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PROSPECTIVE EVALUATION OF AN *IN VITRO* RADIATION RESISTANCE ASSAY IN LOCALLY ADVANCED CANCER OF THE UTERINE CERVIX: A SOUTHWEST ONCOLOGY GROUP STUDY

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Abstract

Objectives—To investigate the feasibility of performing a fresh-tissue, *in vitro* radiation resistance assay (IVRRA) in a cooperative group setting and to assess the association of IVRRA results with clinical outcomes.

Methods—Women with Stages IIB-IVA carcinoma of the uterine cervix without obvious para-aortic lymphadenopathy on imaging were eligible. Primary tumor biopsies were shipped to a central testing facility where agar-based cell suspensions were exposed to 300 cGy of RT ± cisplatin and cultured for 5 days. ^{3}H-thymidine incorporation was used to determine percent cell inhibition (PCI) of test specimen compared to that of the untreated control. Tumors were considered to exhibit extreme radiation resistance (ERR), intermediate radiation resistance (IRR) or low radiation resistance (LRR) based on a standard data set from 39 previously studied specimens. Standardized doses of external beam radiation and intracavitary brachytherapy, when feasible, in addition to platinum-based chemotherapy were mandated. Progression-free survival (PFS) was the primary endpoint. Clinical response and overall survival (OS) were secondary endpoints. Clinical investigators were blinded to assay data and vice versa.

Results—Thirty-six patients were enrolled, but analysis was limited to 17 patients whose specimens were adequate for IVRRA. The median follow-up time was 40 months. There was no association between IVRRA and response. In the Cox model, IRR/ERR tumors showed worse PFS [HR=11.2 (95% CI 1.3–96, p=0.03)] and worse OS [HR=11.7 (95% CI 1.4–99.6, p=0.03)] compared to LRR tumors when IVRRA was performed with RT alone, but there were no associations between IVRRA and PFS or OS when cisplatin was added to the IVRRA.

Conclusions—IVRRA (RT alone) results correlated with PFS and OS in this prospective trial, but follow-up trials are indicated to address feasibility and to confirm results in an expanded cohort. If

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confirmed, IVRRA could potentially direct molecular identification of novel targeted therapeutic approaches which might counteract radiation resistance.

Introduction

An estimated 11,270 United States women will be diagnosed with cancer of the uterine cervix in 2009.[1] Most of these women will present with early-stage disease that is amenable to surgery, but approximately 36% will present with locally advanced disease (LACC)[2] for which radical radiotherapy(RT) is the primary treatment modality. Though women with LACC comprise fewer than half of all cervical cancer cases, they account for a disproportionate number of cancer deaths.[2] The 1973 and 1978 Patterns of Care studies reported 4-year in-field recurrence rates of 20% and 47% in women with Stage IIB and IIIB disease, respectively [3], suggesting that radiation failures contribute to the mortality of women with LACC. Since that time, only one modification to standard RT was instituted in 1999 after five randomized trials demonstrated better progression-free and overall survival when radio-sensitizing cisplatin was given in addition to standard pelvic radiotherapy for LACC.[4–10] Despite this improvement, these trials reported five-year in-field failure rates for all stages ranging from 16% to 25%.

It is postulated that biologic radiation resistance contributes, at least in part, to tumor recurrence and death in locally-advanced tumors such as cervical, breast, lung, head and neck, and colorectal cancers for which radiation is the primary treatment modality. Though tumor hypoxia[11–14], angiogenesis[15–20], and aberrant cellular metabolism[21,22] have been proposed, the mechanisms associated with biologic radiation resistance have yet to be clearly elucidated. Until these mechanisms can be characterized and their impact on patient outcome determined, *in vitro* resistance assays have the potential to empirically predict the failure of radiation prior to its administration.

West, et al was the first to describe a clonogenic assay demonstrating a correlation between an above average *in vitro* response to radiation and prolonged progression-free survival [23], but widespread application of this assay was limited by technical issues including labor intensiveness, long duration (14 days), high cost, and poor standardization due to low plating inefficiencies and clump artifact.[24] An *in vitro* radiation resistance assay based on extreme drug resistance (EDR) technology that overcomes these technical limitations has been previously described. [25,26] The purpose of this study was to determine if results of this assay correlate with outcome in women with locally advanced cervical cancer receiving protocol-mandated treatment on a prospective, multi-center clinical trial.

Materials and Methods

Eligibility

Women with previously-untreated stage IIB-IVA squamous, adeno-squamous, or adenomatous carcinoma (confirmed by central pathologic review) of the uterine cervix with planned primary chemo-radiotherapy were eligible. A complete physical examination by a gynecologist with documentation of pelvic disease measurable in two dimensions, in addition to para-aortic lymph node assessment by either bipedal lymphangiogram, CT scan or MRI of the abdomen was required. Women with findings on imaging suggestive of para-aortic lymph nodes metastases were ineligible unless those lymph nodes were proven to be negative by surgical histo-pathologic examination. All women gave informed consent for participation and signed consent documents that had been approved by the Institutional Review Board of the enrolling and treating institution.

Tumor procurement and IVRRA preparation

Incisional biopsies of 1.5–3 grams of fresh tissue were collected trans-vaginally, shipped in sterile RPMI to a central testing facility (Oncotech, Tustin, CA), and disaggregated mechanically and enzymatically (DNAse/Collagenase Type I) into single-cell agar-based suspensions (RPMI 1640, 15% fetal calf serum, 0.3% agarose). Viability was determined by trypan blue exclusion, and viable specimens were plated onto a 0.4% agarose base layer in 24-well plates at a density of $1–5 \times 10^5$ cells per well. Viable specimens were exposed in triplicate to 3, 6, and 12 Gy of x-ray irradiation (0 Gy as negative control) using a 6 MV X-ray beam from a Cliniac 2300C/D linear accelerator (Varian Oncology Systems, Palo Alto, CA). Irradiated plates were surrounded by tissue equivalent materials to maximize backscatter and to ascertain uniformity of the radiation dose delivered [25]. All plates were then incubated at 37°C, 5% CO2 for 5 days, and [^{3}H]thymidine (5 uCi/well) was added for the last 48 hours. Agarose-tumor cell suspensions were melted at 90°C and harvested onto glass-fiber filters from which radioactivity was counted by liquid scintillation. Duplicate positive (high-dose cisplatin-exposed) and quadruplicate negative (media alone and media plus untreated cells) control cultures were performed with each assay. Results were reported as the percent cell inhibition (PCI) proliferation ([3H]thymidine activity) compared to that of the untreated control. Tumors were considered to exhibit either extreme radiation resistance (ERR), intermediate radiation resistance (IRR) or low radiation resistance (LRR) based on a published data set generated from 39 specimens and 4 cell lines (Figure 1) where PCI at or above the median=LRR, at one standard deviation (SD) below the median PCI= IRR, and 2 SD below the median =ERR [25].

Patient treatment protocol

In an attempt to minimize treatment bias, a standardized chemo-radiation treatment plan was mandated. External beam radiation was delivered to a minimum dose of 45 Gy to the whole pelvis, and a 10.8 Gy parametrial boost was permitted. Intracavitary brachytherapy could be delivered by either low-dose-rate or high-dose-rate to achieve a cumulative dose of 80–90 Gy to Point “A”. Weekly platinum-based chemotherapy was mandated. Progression-free survival (PFS) was the primary endpoint and was measured from date of study entry to first-documented date of progression (defined as unequivocal progression of disease as determined by the examining physician, appearance of new lesions, symptomatic deterioration) or death from any cause. Secondary endpoints included clinical response and overall survival (OS). Response was recorded as complete response defined as disappearance of all disease versus no response which included both stable and progressive disease as described by RECIST criteria.[27] Patients were monitored with physical examination and consistent imaging modality every 3 months for the first 2 years, every 6 months for the next 2 years, and annually for the following 6 years thereafter.

Statistical Considerations

Investigators performing clinical assessments were blinded to assay data and vice versa. An exploratory analysis was performed to investigate in a preliminary manner any associations between clinical outcomes and PCI values. To maximize statistical power, PCI values were dichotomized into two categories LRR versus IRR/ERR. Fisher’s exact test was used to assess the association between response and LRR versus IRR/ERR. Overall survival and progression-free survival were estimated using the method of Kaplan-Meier. Cox regression analysis was used to estimate the hazard ratios (HR) between IVRRA LRR vs. IRR/ERR, and the Chi-square statistic from the score test was used to assess statistical significance.

These analyses were exploratory in nature, with the goal of suggesting hypotheses to be studied in larger patient cohorts. No adjustments were made for multiple comparisons. Thus a p-value less than 0.05 was considered significant. All analyses were performed using SAS version 9.0.

Results

Tumor specimens were submitted for 32 patients. The planned accrual of 100 patients was prematurely interrupted when funding for the SWOG Gynecologic Committee was withdrawn. Seventeen of the 32 tumors collected were viable *in vitro*, and the remaining 15 specimens were contaminated, necrotic, or failed to grow in culture. Viable tumors were slightly larger and had a higher fraction of malignant tumor in the submitted specimen, but these differences were not statistically significant (Table 1). The 15 patients whose tumors were unsatisfactory for the *in vitro* assay were excluded from the final analysis. Clinical characteristics of the 17 patients with adequate assay data are listed in Table 1. Most patients had Stage IIB disease (71%), completed EBRT (94%), and received at least two applications of ICBT (82%). All women received weekly cisplatin. The median follow-up time at the reported analysis was 40 months (range 2–56).

In vitro Assay Data

For quality control purposes, each specimen was exposed *in vitro* to increasing doses of RT± CDDP. These dose-response relationships, depicted in Figure 2, resulted in expected increasing cell growth inhibition of 54%, 68%, and 73% for 300, 600, and 1200 cGy exposure respectively. The corresponding PCI values for CDDP alone, CDDP+300 cGy RT, and CDDP+600 cGy RT were 73%, 90%, and 95% respectively. *In vitro* exposure to 300 cGy categorized 7 tumors as LRR and 10 as IRR/ERR. *In vitro* exposure to CDDP alone yielded 8 LRR specimens and 9 IRR/ERR specimens. *In vitro* exposure to both RT and CDDP categorized 8 specimens as LRR, 8 as IRR/ERR with the exclusion of one uninterpretable result (data not shown).

Clinical Correlative Data

There was no association between IVRRA and clinical response (data not shown). However there was a statistically significant difference in PFS (Figures 3A and 3B) and OS (Figures 4A and 4B) between women whose tumors exhibited LRR vs IRR/ERR to *in vitro* RT alone (100% recurrence-free vs. 32% respectively, p=0.03). No difference was detected, however, for women whose tumors exhibited LRR vs IRR/ERR to *in vitro* RT with *in vitro* CDDP (88 vs. 60%, p=0.38). At 24 months, 1 of 7 women with LRR assay results and 6 of 10 women with IRR/ERR assay results had disease recurrence.

In the univariate Cox model, IRR/ERR tumors showed worse PFS [HR=11.2 (95% CI 1.3–96, p=0.03)] and worse OS [HR=11.7 (95% CI 1.4–99.6, p=0.03)] compared to LRR tumors when IVRRA was performed with RT alone, but there were no associations between IVRRA and PFS or OS when cisplatin was added to the IVRRA (Tables 2 and 3).

Discussion

Chemoradiotherapy (CRT) remains the standard treatment for women with locally advanced carcinoma of the uterine cervix. Based on five randomized, controlled trials [4–10], the addition of “radiosensitizing” cisplatin to standard radiation in 1999 achieved the last widely-accepted improvement in progression-free and overall survival in these women. The largest of these trials [9], which only enrolled women with surgically-proven negative para-aortic lymph nodes, documented recurrence in 42% at 5 years, and half of these were in-field treatment failures. Intrinsic biologic resistance to radiation probably contributes, at least in part, to radiation treatment failures in cervical cancer.

To our knowledge, this is the first prospective evaluation of an *in vitro* assay intended to predict the outcome of women treated with CRT. These data report the provocative finding that women whose tumors exhibit LRR to *in vitro* RT alone have a significantly better outcome relative to recurrence and survival than those whose tumors exhibit IRR/ERR in both the actuarial and

regression models. This effect was not observed when *in vitro* CDDP was added to the assay. Although the positive findings were statistically significant, conclusions regarding the predictive capability of the *in vitro* RT alone assay (IVRRA) must still be drawn with caution, because the small study sample size precluded adjustment for potentially -confounding clinical covariates in the regression analysis. Indeed, the original study design estimated that a sample size of 100 patients would provide an 85% power to detect a progression hazard ratio of 2 between women whose tumors exhibited *in vitro* LRR versus IRR/ERR, with a one-sided log rank test at a significance level of 0.05, assuming a PFS of 75% at one-year in this patient population.]

Though the *in vitro* RT alone assay results were associated with outcome, this association was not observed when CDDP was added to the *in vitro* assay. The laboratory validation of this assay reported *in vitro* synergy between RT and CDDP by Chao analysis. [25] However, tumors in this study exposed to RT+ CDDP *in vitro* had a very high cell-kill fraction with a median PCI of 90% (Table 1). This resulted in a skewed distribution among LRR, IRR, ERR groups as defined in Figure 2, where tumors classified as LRR had PCI values ranging from 95–100% in contrast to *in vitro* RT only LRR values ranging from 63–80%, and though the categories themselves are well distributed in terms of numbers allotted to LRR and IRR/ERR for each assay, the PCI values represented in these categories are skewed toward very high PCI values in the RT+CDDP assay. PCI values in the in vitro RT only group were more “normally” distributed. Therefore, it is not unexpected that no differences could be demonstrated between LRR and IRR/ERR tumors when CDDP was added to the assay.

The clinical limitation to widespread application of the IVRRA is the current lack of a validated alternative treatment to standard CRT. Previously-studied strategies include the administration of neoadjuvant chemotherapy followed by radical surgery for LACC, dose-escalated radiation therapy, and exenterative surgery, none of which have shown survival advantage over standard CRT. High-priority clinical trials that examine the addition of novel agents to standard CRT that are intended to alter or enhance the response to CRT alone are open to accrual. These include GOG 219, a randomized phase III trial investigating the addition of tirapazamine, a bioreductive cytotoxic agent that targets hypoxic tissue hypothesized to significantly contribute to radiation resistance, and RTOG-0417, a phase II trial examining the addition of bevacizumab to CRT, based on the rationale of targeting the abnormal tumor vasculature that is postulated to drive growth factor signaling, evasion of apoptosis, and promote selective survival of the radioresistant fraction of the treated tumor. Both of these strategies have demonstrated modest improvements in patient outcomes in other CRT-treated tumors, but it is possible that the benefit of alternative regimens to CRT might be underestimated due to poor patient selection. Women with radioresistant tumors likely represent a minority of patients treated with CRT on phase III clinical trials which limits the statistical power to detect conclusive treatment differences in these specific patients. IVRRA has the potential to identify these women, allowing stratification within clinical trials and enabling the *in vitro* identification of molecular mechanisms of radiation resistance and testing of novel compounds that target these mechanisms.

Procurement of fresh, sterile tumor tissue is optimal for commercially-available *in vitro* chemotherapeutic assays. Locally-advanced cervical cancers are most easily accessed transvaginally, where significant portions of the tumor are likely necrotic and/or colonized with vaginal flora. *In vitro* testing of tumors in this study was negatively impacted by tumor viability, and the 53% of assays completed did not meet the feasibility endpoint of 74%. There were no statistically significant differences in tumor or patient characteristics between women with viable and non-viable tumors, but sample sizes were insufficient to detect such differences. Though technical considerations such as tissue acquisition, time to arrival at central testing facility and conditions during transport might all account for non-viable specimens, it is

possible that in vitro tumor viability is associated with tumor biology, and that higher fractions of larger tumors are necrotic and non-viable. In this cohort, FIGO IIB tumors tended to be more likely to remain viable in vitro than stages IIIB and IVA (p=0.16; Table 1). Quality-controlled IVRRAs might not be practical for single-institution or even regional use.

In summary, the RT-only IVRRA results were associated with outcome in women with LACC treated on protocol with CRT, but this association was not observed when CDDP was added to the in vitro assay. Though validity of these results is limited by the small sample size studied and lack of control for confounding variables, this study is strengthened by its prospective design, standardized CRT protocol, and exclusion of patients with para-aortic nodal metastases. Validation of the IVRRA in a larger sample size has potential implications for research design (biologic analysis of tumors identified as radioresistant and stratification of patients on CRT trials by assay result) and management of women with LACC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. American Cancer Society. Cancer Facts & Figures 2009. Atlanta: American Cancer Society; 2009.
2. Ries, LAG.; Melbert, D.; Krapcho, M.; Stinchcomb, DG.; Howlader, N.; Horner, MJ.; Mariotto, A.; Miller, BA.; Feuer, EJ.; Altekruse, SF.; Lewis, DR.; Clegg, L.; Eisner, MP.; Reichman, M.; Edwards, BK., editors. SEER Cancer Statistics Review, 1975–2005. National Cancer Institute; Bethesda, MD: 2008. http://seer.cancer.gov/csr/1975_2005/, based on November 2007 SEER data submission, posted to the SEER web site
3. Lanciano RM, Won M, Coia LR, Hanks GE. Pretreatment and treatment factors associated with improved outcome in squamous cell carcinoma of the uterine cervix: a final report of the 1973 and 1978 patterns of care studies. Int J of Rad Onc, Bio, Phys 1991;20:667–76.
4. Keys HM, Bundy BN, Stehman FB, Muderspach LI, Chafe WE, Suggs CL, Walker JL, Gersell D. Cisplatin, radiation, and adjuvant hysterectomy compared with radiation and adjuvant hysterectomy for bulky stage IB cervical carcinoma. NEJM 1999;340:1154. [PubMed: 10202166]
5. Morris M, Eifel PJ, Lu J, Grigsby PW, Levenback C, Stevens RE, Rotman M, Gershenson DM, Mutch DG. Pelvic radiation with concurrent chemotherapy compared with pelvic and para-aortic radiation for high-risk cervical cancer. NEJM 1999;340:1137. [PubMed: 10202164]
6. Rose PG, Ali S, Watkins E, Thigpen JT, Deppe G, Clarke-Pearson DL, Insalaco S. Gynecologic Oncology Group. Long-term follow-up of a randomized trial comparing concurrent single agent cisplatin, cisplatin-based combination chemotherapy, or hydroxyurea during pelvic irradiation for locally advanced cervical cancer: a Gynecologic Oncology Group Study. J Clin Oncol 2007 Jul 1;25 (19):2804–10. [PubMed: 17502627]
7. Eifel PJ, Winter K, Morris M, Levenback C, Grigsby PW, Cooper J, Rotman M, Gershenson D, Mutch DG. Pelvic irradiation with concurrent chemotherapy versus pelvic and para-aortic irradiation for high-risk cervical cancer: an update of radiation therapy oncology group trial (RTOG) 90–01. J Clin Oncol 2004 Mar 1;22(5):872–80. [PubMed: 14990643]
8. Whitney CW, Sause W, Bundy BN, Malfetano JH, Hannigan EV, Fowler WC Jr, Clarke-Pearson DL, Liao SY. Randomized comparison of fluorouracil plus cisplatin versus hydroxyurea as an adjunct to radiation therapy in stage IIB-IVA carcinoma of the cervix with negative para-aortic lymph nodes: a Gynecologic Oncology Group and Southwest Oncology Group study. J Clin Oncol 1999 May;17(5): 1339–48. [PubMed: 10334517]

9. Rose PG, Bundy BN, Watkins EB, Thigpen JT, Deppe G, Maiman MA, Clarke Pearson DL, Insalaco S. Concurrent cisplatin-based radiotherapy and chemotherapy for locally advanced cervical cancer. N Engl J Med 1999 Apr 15;340(15):1144–53. Erratum in: N Engl J Med. 1999 Aug 26;341(9):708. [PubMed: 10202165]
10. Morris M, Eifel PJ, Lu J, Grigsby PW, Levenback C, Stevens RE, Rotman M, Gershenson DM, Mutch DG. Pelvic radiation with concurrent chemotherapy compared with pelvic and para-aortic radiation for high-risk cervical cancer. N Engl J Med 1999 Apr 15;340(15):1137–43. [PubMed: 10202164]
11. Nilsson J, Lind BK, Brahme A. Radiation response of hypoxic and generally heterogeneous tissues. Int J Radiat Biol 2002 May;78(5):389–405. [PubMed: 12020429]
12. Daşu A, Denekamp J. Superfractionation as a potential hypoxic cell radiosensitizer: prediction of an optimum dose per fraction. Int J Radiat Oncol Biol Phys 1999 Mar 15;43(5):1083–94. [PubMed: 10192360]
13. Denekamp J, Daşu A, Waites A, Littbrand B. Hyperfractionation as an effective way of overcoming radioresistance. Int J Radiat Oncol Biol Phys 1998 Nov 1;42(4):705–9. [PubMed: 9845081]
14. Zheng H, Olive PL. Influence of oxygen on radiation-induced DNA damage in testicular cells of C3H mice. Int J Radiat Biol 1997 Mar;71(3):275–82. [PubMed: 9134017]
15. O'Reilly MS. Antiangiogenesis and vascular endothelial growth factor/vascular endothelial growth factor receptor targeting as part of a combined-modality approach to the treatment of cancer. Int J Radiat Oncol Biol Phys 2007;69(2 Suppl):S64–6. Review. [PubMed: 17848299]
16. Brieger J, Schroeder P, Gosepath J, Mann WJ. VEGF-subtype specific protection of SCC and HUVECs from radiation induced cell death. Int J Mol Med 2005 Jan;15(1):145–51. [PubMed: 15583841]
17. Geng L, Donnelly E, McMahon G, Lin PC, Sierra-Rivera E, Oshinka H, Hallahan DE. Inhibition of vascular endothelial growth factor receptor signaling leads to reversal of tumor resistance to radiotherapy. Cancer Res 2001 Mar 15;61(6):2413–9. [PubMed: 11289107]
18. Lee CG, Heijn M, di Tomaso E, Griffon-Etienne G, Ancukiewicz M, Koike C, Park KR, Ferrara N, Jain RK, Suit HD, Boucher Y. Anti-Vascular endothelial growth factor treatment augments tumor radiation response under normoxic or hypoxic conditions. Cancer Res 2000 Oct 1;60(19):5565–70. [PubMed: 11034104]
19. Koukourakis MI, Giatromanolaki A, Fountzilas G, Sivridis E, Gatter KC, Harris AL. Angiogenesis, thymidine phosphorylase, and resistance of squamous cell head and neck cancer to cytotoxic and radiation therapy. Clin Cancer Res 2000 Feb;6(2):381–9. [PubMed: 10690514]
20. Fuks Z, Persaud RS, Alfieri A, McLoughlin M, Ehleiter D, Schwartz JL, Seddon AP, Cordon-Cardo C, Haimovitz-Friedman A. Basic fibroblast growth factor protects endothelial cells against radiation-induced programmed cell death *in vitro* and in vivo. Cancer Res 1994 May 15;54(10):2582–90. [PubMed: 8168084]
23. 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;68:819–23. [PubMed: 8398714]
24. Fruehauf JP, Bosanquet AG. *In vitro* determination of drug response: A discussion of clinical applications. Principles and Pract Oncol Updates 1993;7:1–17.
25. Monk BJ, Burger RA, Parker R, Radany EH, Redpath L, Fruehauf JP. Development of an *in vitro* chemo-radiation response assay for cervical carcinoma. Gynecol Oncol 2002 Nov;87(2):193–9. [PubMed: 12477451]
26. Tewari D, Monk BJ, Al-Ghazi MS, Parker R, Heck JD, Burger RA, Fruehauf JP. Gene expression profiling of *in vitro* radiation resistance in cervical carcinoma: a feasibility study. Gynecol Oncol 2005 Oct;99(1):84–91. [PubMed: 16109440]
27. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors (RECIST Guidelines). J Natl Cancer Inst 2000;92:205–16. [PubMed: 10655437]
28. Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science 2005 Jan 7;307(5706):58–62. Review. [PubMed: 15637262]
29. Winkler F, Kozin SV, Tong RT, Chae SS, Booth MF, Garkavtsev I, Xu L, Hicklin DJ, Fukumura D, di Tomaso E, Munn LL, Jain RK. Kinetics of vascular normalization by VEGFR2 blockade governs

brain tumor response to radiation: role of oxygenation, angiopoietin-1, and matrix metalloproteinases. Cancer Cell 2004 Dec;6(6):553–63. [PubMed: 15607960]

30. Tong RT, Boucher Y, Kozin SV, Winkler F, Hicklin DJ, 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 Jun 1;64(11):3731–6. [PubMed: 15172975]