLRG-targeted nanoparticle drug delivery system causes tumor growth delay *in vivo*. MDA-MB-231 (a) or GL261 (b) tumor cells were implanted in the hind limbs of nude mice or C57/BI6, respectively. Once tumors reached 300 mm<sup>3</sup> in volume, mice were treated with 3 Gy radiation daily for 3 days, or were left as untreated controls. On the 2<sup>nd</sup> day, mice were injected with either systemic paclitaxel, paclitaxel nanoparticle conjugated with the RILGGR scrambled peptide, or paclitaxel nanoparticle conjugated with the GIRLRG targeted peptide at a concentration of 10 mg/kg (n=5) (a) or 20 mg/kg (n=3) (b). Tumor volumes were monitored throughout using calipers. Reprinted with permission [12].

they can be developed using positive and negative screening techniques to improve tumor site specificity.

# Radiation guided-cancer therapy

Once a radiation-induced antigen is identified, the next step is to exploit its presence on tumor cells by utilizing it to for targeted delivery of a therapeutic entity. This can be done in a variety of ways as evidenced by the articles cited in the previous sections. The most common ways of targeting tumor antigens is through the use of antibodies, affibodies, or peptides linked to drug encapsulated nanoparticles [12, 23, 111, 113, 121, 122]. Nanoparticles are advantageous for drug delivery because they offer tumor-specific accumulation when linked to a targeting moiety, reduced nonspecific toxicity, and improved tumor killing efficacy [121]. Of the molecules listed in Table 1, many are excellent candidates for targeted therapeutics and some have already been shown to be useful for such purposes. Recently, new developments in nanoparticle drug delivery have been reported. Lowery et al. demonstrated that liposomeencapsulated doxorubicin nanoparticles can be delivered selectively to irradiated hindlimb tumors in mice when conjugated with the HVGGSSV peptide [115]. Tumor treatment with the targeted liposomes containing doxorubicin in combination with radiation showed moderate tumor growth suppression when compared to other treatment schemes including radiation alone, and free doxorubicin plus radiation [115].

Magnetic iron oxide nanoparticles have also been shown to be useful for combined cancer imaging and drug delivery [123]. These multifunctional nanoparticles have been dubbed "nanotheranostics" and can be conjugated with antibodies, aptamers, peptides, or other molecules like folate to improve tumor targeting capacity [124, 125]. They are useful as MRI contrast enhancement agents, and have been employed for drug delivery either as a carrier for externally conjugated drugs, or through pH dependent drug release of encapsulated anticancer agents like doxorubicin [125-127]. In a preclinical in vivo study, iron oxide nanoparticles conjugated with antibodies targeting mutant EGFR receptors were shown to impart a significant survival benefit in glioblastoma xenografted mice when delivered via MRI-guided convection enhanced delivery [128]. Other metal oxides like zinc oxide nanoparticles are also currently being studied due to their cancer cell cytotoxic properties through reactive oxide species production and their relative biocompatibility [129]. There is great potential for targeting radiation-inducible antigens with new nanoparticle technologies or with the more wellstudied drug delivery vehicles such as albuminbound nanoparticles that have shown success in pre-clinical and clinical trials [130].

Another potential application that has not been the focus of much research to date is radiationguided radioimmunotherapy. The idea is that IR from an external source can be focused on a defined tumor volume to elicit the expression of neoantigens which can then be targeted with radiolabeled antibodies. An advantage to the use of radioimmunoconjugates is that cytotoxic ionizing photons are minimally attenuated by tumor tissue which circumvents the problems of drug distribution to regions of tumors with poor blood supply or drugs that cannot penetrate tumors readily [131]. Internal emitters are therefore capable of irradiating the entire neoplasm even when the biodistribution is nonhomogeneous. Radioimmunoconiugates have been implemented thus far primarily in the treatment of hematologic malignancies [74, 76, 132]. This approach, however, has been limited by suboptimal target-to-nontarget ratio and an inability to deliver sufficient radiation doses to tumors selectively [133]. Pre-targeted radioimmunotherapy is a technique that separates the targeting moiety from the subsequently administered therapeutic radioisotope. Conceptually, sequential administration allows for maximal antibody targeting to occur prior to delivery of the therapeutic radionuclide which is bound to a small molecule to enhance its tumor uptake and rapid excretion [133]. If the radionuclide molecule has higher penetration, clearance, and diffusion rates than the targeting antibody, more rapid tumor radionuclide localization and excretion can occur which minimizes nonspecific radiation to normal tissues. Two main schemes have been proposed for pre-targeted radioimmunotherapy. In one, a tumor-specific antibody tagged with streptavidin is administered followed by a biotin-linked isotope which then binds to the antibody through a biotinstreptavidin interaction [133, 134]. In the other scheme, a molecularly engineered antibody fragment with dual antigen specificity is administered which can bind to both the subsequently delivered isotope and the targeted tumor antigen [133, 134]. A number of clinical studies targeting hematologic malignancies as well as solid tumors like gliomas, small cell lung cancer, medullary thyroid cancer, and colon cancer have been reported with varying levels of success [135-139]. A controlled open nonrandomized study of the effects of adjuvant pretargeted radioimmunotherapy in high grade glioma patients found a median disease-free survival of 28 months versus 8 months in the control group [140]. In another study, the investigators used a bispecific antibody with <sup>131</sup>I in the treatment of medullary thyroid cancer. However, the results of the phase I/II clinical trial indicated that of 17 treated patients, minor tumor response was seen in only 5 and pain relief in 4 patients [136].

# Tumor imaging with radiation induced antigens

In addition to targeted therapeutic applications of radiation-induced antigens, there is also the potential to create new imaging modalities based on the selective expression of these antigens on the tumor cell surface. Current noninvasive imaging methods for staging and candelineation include primarily cer 18fluorodeoxyglucose (18-FDG) positron emission tomography (PET), magnetic resonance imaging (MRI) and computed tomography (CT). FDG-PET requires a significant conglomeration of hypermetabolic tissue in order to yield clinically detectable disease. MRI and CT require significant tissue and density contrasts, respectively, at a

given disease site in order to be observable. As a result, many smaller lesions as well as cellular groups of less active malignant histologies are routinely missed on FDG-PET, MRI and CT. The ultimate result of this imaging insensitivity is inferior disease staging which may lead to treatment with a less aggressive or inappropriate modality. It is postulated that neoantigen-based non-invasive imaging modalities would have the potential to increase sensitivity and specificity for the detection of smaller tumor sites. The neoantigen based radiotracer would localize and respond directly to the neoplastic-specific expression of cellular markers instead of the more variable and less reliable metabolic activity level or tissue and density contrasts [141, 142]. Furthermore, if the guantified level of neoantigen expression in response to radiation administration could be correlated with therapeutic resistance or ultimate prognosis, then there would exist the potential to quickly adapt treatment strategy much earlier in the treatment course than currently possible with existing imaging technology [143].

Historically, the development of neoantigen based imaging or molecular imaging has centered around the primary imaging modalities of PET, single photon emission computed tomography (SPECT), contrast enhanced MRI and contrast enhanced ultrasound. PET and SPECT have the inherent advantage of unlimited depth penetration and conjugation with a wide range of molecular agents [141]. PET provides higher spatial resolution than SPECT, though the resolution of both modalities is inferior to CT and MRI. Additionally, PET requires significant capital equipment investment with a regionally located cyclotron for the generation of the radiotracers. MRI has the distinct advantage of higher resolution and tissue contrast, and does not subject the patient to IR. In order to further improve MR imaging and tumor delineation, a molecular agent is generally coupled with an MRI contrast such as iron nanoparticles or gadolinium. This same strategy may also be employed with ultrasound. Molecular agents may be conjugated with various forms of ultrasound contrast, such as microbubbles or liposomes, in order to provide tumor specific enhancement of the ultrasound images. A limitation of this approach is reduced tissue penetration of the ultrasound contrast-agent assembly due to its relatively large size [141].

The specific technique of employing radiation-

induced neoantigens into new imaging modalities is still in its infancy. While many of the previously mentioned antigens (Table 1) have been incorporated to some extent into molecular imaging research, very little of this research has involved the use of radiation as a trigger for antigen expression prior to targeted imaging. As mentioned previously, specific advantages of such a radiation-triggered molecular imaging approach would include the potential to quickly identify radio-resistant tumor variants and efficiently adapt treatment strategies [143]. Given a small number of studies involving radiationtriggered molecular imaging, this section will also explore basic molecular imaging research and techniques involving the previously mentioned radiation-induced neoantigens.

Perhaps the most successful example of a radiation-induced molecular imaging strategy to date involves the targeted delivery of iron platinum (FePt) nanoparticles to tumors implanted in mouse hindlimbs [114]. The paramagnetic FePt nanoparticles possess similar characteristics to the iron oxide nanoparticles previously discussed, and can therefore function as MRI contrast agents. In the study by Hariri et al. the nanoparticles were conjugated with the HVGGSSV peptide giving them the capacity to bind to TIP-1 which is induced by radiation at the tumor cell surface prior to initiation of apoptosis and cell death [38, 118]. The results showed that irradiated tumors possessed much higher levels of the targeted nanoparticle as compared to nanoparticles conjugated with a scrambled peptide [114]. Further, significantly higher levels of the targeted nanoparticle were observed in tumors treated with 3 Gy versus untreated tumors [114]. This study indicates that paramagnetic nanoparticles have potential application for radiation-guided targeting and imaging of cancers.

Other strategies have focused on conjugating radiotracers to RGD to assess for tumor angiogenesis [144, 145]. The RGD peptide binds to the  $\alpha_{\nu}\beta_3$  integrin, which is known to be overexpressed on the activated endothelial cells of tumor neovasculature and during tumor spread [146, 147]. Numerous studies have evaluated both PET (<sup>18</sup>F and <sup>64</sup>Cu) and SPECT (<sup>99m</sup>Tc) imaging for the evaluation of tumor integrin expression, angiogenesis and metastasis [148, 149]. Out of these various modalities, <sup>18</sup>F PET appears to be the most promising given the high tumor-to-background ratios [146, 150-152]. A

phase I trial of RGD conjugated to a PET radioligand in breast cancer patients was recently shown to be safe, metabolically stable, and retained in tumor tissues. In addition, it was able to detect metastatic breast cancer lesions by PET in most anatomic sites [153].

Another antigen that has been used as a molecular imaging target of tumor vascular changes is P-selectin. Hariri *et al.* explored the *in vivo* expression of P-selectin in Lewis Lung Carcinoma (LLC) tumors implanted in the hind limbs of mice in response to IR with Cy7 and <sup>111</sup>In-DTPA labeled ScFv antibodies to P-selectin [44]. They found significantly increased tumorspecific binding of the labeled ScFv antibodies to irradiated tumors as compared to unirradiated tumors.

Closely related to P-selectin is the cellular adhesion molecule, E-selectin, which also plays a role in the inflammatory response. Funovics et al. conjugated a fluorescent peptide-magnetic nanoparticle to an E-selectin-binding peptide to evaluate the ability of this peptide imaging construct to localize preferentially to LLC as part of an in vivo mouse model [154]. They successfully demonstrated the utility of this approach and also found that E-selectin was expressed in both tumor and endothelial cells. While there has been limited follow-up work on E-selectin imaging in malignancies, there have been several research efforts to develop a clinical molecular imaging technique for benign conditions based on E-selectin. These imaging modalities are primarily based on an <sup>111</sup>In labeled monoclonal antibody to E-selectin for use in the scintigraphic evaluation of arthritis and other inflammatory conditions [155, 156].

Two other cellular adhesion targets with molecular imaging developments and significance are ICAM-1 and V-CAM. As mentioned above, these antigens have demonstrated increased expression in tumors in response to radiation. However, in spite of the possible applications of these antigens in tumor imaging, there has been limited cancer related molecular imaging research. Instead, the antibodies to these targets have been integrated into <sup>18</sup>F PET, photoacoustic imaging with gold nanorods, and contrast enhanced ultrasound for the *in vivo* evaluation of coronary artery disease, cardiac transplant rejection, atherosclerosis, and other benign inflammatory diseases [157-162]. Given the existing molecular imaging techniques for benign diseases, these antigen targets are ripe for further exploration within the malignant disease imaging realm.

In addition to cellular adhesion targets, markers of apoptosis hold special relevance to malignant tumor molecular imaging. The most thoroughly explored marker of apoptosis is PS, which translocates to the cell surface early in apoptosis in response to therapy. The protein annexin V, which binds selectively to PS, has been exploited through PET, SPECT and MR imaging modalities to non-invasively identify areas of apoptosis as an indicator of tumor response [91, 163, 164]. A recent in vivo study of lymphoma tumors within the hind limbs of mice using 99mTc- 6-hydrazinonicotinic annexin V (99mTC-HYNIC-annexin V) imaging found that radiotracer uptake, as an indicator of apoptosis. significantly increased with radiation dose and correlated with terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphatebiotin nick end-labeling (TUNEL) assay quantifications of apoptotic cells [93]. A similar study was performed by Van de Wiele et al. in human patients that successfully correlated 99mTcannexin V uptake in head and neck carcinomas with TUNEL assessed apoptosis in the tumor samples after surgical resection [165].

<sup>99</sup>Tc-Annexin V scintigraphy and SPECT has also been extensively evaluated as a non-invasive indicator of likely treatment response in a variety of tumor sites. Belhocine et al. used 99mTc-Annexin V scintigraphy before and within 3 days after the administration of chemotherapy in lung cancer, breast cancer, and lymphoma patients. They found that overall survival and progression-free survival were significantly correlated with the Annexin V radiotracer uptake after therapy in lung cancer and lymphomas [166]. Similarly, studies by Haas et al. on follicular lymphoma patients and Kartachova et al. on lymphoma, leukemia, non-small cell lung carcinoma and head and neck squamous cell carcinoma patients (HNSCC) found that ultimate tumor response was significantly correlated with annexin V based 99mTc radiotracer uptake following chemotherapy or radiation [94, 167]. In addition to correlation with treatment response. <sup>99m</sup>TC-annexin V has also been shown to display radiation-dose-dependent uptake within parotid glands receiving radiation as part of HNSCC treatment plans [95].

#### Conclusions

The importance of developing targeted approaches for cancer treatment and imaging is underlined by the immense amount of ongoing research dedicated to the discovery and utilization of antigens for such purposes. The use of IR to guide the delivery of therapeutic and imaging agents specifically to tumors is just one facet of the larger picture. However, the characteristics of IR that make it an appealing option for inducing targets are multiple. First, it is already ubiguitously used in cancer treatment protocols. Second, it can be accurately delivered to specific tumor volumes while sparing surrounding normal tissues. Finally, when used at low doses for short periods of time, radiation therapy is associated with relatively few side effects. This review article has provided a discussion of the molecules that have been reported to be differentially induced by IR in tumor cells and tumor vasculature. We believe that further research exploring these targets for therapeutic and imaging purposes as well as in the discovery of novel radiation-induced antigens will aid in improving targeted strategies.

#### Acknowledgements

This work was supported in part by the Emory University School of Medicine Department of Radiation Oncology Medical Student Scholars Program (Corso), the Department of Defense Lung Cancer Research Program Grant W81XWH -10-1-0605 (Ali), start-up funds from Emory University (Diaz), and the Department of Defense Breast Cancer Research Program Grant W81XWH-06-1-0788 (Diaz). We thank Sam Spratt for his artwork.

**Abbreviations:** CTL: Cytotoxic T Lymphocyte; HDMEC: Human dermal microvascular endothelial cell; HMEC: Human microvascular endothelial cell; HNSCC: Head and neck squamous cell carcinoma; HUVEC: Human vascular endothelial cell; IR: Ionizing Radiation; RGD: Arginine-glycine-aspartic acid.

Please address correspondence to: Dr. Roberto Diaz, Department of Radiation Oncology, Emory University School of Medicine, and Winship Cancer Institute of Emory University, Suite A1300, 1365 Clifton Road, NE, Atlanta, GA 30322. Phone: 404-778-3669. Fax: 404-778-1750. E-mail: roberto.diaz@emory.edu

#### References

[1] Hansen EK and Roach M. Handbook of evidence-based radiation oncology. New York, NY: Springer, 2007.

- [2] Govindan R, Bogart J and Vokes EE. Locally advanced non-small cell lung cancer: the past, present, and future. J Thorac Oncol 2008; 3: 917-928.
- [3] Cognetti DM, Weber RS and Lai SY. Head and neck cancer: an evolving treatment paradigm. Cancer 2008; 113: 1911-1932.
- [4] Gold KA, Lee HY and Kim ES. Targeted therapies in squamous cell carcinoma of the head and neck. Cancer. 2009; 115: 922-935.
- [5] Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, Curschmann J, Janzer RC, Ludwin SK, Gorlia T, Allgeier A, Lacombe D, Cairncross JG, Eisenhauer E and Mirimanoff RO. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005; 352: 987-996.
- [6] Eifel PJ, Winter K, Morris M, Levenback C, Grigsby PW, Cooper J, Rotman M, Gershenson D and Mutch DG. Pelvic irradiation with concurrent chemotherapy versus pelvic and paraaortic irradiation for high-risk cervical cancer: an update of radiation therapy oncology group trial (RTOG) 90-01. J Clin Oncol 2004; 22: 872-880.
- [7] Halperin EC, Perez CA and Brady LW. Perez and Brady's principles and practice of radiation oncology. Philadelphia: Wolters Kluwer Health/ Lippincott Williams & Wilkins, 2008.
- [8] Geng L, Osusky K, Konjeti S, Fu A and Hallahan D. Radiation-guided drug delivery to tumor blood vessels results in improved tumor growth delay. J Control Release. 2004; 99: 369-381.
- [9] Hallahan DE, Geng L, Cmelak AJ, Chakravarthy AB, Martin W, Scarfone C and Gonzalez A. Targeting drug delivery to radiation-induced neoantigens in tumor microvasculature. J Control Release. 2001; 74: 183-191.
- [10] Hallahan DE, Qu S, Geng L, Cmelak A, Chakravarthy A, Martin W, Scarfone C and Giorgio T. Radiation-mediated control of drug delivery. Am J Clin Oncol. 2001; 24: 473-480.
- [11] Kiani MF, Yuan H, Chen X, Smith L, Gaber MW and Goetz DJ. Targeting microparticles to select tissue via radiation-induced upregulation of endothelial cell adhesion molecules. Pharm Res. 2002; 19: 1317-1322.
- [12] Passarella RJ, Spratt DE, van der Ende AE, Phillips JG, Wu H, Sathiyakumar V, Zhou L, Hallahan DE, Harth E and Diaz R. Targeted nanoparticles that deliver a sustained, specific release of Paclitaxel to irradiated tumors. Cancer Res. 2010; 70: 4550-4559. Epub 2010 May 4518.
- [13] Pattillo CB, Sari-Sarraf F, Nallamothu R, Moore BM, Wood GC and Kiani MF. Targeting of the antivascular drug combretastatin to irradiated tumors results in tumor growth delay. Pharm Res. 2005; 22: 1117-1120. Epub 2005 Jul 1122.
- [14] Pattillo CB, Venegas B, Donelson FJ, Del Valle L,

Knight LC, Chong PL and Kiani MF. Radiationguided targeting of combretastatin encapsulated immunoliposomes to mammary tumors. Pharm Res. 2009; 26: 1093-1100. Epub 2009 Jan 1027.

- [15] Hall EJ and Giaccia AJ. Radiobiology for the radiologist. Philadelphia: Lippincott Williams & Wilkins, 2006.
- [16] Garnett CT, Palena C, Chakraborty M, Tsang KY, Schlom J and Hodge JW. Sublethal irradiation of human tumor cells modulates phenotype resulting in enhanced killing by cytotoxic T lymphocytes. Cancer Res. 2004; 64: 7985-7994.
- [17] Quarmby S, Hunter RD and Kumar S. Irradiation induced expression of CD31, ICAM-1 and VCAM -1 in human microvascular endothelial cells. Anticancer Res. 2000; 20: 3375-3381.
- [18] Chakraborty M, Abrams SI, Camphausen K, Liu K, Scott T, Coleman CN and Hodge JW. Irradiation of tumor cells up-regulates Fas and enhances CTL lytic activity and CTL adoptive immunotherapy. J Immunol. 2003; 170: 6338-6347.
- [19] Cordes N, Blaese MA, Meineke V and Van Beuningen D. Ionizing radiation induces upregulation of functional beta1-integrin in human lung tumour cell lines in vitro. Int J Radiat Biol. 2002; 78: 347-357.
- [20] Hallahan DE, Staba-Hogan MJ, Virudachalam S and Kolchinsky A. X-ray-induced P-selectin localization to the lumen of tumor blood vessels. Cancer Res. 1998; 58: 5216-5220.
- [21] Hareyama M, Imai K, Kubo K, Takahashi H, Koshiba H, Hinoda Y, Shidou M, Oouchi A, Yachi A and Morita K. Effect of radiation on the expression of carcinoembryonic antigen of human gastric adenocarcinoma cells. Cancer. 1991; 67: 2269-2274.
- [22] Malik AB and Lo SK. Vascular endothelial adhesion molecules and tissue inflammation. Pharmacol Rev. 1996; 48: 213-229.
- [23] Hallahan D, Geng L, Qu S, Scarfone C, Giorgio T, Donnelly E, Gao X and Clanton J. Integrinmediated targeting of drug delivery to irradiated tumor blood vessels. Cancer Cell. 2003; 3: 63-74.
- [24] Curnis F, Sacchi A and Corti A. Improving chemotherapeutic drug penetration in tumors by vascular targeting and barrier alteration. J Clin Invest 2002; 110: 475-482.
- [25] Meineke V, Gilbertz KP, Schilperoort K, Cordes N, Sendler A, Moede T and van Beuningen D. Ionizing radiation modulates cell surface integrin expression and adhesion of COLO-320 cells to collagen and fibronectin in vitro. Strahlenther Onkol. 2002; 178: 709-714.
- [26] Morel A, Fernandez N, de La Coste A, Haddada H, Viguier M, Polla BS, Antoine B and Kahn A. Gamma-ray irradiation induces B7.1 costimulatory molecule neoexpression in various murine tumor cells. Cancer Immunol Immunother. 1998; 46: 277-282.

- [27] Santin AD, Hiserodt JC, Fruehauf J, DiSaia PJ, Pecorelli S and Granger GA. Effects of irradiation on the expression of surface antigens in human ovarian cancer. Gynecol Oncol. 1996; 60: 468-474.
- [28] Sheard MA, Vojtesek B, Janakova L, Kovarik J and Zaloudik J. Up-regulation of Fas (CD95) in human p53wild-type cancer cells treated with ionizing radiation. Int J Cancer 1997; 73: 757-762.
- [29] Baker DG and Krochak RJ. The response of the microvascular system to radiation: a review. Cancer Invest. 1989; 7: 287-294.
- [30] Jain RK and Stylianopoulos T. Delivering nanomedicine to solid tumors. Nat Rev Clin Oncol 2010; 14: 14.
- [31] Tong RT, Boucher Y, Kozin SV, Winkler F, Hicklin DJ and Jain RK. Vascular normalization by vascular endothelial growth factor receptor 2 blockade induces a pressure gradient across the vasculature and improves drug penetration in tumors. Cancer Res. 2004; 64: 3731-3736.
- [32] Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science. 2005; 307: 58-62.
- [33] Netti PA, Berk DA, Swartz MA, Grodzinsky AJ and Jain RK. Role of extracellular matrix assembly in interstitial transport in solid tumors. Cancer Res. 2000; 60: 2497-2503.
- [34] Lugade AA, Moran JP, Gerber SA, Rose RC, Frelinger JG and Lord EM. Local radiation therapy of B16 melanoma tumors increases the generation of tumor antigen-specific effector cells that traffic to the tumor. J Immunol. 2005; 174: 7516-7523.
- [35] Kim JY, Son YO, Park SW, Bae JH, Chung JS, Kim HH, Chung BS, Kim SH and Kang CD. Increase of NKG2D ligands and sensitivity to NK cell-mediated cytotoxicity of tumor cells by heat shock and ionizing radiation. Exp Mol Med. 2006; 38: 474-484.
- [36] Perez CA, Fu A, Onishko H, Hallahan DE and Geng L. Radiation induces an antitumour immune response to mouse melanoma. Int J Radiat Biol. 2009; 85: 1126-1136.
- [37] Ciernik IF, Romero P, Berzofsky JA and Carbone DP. Ionizing radiation enhances immunogenicity of cells expressing a tumor-specific T-cell epitope. Int J Radiat Oncol Biol Phys. 1999; 45: 735-741.
- [38] Wang H, Yan H, Fu A, Han M, Hallahan D and Han Z. TIP-1 translocation onto the cell plasma membrane is a molecular biomarker of tumor response to ionizing radiation. PLoS One. 2010; 5: e12051.
- [39] Baluna RG, Eng TY and Thomas CR. Adhesion molecules in radiotherapy. Radiat Res. 2006; 166: 819-831.
- [40] Cunningham BA. Cell adhesion molecules and the regulation of development. Am J Obstet Gynecol. 1991; 164: 939-948.
- [41] Buckley CD, Rainger GE, Bradfield PF, Nash GB

and Simmons DL. Cell adhesion: more than just glue (review). Mol Membr Biol. 1998; 15: 167-176.

- [42] Skubitz AP. Adhesion molecules. Cancer Treat Res. 2002; 107: 305-329.
- [43] Hallahan DE and Virudachalam S. Accumulation of P-selectin in the lumen of irradiated blood vessels. Radiat Res. 1999; 152: 6-13.
- [44] Hariri G, Zhang Y, Fu A, Han Z, Brechbiel M, Tantawy MN, Peterson TE, Mernaugh R and Hallahan D. Radiation-guided P-selectin antibody targeted to lung cancer. Ann Biomed Eng 2008; 36: 821-830.
- [45] Harada S, Ehara S, Ishii K, Yamazaki H, Matsuyama N, Sato T, Kamiya T and Sera K. Imaging of Metastatic Potential and Inhibition of Metastasis using Two Types of Nanocapsules in Vivo. International Journal of Radiation Oncology Biology Physics 2010; 78: S648-S649.
- [46] Harada S, Ehara S, Sera K, Ishii K, Yamazaki H, Matsuyama N, Sato T, Kamiya T and Yokota S. Liquid-core Nanocapsules Delivered via Radiotherapy Inhibit Metastasis and Enhance Local Antitumor Effects. International Journal of Radiation Oncology Biology Physics 2009; 75: S92 -S93.
- [47] Gaugler MH, Squiban C, van der Meeren A, Bertho JM, Vandamme M and Mouthon MA. Late and persistent up-regulation of intercellular adhesion molecule-1 (ICAM-1) expression by ionizing radiation in human endothelial cells in vitro. Int J Radiat Biol. 1997; 72: 201-209.
- [48] Heckmann M, Douwes K, Peter R and Degitz K. Vascular activation of adhesion molecule mRNA and cell surface expression by ionizing radiation. Exp Cell Res. 1998; 238: 148-154.
- [49] Hallahan DE and Virudachalam S. Ionizing radiation mediates expression of cell adhesion molecules in distinct histological patterns within the lung. Cancer Res. 1997; 57: 2096-2099.
- [50] Prabhakarpandian B, Goetz DJ, Swerlick RA, Chen X and Kiani MF. Expression and functional significance of adhesion molecules on cultured endothelial cells in response to ionizing radiation. Microcirculation. 2001; 8: 355-364.
- [51] Handschel J, Prott FJ, Sunderkotter C, Metze D, Meyer U and Joos U. Irradiation induces increase of adhesion molecules and accumulation of beta2-integrin-expressing cells in humans. Int J Radiat Oncol Biol Phys. 1999; 45: 475-481.
- [52] Hallahan D, Kuchibhotla J and Wyble C. Cell adhesion molecules mediate radiation-induced leukocyte adhesion to the vascular endothelium. Cancer Res. 1996; 56: 5150-5155.
- [53] Santin AD, Rose GS, Hiserodt JC, Fruehauf J, Eck LM, Garcia RI, Schranz V, Disaia PJ, Pecorelli S and Granger GA. Effects of cytokines combined with high-dose gamma irradiation on the expression of major histocompatibility com-

plex molecules and intercellular adhesion molecule-1 in human ovarian cancers. Int J Cancer. 1996; 65: 688-694.

- [54] Santin AD, Hermonat PL, Hiserodt JC, Chiriva-Internati M, Woodliff J, Theus JW, Barclay D, Pecorelli S and Parham GP. Effects of irradiation on the expression of major histocompatibility complex class I antigen and adhesion costimulation molecules ICAM-1 in human cervical cancer. Int J Radiat Oncol Biol Phys. 1997; 39: 737-742.
- [55] Santin AD, Hermonat PL, Ravaggi A, Chiriva-Internati M, Hiserodt JC, Pecorelli S and Parham GP. Effects of retinoic acid combined with irradiation on the expression of major histocompatibility complex molecules and adhesion/ costimulation molecules ICAM-1 in human cervical cancer. Gynecol Oncol. 1998; 70: 195-201.
- [56] Konemann S, Malath J, Bolling T, Kolkmeyer A, Janke K, Riesenbeck D, Hesselmann S, Nguyen TP, Diallo R, Vormoor J, Willich N and Schuck A. Changed adhesion molecule profile of Ewing tumor cell lines and xenografts under the influence of ionizing radiation. Anticancer Res. 2004; 24: 1637-1644.
- [57] Chiriva-Internati M, Grizzi F, Pinkston J, Morrow KJ, D'Cunha N, Frezza EE, Muzzio PC, Kast WM and Cobos E. Gamma-radiation upregulates MHC class I/II and ICAM-I molecules in multiple myeloma cell lines and primary tumors. In Vitro Cell Dev Biol Anim. 2006; 42: 89-95.
- [58] Onoda JM, Kantak SS, Piechocki MP, Awad W, Chea R, Liu B and Honn KV. Inhibition of radiation-enhanced expression of integrin and metastatic potential in B16 melanoma cells by a lipoxygenase inhibitor. Radiat Res. 1994; 140: 410-418.
- [59] Nam JM, Chung Y, Hsu HC and Park CC. beta1 integrin targeting to enhance radiation therapy. Int J Radiat Biol 2009; 85: 923-928.
- [60] Wild-Bode C, Weller M, Rimner A, Dichgans J and Wick W. Sublethal irradiation promotes migration and invasiveness of glioma cells: implications for radiotherapy of human glioblastoma. Cancer Res 2001; 61: 2744-2750.
- [61] Akimoto T, Mitsuhashi N, Saito Y, Ebara T and Niibe H. Effect of radiation on the expression of E-cadherin and alpha-catenin and invasive capacity in human lung cancer cell line in vitro. Int J Radiat Oncol Biol Phys. 1998; 41: 1171-1176.
- [62] Bringuier PP, Umbas R, Schaafsma HE, Karthaus HF, Debruyne FM and Schalken JA. Decreased E-cadherin immunoreactivity correlates with poor survival in patients with bladder tumors. Cancer Res. 1993; 53: 3241-3245.
- [63] Umbas R, Schalken JA, Aalders TW, Carter BS, Karthaus HF, Schaafsma HE, Debruyne FM and Isaacs WB. Expression of the cellular adhesion molecule E-cadherin is reduced or absent in high-grade prostate cancer. Cancer Res. 1992;

52: 5104-5109.

- [64] Ebara T, Mitsuhashi N, Saito Y, Akimoto T and Niibe H. Change in E-cadherin expression after X-ray irradiation of a human cancer cell line in vitro and in vivo. Int J Radiat Oncol Biol Phys. 1998; 41: 669-674.
- [65] Finn OJ. Cancer vaccines: between the idea and the reality. Nat Rev Immunol. 2003; 3: 630-641.
- [66] Hauser SH, Calorini L, Wazer DE and Gattoni-Celli S. Radiation-enhanced expression of major histocompatibility complex class I antigen H-2Db in B16 melanoma cells. Cancer Res. 1993; 53: 1952-1955.
- [67] Reits EA, Hodge JW, Herberts CA, Groothuis TA, Chakraborty M, Wansley EK, Camphausen K, Luiten RM, de Ru AH, Neijssen J, Griekspoor A, Mesman E, Verreck FA, Spits H, Schlom J, van Veelen P and Neefjes JJ. Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy. J Exp Med. 2006; 203: 1259-1271. Epub 2006 Apr 1224.
- [68] Newcomb EW, Demaria S, Lukyanov Y, Shao Y, Schnee T, Kawashima N, Lan L, Dewyngaert JK, Zagzag D, McBride WH and Formenti SC. The combination of ionizing radiation and peripheral vaccination produces long-term survival of mice bearing established invasive GL261 gliomas. Clin Cancer Res. 2006; 12: 4730-4737.
- [69] Bubenik J. Tumour MHC class I downregulation and immunotherapy (Review). Oncol Rep. 2003; 10: 2005-2008.
- [70] Kaminski JM, Shinohara E, Summers JB, Niermann KJ, Morimoto A and Brousal J. The controversial abscopal effect. Cancer Treat Rev. 2005; 31: 159-172.
- [71] Bhat S and Czuczman MS. Galiximab: a review. Expert Opin Biol Ther. 2010; 10: 451-458.
- [72] Cheson BD. Ofatumumab, a novel anti-CD20 monoclonal antibody for the treatment of B-cell malignancies. J Clin Oncol. 2010; 28: 3525-3530. Epub 2010 May 3510.
- [73] Sikder MA and Friedberg JW. Beyond rituximab: The future of monoclonal antibodies in B-cell non-Hodgkin lymphoma. Curr Hematol Malig Rep. 2008; 3: 187-193.
- [74] Goldsmith SJ. Radioimmunotherapy of lymphoma: Bexxar and Zevalin. Semin Nucl Med. 2010; 40: 122-135.
- [75] Tedder TF and Engel P. CD20: a regulator of cell-cycle progression of B lymphocytes. Immunol Today. 1994; 15: 450-454.
- [76] Palanca-Wessels MC and Press OW. Improving the efficacy of radioimmunotherapy for non-Hodgkin lymphomas. Cancer. 2010; 116: 1126 -1133.
- [77] Kunala S and Macklis RM. Ionizing radiation induces CD20 surface expression on human B cells. Int J Cancer. 2001; 96: 178-181.
- [78] Gupta D, Crosby ME, Almasan A and Macklis RM. Regulation of CD20 expression by radia-

tion-induced changes in intracellular redox status. Free Radic Biol Med. 2008; 44: 614-623. Epub 2007 Nov 2001.

- [79] Luce A, Courtin A, Levalois C, Altmeyer-Morel S, Romeo PH, Chevillard S and Lebeau J. Death receptor pathways mediate targeted and nontargeted effects of ionizing radiations in breast cancer cells. Carcinogenesis. 2009; 30: 432-439. Epub 2009 Jan 2006.
- [80] Strasser A, Jost PJ and Nagata S. The many roles of FAS receptor signaling in the immune system. Immunity. 2009; 30: 180-192.
- [81] Ishikawa E, Tsuboi K, Saijo K, Takano S and Ohno T. X-irradiation to human malignant glioma cells enhances the cytotoxicity of autologous killer lymphocytes under specific conditions. Int J Radiat Oncol Biol Phys. 2004; 59: 1505-1512.
- [82] Ogawa Y, Nishioka A, Hamada N, Terashima M, Inomata T, Yoshida S, Seguchi H and Kishimoto S. Expression of fas (CD95/APO-1) antigen induced by radiation therapy for diffuse B-cell lymphoma: immunohistochemical study. Clin Cancer Res. 1997; 3: 2211-2216.
- [83] Abdulkarim B, Sabri S, Deutsch E, Vaganay S, Marangoni E, Vainchenker W, Bongrand P, Busson P and Bourhis J. Radiation-induced expression of functional Fas ligand in EBV-positive human nasopharyngeal carcinoma cells. Int J Cancer. 2000; 86: 229-237.
- [84] Hymowitz SG, Christinger HW, Fuh G, Ultsch M, O'Connell M, Kelley RF, Ashkenazi A and de Vos AM. Triggering cell death: the crystal structure of Apo2L/TRAIL in a complex with death receptor 5. Mol Cell. 1999; 4: 563-571.
- [85] Chinnaiyan AM, Prasad U, Shankar S, Hamstra DA, Shanaiah M, Chenevert TL, Ross BD and Rehemtulla A. Combined effect of tumor necrosis factor-related apoptosis-inducing ligand and ionizing radiation in breast cancer therapy. Proc Natl Acad Sci U S A 2000; 97: 1754-1759.
- [86] Hori T, Kondo T, Kanamori M, Tabuchi Y, Ogawa R, Zhao QL, Ahmed K, Yasuda T, Seki S, Suzuki K and Kimura T. Ionizing radiation enhances tumor necrosis factor-related apoptosisinducing ligand (TRAIL)-induced apoptosis through up-regulations of death receptor 4 (DR4) and death receptor 5 (DR5) in human osteosarcoma cells. J Orthop Res. 2010; 28: 739-745.
- [87] Nagane M, Shimizu S, Mori E, Kataoka S and Shiokawa Y. Predominant antitumor effects by fully human anti-TRAIL-receptor2 (DR5) monoclonal antibodies in human glioma cells in vitro and in vivo. Neuro Oncol 2010;
- [88] Kim H, Folks KD, Guo L, Stockard CR, Fineberg NS, Grizzle WE, George JF, Buchsbaum DJ, Morgan DE and Zinn KR. DCE-MRI Detects Early Vascular Response in Breast Tumor Xenografts Following Anti-DR5 Therapy. Mol Imaging Biol 2010;
- [89] Straughn JM, Jr., Oliver PG, Zhou T, Wang W,

Alvarez RD, Grizzle WE and Buchsbaum DJ. Anti -tumor activity of TRA-8 anti-death receptor 5 (DR5) monoclonal antibody in combination with chemotherapy and radiation therapy in a cervical cancer model. Gynecol Oncol. 2006; 101: 46-54. Epub 2005 Nov 2004.

- [90] Wu F, Hu Y, Long J, Zhou YJ, Zhong YH, Liao ZK, Liu SQ, Zhou FX, Zhou YF and Xie CH. Cytotoxicity and radiosensitization effect of TRA-8 on radioresistant human larynx squamous carcinoma cells. Oncol Rep. 2009; 21: 461-465.
- [91] Lahorte CM, Vanderheyden JL, Steinmetz N, Van de Wiele C, Dierckx RA and Slegers G. Apoptosis-detecting radioligands: current state of the art and future perspectives. Eur J Nucl Med Mol Imaging 2004; 31: 887-919.
- [92] Schutters K and Reutelingsperger C. Phosphatidylserine targeting for diagnosis and treatment of human diseases. Apoptosis 2010; 15: 1072-1082.
- [93] Guo MF, Zhao Y, Tian R, Li L, Guo L, Xu F, Liu YM, He YB, Bai S and Wang J. In vivo99mTc-HYNIC-annexin V imaging of early tumor apoptosis in mice after single dose irradiation. J Exp Clin Cancer Res 2009; 28: 136.
- [94] Haas RL, de Jong D, Valdes Olmos RA, Hoefnagel CA, van den Heuvel I, Zerp SF, Bartelink H and Verheij M. In vivo imaging of radiationinduced apoptosis in follicular lymphoma patients. Int J Radiat Oncol Biol Phys 2004; 59: 782-787.
- [95] Hoebers FJ, Kartachova M, de Bois J, van den Brekel MW, van Tinteren H, van Herk M, Rasch CR, Valdes Olmos RA and Verheij M. 99mTc Hynic-rh-Annexin V scintigraphy for in vivo imaging of apoptosis in patients with head and neck cancer treated with chemoradiotherapy. Eur J Nucl Med Mol Imaging 2008; 35: 509-518.
- [96] Yang DJ, Azhdarinia A, Wu P, Yu DF, Tansey W, Kalimi SK, Kim EE and Podoloff DA. In vivo and in vitro measurement of apoptosis in breast cancer cells using 99mTc-EC-annexin V. Cancer Biother Radiopharm 2001; 16: 73-83.
- [97] Belhocine T, Steinmetz N, Hustinx R, Bartsch P, Jerusalem G, Seidel L, Rigo P and Green A. Increased uptake of the apoptosis-imaging agent (99m)Tc recombinant human Annexin V in human tumors after one course of chemotherapy as a predictor of tumor response and patient prognosis. Clin Cancer Res 2002; 8: 2766-2774.
- [98] Lee HK, Xiang C, Cazacu S, Finniss S, Kazimirsky G, Lemke N, Lehman NL, Rempel SA, Mikkelsen T and Brodie C. GRP78 is overexpressed in glioblastomas and regulates glioma cell growth and apoptosis. Neuro Oncol. 2008; 10: 236-243. Epub 2008 Apr 2010.
- [99] Gonzalez-Gronow M, Selim MA, Papalas J and Pizzo SV. GRP78: a multifunctional receptor on the cell surface. Antioxid Redox Signal. 2009; 11: 2299-2306.
- [100] Fu Y and Lee AS. Glucose regulated proteins in

cancer progression, drug resistance and immunotherapy. Cancer Biol Ther. 2006; 5: 741-744. Epub 2006 Jul 2001.

- [101] Kim Y, Lillo AM, Steiniger SC, Liu Y, Ballatore C, Anichini A, Mortarini R, Kaufmann GF, Zhou B, Felding-Habermann B and Janda KD. Targeting heat shock proteins on cancer cells: selection, characterization, and cell-penetrating properties of a peptidic GRP78 ligand. Biochemistry. 2006; 45: 9434-9444.
- [102] Katanasaka Y, Ishii T, Asai T, Naitou H, Maeda N, Koizumi F, Miyagawa S, Ohashi N and Oku N. Cancer antineovascular therapy with liposome drug delivery systems targeted to BiP/GRP78. Int J Cancer 2010; 22: 22.
- [103] Obeid M, Panaretakis T, Joza N, Tufi R, Tesniere A, van Endert P, Zitvogel L and Kroemer G. Calreticulin exposure is required for the immunogenicity of gamma-irradiation and UVC lightinduced apoptosis. Cell Death Differ. 2007; 14: 1848-1850. Epub 2007 Jul 1827.
- [104] Zitvogel L, Kepp O, Senovilla L, Menger L, Chaput N and Kroemer G. Immunogenic tumor cell death for optimal anticancer therapy: the calreticulin exposure pathway. Clin Cancer Res 2010; 16: 3100-3104.
- [105] Obeid M. ERP57 membrane translocation dictates the immunogenicity of tumor cell death by controlling the membrane translocation of calreticulin. J Immunol 2008; 181: 2533-2543.
- [106] Obeid M. Anticancer activity of targeted proapoptotic peptides and chemotherapy is highly improved by targeted cell surface calreticulin-inducer peptides. Mol Cancer Ther 2009; 8: 2693-2707.
- [107] Duffy MJ. Carcinoembryonic antigen as a marker for colorectal cancer: is it clinically useful? Clin Chem 2001; 47: 624-630.
- [108] Hodge JW. Carcinoembryonic antigen as a target for cancer vaccines. Cancer Immunol Immunother 1996; 43: 127-134.
- [109] Matsumoto H, Takahashi T, Mitsuhashi N, Higuch K and Niibe H. Modification of tumorassociated antigen (CEA) expression of human lung cancer cells by irradiation, either alone or in combination with interferon-gamma. Anticancer Res 1999; 19: 307-311.
- [110] Liu Z, Jin C, Yu Z, Zhang J, Liu Y, Zhao H, Jia B and Wang F. Radioimmunotherapy of human colon cancer xenografts with 131I-labeled anti-CEA monoclonal antibody. Bioconjug Chem 2010; 21: 314-318.
- [111] Hu CM, Kaushal S, Cao HS, Aryal S, Sartor M, Esener S, Bouvet M and Zhang L. Half-antibody functionalized lipid-polymer hybrid nanoparticles for targeted drug delivery to carcinoembryonic antigen presenting pancreatic cancer cells. Mol Pharm 2010; 7: 914-920.
- [112] Alewine C, Olsen O, Wade JB and Welling PA. TIP-1 has PDZ scaffold antagonist activity. Mol Biol Cell 2006; 17: 4200-4211.
- [113] Hariri G, Yan H, Wang H, Han Z and Hallahan

DE. Nanoparticle Albumin Bound Paclitaxel Retargeted to Radiation Inducible TIP-1 in Cancer. Clin Cancer Res 2010; 27: 27.

- [114] Hariri G, Wellons MS, Morris WH, 3rd, Lukehart CM and Hallahan DE. Multifunctional FePt Nanoparticles for Radiation-Guided Targeting and Imaging of Cancer. Ann Biomed Eng 2010;
- [115] Lowery A, Onishko H, Hallahan DE and Han Z. Tumor-targeted delivery of liposomeencapsulated doxorubicin by use of a peptide that selectively binds to irradiated tumors. J Control Release 2010;
- [116] Altmeyer A, Maki RG, Feldweg AM, Heike M, Protopopov VP, Masur SK and Srivastava PK. Tumor-specific cell surface expression of the-KDEL containing, endoplasmic reticular heat shock protein gp96. Int J Cancer. 1996; 69: 340-349.
- [117] Deutscher SL. Phage display in molecular imaging and diagnosis of cancer. Chem Rev 2010; 110: 3196-3211.
- [118] Han Z, Fu A, Wang H, Diaz R, Geng L, Onishko H and Hallahan DE. Noninvasive assessment of cancer response to therapy. Nat Med 2008; 14: 343-349.
- [119] Li ZJ and Cho CH. Development of peptides as potential drugs for cancer therapy. Curr Pharm Des. 2010; 16: 1180-1189.
- [120] Jaboin JJ, Han Z and Hallahan DE. Using in vivo biopanning for the development of radiationguided drug delivery systems. Methods Mol Biol. 2009; 542: 285-300.
- [121] Chen ZG. Small-molecule delivery by nanoparticles for anticancer therapy. Trends Mol Med 2010; 14: 14.
- [122] Shishido T, Mieda H, Hwang SY, Nishimura Y, Tanaka T, Ogino C, Fukuda H and Kondo A. Affibody-displaying bionanocapsules for specific drug delivery to HER2-expressing cancer cells. Bioorg Med Chem Lett. 2010; 20: 5726-5731. Epub 2010 Aug 5726.
- [123] Chertok B, Moffat BA, David AE, Yu F, Bergemann C, Ross BD and Yang VC. Iron oxide nanoparticles as a drug delivery vehicle for MRI monitored magnetic targeting of brain tumors. Biomaterials 2008; 29: 487-496.
- [124] Bhojani MS, Van Dort M, Rehemtulla A and Ross BD. Targeted imaging and therapy of brain cancer using theranostic nanoparticles. Mol Pharm 2010; 7: 1921-1929.
- [125] Wang AZ, Bagalkot V, Vasilliou CC, Gu F, Alexis F, Zhang L, Shaikh M, Yuet K, Cima MJ, Langer R, Kantoff PW, Bander NH, Jon S and Farokhzad OC. Superparamagnetic iron oxide nanoparticle-aptamer bioconjugates for combined prostate cancer imaging and therapy. ChemMedChem 2008; 3: 1311-1315.
- [126] Zou P, Yu Y, Wang YA, Zhong Y, Welton AR, Galban C, Wang S and Sun D. Superparamagnetic iron oxide "nanotheranostics" for targeted cancer cell imaging and pH-dependent intracellular drug release. Mol Pharm 2010; 16: 16.

- [127] Maeng JH, Lee DH, Jung KH, Bae YH, Park IS, Jeong S, Jeon YS, Shim CK, Kim W, Kim J, Lee J, Lee YM, Kim JH, Kim WH and Hong SS. Multifunctional doxorubicin loaded superparamagnetic iron oxide nanoparticles for chemotherapy and magnetic resonance imaging in liver cancer. Biomaterials. 2010; 31: 4995-5006. Epub 2010 Mar 4926.
- [128] Hadjipanayis CG, Machaidze R, Kaluzova M, Wang L, Schuette AJ, Chen H, Wu X and Mao H. EGFRvIII antibody-conjugated iron oxide nanoparticles for magnetic resonance imagingguided convection-enhanced delivery and targeted therapy of glioblastoma. Cancer Res 2010; 70: 6303-6312.
- [129] Rasmussen JW, Martinez E, Louka P and Wingett DG. Zinc oxide nanoparticles for selective destruction of tumor cells and potential for drug delivery applications. Expert Opin Drug Deliv. 2010; 7: 1063-1077.
- [130] Bharali DJ and Mousa SA. Emerging nanomedicines for early cancer detection and improved treatment: Current perspective and future promise. Pharmacol Ther 2010; 10: 10.
- [131] Stacy DR, Lu B and Hallahan DE. Radiationguided drug delivery systems. Expert Rev Anticancer Ther. 2004; 4: 283-288.
- [132] Burdick MJ and Macklis RM. Update on the rational use of tositumomab and iodine-131 tositumomab radioimmunotherapy for the treatment of non-Hodgkin's lymphoma. Onco Targets Ther. 2009; 2: 229-242.
- [133] Walter RB, Press OW and Pagel JM. Pretargeted radioimmunotherapy for hematologic and other malignancies. Cancer Biother Radiopharm. 2010; 25: 125-142.
- [134] Pagel JM. Radioimmunotherapeutic approaches for leukemia: the past, present and future. Cytotherapy 2008; 10: 13-20.
- [135] Breitz HB, Weiden PL, Beaumier PL, Axworthy DB, Seiler C, Su FM, Graves S, Bryan K and Reno JM. Clinical optimization of pretargeted radioimmunotherapy with antibody-streptavidin conjugate and 90Y-DOTA-biotin. J Nucl Med. 2000; 41: 131-140.
- [136] Kraeber-Bodere F, Bardet S, Hoefnagel CA, Vieira MR, Vuillez JP, Murat A, Ferreira TC, Bardies M, Ferrer L, Resche I, Gautherot E, Rouvier E, Barbet J and Chatal JF. Radioimmunotherapy in medullary thyroid cancer using bispecific antibody and iodine 131-labeled bivalent hapten: preliminary results of a phase I/II clinical trial. Clin Cancer Res. 1999; 5: 3190s-3198s.
- [137] Weiden PL and Breitz HB. Pretargeted radioimmunotherapy (PRIT) for treatment of non-Hodgkin's lymphoma (NHL). Crit Rev Oncol Hematol. 2001; 40: 37-51.
- [138] Forero A, Weiden PL, Vose JM, Knox SJ, LoBuglio AF, Hankins J, Goris ML, Picozzi VJ, Axworthy DB, Breitz HB, Sims RB, Ghalie RG, Shen S and Meredith RF. Phase 1 trial of a novel anti-CD20 fusion protein in pretargeted

radioimmunotherapy for B-cell non-Hodgkin lymphoma. Blood. 2004; 104: 227-236. Epub 2004 Mar 2002.

- [139] Vuillez JP, Kraeber-Bodere F, Moro D, Bardies M, Douillard JY, Gautherot E, Rouvier E, Barbet J, Garban F, Moreau P and Chatal JF. Radioimmunotherapy of small cell lung carcinoma with the two-step method using a bispecific anticarcinoembryonic antigen/antidiethylenetriaminepentaacetic acid (DTPA) antibody and iodine-131 Di-DTPA hapten: results of a phase I/II trial. Clin Cancer Res. 1999; 5: 3259s-3267s.
- [140] Grana C, Chinol M, Robertson C, Mazzetta C, Bartolomei M, De Cicco C, Fiorenza M, Gatti M, Caliceti P and Paganelli G. Pretargeted adjuvant radioimmunotherapy with yttrium-90-biotin in malignant glioma patients: a pilot study. Br J Cancer. 2002; 86: 207-212.
- [141] Jaffer FA and Weissleder R. Molecular Imaging in the Clinical Arena. JAMA 2005; 293: 855-862.
- [142] Tempany CM and McNeil BJ. Advances in biomedical imaging. JAMA 2001; 285: 562-567.
- [143] Nimmagadda S, Ford EC, Wong JW and Pomper MG. Targeted molecular imaging in oncology: focus on radiation therapy. Semin Radiat Oncol 2008; 18: 136-148.
- [144] Beer AJ, Niemeyer M, Carlsen J, Sarbia M, Nahrig J, Watzlowik P, Wester HJ, Harbeck N and Schwaiger M. Patterns of alphavbeta3 expression in primary and metastatic human breast cancer as shown by 18F-Galacto-RGD PET. J Nucl Med 2008; 49: 255-259.
- [145] Morrison MS, Ricketts SA, Barnett J, Cuthbertson A, Tessier J and Wedge SR. Use of a novel Arg-Gly-Asp radioligand, 18F-AH111585, to determine changes in tumor vascularity after antitumor therapy. J Nucl Med 2009; 50: 116-122.
- [146] Haubner R and Decristoforo C. Radiolabelled RGD peptides and peptidomimetics for tumour targeting. Front Biosci 2009; 14: 872-886.
- [147] Liu S. Radiolabeled Cyclic RGD Peptides as Integrin alpha(v)beta(3)-Targeted Radiotracers: Maximizing Binding Affinity via Bivalency. Bioconjug Chem 2009;
- [148] Janssen M, Oyen WJ, Massuger LF, Frielink C, Dijkgraaf I, Edwards DS, Radjopadhye M, Corstens FH and Boerman OC. Comparison of a monomeric and dimeric radiolabeled RGDpeptide for tumor targeting. Cancer Biother Radiopharm 2002; 17: 641-646.
- [149] Jung KH, Lee KH, Paik JY, Ko BH, Bae JS, Lee BC, Sung HJ, Kim DH, Choe YS and Chi DY. Favorable biokinetic and tumor-targeting properties of 99mTc-labeled glucosamino RGD and effect of paclitaxel therapy. J Nucl Med 2006; 47: 2000-2007.
- [150] Dumont RA, Hildebrandt I, Su H, Haubner R, Reischl G, Czernin JG, Mischel PS and Weber WA. Noninvasive imaging of alphaVbeta3 func-

tion as a predictor of the antimigratory and antiproliferative effects of dasatinib. Cancer Res 2009; 69: 3173-3179.

- [151] Haubner R, Weber WA, Beer AJ, Vabuliene E, Reim D, Sarbia M, Becker KF, Goebel M, Hein R, Wester HJ, Kessler H and Schwaiger M. Noninvasive visualization of the activated alphavbeta3 integrin in cancer patients by positron emission tomography and [18F]Galacto-RGD. PLoS Med 2005; 2: e70.
- [152] Haubner R, Wester HJ, Weber WA, Mang C, Ziegler SI, Goodman SL, Senekowitsch-Schmidtke R, Kessler H and Schwaiger M. Noninvasive imaging of alpha(v)beta3 integrin expression using 18F-labeled RGD-containing glycopeptide and positron emission tomography. Cancer Res 2001; 61: 1781-1785.
- [153] Kenny LM, Coombes RC, Oulie I, Contractor KB, Miller M, Spinks TJ, McParland B, Cohen PS, Hui AM, Palmieri C, Osman S, Glaser M, Turton D, Al-Nahhas A and Aboagye EO. Phase I trial of the positron-emitting Arg-Gly-Asp (RGD) peptide radioligand 18F-AH111585 in breast cancer patients. J Nucl Med 2008; 49: 879-886.
- [154] Funovics M, Montet X, Reynolds F, Weissleder R and Josephson L. Nanoparticles for the optical imaging of tumor E-selectin. Neoplasia 2005; 7: 904-911.
- [155] Chapman PT, Jamar F, Keelan ETM, Peters AM and Haskard DO. Use of a radiolabeled monoclonal antibody against e-selectin for imaging of endothelial activation in rheumatoid arthritis. Arthritis & Rheumatism 1996; 39: 1371-1375.
- [156] Keelan ETM, Harrison AA, Chapman PT, Binns RM, Peters AM and Haskard DO. Imaging Vascular Endothelial Activation: An Approach Using Radiolabeled Monoclonal Antibodies Against the Endothelial Cell Adhesion Molecule E-Selectin. J Nucl Med 1994; 35: 276-281.
- [157] Kaufmann BA, Sanders JM, Davis C, Xie A, Aldred P, Sarembock IJ and Lindner JR. Molecular Imaging of Inflammation in Atherosclerosis With Targeted Ultrasound Detection of Vascular Cell Adhesion Molecule-1. Circulation 2007; 116: 276-284.
- [158] Kim K, Huang S-W, Ashkenazi S, O'Donnell M, Agarwal A, Kotov NA, Denny MF and Kaplan MJ. Photoacoustic imaging of early inflammatory response using gold nanorods. Applied Physics Letters 2007; 90: 223901.
- [159] Nahrendorf M, Keliher E, Panizzi P, Zhang H, Hembrador S, Figueiredo J-L, Aikawa E, Kelly K, Libby P and Weissleder R. 18F-4V for PET-CT Imaging of VCAM-1 Expression in Atherosclerosis. J Am Coll Cardiol Img 2009; 2: 1213-1222.
- [160] Tsourkas A, Shinde-Patil VR, Kelly KA, Patel P, Wolley A, Allport JR and Weissleder R. In Vivo Imaging of Activated Endothelium Using an Anti -VCAM-1 Magnetooptical Probe. Bioconjug Chem 2005; 16: 576-581.
- [161] Villanueva FS, Jankowski RJ, Klibanov S, Pina ML, Alber SM, Watkins SC, Brandenburger GH

and Wagner WR. Microbubbles Targeted to Intercellular Adhesion Molecule-1 Bind to Activated Coronary Artery Endothelial Cells. Circulation 1998; 98: 1-5.

- [162] Weller GER, Lu E, Csikari MM, Klibanov AL, Fischer D, Wagner WR and Villanueva FS. Ultrasound Imaging of Acute Cardiac Transplant Rejection With Microbubbles Targeted to Intercellular Adhesion Molecule-1. Circulation 2003; 108: 218-224.
- [163] Blankenberg FG. Monitoring of treatmentinduced apoptosis in oncology with PET and SPECT. Curr Pharm Des 2008; 14: 2974-2982.
- [164] Zhao M, Beauregard DA, Loizou L, Davletov B and Brindle KM. Non-invasive detection of apoptosis using magnetic resonance imaging and a targeted contrast agent. Nat Med 2001; 7: 1241-1244.
- [165] Van de Wiele C, Lahorte C, Vermeersch H, Loose D, Mervillie K, Steinmetz ND, Vanderheyden J-L, Cuvelier CA, Slegers G and Dierck RA. Quantitative Tumor Apoptosis Imaging Using

Technetium-99m-HYNIC Annexin V Single Photon Emission Computed Tomography. J Clin Oncol 2003; 21: 3483-3487.

- [166] Belhocine T, Steinmetz N, Hustinx R, Bartsch P, Jerusalem G, Seidel L, Rigo P and Green A. Increased Uptake of the Apoptosis-imaging Agent 99mTc Recombinant Human Annexin V in Human Tumors after One Course of Chemotherapy as a Predictor of Tumor Response and Patient Prognosis. Clinical Cancer Research 2002; 8: 2766-2774.
- [167] Kartachova M, Haas RL, Olmos RA, Hoebers FJ, van Zandwijk N and Verheij M. In vivo imaging of apoptosis by 99mTc-Annexin V scintigraphy: visual analysis in relation to treatment response. Radiother Oncol 2004; 72: 333-339.