Published in "Clinical Cancer Research
doi: 10.1158/1078-0432.CCR-11-1758, 2012"
which should be cited to refer to this work.

Molecular Pathways :

Emerging Pathways Mediating Growth, Invasion And Metastasis Of Tumors Progressing In An Irradiated Microenvironment

François Kuonen [1,2], Chiara Secondini [2,3] and Curzio Rüegg [2,3]

Authors' affiliations:

1. Department of Medicine, Centre Hospitalier Universitaire Vaudois (CHUV) and University of Lausanne (UNIL), Lausanne, Switzerland

2. National Center for Competence in Research (NCCR) Molecular Oncology, Swiss Institute of Experimental Cancer Research, Ecole Polytechnique Fédérale de Lausanne (ISREC-EPFL), Lausanne, Switzerland

3. Pathology, Department of Medicine, Faculty of Science, University of Fribourg, Fribourg (UNIFR), Switzerland

Corresponding author: Curzio Rüegg, Chair of Pathology, Department of Medicine, Faculty of Science, University of Fribourg, 1 Rue Albert Gockel, CH-1700 Fribourg, Switzerland; Phone: +41-26-300-8766; Fax: +41-26-300-9732.

Running title: Radiation-induced tumor progression

Conflicts of interest : CR is cofounder and stock holder of Diagnoplex SA ; FK and CS have no conflicts of interest.

Abstract

Radiotherapy is a well-established therapeutic modality in oncology. It provides survival benefits in several different cancer types. However, cancers relapsing after radiotherapy often develop into more aggressive conditions, which are difficult to treat and are associated with poor prognosis. Cumulating experimental evidence indicates that the irradiated tumor bed contributes to such aggressive behavior. The involved mechanisms have long remained elusive. Recent progress in the field revealed previously unrecognized cellular and molecular events promoting growth, invasion and metastasis of tumors progressing in an irradiated microenvironment. Cellular mechanisms include inhibition of sprouting angiogenesis, generation of hypoxia, activation and differentiation of stromal cells and recruitment of bone marrow-derived cells with vasculogenic and pro-metastatic activities. Identified pathways include TGFβ/ALK5, CXCL12/CXCR4, KITL/KIT and CYR61/αVβ5 integrin. The availability of pharmacological inhibitors impinging on these pathways opens novel opportunities for translational and clinical studies. These experimental results and ongoing work highlight the importance of the irradiated microenvironment in modulating the tumor response to radiotherapy and open new opportunities for the development of novel therapeutic strategies for cancer patients relapsing after radiotherapy. Here we review and discuss recent advances in the field and their translational and therapeutic implications to human cancer treatment.

Background

Radiotherapy plays a prominent role in the treatment of various tumor types and provides significant survival benefits in many cancers, including of the breast, prostate, rectum, brain, lung, and head and neck (1). In breast cancer, radiotherapy, alone or in combination with chemotherapy, is widely used as adjuvant treatment after breast-saving surgery to reduce the incidence of loco-regional and distant recurrences (2). The therapeutic effects of radiotherapy are traditionally considered as due to the induction of double strand DNA breaks in cancer cells causing cell-cycle arrest, senescence or apoptosis (3). Consistent with this view, efforts aimed at understanding and improving the therapeutic efficacy of radiotherapy largely concentrated on the study of mechanisms of DNA damage and repair (4). It is now becoming increasingly evident that ionizing radiation also induces modifications of the tumor microenvironment, which profoundly impact tumor biology (5). This is particularly relevant to cancers relapsing after radiotherapy, which tend to develop into invasive and metastatic conditions with poor prognosis (6). Cumulating experimental evidence indicates that the irradiated tumor microenvironment actively contributes to such aggressive behavior. Here we review and discuss recent advances in unraveling cellular events and molecular pathways of the tumor microenvironment modulated by ionizing radiation and affecting tumor growth, invasion and metastasis.

The tumor bed effect. Experimental tumors implanted in a pre-irradiated bed grow with slower kinetics, an effect originally referred to as the tumor bed effect (TBE) (7, 8). The TBE is dose dependent between 5 and 20 Gy single doses (9), occurs with

fractionated therapy (e.g. daily doses of 2Gy) (10) and within broad time intervals (0-90 days) between irradiation and tumor implantation (11) and is cell line dependent (10). A second element of the TBE is enhanced invasion and metastasis, which might seem paradoxical considering the reduced primary tumor growth (6 , 12). Enhanced metastasis was observed regardless of whether the analysis was metachronic (i.e. equivalent tumor sizes at different time points) (12) or synchronic (i.e. different tumor sizes at the same time point) (13 , 14). The TBE is a local effect, as it is only observed for tumors injected inside of the irradiated bed (14). These observations are of clinical relevance, since adjuvant radiotherapy improves local tumor control but tumor recurrences within a pre-irradiated field are often associated with an elevated risk of metastasis and poor prognosis (2, 15-17).

Inhibition of sprouting angiogenesis. Several microenvironmental events have been linked to the TBE, most notably the decrease in tumor vascularity (18-20). We know today that radiotherapy-induced modifications of tumor vasculature are due to direct effects of ionizing radiations on endothelial cells. Garcia-Barros *et al.,* showed that high-dose ionizing radiation induces ceramide-mediated apoptosis of tumor-associated endothelial cells, causing tumor vessel disruption and delayed tumor growth. Preventing ceramide-mediated endothelial cell apoptosis attenuated these effects, indicating that endothelial cells are a therapeutically-relevant target of radiotherapy (21). We showed that ionizing radiation suppresses *de novo* angiogenesis by inhibiting endothelial cell proliferation, migration and sprouting and by causing premature senescence, in part mediated by the TGFβ/ALK5 pathway (22). ALK5 inhibition rescued radiation-induced cell sprouting and migration defects *in vitro* and restored angiogenesis *in vivo* (22). In spite of inhibited sprouting angiogenesis,

vessels can still form, to a certain extent, in irradiated tumors. Hlushchuk *et al.,* demonstrated that intussusceptive angiogenesis replaces sprouting angiogenesis in irradiated tumors to support growth of surviving tumor cells (23). The resulting microvascular density is, however, reduced by 30% to 40% compared to non-irradiated tumors. The molecular mechanisms of intussusceptive angiogenesis remain largely unknown.

Hypoxia, vasculogenesis, invasion and metastasis. Hypoxia consequent to impaired angiogenesis is considered as a major cause of the TBE (13, 14, 24). Indeed, tumor hypoxia is associated with a shorter disease-free survival in many human cancers (25). Many molecular mechanisms induced by hypoxia and promoting cancer progression have been unraveled (26), including in tumor growing in irradiated beds. The Brown laboratory reported that hypoxia-mediated activation of hypoxia inducible factor (HIF) -1 in glioblastoma cells after radiotherapy leads to increased expression of the chemokine CXCL12 (27). CXCL12 stimulates the recruitment of $CXCR4^+CD11b^+$ BMDC into irradiated tumors to promote MMP-9-dependent blood vessel formation by vasculogenesis, which sufficient to support the growth of recurring tumors (27, 28). Using an orthotopic TBE model of breast cancer, we observed HIF-dependent induction of KitL in hypoxic tumors, causing the recruitment of bone marrow-derived pro-metastatic Kit^+CD11b^+ BMDC to primary tumors and pre-metastatic lungs (29). Using a subcutaneous model of TBE we observed that recovered tumor cells expanded *in vitro* and re-injected in non-irradiated mice retained enhanced metastatic capacity (14). The cysteine-rich protein 61 (CYR61), a matricellullar protein that regulates cell growth, differentiation, survival, and migration (30), and the adhesion receptor integrin $\alpha V\beta 5$ emerged as two

molecules cooperating to enhance metastasis. Importantly, CYR61 and αVβ5 promoted resistance to hypoxia, although they were not induced by hypoxia (14). These results support the notion that besides HIF-depended adaptive reactions to hypoxia, selection of hypoxia-resistant cancer cells with superior metastatic capacities also contributes to the TBE (31).

Hypoxia-independent mechanisms. Ionizing radiation induces the generation of reactive oxygen/nitrogen species (ROS/RNS). When exceeding the cellular anti-oxidants defense, ROS/RNS induce damages to DNA, proteins and lipids resulting in cell cycle arrest, apoptosis, cell activation or differentiation (32). ROS/RNS also regulate cellular functions by acting as messenger molecules in signaling pathways and by direct effects on transcription (33). ROS/RNS also modify production and activation of transforming growth factor (TGF) β1 (34, 35). Since ROS/RNS are rapidly induced, and persist over time by self-amplification, they are considered as main mediators of sustained radiation-modifications of the microenvironment, in particular fibrosis (desmoplastic reaction) (36). Ionizing radiation induces expression of growth and inflammatory factors and matrix proteins (5). For example, ionizing radiation induces endothelial cell activation resulting in the activation of the pro-inflammatory pathways NF-kB (37), the expression of pro-coagulant proteins (e.g. thrombomodulin, von Willebrand factor), and leukocyte endothelial-cell adhesion molecules (e.g. intercellular adhesion molecule-1, vascular endothelial cell adhesion molecule-1) (6, 38). Irradiated fibroblasts differentiate into myofibroblasts (39) producing tissue-specific collagens, growth factors and cytokines, such as platelet-derived growth factor (PDGF), interleukin (IL) 1β, tumor necrosis factor (TNF) and TGFβ (5, 6, 40). Irradiated fibroblasts enhance the invasive capacity of co-cultured

pancreatic cancer cells (41). This effect was attributed to fibroblast-induced activation of MET, a receptor tyrosine kinase promoting cell growth and invasiveness through the Ras/mitogen activated protein kinase (MAPK) pathway (42). While further experiments are needed to confirm the role of MET in the TBE, recent evidence indicates that ionizing radiations induces MET expression in cancer cells, via the ataxia telangiectasia mutated (ATM) and nuclear factor (NF) -κB signaling pathways, resulting in ligand-independent MET activation and enhanced invasiveness (43). Among all radiation-induced cytokines, TGFβ is of particular relevance, since it elicits strong and long-lasting microenvironmental changes (*e.g.* suppressed angiogenesis, inhibited immune response, fibrosis) and tumor reactions (*e.g.* invasiveness, epithelial-to-mesenchymal transition) concurring to promote carcinogenesis and accelerating the development of highly malignant phenotypes (44, 45).

Induction of angiogenesis by low dose radiotherapy. While high doses of ionizing radiation inhibit sprouting angiogenesis, low doses stimulate it. Sonveaux *et al.,* originally reported that low doses ionizing radiation stimulates endothelial cell migration and tubologenesis *in vitro* and angiogenesis *in vivo* of by activating the nitric oxide pathway (46). More recently, Sofia Vala *et al.,* observed that doses lower or equal to 0.8 Gy cause ligand-independent activation of vascular endothelial growth factor receptor (VEGFR) -2, resulting in enhanced endothelial cell migration, survival and angiogenesis (47). Low-dose ionizing radiation promotes cancer cell dissemination and metastasis of leukemia and breast cancer cells, which are prevented by blocking VEGFR-2 activation (47). These observations might be relevant to human cancer therapy, especially in hyper-fractionation protocols, since

the tissue and the border of the irradiated field is exposed to lower doses compared to the bulk of the tumor (48).

Clinical-translational advances

The fact that tumor bed irradiation promotes metastasis in experimental models is well established. In contrast whether this also occurs in patients treated with radiotherapy is still matter of debate (6). Increased risk of developing distant metastases upon local recurrences after radiotherapy has been reported in some (15-17) but not all (49) studies. Since these analyses were retrospective and non-randomized, it will be necessary to perform prospective randomized studies in order to obtain conclusive results. Translational studies will be necessary to validate or invalidate whether cellular and molecular events observed in preclinical models also apply to human cancer. The fact that different and complementary mechanisms are at play in the TBE, that some of them may be tumor specific or dominant over others, and that tumors are highly heterogeneous tissues may complicate the interpretation of the results issued from these translational studies. Nevertheless, it is important to start considering which pathways emerging from pre-clinical studies may be relevant targets for future therapeutic approaches to blunt invasion and metastasis of cancers relapsing after radiotherapy (See Figure 1 and Table 1).

***HIF-1 pathway*.** Preclinical models of the TBE point to HIF-1 activation as an important event in promoting tumor invasion and metastasis, suggesting that HIF-1 inhibition might provide therapeutic benefits. Blocking HIF-1 transcriptional activity

prevented the release of CXCL12 and attraction of $CXCR4^+$ myelomonocytic cells promoting the formation a novel vessels by vasculogenesis, the mobilization of pro-metastatic $cKit^+CD11b^+$ cells, and the expression of HIF1-dependent pro-metastatic genes, including the urokinase-type of plasminogen activator receptor (uPAR), lysil oxydase (LOX), plasminogen activator inhibitor (PAI) 1, matrix metallo proteinase (MMP) 2, snail or fibronectin (26). However, recent preclinical studies warrant caution in the use of HIF inhibitors in cancer as different HIF isoforms (e.g. HIF-1, -2, -3) have pleiotropic and sometimes opposing effects (50). For example, in contrast to its well-known tumor-promoting activity, HIF-1 has growth inhibitory and pro-apoptotic effects in some cancers (51, 52). At this point it might be prudent to target pro-metastatic pathways downstream of HIF-1 rather than HIF-1 itself.

CXCL12/CXCR4 pathway. Inhibition of CXCR4 activation using a small molecule (AMD3100) or neutralizing antibodies to CXCR4, prevented the recruitment of vasculogenic $CD11b^+$ cells to glioblastoma relapsing after radiotherapy and inhibited tumor re-growth (27). These pre-clinical results are corroborated by the increased accumulation of $CD11b^+$ cells observed in recurrent human glioblastoma compared to untreated tumors. Thus, targeting the CXCL12/CXCR4 axis might add potential benefits to standard radiotherapy. This hypothesis could be rapidly tested in patients, as CXCR4 inhibitors are available for clinical use (53). It should be noted, however, that CXCR4 inhibition leads to a rapid mobilization of hematopoietic stem cells into the peripheral circulation, some of which may have pro-tumoral activities.

***KITL/KIT pathway*.** In our orthotopic breast cancer model of TBE, KitL silencing in tumor cells and systemic Kit inhibition with a blocking antibody or the tyrosine kinase

inhibitor nilotinib (54), reduced the mobilization of Kit^+CD11b^+ cells, and their recruitment to primary tumors and lungs and attenuated lung metastasis (29). Whether mobilized KIT^+CD11b^+ cells are present in the blood of breast cancer patients treated with radiotherapy and their relationship to $CD11b^+$ cells infiltrating the primary tumor and metastases is currently under investigation. Of interest, increased expression of KITL was reported in peri-necrotic regions of glioblastoma and breast cancer tissues in association with poor prognosis (55). Several small molecular tyrosine kinase inhibitors targeting KIT are already approved for clinical use (56) thereby facilitating the planning of clinical studies aimed at inhibiting KIT in patients with post-radiation recurrences.

***$CD11b^+$ cells*.** Zoledronic acid and liposomal clodronate were successfully used in post-irradiation settings to target MMP-9-expressing tumor-recruited $CD11b^+$ monocytes/macrophages and to deplete tumor-mobilized Kit^+CD11b^+ populations resulting in reduced tumor growth and metastasis, respectively (28, 29). Whether biphosphonates may also be used in cancer patients for the same purpose remains to be demonstrated. Carrageenan, a sulfated polysaccharides extracted from red seaweeds, was also used to deplete monocytes/macrophages with similar inhibitory effects on tumor re-growth after radiotherapy (27). Inhibition of the CD11b/CD18 complex using function-blocking antibodies effectively inhibited the recruitment of $CD11b^+$ myeloid cells into irradiated tumors and prevented tumor growth (57). Humanized antibodies to the CD11b/CD18 complex (β2 integrin subfamily) have been developed for human use (58) and could be tested in patients with post-radiotherapy recurrences. In contrast, humanized, CD11b-selective antibodies are not available yet. Considering their essential role in mediating leukocyte recruitment

to sites of infection, sustained inhibition of β2 integrins bears the potential risk of increased infections.

HGF/MET pathway. A specific antagonist of hepatocyte growth factor (HGF), the MET ligand, suppressed growth, invasion and metastasis of pancreatic cancer cells co-cultured with irradiated fibroblasts (41). MET kinase inhibitors enhanced the efficacy of radiotherapy to halt tumor growth and prevent radiation-induced invasiveness (43). Considering the well-established role of the HGF/MET pathway in mediating invasive tumor growth (42) and the clinical development of novel MET inhibitors (59), MET should be considered as an attractive target to prevent or treat invasive growth of post-radiation tumor relapses.

TGFβ pathway. Because of its multiple tumor-promoting effects, the TGFβ pathway has been long considered as an appealing target in cancer (44). For example, genetic and pharmacological targeting of the TGFβ receptor activin receptor-like kinase (ALK) 1, impaired tumor growth and angiogenesis in the Rip1Tag2 model (60), and TGFβ neutralizing antibody enhanced efficacy of radiotherapy in a breast cancer model (61). Since TGFβ signaling is induced by ionizing radiation (62), and TGFβ receptors inhibitors are in clinical development, it may be tempting to consider this pathway as a valid therapeutic target to prevent invasion and metastasis in post-radiotherapy recurrences. In view of the complexity, and sometimes opposing effects of TGFβ in cancer and its role in healthy tissue homeostasis, however, it may be wise to focus on the inhibition of molecules up or downstream of TGFβ, such as AKT (63).

***αV integrins*.** The function-blocking anti-αV mAb 17E6 and the αVβ3/αVβ5-specific peptidic antagonist cilengitide (EMD12197) prevented metastasis induced by tumor bed irradiation or CYR61 overexpression (14). Since cilengitide is currently in advanced clinical testing in glioblastoma patients in combination with radio- and chemotherapies (64), it seems attractive to assess the effect of cilengitide in patients at high risk for post radiation relapses in other cancers. Of note, cilengitide, alone or in combination with radio- and chemotherapy, has shown high tolerability and low toxicity making it an ideal drug for combination therapies (65).

Conclusion

In recent time we have gained insights in some of the cellular events and molecular pathways responsible for the TBE. These results represent a conceptual advance to the understanding of the TBE and provide some rationales to the development of new therapeutic approaches to prevent invasion and metastasis of tumors locally relapsing after radiotherapy. However, they may only represent the tip of the iceberg of the radiation-induced microenvironmental modifications modulating cancer progression, and many more mechanisms are likely to be discovered. Drugs targeting some of the uncovered pathways are already available for human use. We need now to move on and perform innovative translational studies and combination trials to validate or invalidate in patients the molecular pathways uncovered in preclinical models. In addition, we need to determine predictive biomarkers, such as gene expression signatures in primary tumors, circulating molecules or circulating cell populations, in order to identify patients at risk for relapse and progression after

radiotherapy. Such patients would be the ones to benefit most from a concomitant therapy blunting tumor escape and evasive growth. The TBE may also serve as a model relevant to unravel pathways mediating evasive resistance to anti-angiogenic therapies. Indeed, hypoxia and metabolic starvation caused by anti-angiogenic treatments (66), much alike hypoxia caused by radiotherapy, may initiate microenvironmental modifications eliciting adaptive tumor responses or the selection of highly aggressive tumor cell populations. This would be an unexpected but welcome contribution to research in anti-angiogenesis for an effect originally described nearly 100 years ago!

Acknowledgements

Work in our laboratory is supported by grants from the Swiss National Science Foundation (SNF), the National Center for Competence in Research (NCCR, molecular Oncology, a research instrument of the SNF), Swiss Cancer League, the CCRP program from Oncosuisse, the ISREC Foundation, the Medic Foundation, and the 7th Frame program from the European Union. We apologize to those colleagues whose work could not be cited due to space limitations.

References

1. Bernier J, Hall EJ, Giaccia A. Radiation oncology: a century of achievements. Nat Rev Cancer 2004;4: 737-47.
2. Bartelink H, Horiot JC, Poortmans P, Struikmans H, Van den Bogaert W, Barillot I*, et al.* Recurrence rates after treatment of breast cancer with standard radiotherapy with or without additional radiation. N Engl J Med 2001;345: 1378-87.
3. Nunez MI, McMillan TJ, Valenzuela MT, Ruiz de Almodovar JM, Pedraza V. Relationship between DNA damage, rejoining and cell killing by radiation in mammalian cells. Radiother Oncol 1996;39: 155-65.
4. Kinsella TJ. Coordination of DNA mismatch repair and base excision repair processing of chemotherapy and radiation damage for targeting resistant cancers. Clin Cancer Res 2009;15: 1853-9.
5. Barcellos-Hoff MH, Park C, Wright EG. Radiation and the microenvironment - tumorigenesis and therapy. Nat Rev Cancer 2005;5: 867-75.
6. Madani I, De Neve W, Mareel M. Does ionizing radiation stimulate cancer invasion and metastasis? Bull Cancer 2008;95: 292-300.
7. Stenstrom KW, Vermund H, Mosser DG, Marvin JF. Effects of roentgen irradiation on the tumor bed. I. The inhibiting action of local pretransplantation roentgen irradiation (1500 r alpha) on the growth of mouse mammary carcinoma. Radiat Res 1955;2: 180-91.
8. Milas L. Tumor bed effect in murine tumors: relationship to tumor take and tumor macrophage content. Radiat Res 1990;123: 232-6.

9. Camplejohn RS, Penhaligon M. The tumour bed effect: a cell kinetic and histological investigation of tumours growing in irradiated mouse skin. Br J Radiol 1985;58: 443-51.

10. Milas L, Ito H, Hunter N, Jones S, Peters LJ. Retardation of tumor growth in mice caused by radiation-induced injury of tumor bed stroma: dependency on tumor type. Cancer Res 1986;46: 723-7.

11. Wondergem J, Begg AC, Haveman J. Effects of hyperthermia and X-irradiation on mouse stromal tissue. Int J Radiat Biol Relat Stud Phys Chem Med 1986;50: 65-76.

12. Milas L, Hirata H, Hunter N, Peters LJ. Effect of radiation-induced injury of tumor bed stroma on metastatic spread of murine sarcomas and carcinomas. Cancer Res 1988;48: 2116-20.

13. Rofstad EK, Mathiesen B, Henriksen K, Kindem K, Galappathi K. The tumor bed effect: increased metastatic dissemination from hypoxia-induced up-regulation of metastasis-promoting gene products. Cancer Res 2005;65: 2387-96.

14. Monnier Y, Farmer P, Bieler G, Imaizumi N, Sengstag T, Alghisi GC*, et al.* CYR61 and alphaVbeta5 integrin cooperate to promote invasion and metastasis of tumors growing in preirradiated stroma. Cancer Res 2008;68: 7323-31.

15. O'Brien CJ, Lahr CJ, Soong SJ, Gandour MJ, Jones JM, Urist MM*, et al.* Surgical treatment of early-stage carcinoma of the oral tongue--wound adjuvant treatment be beneficial? Head Neck Surg 1986;8: 401-8.

16. Vicini FA, Kestin L, Huang R, Martinez A. Does local recurrence affect the rate of distant metastases and survival in patients with early-stage breast carcinoma treated with breast-conserving therapy? Cancer 2003;97: 910-9.

17. Vikram B, Strong EW, Shah JP, Spiro R. Failure at distant sites following multimodality treatment for advanced head and neck cancer. Head Neck Surg 1984;6: 730-3.

18. Clifton KH, Jirtle R. Mammary carcinoma cell population growth in preirradiated and unirradiated transplant sites. Viable tumor growth, vascularity, and the tumor-bed effect. Radiology 1975;117: 459-65.

19. Jirtle R, Clifton KH. Effect of preirradiation of the tumor bed on the relative vascular space of mouse gastric adenocarcinoma 328 and mammary adenocarcinoma CA755. Cancer Res 1973;33: 764-8.

20. Saeki Y, Shimazaki S, Urano M. Radiation effect on the vascularization of a C3H mouse mammary carcinoma. Microangiographic studies of the tumor in preirradiated tissue and of the recurrent tumor. Radiology 1971;101: 175-80.

21. Garcia-Barros M, Paris F, Cordon-Cardo C, Lyden D, Rafii S, Haimovitz-Friedman A*, et al.* Tumor response to radiotherapy regulated by endothelial cell apoptosis. Science 2003;300: 1155-9.

22. Imaizumi N, Monnier Y, Hegi M, Mirimanoff RO, Ruegg C. Radiotherapy suppresses angiogenesis in mice through TGF-betaRI/ALK5-dependent inhibition of endothelial cell sprouting. PLoS One 2010;5: e11084.

23. Hlushchuk R, Riesterer O, Baum O, Wood J, Gruber G, Pruschy M*, et al.* Tumor recovery by angiogenic switch from sprouting to intussusceptive angiogenesis after treatment with PTK787/ZK222584 or ionizing radiation. Am J Pathol 2008;173: 1173-85.

24. Penhaligon M, Courtenay VD, Camplejohn RS. Tumour bed effect: hypoxic fraction of tumours growing in preirradiated beds. Int J Radiat Biol Relat Stud Phys Chem Med 1987;52: 635-41.

25. Wilson WR, Hay MP. Targeting hypoxia in cancer therapy. Nat Rev Cancer 2011;11: 393-410.

26. Gordan JD, Simon MC. Hypoxia-inducible factors: central regulators of the tumor phenotype. Curr Opin Genet Dev 2007;17: 71-7.

27. Kioi M, Vogel H, Schultz G, Hoffman RM, Harsh GR, Brown JM. Inhibition of vasculogenesis, but not angiogenesis, prevents the recurrence of glioblastoma after irradiation in mice. J Clin Invest 2010;120: 694-705.

28. Ahn GO, Brown JM. Matrix metalloproteinase-9 is required for tumor vasculogenesis but not for angiogenesis: role of bone marrow-derived myelomonocytic cells. Cancer Cell 2008;13: 193-205.

29. Kuonen F, Laurent J, Secondini C, Lorusso G, Stehle JC, Rausch T*, et al.* Inhibition of the Kit ligand/c-Kit axis attenuates metastasis in a mouse model mimicking local breast cancer relapse after radiotherapy. Clin Cancer Res 2012, Jun 18. [Epub ahead of print]

30. Lau LF. CCN1/CYR61: the very model of a modern matricellular protein. Cell Mol Life Sci 2011;68: 3149-63.

31. Sullivan R, Graham CH. Hypoxia-driven selection of the metastatic phenotype. Cancer Metastasis Rev 2007;26: 319-31.

32. Mikkelsen RB, Wardman P. Biological chemistry of reactive oxygen and nitrogen and radiation-induced signal transduction mechanisms. Oncogene 2003;22: 5734-54.

33. Poli G, Leonarduzzi G, Biasi F, Chiarpotto E. Oxidative stress and cell signalling. Curr Med Chem 2004;11: 1163-82.

34. Jobling MF, Mott JD, Finnegan MT, Jurukovski V, Erickson AC, Walian PJ*, et al.* Isoform-specific activation of latent transforming growth factor beta (LTGF-beta) by reactive oxygen species. Radiat Res 2006;166: 839-48.

35. Vodovotz Y, Chesler L, Chong H, Kim SJ, Simpson JT, DeGraff W*, et al.* Regulation of transforming growth factor beta1 by nitric oxide. Cancer Res 1999;59: 2142-9.

36. Spitz DR, Azzam EI, Li JJ, Gius D. Metabolic oxidation/reduction reactions and cellular responses to ionizing radiation: a unifying concept in stress response biology. Cancer Metastasis Rev 2004;23: 311-22.

37. Hallahan D, Clark ET, Kuchibhotla J, Gewertz BL, Collins T. E-selectin gene induction by ionizing radiation is independent of cytokine induction. Biochem Biophys Res Commun 1995;217: 784-95.

38. Nicolson GL, Custead SE, Dulski KM, Milas L. Effects of gamma irradiation on cultured rat and mouse microvessel endothelial cells: metastatic tumor cell adhesion, subendothelial matrix degradation, and secretion of tumor cell growth factors. Clin Exp Metastasis 1991;9: 457-68.

39. Lara PC, Russell NS, Smolders IJ, Bartelink H, Begg AC, Coco-Martin JM. Radiation-induced differentiation of human skin fibroblasts: relationship with cell survival and collagen production. Int J Radiat Biol 1996;70: 683-92.

40. Herskind C, Bamberg M, Rodemann HP. The role of cytokines in the development of normal-tissue reactions after radiotherapy. Strahlenther Onkol 1998;174 Suppl 3: 12-5.

41. Ohuchida K, Mizumoto K, Murakami M, Qian LW, Sato N, Nagai E*, et al.* Radiation to stromal fibroblasts increases invasiveness of pancreatic cancer cells through tumor-stromal interactions. Cancer Res 2004;64: 3215-22.

42. Boccaccio C, Comoglio PM. Invasive growth: a MET-driven genetic programme for cancer and stem cells. Nat Rev Cancer 2006;6: 637-45.

43. De Bacco F, Luraghi P, Medico E, Reato G, Girolami F, Perera T*, et al.* Induction of MET by ionizing radiation and its role in radioresistance and invasive growth of cancer. J Natl Cancer Inst 2011;103: 645-61.

44. Meulmeester E, Ten Dijke P. The dynamic roles of TGF-beta in cancer. J Pathol 2011;223: 205-18.

45. Nguyen DH, Oketch-Rabah HA, Illa-Bochaca I, Geyer FC, Reis-Filho JS, Mao JH*, et al.* Radiation acts on the microenvironment to affect breast carcinogenesis by distinct mechanisms that decrease cancer latency and affect tumor type. Cancer Cell 2011;19: 640-51.

46. Sonveaux P, Brouet A, Havaux X, Gregoire V, Dessy C, Balligand JL*, et al.* Irradiation-induced angiogenesis through the up-regulation of the nitric oxide pathway: implications for tumor radiotherapy. Cancer Res 2003;63: 1012-9.

47. Sofia Vala I, Martins LR, Imaizumi N, Nunes RJ, Rino J, Kuonen F*, et al.* Low doses of ionizing radiation promote tumor growth and metastasis by enhancing angiogenesis. PLoS One 2010;5: e11222.

48. Purdy JA. Dose to normal tissues outside the radiation therapy patient's treated volume: a review of different radiation therapy techniques. Health Phys 2008;95: 666-76.

49. Woodward WA, Truong PT, Yu TK, Tereffe W, Oh J, Perkins G*, et al.* Among women who experience a recurrence after postmastectomy radiation therapy

irradiation is not associated with more aggressive local recurrence or reduced survival. Breast Cancer Res Treat 2010;123: 597-605.

50. Keith B, Johnson RS, Simon MC. HIF1alpha and HIF2alpha: sibling rivalry in hypoxic tumour growth and progression. Nat Rev Cancer 2012;12: 9-22.
51. Sowter HM, Ratcliffe PJ, Watson P, Greenberg AH, Harris AL. HIF-1-dependent regulation of hypoxic induction of the cell death factors BNIP3 and NIX in human tumors. Cancer Res 2001;61: 6669-73.
52. Carmeliet P, Dor Y, Herbert JM, Fukumura D, Brusselmans K, Dewerchin M*, et al.* Role of HIF-1alpha in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. Nature 1998;394: 485-90.
53. Uy GL, Rettig MP, Cashen AF. Plerixafor, a CXCR4 antagonist for the mobilization of hematopoietic stem cells. Expert Opin Biol Ther 2008;8: 1797-804.
54. Manley PW, Drueckes P, Fendrich G, Furet P, Liebetanz J, Martiny-Baron G*, et al.* Extended kinase profile and properties of the protein kinase inhibitor nilotinib. Biochim Biophys Acta 2010;1804: 445-53.
55. Sihto H, Tynninen O, Halonen M, Puputti M, Karjalainen-Lindsberg ML, Kukko H*, et al.* Tumour microvessel endothelial cell KIT and stem cell factor expression in human solid tumours. Histopathology 2009;55: 544-53.
56. Lennartsson J, Ronnstrand L. The stem cell factor receptor/c-Kit as a drug target in cancer. Curr Cancer Drug Targets 2006;6: 65-75.
57. Ahn GO, Tseng D, Liao CH, Dorie MJ, Czechowicz A, Brown JM. Inhibition of Mac-1 (CD11b/CD18) enhances tumor response to radiation by reducing myeloid cell recruitment. Proc Natl Acad Sci U S A 2010;107: 8363-8.
58. Jones R. Rovelizumab (ICOS Corp). IDrugs 2000;3: 442-6.

59. Eder JP, Vande Woude GF, Boerner SA, LoRusso PM. Novel therapeutic inhibitors of the c-Met signaling pathway in cancer. Clin Cancer Res 2009;15: 2207-14.

60. Cunha SI, Pardali E, Thorikay M, Anderberg C, Hawinkels L, Goumans MJ*, et al.* Genetic and pharmacological targeting of activin receptor-like kinase 1 impairs tumor growth and angiogenesis. J Exp Med 2010;207: 85-100.

61. Bouquet F, Pal A, Pilones KA, Demaria S, Hann B, Akhurst RJ*, et al.* TGFbeta1 inhibition increases the radiosensitivity of breast cancer cells in vitro and promotes tumor control by radiation in vivo. Clin Cancer Res 2011;17: 6754-65.

62. Barcellos-Hoff MH, Derynck R, Tsang ML, Weatherbee JA. Transforming growth factor-beta activation in irradiated murine mammary gland. J Clin Invest 1994;93: 892-9.

63. Xue G, Restuccia DF, Lan Q, Hynx D, Dirnhofer S, Hess D*, et al.* Akt/PKB-mediated phosphorylation of Twist1 is crucial for tumor metastasis. Cancer Discovery 2012;2: 193.

64. Stupp R, Hegi ME, Neyns B, Goldbrunner R, Schlegel U, Clement PM*, et al.* Phase I/IIa study of cilengitide and temozolomide with concomitant radiotherapy followed by cilengitide and temozolomide maintenance therapy in patients with newly diagnosed glioblastoma. J Clin Oncol 2010;28: 2712-8.

65. Stupp R, Ruegg C. Integrin inhibitors reaching the clinic. J Clin Oncol 2007;25: 1637-8.

66. Steeg PS. Angiogenesis inhibitors: motivators of metastasis? Nat Med 2003;9: 822-3.

Legend

Figure 1. Emerging pathways mediating growth, invasion and metastasis of tumors progressing after radiotherapy. High dose ionizing radiation (IR) kills tumor -associated endothelial cells and inhibits sprouting angiogenesis. Tumor cells growing within this angiogenesis-suppressed microenvironment cause tissue hypoxia, which elicits HIF-dependent and HIF-independent responses. Hypoxia induces HIF-dependent expression of CXCl12 and KITL promoting the mobilization from the bone marrow and the recruitment to tumors or pre-metastatic sites of cells promoting vasculogenesis ($CXCR4^+CD11b^+$ cells) or metastasis (KIT^+CD11b^+ cells). Hypoxia selects for invasive and metastatic tumor cells expressing high levels of αV integrins and CYR61. Hypoxia also stimulates the expression of additional pro-metastatic factors, such as uPAR, LOX, PAI-1, MMP-2. Ionizing radiation also induces the expression of tumor-promoting cytokine and growth factor by stromal cells (TGFβ, PDGF, IL1β) directly or indirectly (e.g. thought ROS/NOS). Irradiated tumor cells increase expression of the pro-invasive receptor MET, through the ATM/NF-κB pathway. Together, these events concur to promote growth, invasion and metastasis of tumors progressing in a pre-irradiated microenvironment. The “stop” signs indicate candidate target molecules that can be blocked with available inhibitors in order to suppress growth and metastasis of tumors growing in an irradiated bed. See table 1 for a selection of inhibitory molecules. The pro-angiogenic effects of low dose IR are not depicted here.

Table 1. Synopsis of molecules promoting tumor growth and metastasis in a pre-irradiated bed and selected inhibitory drugs. This table lists candidate

therapeutic targets and a selection of inhibitory molecules to consider in preclinical and clinical studies to impinge on pro-metastatic pathways activated by ionizing radiation.

Table 1. Synopsis of molecules promoting tumor growth and metastasis in a pre-irradiated bed and selected inhibitory drugs.

Target		Inhibitor		Targeted event
Molecule	**Class**	**Molecule**	**Class**	
αVβ3 integrin	Adhesion receptor	Celengitide; 17E6	Peptide (αVβ3/αVβ5) Antibody (pan anti-αV)	Tumor cell adhesion, migration, invasion, metastasis
β2 integrins (CD11/18 complex)	Adhesion receptor	Rovelizumab	Antibody	Recruitment of $CD11/18^{+}$ monocytes
KIT	Receptor tyrosine kinase	Imatinib; Nilotinib	Ttyrosine kinase inhibitors	Recruitment of $KIT^{+}CD11b^{+}$ BMDC
MET	Receptor tyrosine kinase	PHA665752; JNJ-38877605; SU11274; PF-02341066; XL880;	Ttyrosine kinase inhibitors	Tumor cell growth, survival and invasion
CXCR4	Chemokine receptor	BKT140; ALX40-4C; Plerixafor	Small molecular inhibitors	Recruitment of $CD11b^{+}$ BMDC
ALK5 ALK1	TGFβ receptors	EW-7195; SB-431542; PF-03446962	Kinase inhibitors Antibody	Inhibition of angiogenesis, invasion, epithelial-to-mesenchymal transition
HIF-1	Transcription factor	NSC-134754; PX-478; PX-12; YC-1; Topotecan;	Small molecular inhibitors Camptothecin analogue	Metabolism, migration, invasion, angiogenesis, survival

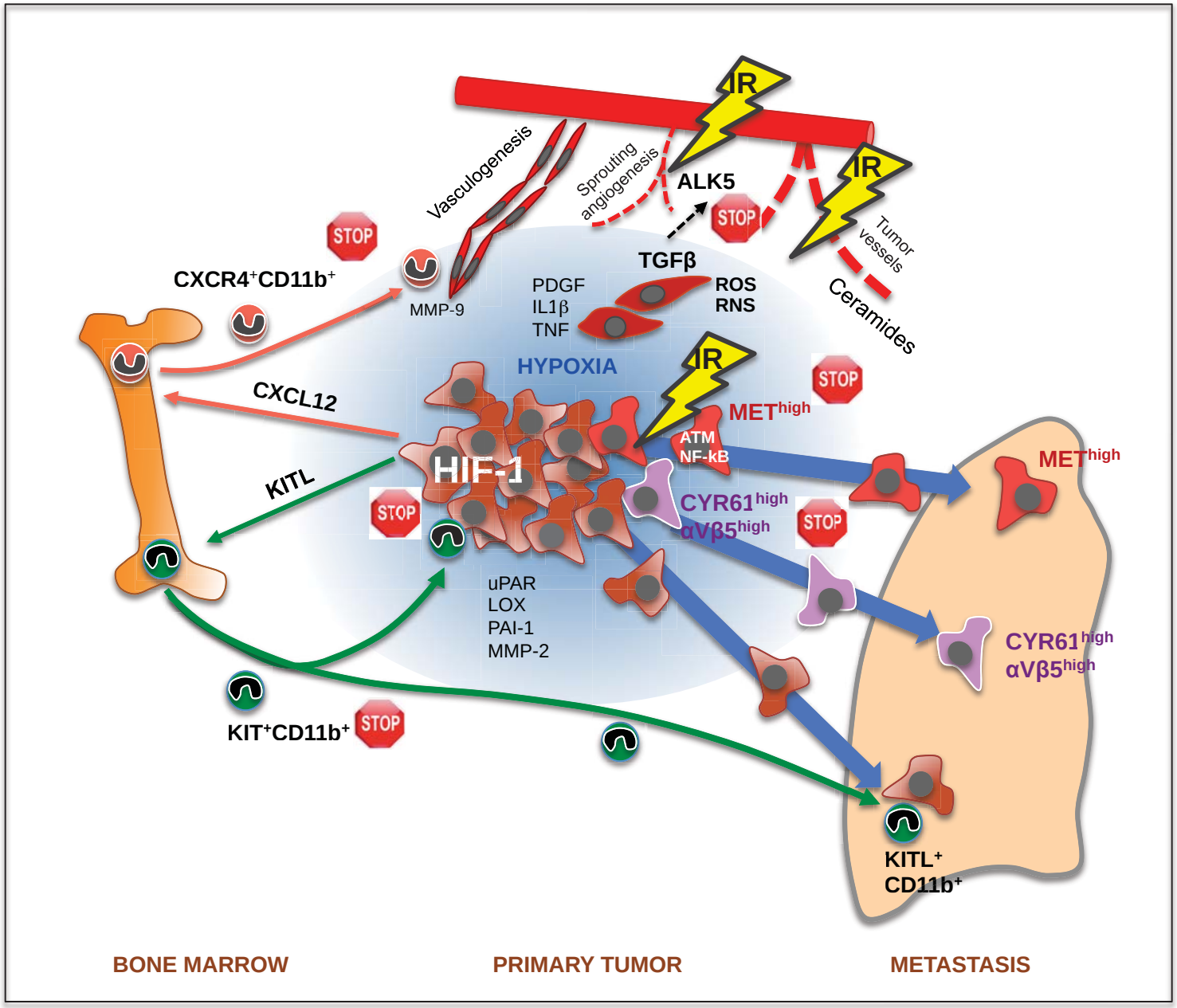
IR
IR
IR
Vasculogenesis
Sprouting angiogenesis
ALK5
STOP
STOP
Tumor vessels
Ceramides
TGFβ
PDGF
IL1β
TNF
ROS
RNS
CXCR4+CD11b+
MMP-9
HYPOXIA
CXCL12
KITL
HIF-1
STOP
STOP
STOP
METhigh
ATM
NF-kB
CYR61high
αVβ5high
METhigh
CYR61high
αVβ5high
uPAR
LOX
PAI-1
MMP-2
KIT+CD11b+
STOP
KITL+
CD11b+
BONE MARROW
PRIMARY TUMOR
METASTASIS

Figure 1