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A molecular assay of tumor radiosensitivity: a roadmap towards biology-based personalized radiation therapy

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Abstract

The last two decades have seen technological developments that have led to more accurate delivery of radiation therapy (RT), which has resulted in clinical gains in many solid tumors. However, a fundamental question and perhaps the next major hurdle is whether biological strategies can be developed to further enhance the effectiveness and efficiency of RT. This article addresses the development of a novel genomics-based molecular assay to predict tumor radiosensitivity, and proposes that this assay may prove pivotal in the development of biologically guided RT.

Keywords

gene expression; molecular signatures; radiation therapy; radiosensitivity; systems biology

Radiation therapy (RT) plays a central role in cancer therapeutics. A recent analysis concluded that RT was a necessary treatment component for 52% of newly diagnosed cancer patients [1]. RT is utilized in virtually every solid tumor as a curative approach. In addition, in patients with metastatic cancer, RT serves as an excellent palliative therapy. Importantly, the impact of RT on overall cancer-cure rates has been estimated at 40% compared with 49% for surgery and 11% for systemic treatments [2,3]. Furthermore, health economics studies have shown that RT is a highly cost-effective therapy [4,5].

Over the last two decades, technological advances in radiation treatment planning and delivery have resulted in more precise delivery of RT. Intensity-modulated RT along with inverse treatment planning delivers higher radiation doses to the tumor, while sparing normal tissue. In addition, the integration of 4D anatomy (including tumor motion) into treatment planning has refined the ability to personalize radiation treatment fields [6].

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Although technological developments have significantly improved the quality of RT delivery, a fundamental question that remains unanswered is whether it is possible to identify individuals that are more likely to benefit from RT based on their tumor biology. Furthermore, clinical decision-making regarding RT is still based on classic clinico-pathological features, which estimate overall level of tumor aggressiveness, but are not specific for predicting benefit from RT. In addition, there is also evidence that RT benefit is nonuniform. For example, a recent study showed that in breast cancer patients receiving breast conservation therapy, locoregional recurrence risk was higher in HER2-enriched and basal subtypes, whereas after mastectomy, luminal B, luminal-HER2, HER2-enriched and basal subtypes were all associated with an increase risk of locoregional recurrences [7]. A similar observation was made in triple-negative breast cancer with a higher risk for locoregional recurrence in mastectomy patients when compared with breast conservation therapy [8]. Therefore a molecular assay that can predict which patients are more likely to benefit from treatment with RT would be a great clinical asset for radiation oncologists. An accurate predictor of RT benefit may lead to the refinement of treatment decision algorithms and may lead to novel paradigms to test in signature-directed clinical trials.

Historical perspective on the development of radiosensitivity assays

The development of a successful clinical assay to predict response to RT has been a major clinical goal in radiation oncology [9,10]. Past efforts in developing a predictive assay of tumor radio-sensitivity have been recently reviewed [11] and can be grouped into three categories: assays to determine intrinsic radiosensitivity (*ex vivo* determination of tumor survival fraction at 2 Gy [SF2]) [12–18]; assays to determine tumor oxygen levels (electrodes to measure tumor pO₂) [19,20]; and determination of tumor proliferative potential [21–23]. Unfortunately, although initial clinical data supported each of the approaches, none of them have become routine. A central reason has been that all approaches developed were highly impractical for routine clinical application. For example, *ex vivo* determination of SF2 required clonogenic culture of primary tumor cells *in vitro*. This process had relatively low yield and would take several weeks to complete, preventing its use in the clinical decision process. Furthermore, measuring tumor pO₂ with electrodes required a readily accessible tumor and thus limited its widespread application. Finally, tumor proliferative potential was tested in a multicenter European Organisation for Research and Treatment of Cancer (Brussels, Belgium) analysis of 476 patients with head and neck cancer where it was found not to be a significant predictor of clinical outcome [21]. However, these efforts, at least in the first two instances (SF2 and pO₂) established that it was possible to develop assays of radiosensitivity that would predict clinical outcome in patients treated with RT.

Diagnostic approaches to personalizing cancer therapy

A central goal of personalized medicine is the development of treatment strategies that are developed to fit an individual's tumor biology. Thus, there is significant emphasis on the development of companion diagnostics and/or molecular signatures to guide therapeutic decisions [24,25]. For example, two recurrence-risk signatures (Oncotype DX[®] [Genomic Health, Inc., CA, USA] and MammaPrint[®] [Agendia, Amsterdam, The Netherlands]) are commonly used to guide chemotherapy in women with node-negative breast cancer [26–28]. In addition, *KRAS* mutation has been shown to be predictive of panitumumab and cetuximab nonbenefit in colorectal cancer [29,30]. Furthermore, *EGFR* mutations have been shown to predict benefit from tyrosine kinase inhibitors and more recently, the *ALK* gene rearrangement has shown to be predictive for crizotinib benefit in non-small-cell lung cancer [31–33]. By contrast, as discussed above, clinical decision-making in radiation oncology is

still largely based on clinico-pathological features. Thus, there is a great need to develop molecular diagnostics to more efficiently utilize RT.

A systems biology approach to identifying radiation-specific biomarkers

The generation of high-throughput data sets in the 'omics era has provided an opportunity to address the identification of biomarkers from a different perspective. For example, gene-expression signatures have been shown to be prognostic in breast, lung, head and neck and colon cancers [27,28,34–38]. In addition, these high-throughput technologies have been central to the development of a systems view of complex biological systems [39,40]. In systems biology, regulatory pathways are proposed to be organized as complex interacting networks similar to the worldwide web [39–42]. An advantage of systems biology is that it can incorporate complexity in multiple scales into the modeling strategies. Cancer is considered the most complex of all human diseases, and this complexity may play a central role in determining the success of novel therapeutic and diagnostic approaches [43]. Figure 1 shows a systems biology approach to modeling disease. As discussed above, one important feature of systems biology is that it integrates biological scales (e.g., molecular, regulatory network, cellular, tissue and organism) when modeling disease, thus representing a more global approach to modeling.

It was hypothesized that developing a systems- biology model of cellular radiosensitivity would lead to the discovery of novel radiation-specific biomarkers. Furthermore, it was reasoned that those biomarkers could then be used to generate a model to predict cellular radiosensitivity.

Modeling strategy: a classifier to predict cellular radiosensitivity

We chose to model cellular radiosensitivity in a cohort of cell lines from the National Cancer Institute (MD, USA) panel of 60 [44]. We defined radiosensitivity based on cellular clonogenic survival after 2 Gy (SF2) for the 35 cell lines. Since gene-expression profiles were available for all cell lines, we used gene expression as the basis of our model. The schema for the classifier is shown in Figure 2, which used a linear regression algorithm to screen for genes associated with radio sensitivity. Gene selection was based on the individual gene's ability to fit a linear regression model of gene expression versus cellular radiosensitivity (SF2) using significance analysis of microarrays with a false discovery rate of 5%. Genes selected by significance analysis of microarrays were then used to build a multivariate linear regression model to predict SF2. Using a leave-one-out cross-validation approach, we showed that cellular radiosensitivity was predictable based on gene expression (accuracy: 62%; continuous variable; $p = 0.002$). Moreover, we biologically validated the linear regression approach, by demonstrating that the classifier led to biological discovery. Two out of three novel genes (*RBAP48* and *RGS19*) identified by the classifier were validated as mechanistically involved in radiation response and a third gene was a known clinical target for radiosensitization (*TOP1*).

By developing the classifier we showed it was possible to relate the molecular and cellular scales within an accurate radiosensitivity model and that this approach led to the discovery of novel genes associated with radiation response. However the classifier identified only few genes and a better approach was needed to model a larger regulatory network.

Modeling the radiosensitivity network

Figure 3 shows the strategy involved in identifying the radiosensitivity gene network. We integrated genomic expression and cellular radiosensitivity in a database of 48 cancer cell lines to identify a ten-gene network that we propose plays a central role in determining

radiophenotype (Figure 3B) [45]. A linear regression model including gene expression, tissue of origin, *RAS* status (mutant/wild-type) and *p53* status (mutant/wild-type) was developed to identify genes associated with radiosensitivity. A total of 7159 gene-based models were developed, and the top 500 genes were identified based on linear fit. Since our hypothesis was that the genes would be represented within a network, we interconnected all 500 genes using Metacore™ software (GeneGo, MI, USA) and then identified major hubs within the network. This resulted in the ten-gene network shown in Figure 3. Importantly, seven out of ten nodes have been proposed as targets for the development of radiosensitizers [46–50] and several of them have been reported to be involved in radiation signaling [51–60]. In addition pathways represented in the network included: DNA damage response, histone deacetylation, cell cycle, apoptosis and proliferation, all of which have been previously indicated in regulating radiation response [47,61,62].

Modeling cellular radiosensitivity using the hub-based network

The systems biology-based modeling approach uncovered a ten-hub gene network that we proposed was central in determining cellular radiophenotype. If the ten identified hubs were critical to radiophenotype, we hypothesize it should be possible to develop a model to predict cellular radiosensitivity based on the expression of the ten hubs. Therefore we modeled cellular radiosensitivity based on SF2 and defined as radiosensitivity index (RSI) as a linear function of genes expression for the ten genes. Since RSI is modeled on the cellular survival after RT in cell lines, a low RSI indicates a higher response to RT (more radiosensitive). The final model is given by the equation in Box 1.

Box 1

Linear algorithm defining radiosensitivity index

- Radiosensitivity index = $(-0.0098009 \times AR) + (0.0128283 \times cJun) + (0.0254552 \times STAT1) - (0.0017589 \times PKC) - (0.0038171 \times RelA) + (0.1070213 \times cABL) - (0.0002509 \times SUMO1) - (0.0092431 \times CDK1) - (0.0204469 \times HDAC1) - (0.0441683 \times IRF1)$

To validate RSI we decided to test the model directly in patients treated with RT. However, it should be noted that other authors have shown that testing signatures in tumour xenograft models is a reasonable intermediate step, which can help fine tune the signature before definitive clinical testing [63]. We tested the gene-expression model or signature in five applied in each of the data sets without modification and was validated independently in each of these cohorts with no tuning or readjustment.

Clinical validation of RSI

The published clinical validation data sets are presented in Figures 4 & 5 [64]. RSI was initially tested as a predictor of response in rectal (n = 14) and esophageal (n = 12) cancer patients undergoing preoperative chemoradiation at the Moffitt Cancer Center (FL, USA). In both cases, responders to preoperative chemoradiation had a lower RSI (i.e., were more radiosensitive) than nonresponders and this difference was statistically significant. We further tested RSI in 92 head and neck cancer patients treated with definitive cisplatin-based chemo-radiation in The Netherlands Cancer Institute (Amsterdam, The Netherlands). Since our signature was developed based on cellular radio-sensitivity, we chose 2-year locoregional failure as our clinical end point, which is directly associated with the therapeutic effect of RT. In addition, the majority of locoregional failures in head and neck cancer patients occur within the first 2 years after completion of treatment. RSI-low patients (i.e., radiosensitive; cut-off point 25th percentile) had an improved 2-year locoregional

recurrence-free survival when compared with RSI-high patients (i.e., radioresistant), which was statistically significant (86 vs 61%; $p = 0.05$).

In addition, in unpublished work RSI has been validated in two independent data sets of breast cancer patients in a total of 503 patients [Eschrich SA *et al.* Validation of a radiosensitivity molecular signature in breast cancer (2012), Submitted] [65]. In both data sets it is shown that RSI predicts distant metastasis risk in breast cancer patients treated with RT. By contrast, RSI did not predict outcome in patients treated without RT, consistent with RSI being RT specific (i.e., a predictive biomarker). In summary, RSI has been validated in five distinct independent data sets, in a total of 621 patients, and represents to our knowledge the most thoroughly examined and validated signature of radiosensitivity. It should be emphasized that RSI was developed in cell lines and then applied without modification or readjustments in each of the clinical cohorts. While many studies involve the development of a signature in a single training set and validated in a single independent data set, we have demonstrated the validation of a single signature in five distinct, independent data sets. The successful translation from cell lines to patients in multiple disease sites (e.g., breast, rectal, esophageal and head and neck cancer) strongly argues that the biological basis of cellular radiosensitivity is conserved between cell lines and patients and across epithelial tumors (cross-tissue platform). Finally, a central implication of this work is that cellular radiosensitivity as determined in a clonogenic assay in a large group of cancer cell lines is relevant to clinical radiosensitivity in patients.

Clinical applications of biology-based personalized RT: a roadmap

As discussed above it has been estimated that RT alone or in association with other therapies is responsible for 40% of cures in clinical oncology. This estimate, which was generated in an extensive analysis published in the mid 1990s, does not account for improvements in RT delivery, dose intensification and/or concurrent chemo-radiation protocols which have been shown to impact on the curative potential of RT. Thus, there is great potential to improve the therapeutic impact of RT by better understanding the molecular fingerprints that determine RT benefit in cancer patients.

One of the difficulties of developing a clinically relevant radiosensitivity molecular signature stems from the broad applicability of RT as a therapeutic agent in cancer. Since RT is used in different settings depending on disease site, the clinical utility of the signature would vary depending on the clinical application. By contrast, other successful molecular signatures have been developed to specifically fit a clinical situation (i.e., need for adjuvant chemotherapy in node-negative breast cancer) [26,28]. However a central advantage of focusing on a RT-specific signature is that the resulting technology could function as a predictive biomarker rather than a prognostic biomarker. These data are consistent with RSI serving this function.

Predictive biomarkers are considered the gatekeepers to personalized medicine [66]. A central characteristic of these biomarkers is that they are therapy-specific and thus predict therapy benefit or nonbenefit. Therefore they are clinically actionable and can guide therapeutic decision-making [67]. For example and as discussed above, *KRAS* mutation has been shown to be predictive of panitumumab and cetuximab nonbenefit in colorectal cancer [29,30]. Furthermore, *EGFR* mutations have been shown to predict benefit from tyrosine kinase inhibitors and more recently *ALK* gene rearrangement has shown to be predictive for crizotinib benefit in non-small-cell lung cancer [31–33]. In similar fashion, a successful radiosensitivity clinical test may have great potential to significantly improve RT use in clinical oncology.

Clinical utility applications for RSI: rectal cancer & head & neck cancer

As discussed above, the clinical application of a radiosensitivity signature would vary depending on disease site. However, we think one of the most direct applications would be in rectal cancer. Preoperative concurrent chemoradiation followed by surgery (lower anterior resection or abdomino-perineal resection) is the standard of care for patients with locally advanced rectal adenocarcinoma [68]. Approximately 20% of patients experience a complete pathological response to preoperative chemoradiation [69] and there is emerging data from two independent groups that these patients can be spared definitive surgery and closely followed [70,71]. However, approximately 40–45% of patients do not respond to preoperative chemoradiation, emphasizing the critical role that a successful radiosensitivity signature could play in this disease. While patients likely to respond can be guided towards treatment with preoperative chemoradiation, patients deemed unlikely to respond could be operated on immediately. A reasonable discussion would be the level of accuracy required for such technology. Sensitivity and specificity in the 90% range is probably desirable, but this should be taken in the context of the current overall accuracy of this clinical decision (in which all patients are treated with approximately 50% responding and 20% experiencing complete pathological response). Another potential clinical approach would be to try to enhance complete pathological response by delivering higher RT doses (54 Gy) to patients predicted to be radiosensitive. Since complete pathological response is an early surrogate for good outcome this could lead to better outcomes for these patients.

Another potential clinical utilization for the signature would be in head and neck cancer. RSI in combination with known prognostic factors in the disease (e.g., human papillomavirus) could be used to identify patients that may benefit from minimizing the use of aggressive concurrent chemoradiation protocols. By contrast, patients with radioresistant tumors could be selected for more aggressive radiation dosing approaches or for testing novel radiosensitizing agents.

Future perspective

In this article I have discussed the development of a radiosensitivity molecular signature that has been clinically validated in five independent data sets in four disease sites. It should be noted that all the validation has been performed using publically available data sets and further clinical validation and platform developments are required before this signature can be applied in routine clinical care. A critical issue to address is standardizing the process for tissue acquisition, RNA isolation and gene-expression measurement. A locked-down protocol for these steps as well as analytical validation of the process is required to meet regulatory body requirements and is currently under development. In addition, I think that transferring the diagnostic platform to work with formalin-fixed paraffin-embedded tissue will be critical to the success of this assay.

In conclusion, technological developments have been the main driving force of innovation in RT over the last two decades. These developments have significantly improved the quality and precision of RT delivery, allowing RT dose escalation and decreased side effects. However, I think that biologically guided RT holds the promise to identify novel clinical approaches to more efficiently and effectively utilize RT in cancer patients. Although not ready for routine clinical use yet, the successful development of RSI shows that it is possible to develop technologies that will make biologically guided RT a reality.

References

Papers of special note have been highlighted as:

■ of interest

1. Delaney G, Jacob S, Featherstone C, Barton M. The role of radiotherapy in cancer treatment: estimating optimal utilization from a review of evidence-based clinical guidelines. *Cancer*. 2005; 104(6):1129–1137. [PubMed: 16080176]
2. Lievens Y, Grau C. Health economics in radiation oncology: introducing the ESTRO HERO project. *Radiother Oncol*. 2012; 103:109–112. [PubMed: 22325993]
3. Bentzen SRM, Heeren G, Cottier B, et al. Towards evidence-based guidelines for radiotherapy infrastructure and staffing needs in Europe: the ESTRO QUARTS project. *Radiother Oncol*. 2005; 75(3):355–365. [PubMed: 16086915]
4. Sher DJ. Cost-effectiveness studies in radiation therapy. *Expert Rev Pharmacoecon Outcomes Res*. 2010; 10(5):567–582. [PubMed: 20950072]
5. Verellen D, De Ridder M, Linthout N, Tournel K, Soete G, Storme G. Innovations in image-guided radiotherapy. *Nat Rev Cancer*. 2007; 7(12):949–960. Excellent review that discusses the technological advances in radiation therapy delivery. [PubMed: 18034185]
6. Bucci MK, Bevan A, Roach M 3rd. Advances in radiation therapy: conventional to 3D, to IMRT, to 4D, and beyond. *CA Cancer J Clin*. 2005; 55(2):117–134. [PubMed: 15761080]
7. Voduc KD, Cheang MC, Tyldesley S, Gelmon K, Nielsen TO, Kennecke H. Breast cancer subtypes and the risk of local and regional relapse. *J Clin Oncol*. 2010; 28(10):1684–1691. [PubMed: 20194857]
8. Abdulkarim BS, Cuartero J, Hanson J, Deschenes J, Lesniak D, Sabri S. Increased risk of locoregional recurrence for women with T1–2N0 triple-negative breast cancer treated with modified radical mastectomy without adjuvant radiation therapy compared with breast-conserving therapy. *J Clin Oncol*. 2011; 29(21):2852–2858. [PubMed: 21670451]
9. Peters LJ. The ESTRO Regaud lecture. Inherent radio sensitivity of tumor and normal tissue cells as a predictor of human tumor response. *Radiother Oncol*. 1990; 17(3):177–190. [PubMed: 2181561]
10. Peters LJ, Brock WA, Chapman JD, Wilson G. Predictive assays of tumor radiocurability. *Am J Clin Oncol*. 1988; 11(3):275–287. [PubMed: 3289361]
11. Torres-Roca JF, Stevens CW. Predicting response to clinical radiotherapy: past, present, and future directions. *Cancer Control*. 2008; 15(2):151–156. [PubMed: 18376382]
12. West CM, Davidson SE, Roberts SA, Hunter RD. Intrinsic radiosensitivity and prediction of patient response to radiotherapy for carcinoma of the cervix. *Br J Cancer*. 1993; 68(4):819–823. [PubMed: 8398714]
13. West CM, Davidson SE, Roberts SA, Hunter RD. The independence of intrinsic radiosensitivity as a prognostic factor for patient response to radiotherapy of carcinoma of the cervix. *Br J Cancer*. 1997; 76(9):1184–1190. [PubMed: 9365167]
14. Buffa FM, Davidson SE, Hunter RD, Nahum AE, West CM. Incorporating biologic measurements (SF(2), CFE) into a tumor control probability model increases their prognostic significance: a study in cervical carcinoma treated with radiation therapy. *Int J Radiat Oncol Biol Phys*. 2001; 50(5):1113–1122. [PubMed: 11483320]
15. Bjork-Eriksson T, West C, Karlsson E, Mercke C. Tumor radiosensitivity (SF2) is a prognostic factor for local control in head and neck cancers. *Int J Radiat Oncol Biol Phys*. 2000; 46(1):13–19. [PubMed: 10656366]
16. Stausbol-Gron B, Overgaard J. Relationship between tumour cell *in vitro* radiosensitivity and clinical outcome after curative radiotherapy for squamous cell carcinoma of the head and neck. *Radiother Oncol*. 1999; 50(1):47–55. [PubMed: 10225557]
17. Taghian A, Ramsay J, Allalunis-Turner J, et al. Intrinsic radiation sensitivity may not be the major determinant of the poor clinical outcome of glioblastoma multiforme. *Int J Radiat Oncol Biol Phys*. 1993; 25(2):243–249. [PubMed: 8380568]
18. Eschwege F, Bourhis J, Girinski T, et al. Predictive assays of radiation response in patients with head and neck squamous cell carcinoma: a review of the Institute Gustave Roussy experience. *Int J Radiat Oncol Biol Phys*. 1997; 39(4):849–853. [PubMed: 9369133]

19. Movsas B, Chapman JD, Hanlon AL, et al. Hypoxic prostate/muscle pO₂ ratio predicts for biochemical failure in patients with prostate cancer: preliminary findings. *Urology*. 2002; 60(4): 634–639. [PubMed: 12385924]
20. Fyles A, Milosevic M, Hedley D, et al. Tumor hypoxia has independent predictor impact only in patients with node-negative cervix cancer. *J Clin Oncol*. 2002; 20(3):680–687. [PubMed: 11821448]
21. Begg AC, Haustermans K, Hart AA, et al. The value of pretreatment cell kinetic parameters as predictors for radiotherapy outcome in head and neck cancer: a multicenter analysis. *Radiother Oncol*. 1999; 50(1):13–23. [PubMed: 10225552]
22. Bourhis J, Dendale R, Hill C, et al. Potential doubling time and clinical outcome in head and neck squamous cell carcinoma treated with 70 GY in 7 weeks. *Int J Radiat Oncol Biol Phys*. 1996; 35(3):471–476. [PubMed: 8655369]
23. Corvo R, Giaretti W, Sanguineti G, et al. *In vivo* cell kinetics in head and neck squamous cell carcinomas predicts local control and helps guide radiotherapy regimen. *J Clin Oncol*. 1995; 13(8): 1843–1850. [PubMed: 7636527]
24. Ramalingam SS, Owonikoko TK, Khuri FR. Lung cancer: new biological insights and recent therapeutic advances. *CA Cancer J Clin*. 2011; 61(2):91–112. [PubMed: 21303969]
25. Krause M, Baumann M. Clinical biomarkers of kinase activity: examples from *EGFR* inhibition trials. *Cancer Metastasis Rev*. 2008; 27(3):387–402. [PubMed: 18427731]
26. Van De Vijver MJ, He YD, Van't Veer LJ, et al. A gene-expression signature as a predictor of survival in breast cancer. *N Engl J Med*. 2002; 347(25):1999–2009. Along with [27], original work describing the successful development of a gene-expression signature to predict clinical outcome in breast cancer. This work led to the development of the commercial diagnostic assay, Mammaprint[®], by Agendia (Amsterdam, The Netherlands). [PubMed: 12490681]
27. Van't Veer LJ, Dai H, Van De Vijver MJ, et al. Gene expression profiling predicts clinical outcome of breast cancer. *Nature*. 2002; 415(6871):530–536. [PubMed: 11823860]
28. Paik S, Shak S, Tang G, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med*. 2004; 351(27):2817–2826. Original work describing the development of Oncotype DX[®] by Genomic Health, Inc. (CA, USA), another successful commercial assay. [PubMed: 15591335]
29. Amado RG, Wolf M, Peeters M, et al. Wild-type *KRAS* is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J Clin Oncol*. 2008; 26(10):1626–1634. [PubMed: 18316791]
30. Karapetis CS, Khambata-Ford S, Jonker DJ, et al. *K-ras* mutations and benefit from cetuximab in advanced colorectal cancer. *N Engl J Med*. 2008; 359(17):1757–1765. [PubMed: 18946061]
31. Mitsudomi T, Morita S, Yatabe Y, et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised Phase 3 trial. *Lancet Oncol*. 2010; 11(2):121–128. [PubMed: 20022809]
32. Maemondo M, Inoue A, Kobayashi K, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated *EGFR*. *N Engl J Med*. 2010; 362(25):2380–2388. [PubMed: 20573926]
33. Shaw AT, Yeap BY, Solomon BJ, et al. Effect of crizotinib on overall survival in patients with advanced non-small-cell lung cancer harbouring *ALK* gene rearrangement: a retrospective analysis. *Lancet Oncol*. 2011; 12(11):1004–1012. [PubMed: 21933749]
34. Beer DG, Kardia SL, Huang CC, et al. Gene-expression profiles predict survival of patients with lung adenocarcinoma. *Nat Med*. 2002; 8(8):816–824. [PubMed: 12118244]
35. Chung CH, Parker JS, Karaca G, et al. Molecular classification of head and neck squamous cell carcinomas using patterns of gene expression. *Cancer Cell*. 2004; 5(5):489–500. [PubMed: 15144956]
36. Eschrich S, Yang I, Bloom G, et al. Molecular staging for survival prediction of colorectal cancer patients. *J Clin Oncol*. 2005; 23(15):3526–3535. [PubMed: 15908663]
37. Pawitan Y, Bjohle J, Amler L, et al. Gene expression profiling spares early breast cancer patients from adjuvant therapy: derived and validated in two population-based cohorts. *Breast Cancer Res*. 2005; 7(6):R953–R964. [PubMed: 16280042]

38. Wang Y, Klijn JG, Zhang Y, et al. Gene-expression profiles to predict distant metastasis of lymph-node-negative primary breast cancer. *Lancet*. 2005; 365(9460):671–679. [PubMed: 15721472]
39. Hood L, Heath JR, Phelps ME, Lin B. Systems biology and new technologies enable predictive and preventative medicine. *Science*. 2004; 306(5696):640–643. Along with [40,41], an excellent review on systems biology and its applications in the development of novel technologies. [PubMed: 15499008]
40. Hood L, Perlmutter RM. The impact of systems approaches on biological problems in drug discovery. *Nat Biotech*. 2004; 22(10):1215–1217.
41. Kitano H. Systems biology: a brief overview. *Science*. 2002; 295(5560):1662–1664. [PubMed: 11872829]
42. Kitano H. Computational systems biology. *Nature*. 2002; 420(6912):206–210. [PubMed: 12432404]
43. Goh KI, Cusick ME, Valle D, Childs B, Vidal M, Barabasi AL. The human disease network. *Proc Natl Acad Sci USA*. 2007; 104(21):8685–8690. [PubMed: 17502601]
44. Torres-Roca JF, Eschrich S, Zhao H, et al. Prediction of radiation sensitivity using a gene expression classifier. *Cancer Res*. 2005; 65(16):7169–7176. Original work describing the development of the radiosensitivity index (RSI), showing proof-of-principle that models based on gene expression can be developed to predict cellular radiosensitivity. [PubMed: 16103067]
45. Eschrich S, Zhang H, Zhao H, et al. Systems biology modeling of the radiation sensitivity network: a biomarker discovery platform. *Int J Radiat Oncol Biol Phys*. 2009; 75(2):497–505. Original work describing the development of RSI, showing the development of a systems-biology approach that integrates genomic expression with biological indicators to model the radiosensitivity network. [PubMed: 19735874]
46. Russell JS, Brady K, Burgan WE, et al. Gleevec-mediated inhibition of Rad51 expression and enhancement of tumor cell radiosensitivity. *Cancer Res*. 2003; 63(21):7377–7383. [PubMed: 14612536]
47. Ma BBY, Bristow RG, Kim J, Siu LL. Combined-modality treatment of solid tumors using radiotherapy and molecular targeted agents. *J Clin Oncol*. 2003; 21(14):2760–2776. [PubMed: 12860956]
48. Cerna D, Camphausen K, Tofilon PJ. Histone deacetylation as a target for radiosensitization. *Curr Top Dev Biol*. 2006; 73:173–204. [PubMed: 16782459]
49. Milas L, Mason KA, Liao Z, Ang KK. Chemoradiotherapy: emerging treatment improvement strategies. *Head Neck*. 2003; 25(2):152–167. [PubMed: 12509799]
50. Kaminski JM, Hanlon AL, Joon DL, Meistrich M, Hachem P, Pollack A. Effect of sequencing of androgen deprivation and radiotherapy on prostate cancer growth. *Int J Radiat Oncol Biol Phys*. 2003; 57(1):24–28. [PubMed: 12909211]
51. Deng X, Hofmann Er, Villanueva A, et al. *Caenorhabditis elegans* ABL-1 antagonizes p53-mediated germline apoptosis after ionizing irradiation. *Nature Genet*. 2004; 36(8):906–912. [PubMed: 15273685]
52. Hallahan DE, Dunphy E, Kuchibhotla J, Kraft A, Unlap T, Weichselbaum RR. Prolonged *c-jun* expression in irradiated ataxia telangiectasia fibroblasts. *Int J Radiat Oncol Biol Phys*. 1996; 36(2):355–360. [PubMed: 8892460]
53. Kao GD, McKenna WG, Muschel RJ. p34(Cdc2) kinase activity is excluded from the nucleus during the radiation-induced G(2) arrest in HeLa cells. *J Biol Chem*. 1999; 274(49):34779–34784. [PubMed: 10574948]
54. Li N, Karin M. Ionizing radiation and short wavelength UV activate NF-kappa B through two distinct mechanisms. *Proc Natl Acad Sci USA*. 1998; 95(22):13012–13017. [PubMed: 9789032]
55. Liu J, Yang D, Minemoto Y, Leitges M, Rosner MR, Lin A. NF-[kappa]B is required for UV-induced JNK activation via induction of PKC[delta]. *Molecular Cell*. 2006; 21(4):467–480. [PubMed: 16483929]
56. Fryknäs M, Dhar S, Oberg F, et al. STAT1 signaling is associated with acquired crossresistance to doxorubicin and radiation in myeloma cell lines. *Int J Cancer*. 2007; 120(1):189–195. [PubMed: 17072862]

57. Nakajima T, Yukawa O, Azuma C, et al. Involvement of protein kinase C-related anti-apoptosis signaling in radiation-induced apoptosis in murine thymic lymphoma (3SBH5) cells. *Radiat Res.* 2004; 161(5):528–534. [PubMed: 15161371]
58. Pamment J, Ramsay E, Kelleher M, Dornan D, Ball KL. Regulation of the IRF-1 tumour modifier during the response to genotoxic stress involves an ATM-dependent signalling pathway. *Oncogene.* 2002; 21(51):7776–7785. [PubMed: 12420214]
59. Terzoudi, Gi; Jung, T.; Hain, J., et al. Increased G2 chromosomal radiosensitivity in cancer patients: the role of cdk1/cyclin-B activity level in the mechanisms involved. *Int J Radiat Biol.* 2000; 76(5):607–615. [PubMed: 10866282]
60. Wang QE, Zhu Q, Wani G, El-Mahdy MA, Li J, Wani AA. DNA repair factor XPC is modified by SUMO-1 and ubiquitin following UV irradiation. *Nucleic Acids Res.* 2005; 33(13):4023–4034. [PubMed: 16030353]
61. Marples B, Collis SJ. Low-dose hyper-radiosensitivity: past, present, and future. *Int J Radiat Oncol Biol Phys.* 2008; 70(5):1310–1318. [PubMed: 18374221]
62. Lindsay KJ, Coates PJ, Lorimore SA, Wright EG. The genetic basis of tissue responses to ionizing radiation. *Br J Radiol* 80 Spec No. 2007; 1:S2–S6.
63. Starmans MH, Zips D, Wouters BG, Baumann M, Lambin P. The use of a comprehensive tumour xenograft dataset to validate gene signatures relevant for radiation response. *Radiother Oncol.* 2009; 92(3):417–422. [PubMed: 19615772]
64. Eschrich SA, Pramana J, Zhang H, et al. A gene expression model of intrinsic tumor radiosensitivity: prediction of response and prognosis after chemoradiation. *Int J Radiat Oncol Biol Phys.* 2009; 75(2):489–496. Original work describing the development of RSI, demonstrating independent clinical validation for RSI in three data sets of rectal, esophageal and head and neck cancer. [PubMed: 19735873]
65. Torres Roca, JF.; Fulp, WJ.; Pawitan, Y., et al. RSI: a novel biomarker predicts RT therapeutic benefit in breast cancer. Program and Abstracts of ASTRO 53rd Annual Meeting; Miami Beach, FL, USA. 2–6 October 2011; p. Abstract 122
66. Dalton WS, Friend SH. Cancer biomarkers – an invitation to the table. *Science.* 2006; 312(5777): 1165–1168. [PubMed: 16728629]
67. Clark GM. Prognostic factors versus predictive factors: examples from a clinical trial of erlotinib. *Mol Oncol.* 2008; 1(4):406–412. [PubMed: 19383314]
68. Sauer R, Becker H, Hohenberger W, et al. Preoperative versus postoperative chemoradiotherapy for rectal cancer. *N Engl J Med.* 2004; 351(17):1731–1740. [PubMed: 15496622]
69. O'Neill BD, Brown G, Heald RJ, Cunningham D, Tait DM. Non-operative treatment after neoadjuvant chemoradiotherapy for rectal cancer. *Lancet Oncol.* 2007; 8(7):625–633. [PubMed: 17613424]
70. Habr-Gama A, Perez Ro, Nadalin W, et al. Operative versus nonoperative treatment for stage 0 distal rectal cancer following chemoradiation therapy: long-term results. *Ann Surg.* 2004; 240(4): 711–717. discussion 717–718. [PubMed: 15383798]
71. Maas M, Beets-Tan RG, Lambregts DM, et al. Wait-and-see policy for clinical complete responders after chemoradiation for rectal cancer. *J Clin Oncol.* 2011; 29(35):4633–4640. [PubMed: 22067400]
72. Butcher EC, Berg EL, Kunkel EJ. Systems biology in drug discovery. *Nature Biotech.* 2004; 22(10):1253–1259.

Executive summary

Background

- Radiation therapy (RT) is the most commonly prescribed therapeutic agent in clinical oncology.
- Approximately 50% of cancer patients receive RT.
- The development of a successful molecular diagnostic to more efficiently utilize RT would be of significant clinical importance.

Historical perspective to the development of radiosensitivity assays

- Past efforts aimed at developing diagnostics to predict tumor radiosensitivity have focused in three areas: *ex vivo* determination of tumor survival at 2 Gy (SF2); measurement of tumor pO₂; and measurement of tumor proliferative potential.
- None of the diagnostics became routine.
- However, this research effort established that it was possible to develop diagnostics to predict tumor radiosensitivity.

Diagnostic approaches to personalized cancer therapy

- A central tenet of the personalized cancer therapy era is the successful development of molecular diagnostics to treat the right tumor with the right drug.
- Examples of successful molecular diagnostics in clinical use include: Oncotype DX[®] (Genomic Health, Inc., CA, USA); Mammaprint[®] (Agendia, Amsterdam, The Netherlands); *EGFR* mutational analysis; *RAS* mutational analysis; and the *ALK* gene rearrangement.
- It was hypothesized that it would be possible to generate a successful molecular diagnostic for tumor radiosensitivity using a genomic approach.

Radiosensitivity model development & clinical validation

- A radiosensitivity classifier was generated in 35 cell lines as proof-of-principle that gene expression and linear regression could be used to build predictive models of cellular radiosensitivity.
- Genes identified by this initial classifier were biologically validated.
- A systems-biology model was developed incorporating biological features (*RAS* mutational status, *p53* mutational status and tissue of origin) along with gene expression. This model was used to identify the ten-gene network in a 48 cell line database.
- The ten-gene network was applied to the development of a radiosensitivity molecular signature (radiosensitivity index [RSI]) using linear regression. This final model was trained and developed in a 48-cell line database.
- RSI has been clinically validated in five independent data sets in four disease sites in a total of 621 patients.

Clinical utility applications for RSI: rectal cancer & head & neck cancer

- The clinical applications for RSI vary greatly based on disease site.

- Potential clinical applications include:
 - Rectal and esophageal cancer: to identify patients unlikely to benefit from preoperative treatment with concurrent chemoradiation.
 - Head and neck cancer: to identify patients that might benefit from treatment de-intensification and/or patients that may benefit from more aggressive treatment.

Future perspective

- Biologically guided RT will lead to clinical strategies to more effectively and efficiently utilize RT in cancer patients.
- The development of RSI is the first step towards the fulfillment of this important clinical goal.

Figure 1. Systems biology approaches to modeling disease
Adapted with permission from [72].

Figure 2. Schema for a radiosensitivity classifier

A leave-one-out cross-validation approach was utilized to develop and test the classifier. Linear regression was utilized to identify radiation-specific biomarkers to build the classifier. The classifier was based on a linear regression equation where y_x stands for expression for the selected genes and k_x are coefficients generated during the training process. Accuracy was 62%, based on continuous variable prediction (not binary; $p = 0.002$).

SF2: Survival fraction at 2 Gy.

Data taken from [44].

independent clinical data sets in four disease sites in a total of 621 patients. Importantly, RSI was

Figure 3. Strategy for development of a systems biology model of radiosensitivity

(A) Details the step-by-step approach in the development process. (B) Identification of ten genes in the systems model. A linear regression equation was developed to correlate gene expression and cellular radiosensitivity in a database of 48 cell lines. The final algorithm for RSI was developed in the 48-cell line database as a linear function of gene expression for the ten genes identified. This final lock-down algorithm was then validated in five independent data sets, totaling 621 patients.

[†]Data set originated from the Karolinska Institute (Stockholm, Sweden).

[‡]Data set originated from the Erasmus Medical Center (Rotterdam, The Netherlands).

RSI: Radiosensitivity index; TO: Tissue of origin.

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Figure 4. Radiosensitivity index is correlated with clinical response to concurrent radiochemotherapy in rectal and esophageal cancer patients

Radiosensitivity index (RSI) for each patient was generated using a rank-based linear regression model built from the cell line data. Statistical significance was determined using a one-sided Mann–Whitney test for differences. **(A)** The mean RSI of responders is significantly lower than in nonresponders in both clinical cohorts (esophageal: $p = 0.05$; rectal: $p = 0.03$). **(B)** RSI for each individual patient in the cohorts (combined: $p = 0.002$). PPV: Positive predictive value; Sens.: Sensitivity; Spec.: Specificity; SF2: Survival fraction at 2 Gy.

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Figure 5. Radiosensitivity index distinguishes clinical populations with different disease-related outcomes in head and neck cancer

Radiosensitivity predictions were generated with the gene-expression model as described in 92 patients treated with definitive concurrent radiochemotherapy at The Netherlands Cancer Institute (Amsterdam, The Netherlands). Using the 25th percentile as the cutoff point, there is a superior 2-year locoregional control in the predicted radiosensitive group (green vs red, 86 vs 61%; $p = 0.05$)

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