Predictive Assays in Radiation Therapy
Immunotherapy in Cancer Treatment

Radiation Biology

Lecture 4-27-2020

Outline

- Introduction: Predictive assays in radiation therapy
- Examples for specific tumors
- Immunotherapy
- Summary

Introduction

- Absolute radioresistance does not exist: if a sufficiently high dose is delivered, all cells can be sterilized
- Radiation therapy objective is to optimize treatment for a higher probability of cure and minimal normal tissue damage
- Predictive assays are needed due to the potential role they could have in selecting individually tailored therapy course

Current clinical practice

- The radiation oncologist writes a prescription for
  - the total radiation dose in Gy
  - the dose per fraction
  - the number of fractions needed to deliver the total dose (and their temporal separation)
- These variables are mostly dictated by the primary site of disease, the histology and the stage of the cancer
- Geometrical factors are of utter importance: target should be fully covered, volume of exposed normal tissues minimized

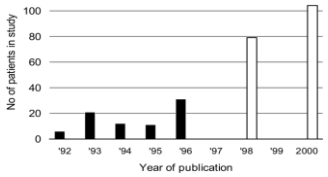
Biological factors determining tumor response to radiotherapy

- There are three widely acknowledged radiobiological factors involved in determining tumor response to radiotherapy:
  - Cellular radiosensitivity
  - Tumor hypoxia
  - Cell proliferation rate
- Studies suggesting the potential of all three as prognostic factors for radiotherapy

Cellular Radiobiology Assays

- Not only tumors, but also normal tissues of individuals, differ in their intrinsic radiosensitivity
- Correlation between cellular radiosensitivity of skin fibroblasts and severe reaction to radiotherapy in an individual with the genetic disorder ataxia telangiectasia (A-T) was initially discovered in 1975
- Several independent studies shown a correlation between the in vitro radiosensitivity of skin fibroblasts and the severity of late complications
- A promising predictive assay?

Cellular Radiobiology Assays



- In the early 1990s, 1 study per year was published (black bars), all of them showing a significant relationship between *in vitro* radiosensitivity of fibroblasts and late effects of radiotherapy
- Two large confirmatory studies (white bars) published in 1998 and 2000 showed no significant predictive value of this assay for late effects

Early predictive assays

- Inherent radiosensitivity for normal tissue side effects is predictive in only small subset of tumors
- Proliferation rate (doubling time) looked promising in many small studies but turned out not to be a significant predictor of radiotherapy outcome in a larger multi-center analysis of 476 patients with head and neck squamous-cell carcinoma (HNSCC)
- Only the Eppendorf microelectrode measurement of partial oxygen tension has consistently shown to have prognostic value, recently confirmed in a joint analysis of outcome after radiotherapy in 397 patients with HNSCC from 7 centers

New era of predictive assays

- The cellular-based assays lacked the sensitivity and specificity
- New opportunity emerged through the Human Genome Project (2001 – 2003)
- Accompanying development of new high-throughput techniques provide extensive capabilities for the analysis of a large number of genes

New era of predictive assays

- Molecular (biomarker) tests have the potential to be more robust, comprehensive, and capable of better standardization between centers
- These assays can be carried out in various clinical samples at the DNA (genome), RNA (transcriptome) or protein (proteome) level

DNA assays for normal tissue radiosensitivity

- It is now recognized that DNA mutations in a single or even a few genes are unlikely to be responsible for the patient-to-patient variability in sensitivity to radiation

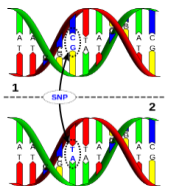


Image from: http://en.wikipedia.org/wiki/Single-nucleotide_polymorphism

- Single nucleotide polymorphisms (SNP) account for ~90% of the naturally occurring sequence variation within a population
  - up to 1% of the total of 3 billion bps
- Database of >10^8 SNPs at http://www.ncbi.nlm.nih.gov/snp

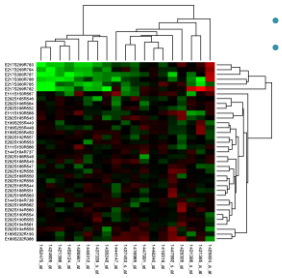
DNA assays for tissue response

- Work carried out to date exploring genotyping to predict normal tissue and tumor response to radiotherapy has involved a candidate gene approach
  - uses a priori knowledge of SNP and gene functions
- Such approaches require smaller sample sizes and benefit from reduced complexity by targeting relevant genes

RNA microarrays

- Gene expression microarrays provide the ability to monitor, rapidly and simultaneously, the RNA expression levels of thousands of genes or the whole genome
- Allows investigation of gene expression profiles associated with the radioresponse of tumors and normal tissues for the derivation of biomarkers to predict local control and toxicity after radiotherapy

DNA/RNA microarrays



- Done on "chips"
- Gene expression values from microarray experiments can be represented as heat maps to visualize the result of data analysis.

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RNA microarrays

Table 4 — Transcriptional response to irradiation

Reference	Cell/tissue	Gy	Time	Up-regulated genes*	Down-regulated genes*
[58]	Human lymphoblastoid	0.5	4 h	***STAT3***, *CAMKK2, SIRT1, CREM, MAPK3K7IP2, GPR56, CHD6, CRSP3, SNURF, CTPP, MLZF*	*WASF2, LCP1, MSH, NIPSNAP1, KIF2C, MCM2, MCM3, MCM7, XRCC4*
[59]	Mouse bone marrow	6.5†	6 h	Jun, Bax, Apaf1, Ccng1, p21, **Stat3**	Rb1, Sirt1, Ccne1, Cdk9, Cdk4, Cdk2, Mcm5, Mcm4, Rad1, Top2A, Top2B, Rad541, Pold2
[60]	Human fibroblasts	2	2 h	***GADD45A***, *BTG2, PCNA, IER5, CDKN1A, PPM1D, SERTAD1, PLK2, PLK3, BBC3, TP53INP1, SH2D2A, SLIC1, CDF15, THSD1*	*CYR61, AMOTL2*
[61]	Rat hepatocytes	8	6 h	*Mif, Mdr1, Grb2, Prl1, Cabp2, Elf2a*	None detected
[62]	Human fibroblasts	3.5/3 + 3.5	2, 24 h	***TP53INP1***, ***CDKN1A***, ***DDB2***, *SOD2, SOD3, CYP1B1, COL15A1*	*H2AX, TOP2A, CCNA2, EGR1, MMP3*
[63]	Mouse kidney	16	1–30 weeks	*Gulo, Klf5, Jag1, Gpp1, Pcm1, Xpo4, Cyclin*G, *Tic22d1*	*Slc22a7, Hba-a1, Syn1, Akp2*
[64]	Mouse rectum	16	1–30 weeks	*Klf5, Jag1, Xpo4,* **RhoB**	*Hba-a1, Syn1, Akp2*
[65]	Bowel tissue	45–60	1–75 months	***RHOB***, *CD47, MMP1, MMP3, MMP14, TIMP1, TIMP2, IGFBP2, ERF1, PDL4, CD27*	*TNF, ITGB4, EPHA1, DPP4, PTPRF*
[66]	Human lymphocytes	1.5–3†	6, 24 h	***CDKN1A***, ***GADD45A***, ***DDB2***	
[67]	Human lymphoblastoid	3, 10	1–24 h	***CDKN1A***, ***GADD45A***, ***DDB2***, *TNFRSF10B, TNFRSF6, PIG3, FDXR, HSPCB, HSPE1, ATF3, PPM1D*	*CCNB1*
[68]	Human lymphoblastoid	5	4 h	***CDKN1A***, ***GADD45A***, *FAS, PCNA, CCNG1, MDM2*	*TNF, KIF23, MYC*
[69]	Human lymphoblastoid	1	4 h	*SRPK1, RENBP, TAN1, F2R, ETV3, MYB, MAPK1, CCNE1, MAPK3, HTR3A, TNK1*	*CSK, VEGFB, MT3, FLT3, DLX1, SFRP2, IGHMBP2, ICSBP1*

Genes highlighted in bold were up- or down-regulated in more than one study. *Selected genes — for full lists see reference. †Total body irradiation.

Proteomics and Tissue Microarrays

- The study of the function of all expressed proteins
- The promise of proteomics lies in the identification of biomarkers that could favorably affect disease diagnosis, as well as our ability to assess the response to treatment and, thereby, the prognosis
- Radioresistance-related proteins were identified in a proteomic study of pre-radiotherapy tumor biopsies from 17 patients with rectal cancer

Biomarker predictive assays

Table 3 — Large national/international genotyping studies

Study name	Full title	Planned recruitment	Primary	Based
Gene-PARE	Genetic Predictors of Adverse Radiotherapy Effects	>2000	Breast, prostate, head and neck	USA, Israel, France, Switzerland
GENEPI	GENEtic pathways for the Prediction of the effects of Irradiation	3000–4000	Breast, prostate, head and neck, rectal	Europe
RadGenomics	Japanese RadGenomics study	1071	Breast, cervix, prostate, head and neck	Japan
RAPPER	Radiogenomics: Assessment of Polymorphisms for Predicting the Effects of Radiotherapy	2200	Breast, prostate, gynaecological	UK

- Large studies are required with exploratory and validation cohorts of patients, associated with the collection of high-quality physics, clinical and outcome data

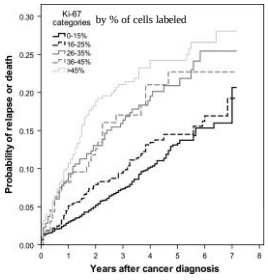
Controversial observations

- Example: the tumor suppresser gene p53
  - Mutations of p53 generally lead to deregulation of cell cycle by eliminating the G1 checkpoint, and impairment of DNA repair process
- Reported to be associated with increased cellular resistance to irradiation and tumor relapse after therapy
- The loss of p53 also shown to either increase or not change radiosensitivity of cells
- Current trend: the p53 protein is analyzed in normal and tumor cells for its functional quality

Controversial observations

- Ki-67 protein is associated with proliferation, cell does not progress through division without this protein generated
- It is a *prognostic* parameter, related to disease-free and overall survival, especially for breast cancer patients
- It is not a *predictive* parameter, so far no correlation with efficacy of a specific chemo agent, etc. has been established

Controversial observations



- Kaplan–Meier plot of disease-free survival in years based on Ki-67 categories
- A total of 3,658 patients were eligible for this retrospective population-based analysis; the mean age was 62 years (median: 63 years; range: 24–99 years)

E. C. Inwald, M. Klinkhammer-Schalke, F. Hofstädter, F. Zeman, M. Koller, M. Gerstenhauer, and O. Ortmann, Ki-67 is a prognostic parameter in breast cancer patients: results of a large population-based cohort of a cancer registry, Breast Cancer Res Treat. 2013; 139(2): 539–552.

Example: breast cancer

Table 1 Local and Regional Recurrence Rates by Breast Cancer Subtype

Study	n	Median Follow-up (mo)	Luminal A (%)	Luminal B (%)	HER2 (%)	Basal (%)
BCS + RT (Nguyen[16])						
5-year LR	793	70	0.8	1.5	8.4	7.1
BCS + RT (Millar[17])						
5-year LR	498	84	1.0	4.3	7.7	9.6
5-year LRR			2.0	4.3	15.3	14.8
BCS + RT (Voduc[18])						
10-year LR	1461	144	8	10	21	14
10-year RR			3	8	16	14
Mastectomy + RT (Kyndi[20])						
5-year LRR	489	204	2	3	13	21
Neoadjuvant chemotherapy + BCS + RT (Yu[19]) 5-year LRR						
0-3 LN	514	65	2	2	14	9
≥4 LN	77		7	0	34	44

Abbreviations: BCS, breast-conserving surgery; LR, local recurrence; LRR, local-regional recurrence; RR, regional recurrence; LN, lymph node.

- At least 4 biologically distinct molecular subtypes of breast cancer were identified, which correlated to different clinical outcomes: luminal A (ER+, and/or PR+, HER2-), luminal B (ER+, and/or PR+, HER2+), HER2+(ER-, PR-, HER2+), and basal-like (ER-, PR-, HER2-) – also called "triple negative"

Example: prostate cancer

- Novel gene-based tests have been developed to improve the prediction accuracy at various phases within the prostate cancer (PCa) disease course
- Urine-based assays (expression levels of PCA3 and TMPRSS2:ERG) aim to refine the selection for both initial and repeat prostate biopsy
- Tissue-based gene expression tests: to predict the occurrence of subsequent PCa events, including adverse characteristics, biochemical recurrence, metastatic progression, and mortality

Immunological markers that predict radiation toxicity

- Radiotoxicities can be generally classified into two major groups, 'early', and 'late' (months to years following treatment)
- Late adverse effects are more critical
  - They are persistent and often progressive
  - May have severe and debilitating effects (e.g. fibrosis, necrosis, atrophy, vascular changes, telangiectasia, secondary malignancies)
  - Can be fatal in some instances

Immunological markers that predict radiation toxicity

- Therapeutic doses of radiation lead to large amounts of cellular damage; the immune response plays a major role in dealing with it
- The resident immune cells produce pro-inflammatory cytokines and growth factors, eventually leading to chronic inflammation, which may induce the genomic instability which in turn perpetuates the inflammation

Immunological markers that predict radiation toxicity

- Modulating immune cells during the radiation-induced inflammatory response may provide benefits to avoid a severe fibrosis outcome
- Several studies for different cancer types implicate immunological markers for radiation sensitivity such as transforming growth factor TGFβ and associated genes

Current (2002) status of various predictive assays

Assay	Brief description	Status (under study/clinical applicable)
Tumour clonogenic survival (SF_2)	♦ Proof of reproductive integrity, usually in semi-solid agar supplemented with growth factors ♦ Assay of fresh tumour biopsies	Clinical
Tumour growth assay (CAM)	♦ Assay of fresh tumour biopsies for fibronectin- coated plates, using crystal violet	Clinical
Chromosome aberrations (PCC & FISH)	♦ Target cells fused with mitotic cells ♦ Assessment of interphase chromosome malformations	Study
Micronucleus assay	♦ Acentric fragments or aborted whole chromosomes detected by Cytokinesis-block method	Clinical
Apoptotic assay	♦ Quantitative index of radiation injury: Apoptotic body or fragments	Study
Oncogene expression	♦ Alteration in either expression or function of cellular genes like c-erb B-2, p53 expression, ras gene, p21 product, c-myc oncogene	Study/Clinical
BUdR labelling index	♦ Fresh tumour biopsy incubated with BUdR and analysed by flow cytometry	Clinical
Growth Fraction	♦ Heat processed immunostaining with MIB1	Clinical
pMI	♦ Ratio of the Mitotic cells to Ki-67 positive cells	Study/Clinical
Mn-SOD	♦ Paraffin section, Immunostaining with anti-Mn-SOD antibody	Study

Current (2002) status of various predictive assays

Assay	Brief description	Status (under study/clinical applicable)
Serial Cytology	♦ Real time assay; evaluation of nuclear changes (micro- or multinucleation)	Clinical
Lymphocyte clonogenic survival	♦ Separation of peripheral blood sample and lymphocyte cultured in medium supplemented with PHA and IL2	Clinical
Microvessel density (MVD)	♦ Evaluation of tumour specimens using a variety of stains (CD31, factor VIII)	Clinical
DNA dsb rejoining assay by Pulsed Field Gel Electro-Phoresis (PFGE)	♦ Estimation of amount of residual DNA double strand breaks	Clinical
Biochemical	Determination of thiols (GSH, CySH) in tissue and plasma	Study/Clinical
Polarographic pO_2 Measurement	♦ Microelectrode sequentially moved through tissue	Clinical
Markers	♦ Nitroimidazole binding in hypoxic cells, detected by immunohistochemistry or physical method (eg PET)	Clinical
Comets	♦ DNA breaks are enhanced by O_2	Study/Clinical

Technical aspects and costs

Method	Technical difficulties	Grade of difficulties (high/low)	Time to obtain results (days)	Initial cost (US$)	Running cost per sample (US$)
Tumour clonogenic survival (SF_2)	Poor PE	high	28	32,000	200
CAM assay	Success rate 70%	high	21	32,000	400
Lymphocyte clonogenic survival	Success rate 95%	high	14	32,000	80
Chromosome aberrations (PCC & FISH)	Difficulty of fusion	high	15	36,000	1,000
Micronucleus assay	Not automated	low	7	27,000	20
Apoptotic assay		low	5	27,000	100
Oncogene expression	Reproducibility	low	1- 5	30,000	500
Growth Fraction (MIB1)		low	3	32,000	100
pMI		low	3	22,000	50
Mn-SOD		low	3	20,000	50
DNA dsb rejoining assay by PFGE	Requires a large tumour sample. Quantitation is complicated.	high	6–7	20,000 in a well equipped lab	50
MVD	no. success rate 100%	low	1 hour	16,000	16
Polarographic pO_2 measurement	Probe consistency, sterilisation, calibration	high	1 hour	80,000	200

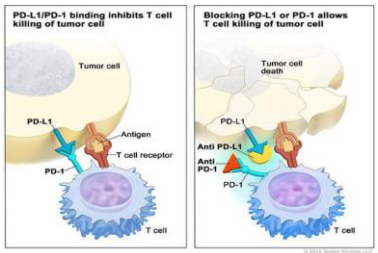
Current (2019) breast cancer genomic tests

- The Breast Cancer Index test analyzes the activity of 7genes that influence how likely the cancer is to recur in 5 to 10 years after diagnosis, and how likely a woman is to benefit from 5 additional years of hormonal therapy
- The EndoPredict test is used to predict the risk of distant recurrence of early-stage, hormone-receptor-positive, HER2-negative breast cancer that is either node-negative or has up to three positive lymph nodes
- The MammaPrint test is used to predict the risk of recurrence within 10 years after diagnosis of stage I or stage II breast cancer that is hormone-receptor-positive or hormone-receptor-negative.
- The Oncotype DX test is used to predict the risk of recurrence of early-stage, hormone-receptor-positive breast cancer, and benefits from chemotherapy after surgery. The Oncotype DX DCIS predicts the risk of recurrence of DCIS and/or the risk of a new invasive cancer developing in the same breast, and benefits from radiation after surgery
- The Prosigna Breast Cancer Prognostic Gene Signature Assay (formerly the PAM50 test) predicts the risk of distant recurrence for postmenopausal women within 10 years of diagnosis of early-stage, hormone-receptor-positive disease with up to 3 positive lymph nodes after 5 years of hormonal therapy

Immunotherapy in treatment of cancer

- Body has a natural mechanism to limit the strength and duration of immune responses with immune checkpoint proteins (e.g., located on the surface of activated T-cells)
- Some tumors can commandeer these proteins and use them to suppress immune responses
- Blocking the activity of immune checkpoint proteins releases the "brakes" on the immune system, increasing its ability to destroy cancer cells

Immunotherapy in treatment of cancer



- Test for levels of PD-L1 (programmed death ligand 1) in tumor, if >50% of cells, use of anti-PD-L1 agents should be successful

Immunotherapy in treatment of cancer

- Several immune checkpoint inhibitors have been approved by the FDA
- The first such drug to receive approval, ipilimumab (Yervoy), for the treatment of advanced melanoma
- Other drugs, targeting different checkpoint inhibitors are: nivolumab (Opdivo) and pembrolizumab (Keytruda); approved for treatments of advanced melanoma or advanced lung cancer

Immunotherapy in treatment of cancer

- Drugs acting through other mechanisms are under development
  - Adaptive cell transfer (ACT) – patient cells with abilities to recognize tumor cells are grown in a lab and re-introduced into the patient in massive quantities
  - Therapeutic antibodies – designed and grown in a lab; several antibody–drug conjugates (ADCs) were FDA approved: ado-trastuzumab emtansine (Kadcyla) for the treatment of some types of breast cancer; brentuximab vedotin (Adcetris) for Hodgkin lymphoma and a type of non-Hodgkin T-cell lymphoma; ibritumomab tiuxetan (Zevalin) for a type of non-Hodgkin B-cell lymphoma

Immunotherapy: new role of radiation therapy

- Standard approach: radiotherapy effects on survival of cancer patients are generally interpreted as the consequence of improved local control of the tumor, directly decreasing systemic spread
- Experimental data from multiple cancer models have provided sufficient evidence to propose a paradigm shift: some of the effects of ionizing radiation are recognized as contributing to *systemic antitumor immunity*

Immunotherapy: new role of radiation therapy

- Example: two metaanalyses of prospective, randomized trials in breast cancer demonstrated a direct contribution of adjuvant radiotherapy to patients' long-term survival; the effect was independent of stage and extent of surgery
- Possible explanation: Radiotherapy engages both the innate and adaptive arms of the immune system, with the potential to convert the irradiated cancer into an in situ vaccine that elicits tumor-specific T cells

Immunotherapy: abscopal effect with RT

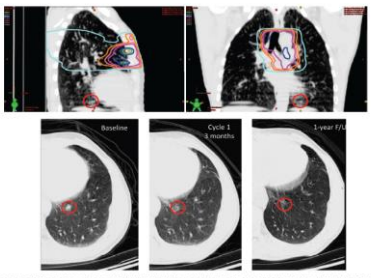
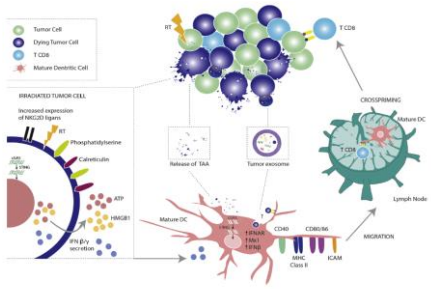


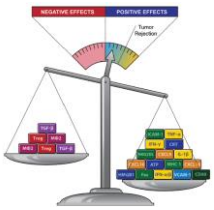
Figure 2. A case of an abscopal response in a patient with metastatic, poorly differentiated thymic carcinoma treated in a trial of granulocyte-macrophage colony-stimulating factor (GM-CSF) and radiation (51). **Top panels**, computed tomography cuts with dose overlays of radiotherapy to the mediastinum. At the base of the left lung, outside the radiation field, a biopsy-proven metastasis is visualized (**red circle**). **Bottom panels**, this lesion regressed after radiotherapy and GM-CSF. Although the patient eventually progressed elsewhere in the body, this area never recurred, and the patient is alive with stable disease 38 months later. F/U = follow-up.

Immunotherapy: abscopal effect with RT



Immunotherapy: abscopal effect with RT

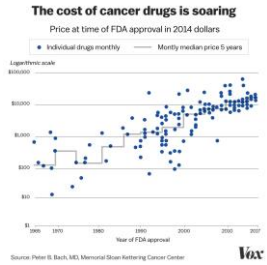
- Why does it work so rarely? Progression of cancer is possible through escape from immune system
- Balance between pro-immunogenic and immunosuppressive effects
- Current status: pre-clinical



Immunotherapy in treatment of cancer

- Cost is prohibitive for many patients:
  - 12 new oncology treatments approved in 2012, 11 were priced above $100,000 for one year of treatment
  - Opdivo, approved for both melanoma and lung cancer, is priced at $12,500 a month, or about $150,000 for a year of treatment; Keytruda, approved for the treatment of metastatic melanoma, costs about the same
  - Provenge (sipuleucel-T), a series of 3 immunotherapy vaccines approved in 2010; improves median overall survival of men with advanced prostate cancer by 4.1 months, is priced at $93,000 per patient
- Patients take the drug until disease progression or unacceptable toxicity

Immunotherapy in treatment of cancer



- Y-axis is log scale
- The average cost of cancer drugs has increased from $50,000 per patient in the mid-1990s to $250,000 today (four times the median US household annual income)

https://www.vox.com/science-and-health/2018/10/1/17923720/immunotherapy-cancer-cost

Summary

- Despite a substantial research effort over 25 years, very few prognostic markers and virtually no predictive assays have been established in routine clinical radiation oncology
- New approaches concentrating on biological markers as opposed to cellular assays are promising due to possibility of acquiring large datasets
- Immunotherapy is a fast-growing and promising field; so far works only for limited number of patients

References

- Predictive assays and their role in selection of radiation as the therapeutic modality, IAEA, VIENNA, 2002
- C.M. L. West et al., Molecular markers predicting radiotherapy response: report and recommendations from an international atomic energy agency technical meeting, Int. J. Radiation Oncology Biol. Phys., Vol. 62, No. 5, pp. 1264–1273, 2005
- C.M. L. West , The Genomics Revolution and Radiotherapy, Clinical Oncology (2007) 19: 470-480
- S.N. Bentzen, From Cellular to High-Throughput Predictive Assays in Radiation Oncology: Challenges and Opportunities, Semin Radiat Oncol 18:75-88, 2008
- Michael S. Leapman and Peter R. Carroll, New Genetic Markers for Prostate Cancer, Urologic Clinics of North America 43, 2016, pp. 7-15
- Carl N. Sprung , Helen B. Forrester, Shankar Siva, Olga A. Martin, Immunological markers that predict radiation toxicity, Cancer Letters 368 (2015) 191–197
- http://www.cancer.gov/research/areas/treatment/immunotherapy-using-immune-system
- http://www.curetoday.com/publications/cure/2015/immunotherapy/considering-cost-whats-an-immunotherapy-worth?p=1
- E.A. Repasky, Cancer and the Immune System: The Basics, AAPM Annual meeting 2016

Beware of the bystander effect!

- Richard W. Smith, Colin B. Seymour, Richard D. Moccia, Carmel E. Mothersill, **Irradiation of rainbow trout at early life stages results in trans-generational effects including the induction of a bystander effect in non-irradiated fish**, Environmental Research 145, 2016, pp. 26–38.