Radiation Therapy and Genomic Interactions in Breast Cancer Patients

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Radiation Therapy in Oncology

- Why talk about RT?
  - It is the most commonly utilized single therapeutic agent in oncology (up to 60% of all cancer patients)
  - Responsible for 40% of all cancer cures
  - Highly cost effective

The empiric basis of modern radiotherapy

1911

Claudius Regaud –Fractionated RT more effective than single dose

1920-30

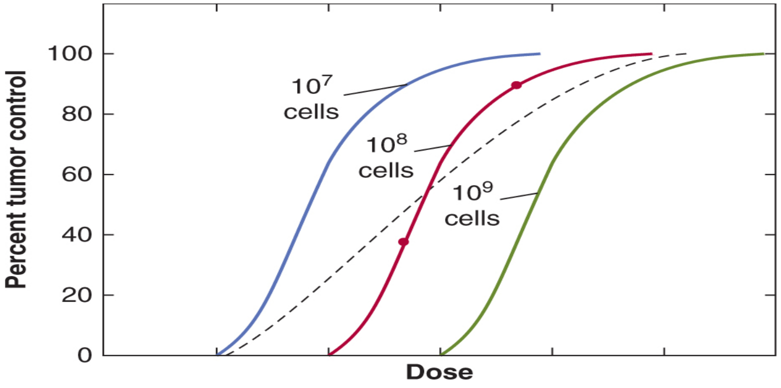
THE RESULTS AND METHODS OF TREATMENT OF CANCER BY RADIATION

PROFESSOR HENRI COUTARD, M.D.

PARIS, FRANCE

Coutard reports fractionated RT cures head and neck cancer

1940-60



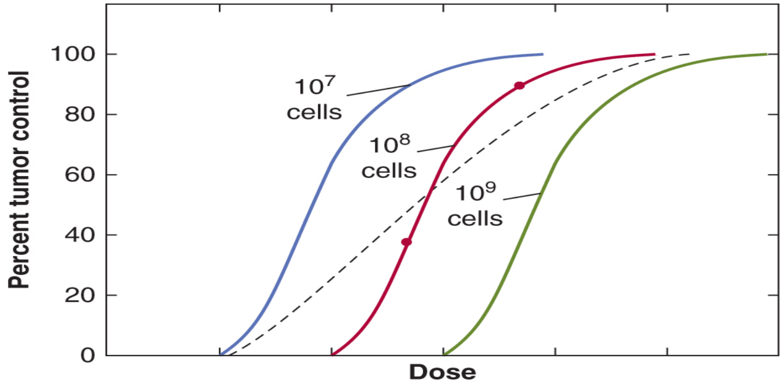
Fletcher summarizes required doses for optimized tumor control in head and neck

50 Gy – Subclinical disease

60 Gy – Microscopic disease

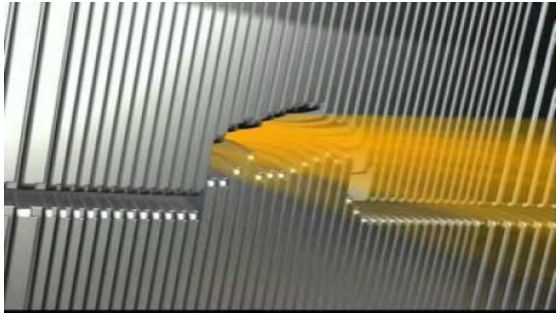
70 Gy – Macroscopic disease

The empiric basis of radiotherapy

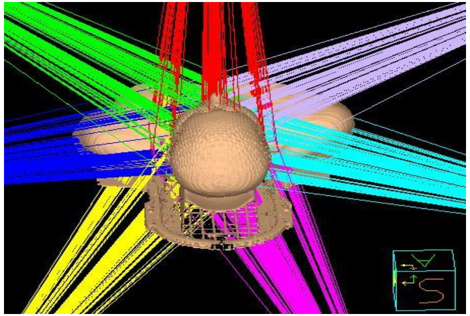


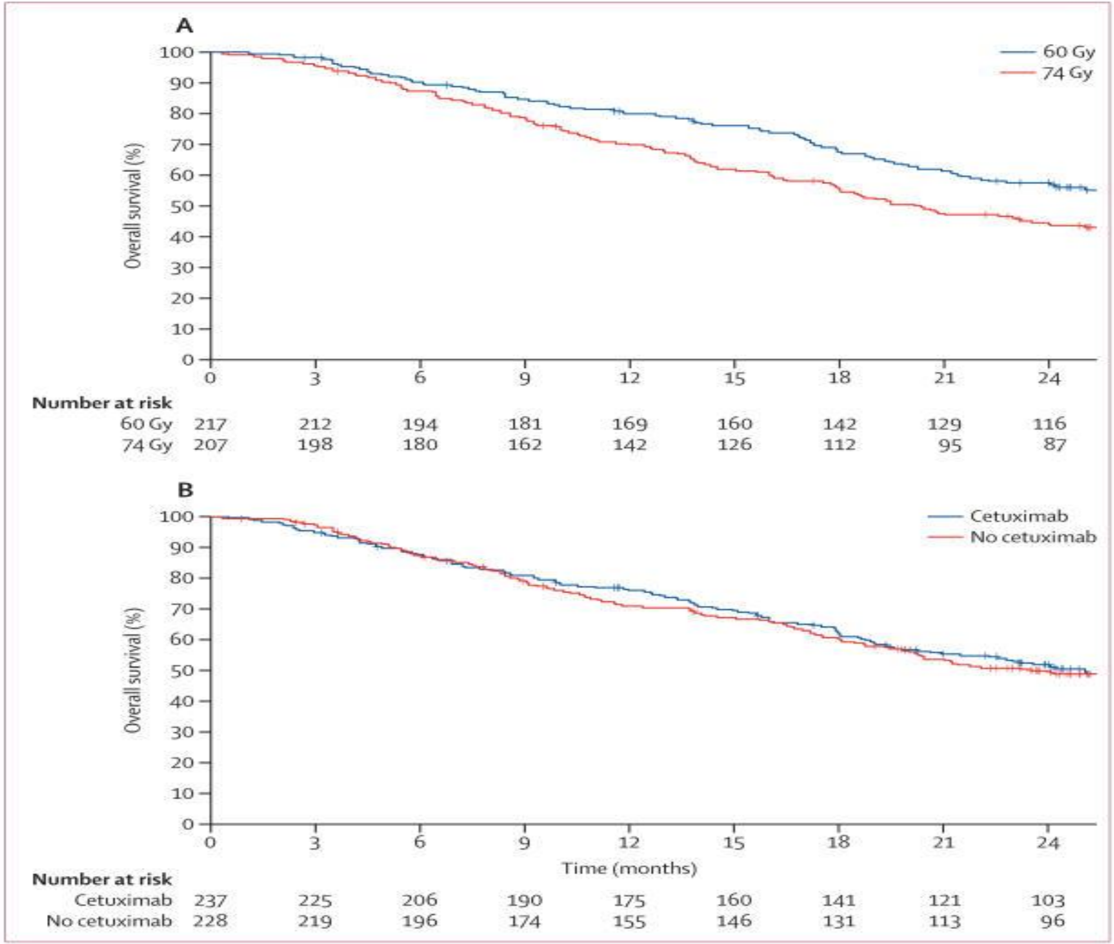
- Radiation damage is probabilistic
- Assumption tumors are homogenous
- Everyone has the same opportunity to benefit

Dogma – "It is axiomatic that a higher dose results in a higher effect"
~Perez and Brady – Textbook of Radiation Oncology



<u>Simple idea</u>: If we increase the total dose we will cure more patients





RTOG 06-17

- Randomized over 400 pts
- 60 Gy vs. 74 Gy
- 60 Gy was superior to 74

Other negative studies:

RTOG 0126

- 70.2 vs. 79.2
- No difference in 10-yr OS

RTOG (Esophagus)

- 50.4 vs. 64.8
- No difference in OS

Bradley J et al. Lancet Oncol. 2015 Feb; 16(2): 187–199.

Modern oncology is genomics-based

- Medical Oncology

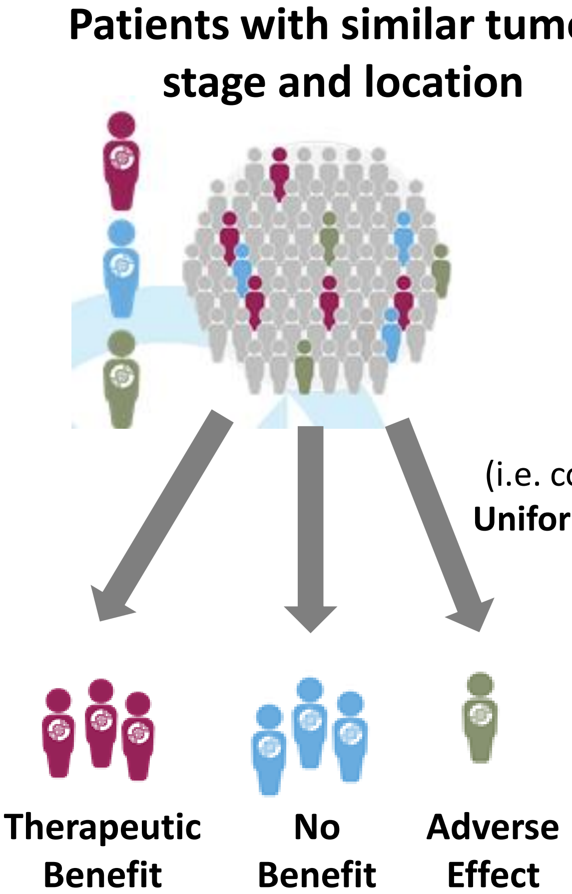
- Field has transitioned from an empiric basis to a scientific basis

- Biology guides decisions

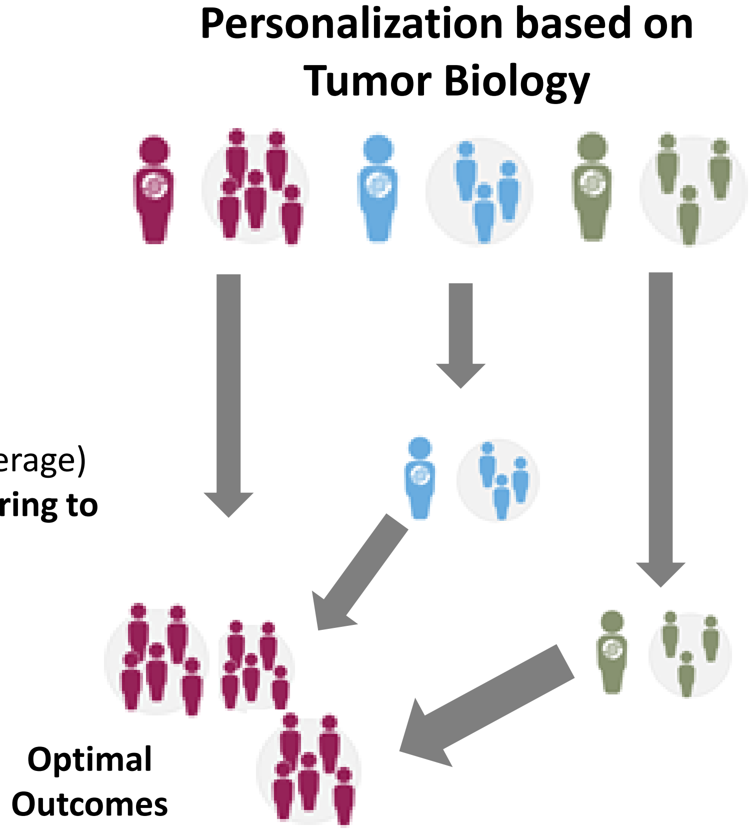
    Oncotype DX – no chemotherapy breast
    HER-2 neu - Herceptin
    ALK fusion gene – crizotinib
    Sequencing – Personalized Genomic Reports

- Radiation Oncology is still mostly empiric

A Need for a Personalized Approach in Radiation Oncology



High quality radiation
(i.e. conformal, excellent volume coverage)
Uniform empiric dosing without tailoring to patient tumor biology



Scott JG, et al. Lancet Oncology (2017) 18(2): 202-211
Caudell JJ, et al. Lancet Oncology (2017) 18(5): e266-e273

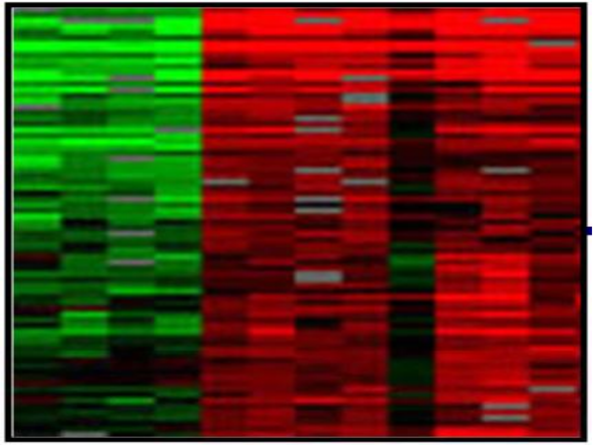
Development of the Radiosensitivity Index (RSI)

Validated Surviving Fraction at 2 Gy

Cell Line	Recorded SF2	Cell Line	Recorded SF2
BREAST_HS578T	0.79	COLON_COLO205	0.69
BREAST_MDAMB231	0.82	COLON_HCC-2998	0.44
COLON_HCT116	0.38	COLON_HT29	0.79
COLON_HCT15	0.4	COLON_KM12	0.42
COLON_SW620	0.62	MELAN_LOXIMVI	0.68
LEUK_CCRFCEM	0.185	MELAN_M14	0.42
LEUK_HL60	0.315	MELAN_MALME3M	0.8
LEUK_MOLT4	0.05	MELAN_SKMEL28	0.74
MELAN_SKMEL2	0.66	MELAN_SKMEL5	0.72
NSCLC_A549ATCC	0.61	MELAN_UACC257	0.48
NSCLC_H460	0.84	MELAN_UACC62	0.52
NSCLC_HOP62	0.164	NSCLC_EKVX	0.7
NSCLC_NCIH23	0.086	NSCLC_HOP92	0.43
OVAR_OVCAR5	0.408	OVAR_OVCAR3	0.55
RENAL_SN12C	0.62	OVAR_OVCAR4	0.29
BREAST_BT549	0.632	OVAR_OVCAR8	0.6
BREAST_MCF7	0.576	OVAR_SKOV3	0.9
BREAST_MDAMB435	0.1795	PROSTATE_DU145	0.52
BREAST_T47D	0.52	PROSTATE_PC3	0.484
CNS_SF268	0.45	RENAL_7860	0.66
CNS_SF539	0.82	RENAL_A498	0.61
CNS_SNB19	0.43	RENAL_ACHN	0.72
CNS_SNB75	0.55	RENAL_CAKI1	0.37
CNS_U251	0.57	RENAL_UO31	0.62

Gene mutation	Wild Type	Mutant	Total
RAS (H,N, or K)	33	15	48
TP53	17	31	48

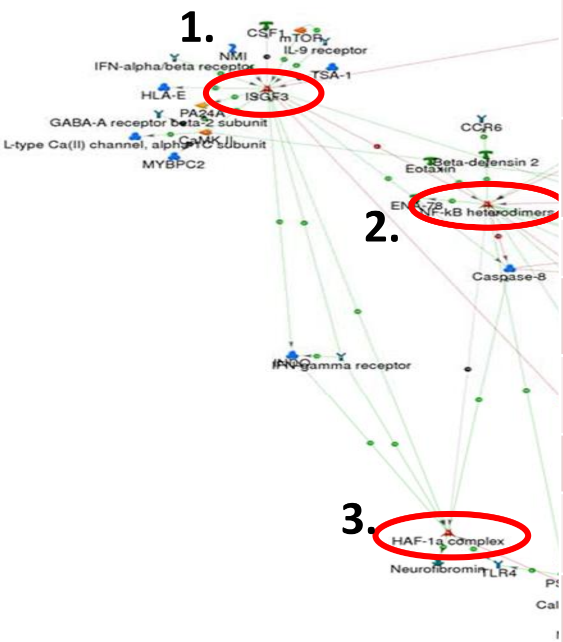
Basal transcriptome of cell lines



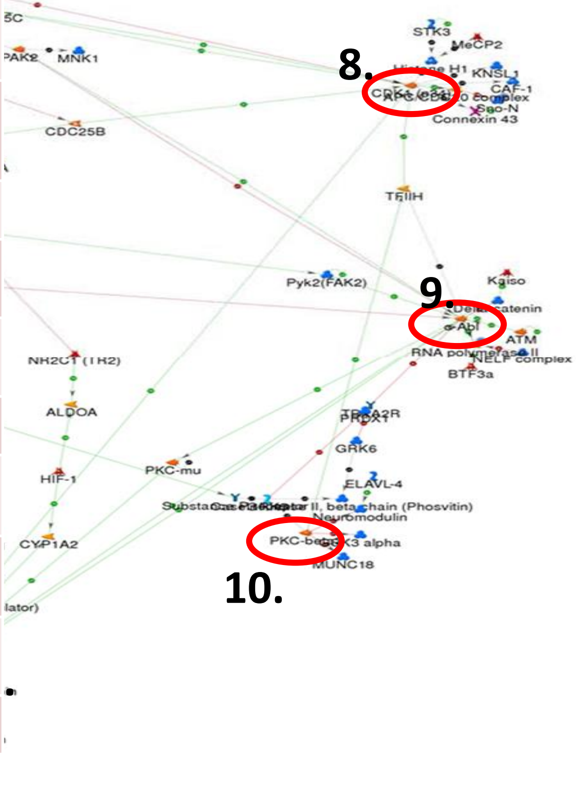
$SF2_x = k_0 + k_1(y_x) + k_2(TO) + k_3(\text{ras status}) + k_4(\text{p53 status}) + k_5(y_x)(TO) + k_6(y_x)(\text{ras status}) + k_7(TO)(\text{ras status}) + k_8(y_x)(\text{p53 status}) + k_9\,(TO)(\text{p53}) + k_{10}\,(\text{ras status})(\text{p53 status}) + k_{11}(y_x)(TO)(\text{ras status}) + k_{12}(y_x)(\text{ras status})(\text{p53 status}) + k_{13}(TO)(\text{ras status})(\text{p53status}) + k_{14}\,(y_x)(TO)(\text{ras status})(\text{p53}) \ldots$

(Eschrich et al. IJROBP 2009)

500 Gene Radiosensitivity Network



10 Gene Hub Defining RSI Signature		
	Gene	Function
1	**IFSG3 (Interferon [IFN}-stimulated gene factor 3)/STAT1**	A transcription factor complex that consists of a STAT1/STAT2 heterodimer and IFN-regulatory factor 9 (IRF9): translocates to nucleus to modulate IFN-responsive genes
2	**RelA (Transcription factor p65)**	An essential and rapid-acting transcriptional factor, which is a functional component of Class II NFκβ complexes
3	**IRF-1 (IFN regulatory factor 1)**	A transcription factor involved in immune response (Type I IFN), DNA damage
4	**HDAC1 (Histone Deacetylase 1)**	Enzyme that removes acetyl groups from histones and non-histone proteins to regulate gene expression
5	**SUMO-1 (Small Ubiquitin-like modifier 1)**	A dynamic protein modifier that covalently links to proteins (sumoylation): maintains genomic integrity
6	**AR (Androgen receptor)**	A steroid-binding transcription factor that coordinates changes in gene expression
7	**c-Jun**	Forms heterodimer with c-Fos to form the transcription factor complex AP-1, which regulates gene expression
8	**PAK2 (p21 activated kinase 2)**	Serine/threonine kinase which regulates cell cycle progression, proliferation, cytoskeletal dynamics and motility
9	**c-Abl**	Non-receptor tyrosine kinase regulating gene expression, DNA synthesis and cell signaling pathways
10	**PKC-β (Protein Kinase C-beta)**	Serine/threonine kinase involved in multiple cellular processes



500 gene network identified by least sum squares approach

Network hubs identified by having > 5 connections

(Eschrich et al. IJROBP 2009)

Next, the expression levels of the 10 hub genes were ranked (greatest to least) and modeled with regression analysis to predict SF_2 in each of the 48 cell lines

RSI = -0.0098009*AR + 0.0128283*cJun + 0.0254552*STAT1- 0.0017589*PKC - 0.0038171*RelA + 0.1070213*cABL - 0.0002509*SUMO1 - 0.0092431*PAK2 - 0.0204469*HDAC1 -0.0441683* IRF1

RSI provides a continuous score from 0-1
Lower the score= radiosensitive

Clinical Validation of RSI

Radiation Treatment N=1442

Disease Site	N	Endpoint	Hazard Ratio (Ref. Radioresistance)	Clinical Outcome RS vs RR	p-value
Breast (Karolinska)	77	RFS	0.13 (0.02-1.0)	95% vs. 75% (5-yr)	**0.02**
Breast (Erasmus)	288	DMFS	0.57 (0.33-0.98)	77% vs. 64% (5-yr)	**0.04**
Breast (Curie,NKI	343	LRFS	0.23 (0.1-0.53)	--	**0.0006**
Lung (Moffitt)	53	DFS	0.42 (0.25-0.92)	63% vs. 22% (5-yr)	**0.02**
Lung (Dir Chall)	27	DFS	0.44 (0.16-1.18)	--	0.09
Lung (Korea)	16	DFS	0.27 (0.03-2.17)	75% vs. 25% (5-yr)	0.18
GBM (TCGA)	214	OS	0.57 (0.38-0.85)	--	**0.005**
Pancreas (Moffitt)	48	OS	0.42 (0.19-0.94)	--	**0.04**
Prostate (Mayo)	82	DMFS	--	94% vs. 72% (10-yr)	**0.03**
Prostate (TJU)	132	BFFS	--	80% vs. 60% (5-yr)	**0.026**
Head and Neck (NKI)	92	LRFS	--	86% vs. 61% (2-yr)	**0.05**
Rectal	14	Response			**0.03**
Esophageal	12	Response			**0.05**
Melanoma	11	OS	0.09 (0.01-0.81)	100% vs. 14.3% (2-yr)	**0.009**
Liver metastasis	33	LC (s/p SBRT)		100% vs. 59% (2-yr)	**0.019**

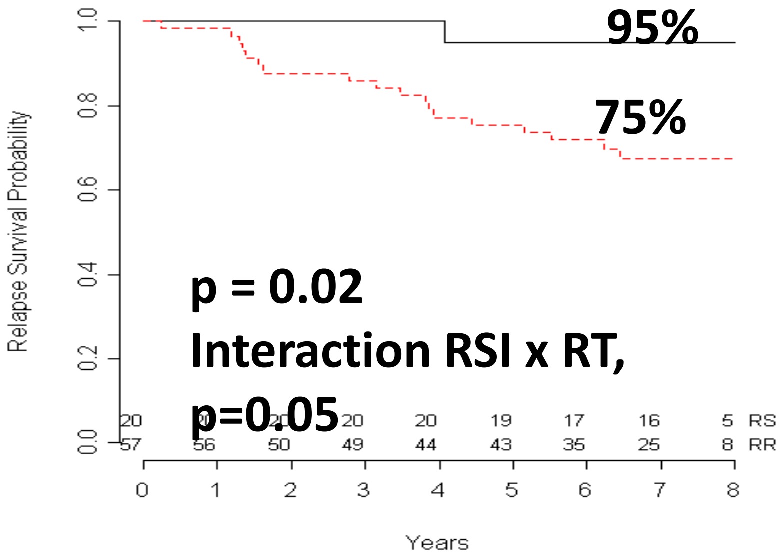
No Radiation Treatment N=877

Disease Site	N	Endpoint	Hazard Ratio (Ref. Radioresistance)	Clinical Outcome RS vs RR	p- value
Breast (Karolinksa)	82	RFS	1.21 (0.50-2.91)	77% vs. 71% (5-yr)	0.67
Breast (Erasmus)	62	DMFS	1.06 (0.23-4.83)	80% vs. 81% (5-yr)	0.94
Lung (Moffitt)	42	RFS	1.09 (0.45-2.65)	--	0.98
Lung (Dir Chall)	47	DFS	0.93 (0.50-1.79)	19% vs. 14% (5-yr)	0.84
GBM (TCGA)	52	OS	--	5% vs. 5% (1-yr)	0.64
Pancreas (Moffitt)	25	OS	0.76 (0.29-1.99)	69% vs. 67% (2-yr)	0.58
Prostate (Mayo)	536	DMFS	--	70% vs. 71% (10-yr)	0.58
Melanoma	31	OS		91.7% vs. 63% (2-yr)	0.19

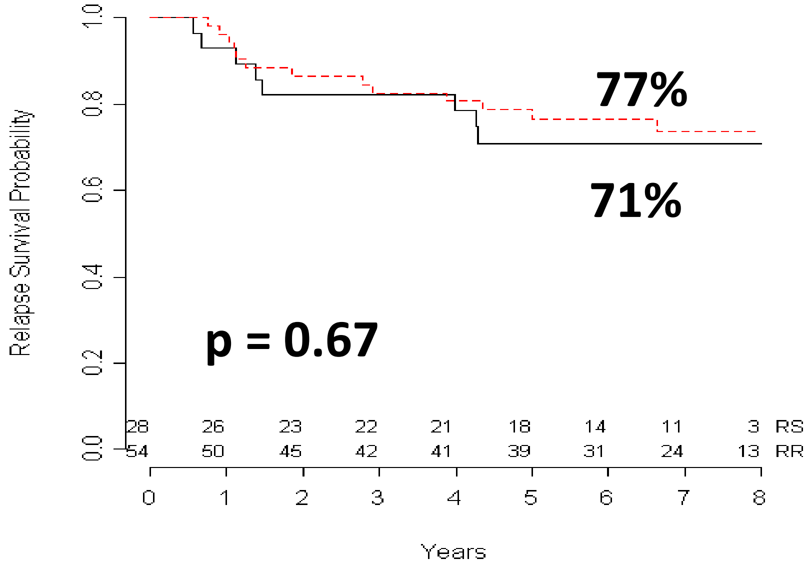
Abbreviations: RS, radiosensitive; RR, radioresistant; RFS, relapse-free survival; DMFS, distant metastasis-free survival; LRFS, local-regional relapse-free survival; DFS, disease-free survival; BFFS, biochemical failure-free survival; LC, local control; OS, overall survival

RSI: Predicts Outcome Only in RT-Treated Patients

Segmentectomy/Lumpectomy + RT
N=77



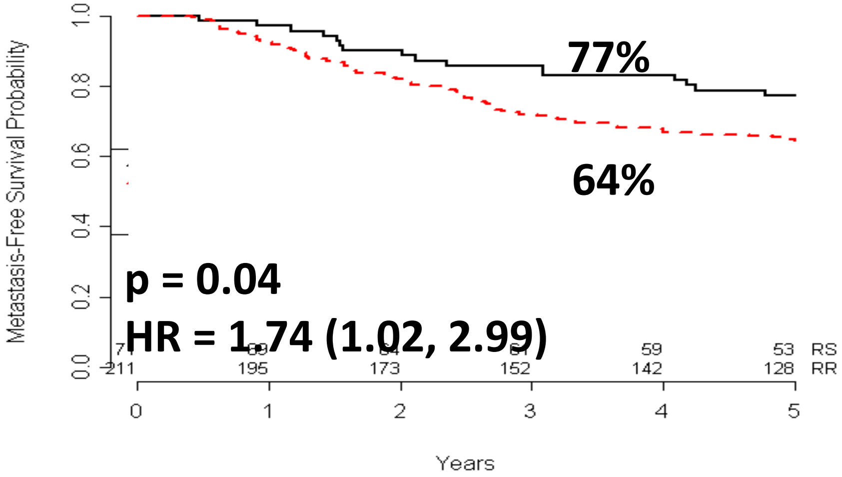
Mastectomy No RT
N=82



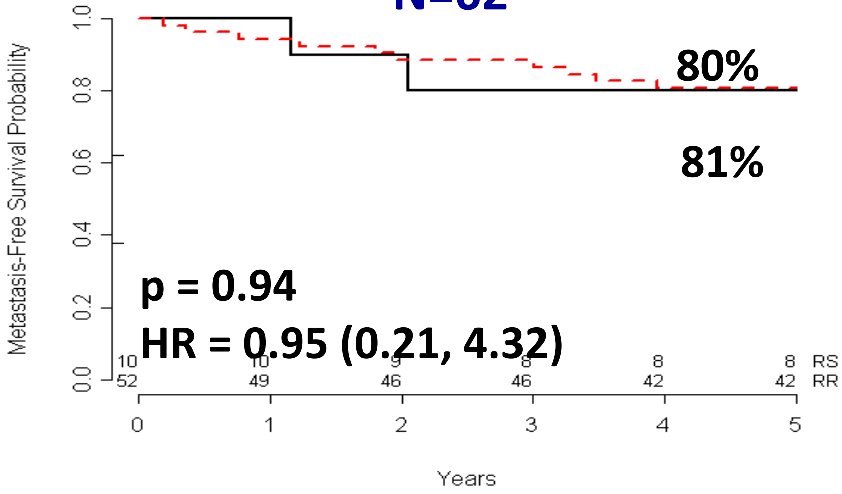
Eschrich SA et al (2012) Clin Can Res 18:5134-43

RSI Predicts Distant Metastasis Risk Only in RT-Treated Patients

Lumpectomy + RT
N=282



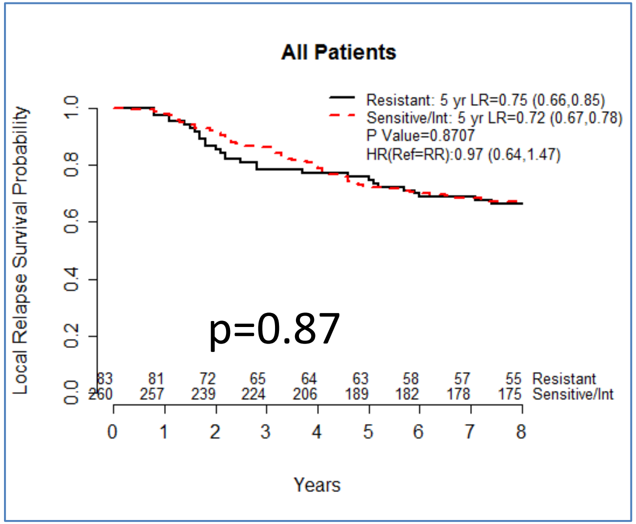
Mastectomy No RT
N=62



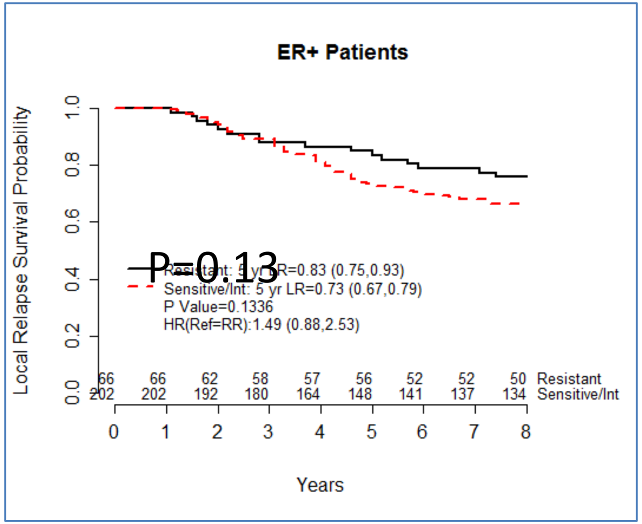
Eschrich SA et al (2012) Clin Can Res 18:5134-43

RSI predicts for local recurrence in breast cancer only in ER negative

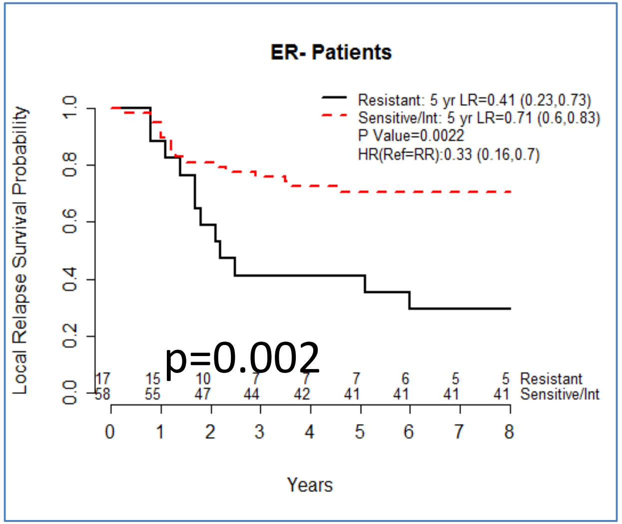
All patients



ER + Patients

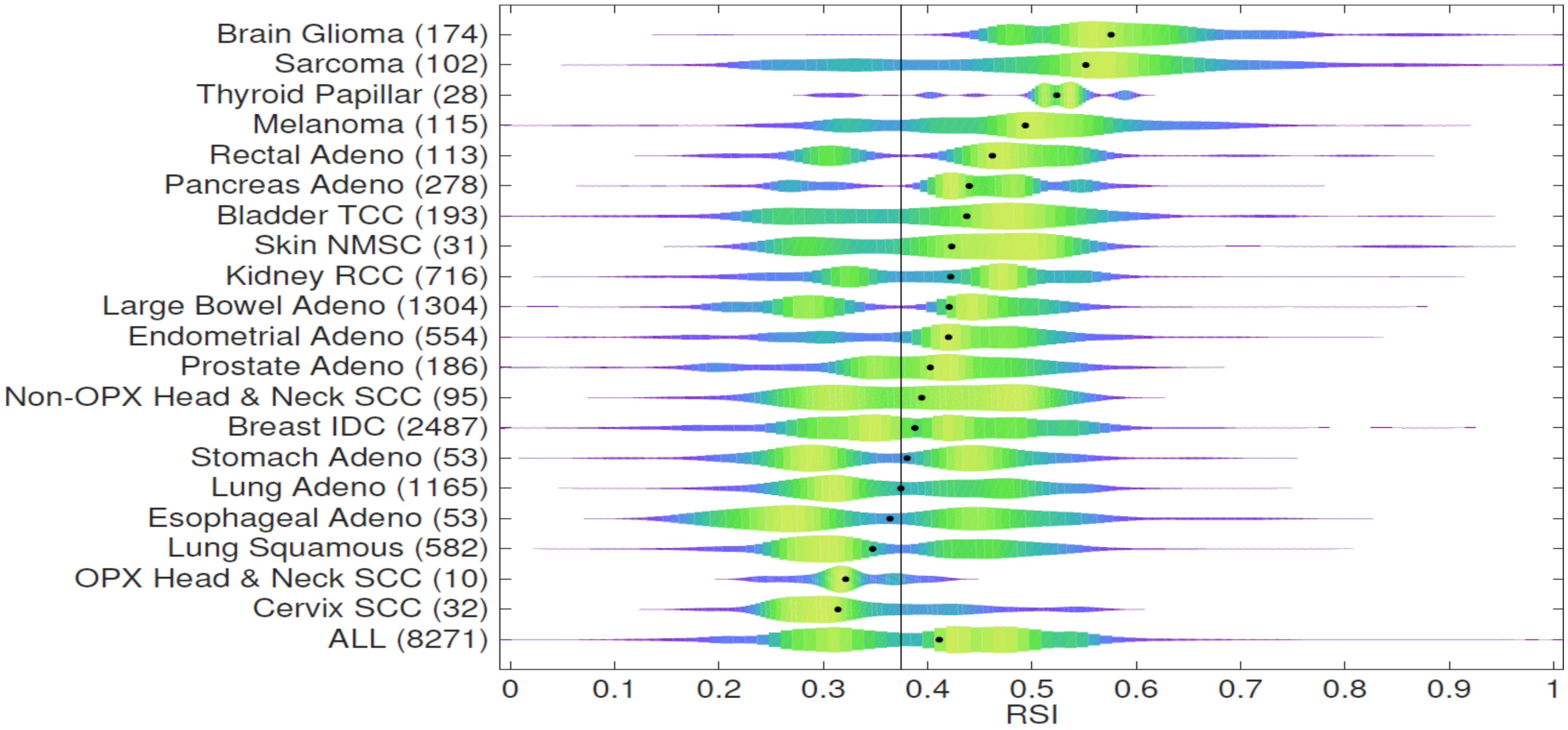


ER - Patients



Torres-Roca JF et al (2015) IJROBP 93:631-8

RSI Distribution in 8,271 tissue samples



Grass, D et al (ASTRO 2017) (in preparation)

RSI: A Novel Assay of Tumor Radiosensitivity

- RSI
  - Biomarker of radiosensitivity
    - Developed based on SF2
  - Clinically Validated
    - RT-specific, Disease-site independent
    - 13 independent datasets, 2,319 patients
    - Assessed in 13,343 patient samples

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RSI: Integrating Genomics Into Clinical Practice

- **RSI**
  - RT benefit (response) is not uniform
  - RT Dosing is Uniform. Why?
- **Physical Dose (same) vs. Tumor effect (different)**
  - A given RT dose has varied effects on individual tumors
  - Uniform physical dose is biologically imprecise
- **How about prescribing RT to tumor effect?**
  - GARD – Genomic Adjusted Radiation Dose
  - Dose Can be adjusted to account for biological heterogeneity

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Deriving GARD by combining the LQ model and RSI

Deriving GAD from individual patient RSI

(1)

Linear-Quadratic model of survival after n doses of d Gray

$$SF = e^{-n(\alpha d + \beta d^2)}$$

in experiments
RSI ~ SF2
(n=1 and d=2)

(2)

Patient specific radiobiological parameters

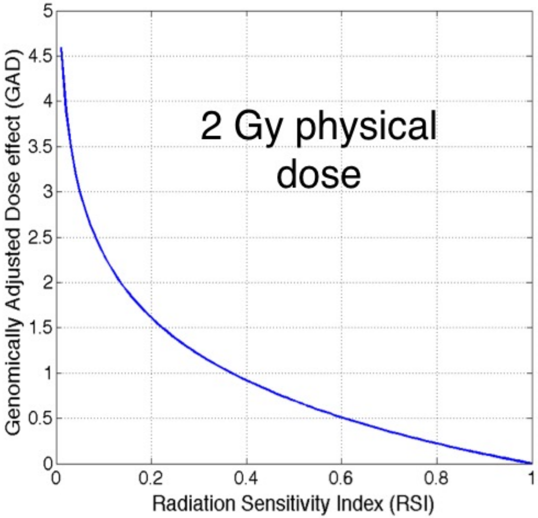
$$\alpha = \frac{\ln \mathrm{RSI} + \beta n d^2}{-nd}$$

(3)

Genomically Adjusted Dose effect

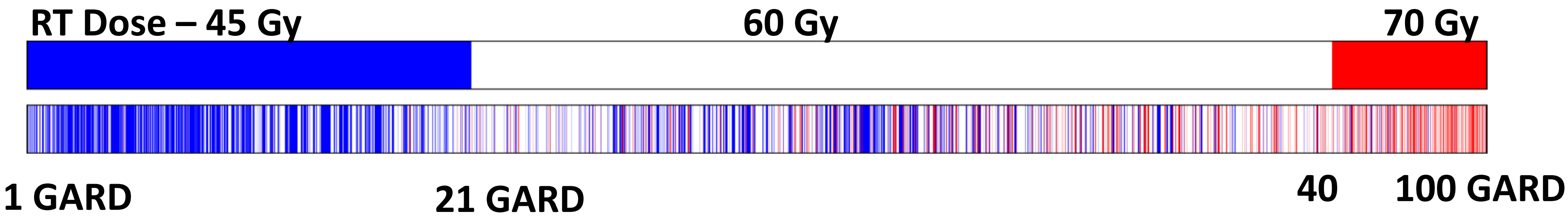
$$\mathrm{GAD} = nd(\alpha + \beta d)$$

n & d trial specific,
α patient specific,
β constant



Scott JG (2017) Lancet Oncology 18:202-11

From Physical to Genomic RT Dose



N=8,271
Doses are assigned

Physical Dose – Discrete

GARD – Continuous

Higher Dose is not always associated with higher effect

Scott JG (2017) Lancet Oncology 18:202-11

GARD: Clinical Validation

	Median follow-up (months)	Events	Radiotherapy dose range (Gy)	GARD range	Endpoint	HR from multivariable analysis (95% CI)	p value	Adjustment factors
Erasmus Breast Cancer Cohort (n=263)	60	23	40–74	4·01–104·25	Distant-metastasis-free survival*	2·11 (1·1–3·9)	0·018	Oestrogen and progesterone receptor status, age, surgery (vs lumpectomy), and T-stage
Karolinska Breast Cancer Cohort (n=77)	87	19	50	8–60	Relapse-free survival†	7·4 (1·4–138)	0·014	Hormonal therapy, chemotherapy, and oestrogen and progesterone receptor status
Moffitt Lung Cancer Cohort (n=60)	37	23	45–70	15–125	Local control‡	3·4 (1·3–9·1)	0·016	Surgery, stage, histology, lymphovascular invasion
TCGA Glioblastoma Cohort (n=98)	11	76	12·6–97·0	0·4–46·0	Overall survival§	1·9 (1·1–3·3)	0·019	Age, performance status
Moffitt Pancreas Cancer Cohort (n=40)	68	27	45–54	16–40	Overall survival§	2·6 (1·1–6·0)	0·029	CA 19·9, margin lymph nodes

TCGA=The Cancer Genome Atlas. *Primary endpoint defined as distant recurrence in the first 5 years following completion of primary treatment. †Primary endpoint defined as any relapse distant, regional, or local from the end of primary treatment. ‡Defined in this study as time from surgical resection to cancer recurrence within the irradiated field. If no event occurred, then cases were censored at the date of last clinical evaluation. Cases in which more than 4 months elapsed without a clinical evaluation were censored at the date of antecedent clinical evaluation.[22] §Defined in this study as the interval from surgery to date of death.

Table 2: **Clinical cohort description and multivariate analysis for the effect of GARD on selected endpoints**

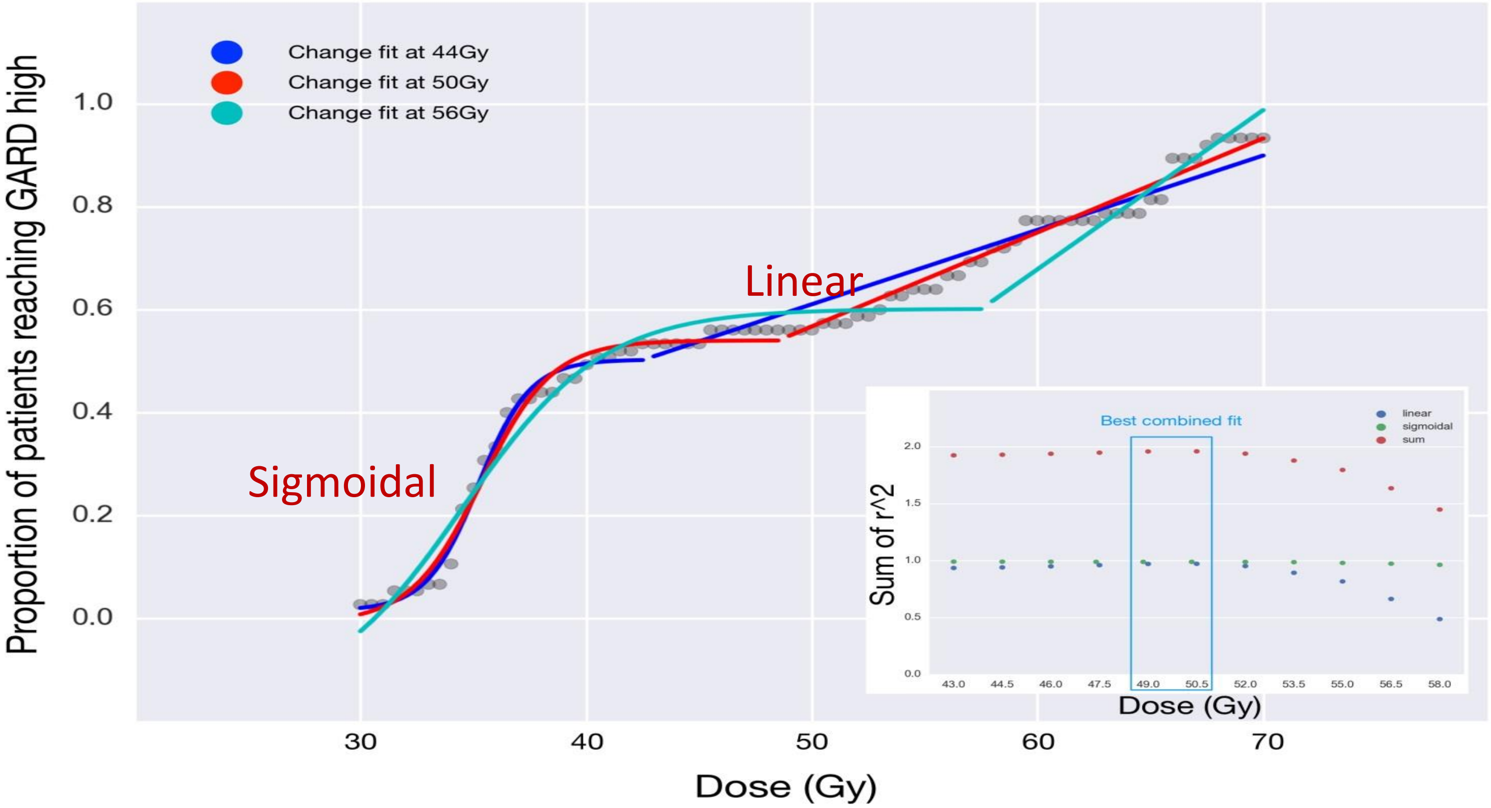
Scott JG (2017) Lancet Oncology 18:202-11

GARD predicts local control in ER negative breast cancer

ER-				
		HR	95% CI	P-value
GARD		0.91	0.84-0.98	0.0082
Node status (ref: pN-)	pN+	2.02	0.93-4.55	0.08
LVSI (ref: No)	Yes	2.11	0.89-4.66	0.09
ER +				
Age		0.96	0.93-1.0	0.08
Grade (ref: I & II)	III	1.62	1.01-2.6	0.04
DCIS (ref: No DCIS)	DCIS	1.54	0.95-2.59	0.08
LVSI (ref: No)	Yes	1.25	0.76-2.0	0.37

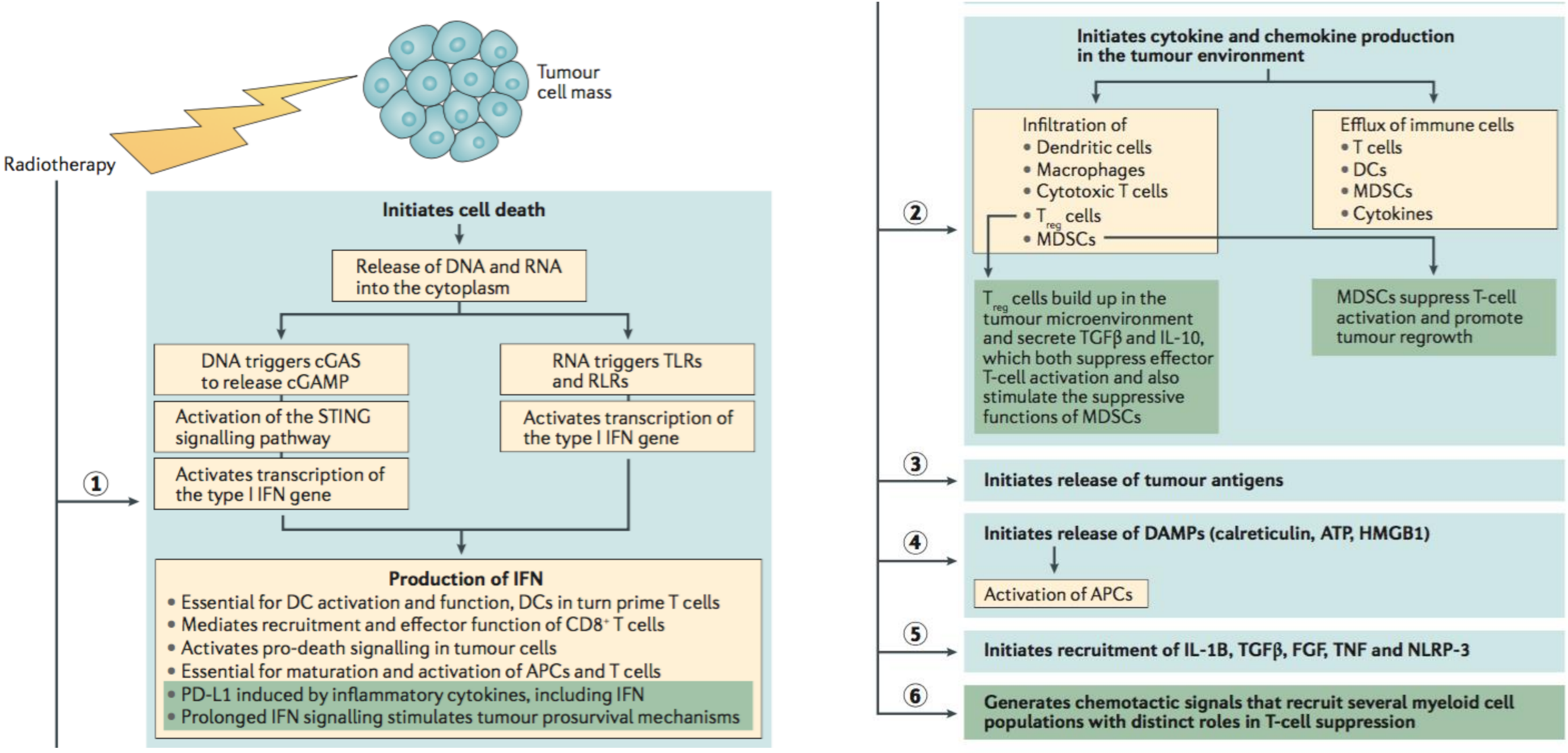
Ahmed KA et al (2017) Submitted

Personalized Genomic-Based RT Dose for ER negative breast cancer



Ahmed KA et al (2018) Submitted

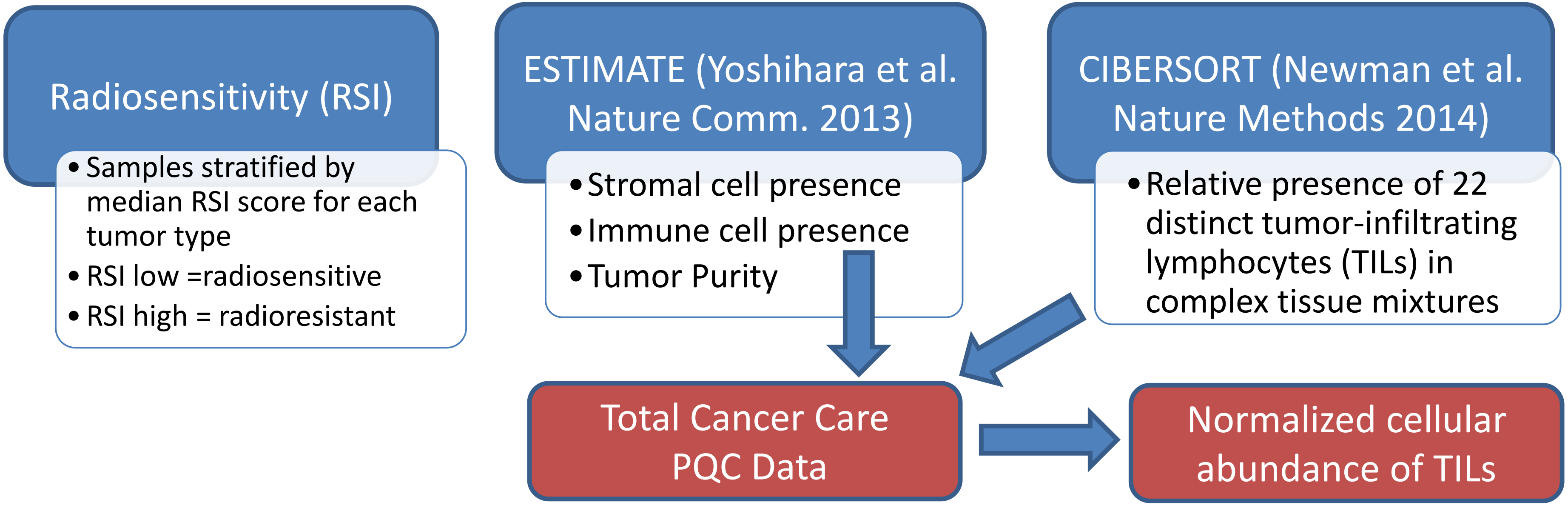
Radiation Therapy & Immune Response

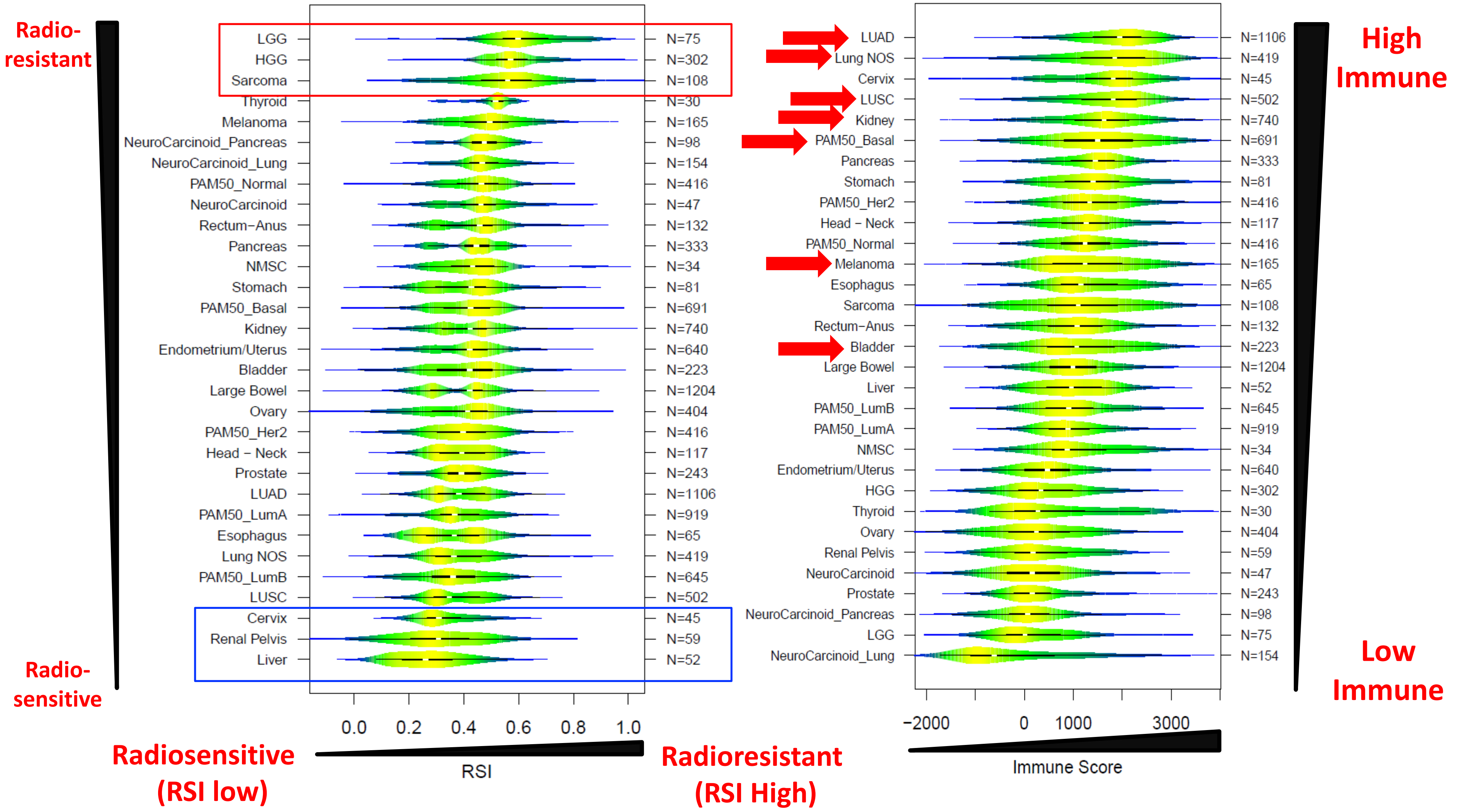


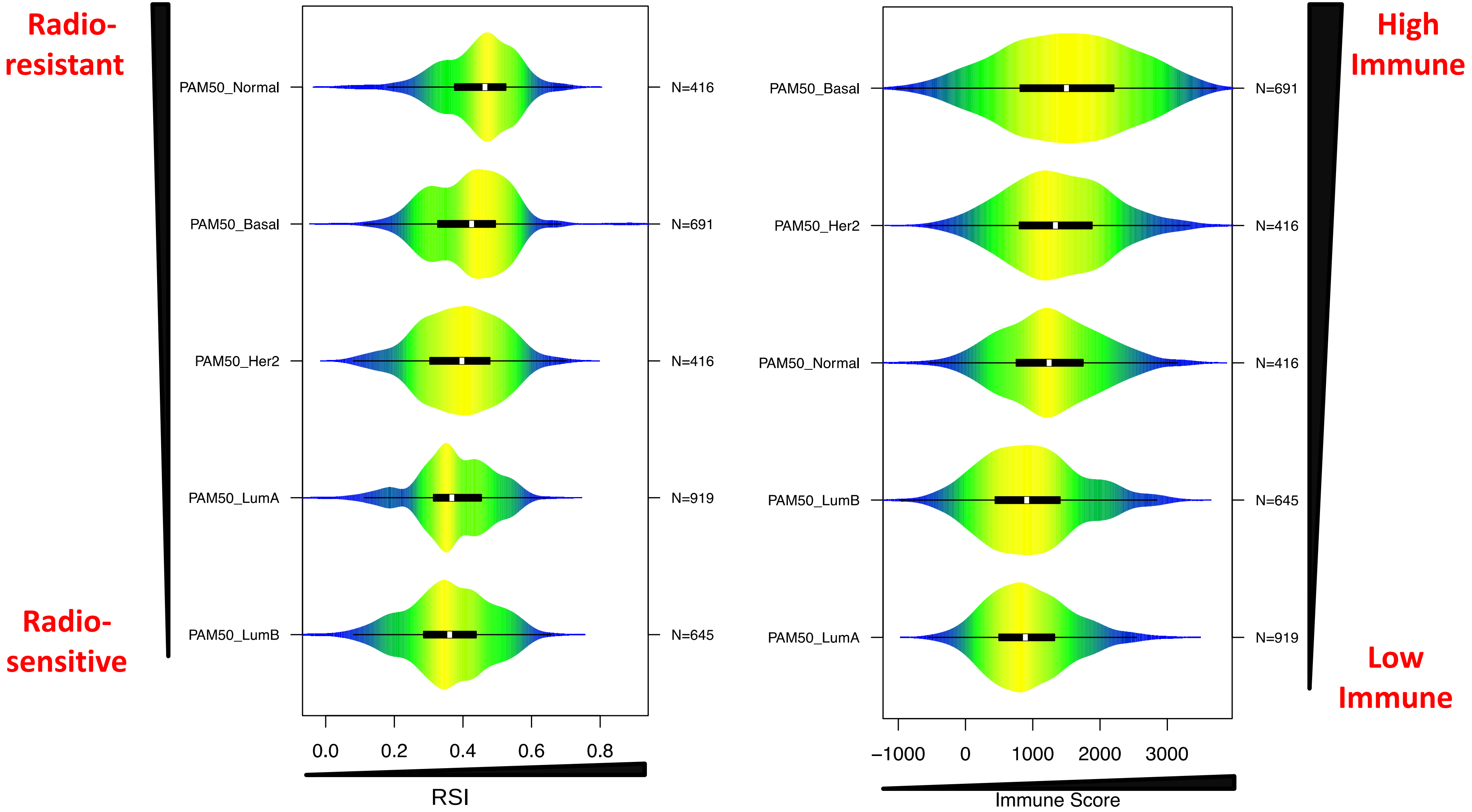
Weichselbaum RR, et al. Nat Rev Clin Oncol 2017

Experimental Approach:

- 10,469 primary macrodissected tumors were analyzed via an IRB-approved prospective de-identified tissue collection protocol at Moffitt since 2006 (Total Cancer Care)
  - Prospective pathology quality control (PQC) data of percent cellularity, stroma, malignancy & necrosis in each sample
  - All samples with gene expression data - Affymetrix GeneChips (60,607 probe sets representing ~30,000 unique genes

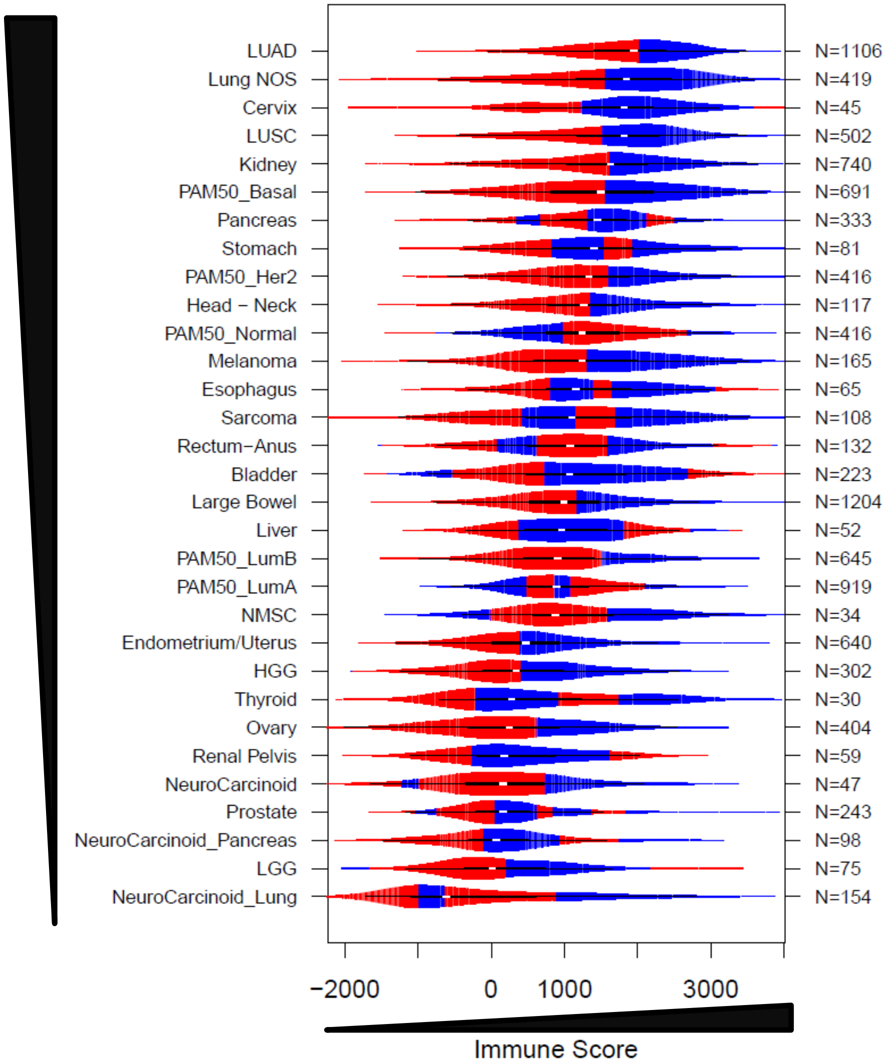



Radio-resistant
Radio-sensitive
LGG
HGG
Sarcoma
Thyroid
Melanoma
NeuroCarcinoid_Pancreas
NeuroCarcinoid_Lung
PAM50_Normal
NeuroCarcinoid
Rectum−Anus
Pancreas
NMSC
Stomach
PAM50_Basal
Kidney
Endometrium/Uterus
Bladder
Large Bowel
Ovary
PAM50_Her2
Head − Neck
Prostate
LUAD
PAM50_LumA
Esophagus
Lung NOS
PAM50_LumB
LUSC
Cervix
Renal Pelvis
Liver
N=75
N=302
N=108
N=30
N=165
N=98
N=154
N=416
N=47
N=132
N=333
N=34
N=81
N=691
N=740
N=640
N=223
N=1204
N=404
N=416
N=117
N=243
N=1106
N=919
N=65
N=419
N=645
N=502
N=45
N=59
N=52
0.0
0.2
0.4
0.6
0.8
1.0
RSI
Radiosensitive (RSI low)
Radioresistant (RSI High)
LUAD
Lung NOS
Cervix
LUSC
Kidney
PAM50_Basal
Pancreas
Stomach
PAM50_Her2
Head − Neck
PAM50_Normal
Melanoma
Esophagus
Sarcoma
Rectum−Anus
Bladder
Large Bowel
Liver
PAM50_LumB
PAM50_LumA
NMSC
Endometrium/Uterus
HGG
Thyroid
Ovary
Renal Pelvis
NeuroCarcinoid
Prostate
NeuroCarcinoid_Pancreas
LGG
NeuroCarcinoid_Lung
N=1106
N=419
N=45
N=502
N=740
N=691
N=333
N=81
N=416
N=117
N=416
N=165
N=65
N=108
N=132
N=223
N=1204
N=52
N=645
N=919
N=34
N=640
N=302
N=30
N=404
N=59
N=47
N=243
N=98
N=75
N=154
−2000
0
1000
3000
Immune Score
High Immune
Low Immune


Radio-
resistant
Radio-
sensitive
PAM50_Normal
PAM50_Basal
PAM50_Her2
PAM50_LumA
PAM50_LumB
N=416
N=691
N=416
N=919
N=645
0.0
0.2
0.4
0.6
0.8
RSI
PAM50_Basal
PAM50_Her2
PAM50_Normal
PAM50_LumB
PAM50_LumA
N=691
N=416
N=416
N=645
N=919
−1000
0
1000
2000
3000
Immune Score
High
Immune
Low
Immune

High Immune

Low Immune

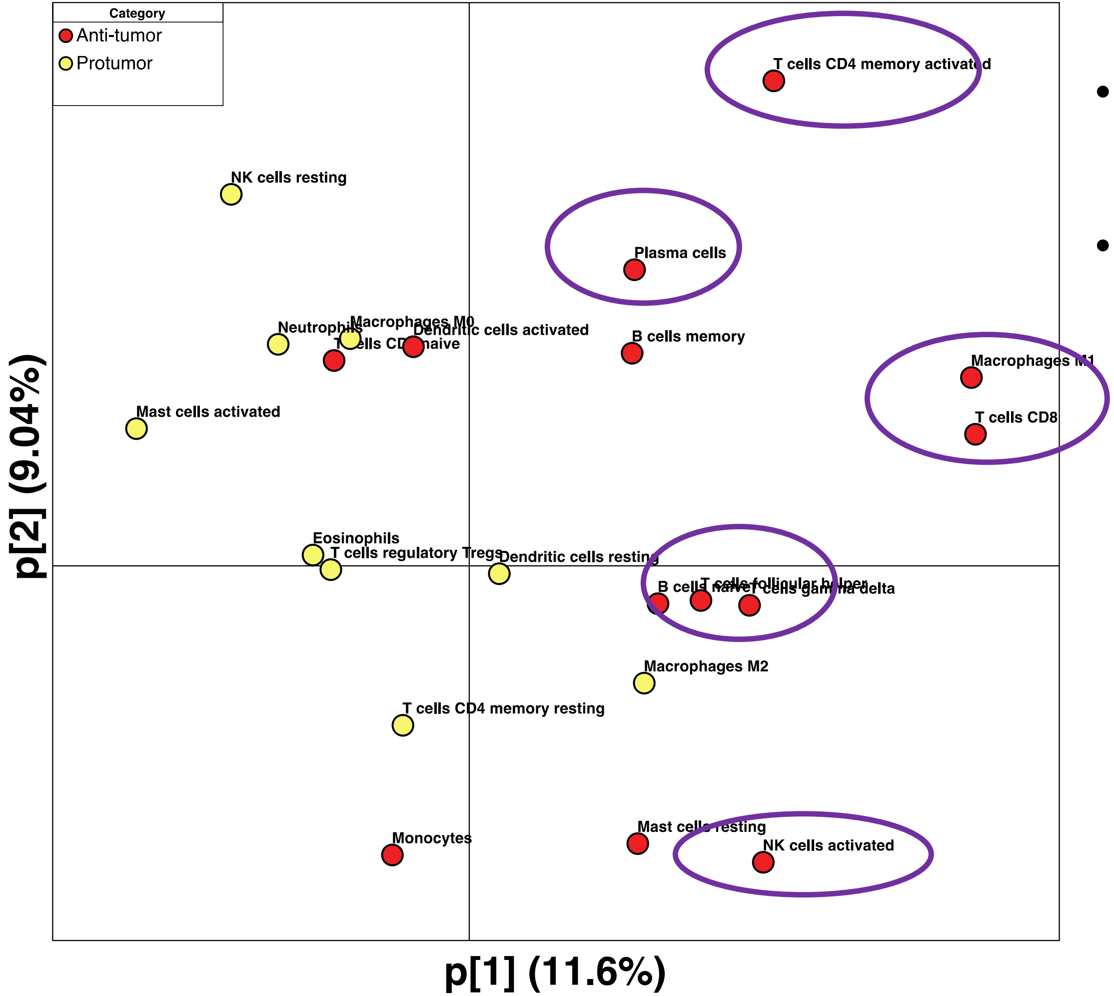


Radiosensitive= BLUE

Radioresistant = RED

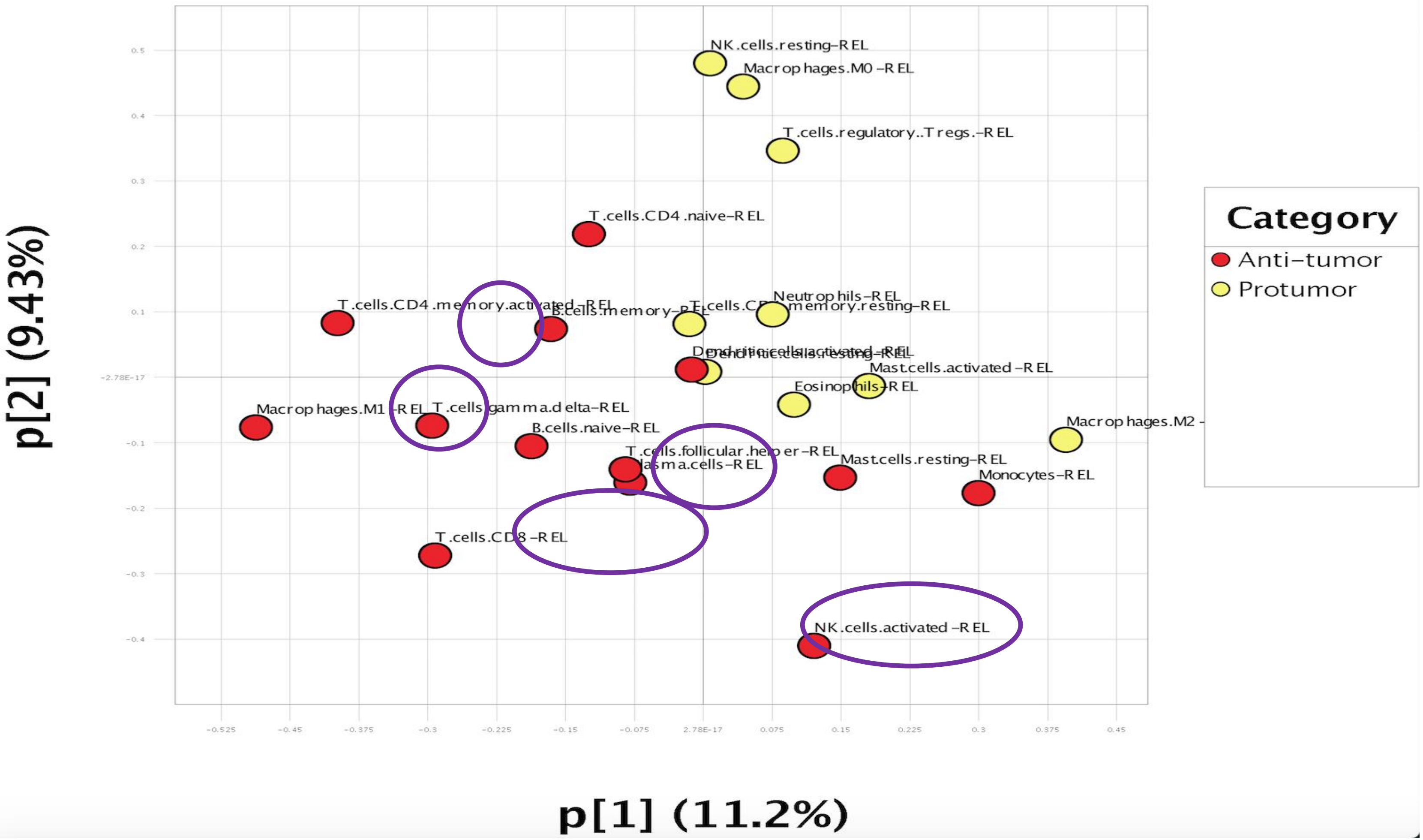
For a given immune score there is an enrichment of radiosensitive tumors in samples also defined to have the highest immune cell presence, though with some heterogeneity

Principal Component Analysis and TIL Analysis



- Analysis of 10,469 samples across all tumor types
- Combination of laboratory-based descriptions of TIL activity (color-coded) and unsupervised clustering demonstrates TIL groupings with similar biology <u>*independent of radiosensitivity*</u>
  - **$CD4^+$ memory activated T cells**
  - **Plasma cells**
  - **M1 Macrophages**
  - **$CD8^+$ T cells**
  - **NK cells (activated)**
  - **T cell follicular helper**

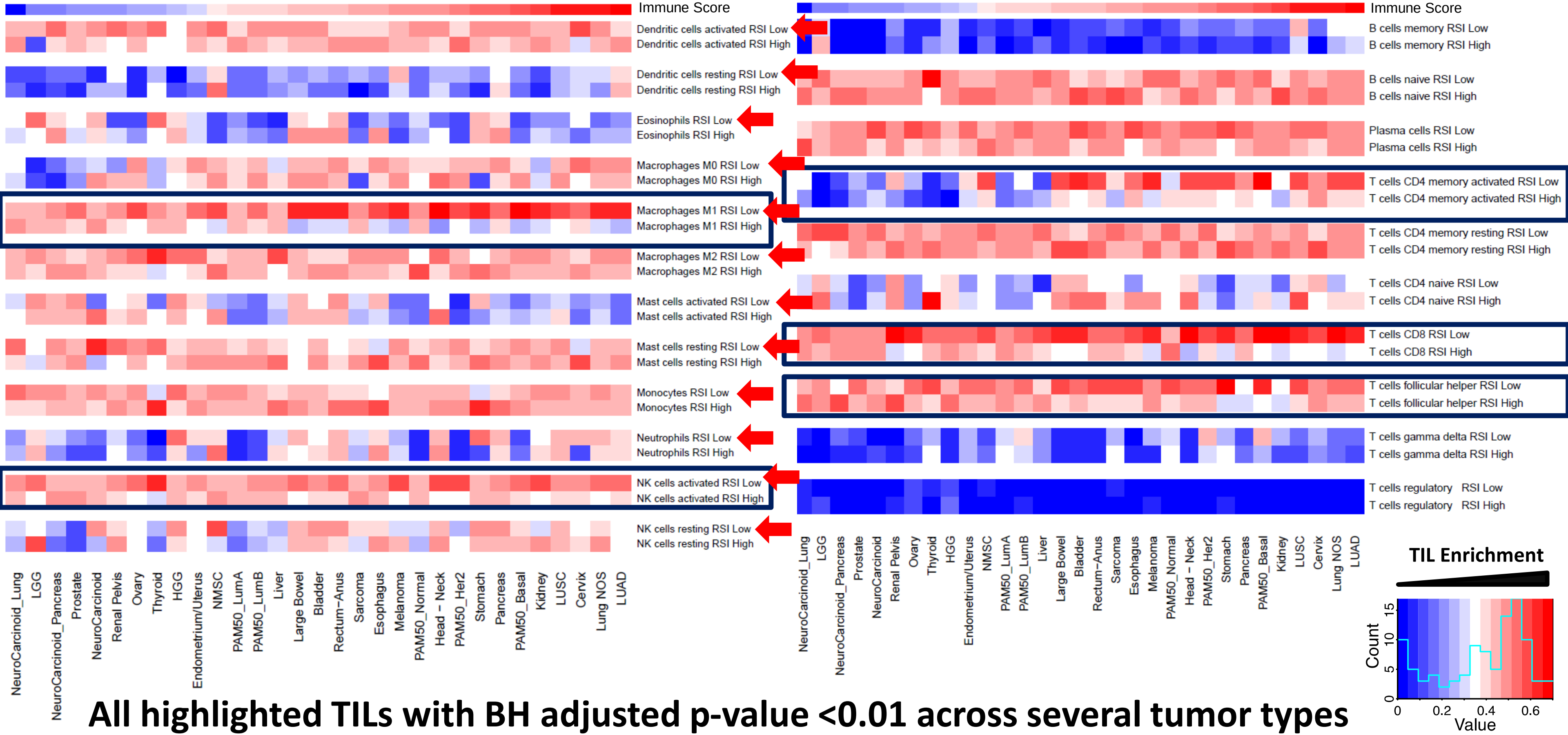
All Breast Cancer Types



TIL Enrichment by Radiosensitivity

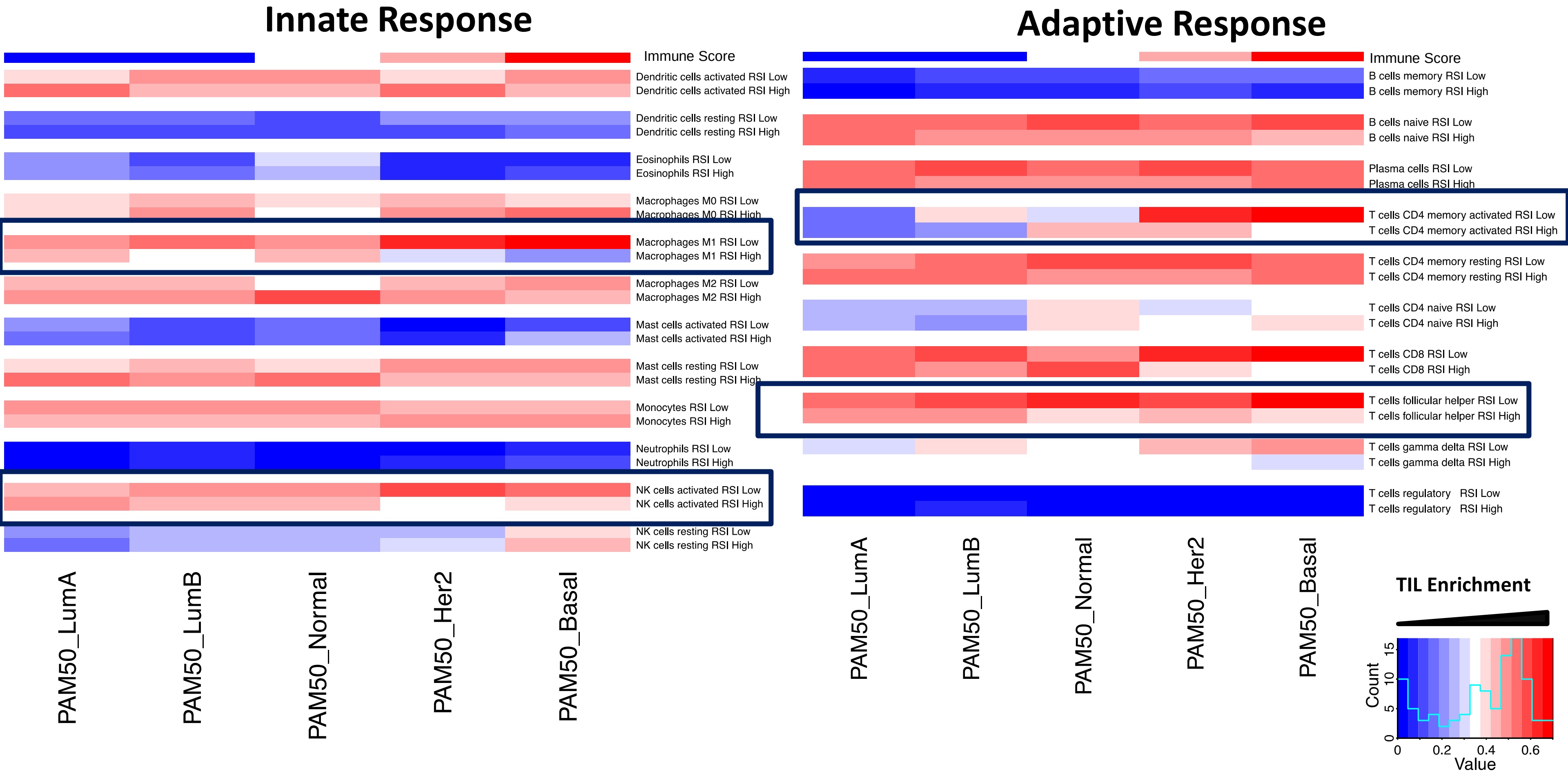
Innate Response

Adaptive Response

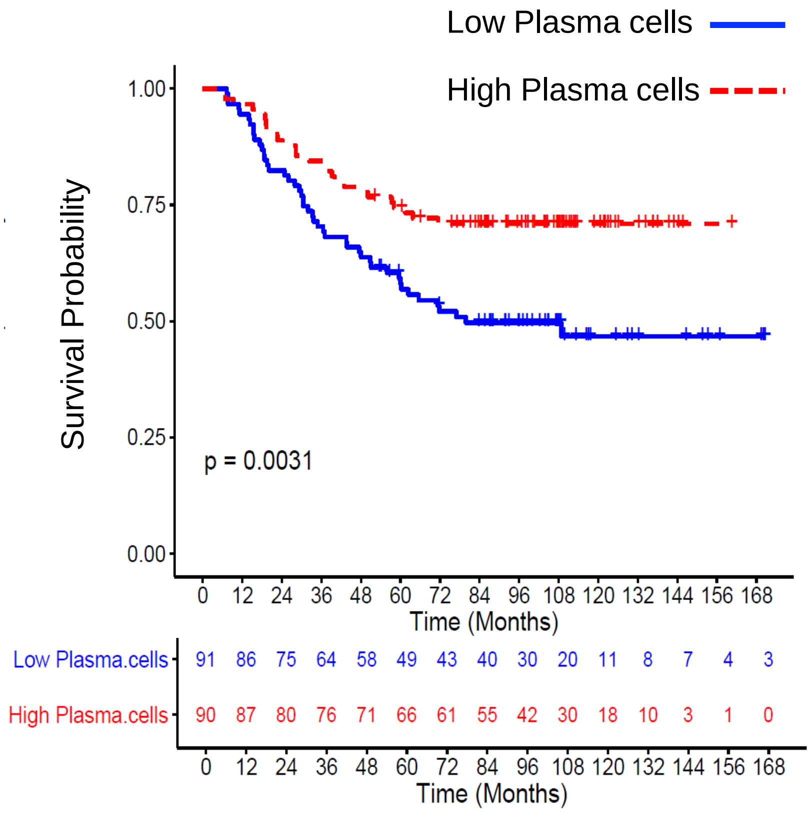


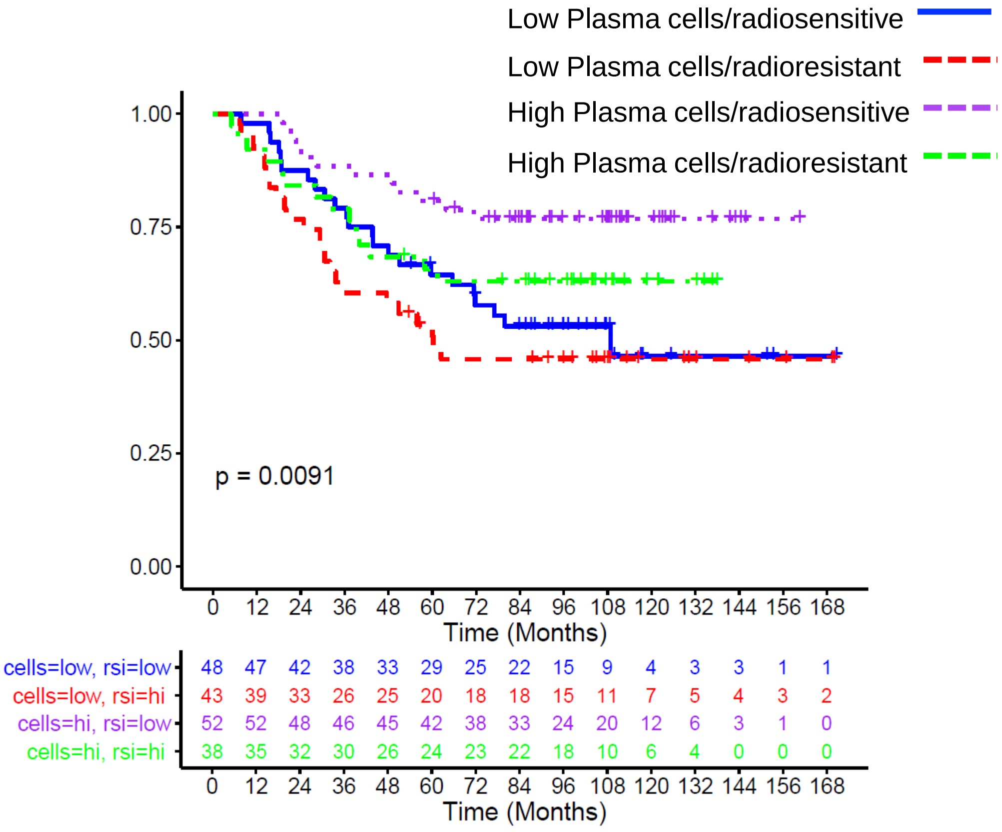
All highlighted TILs with BH adjusted p-value <0.01 across several tumor types

Breast Cancer TIL Enrichment by Radiosensitivity

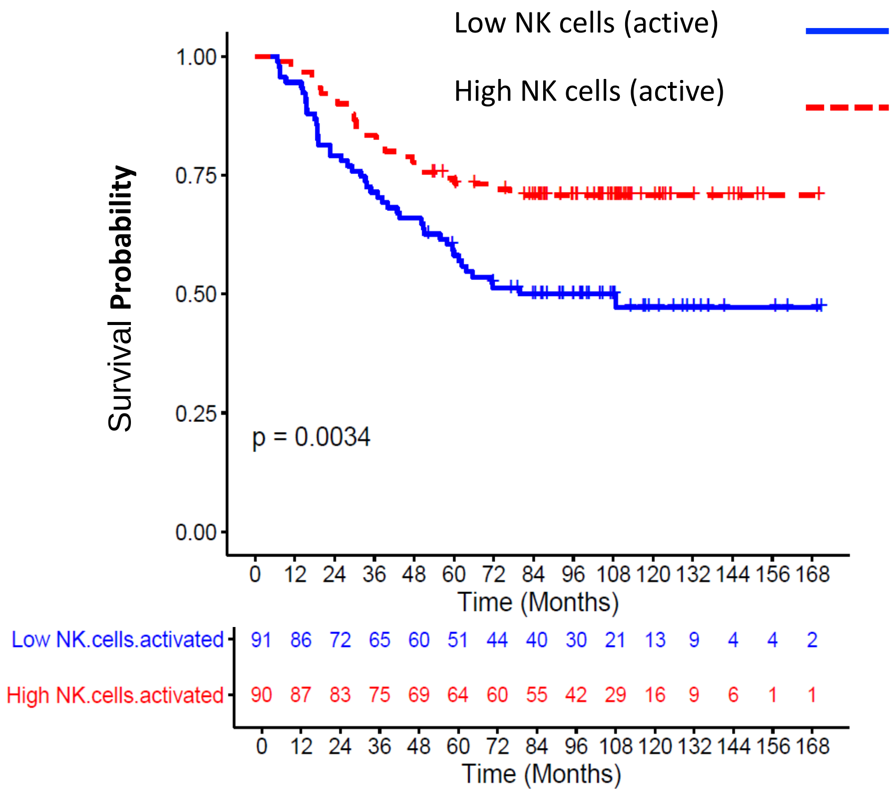


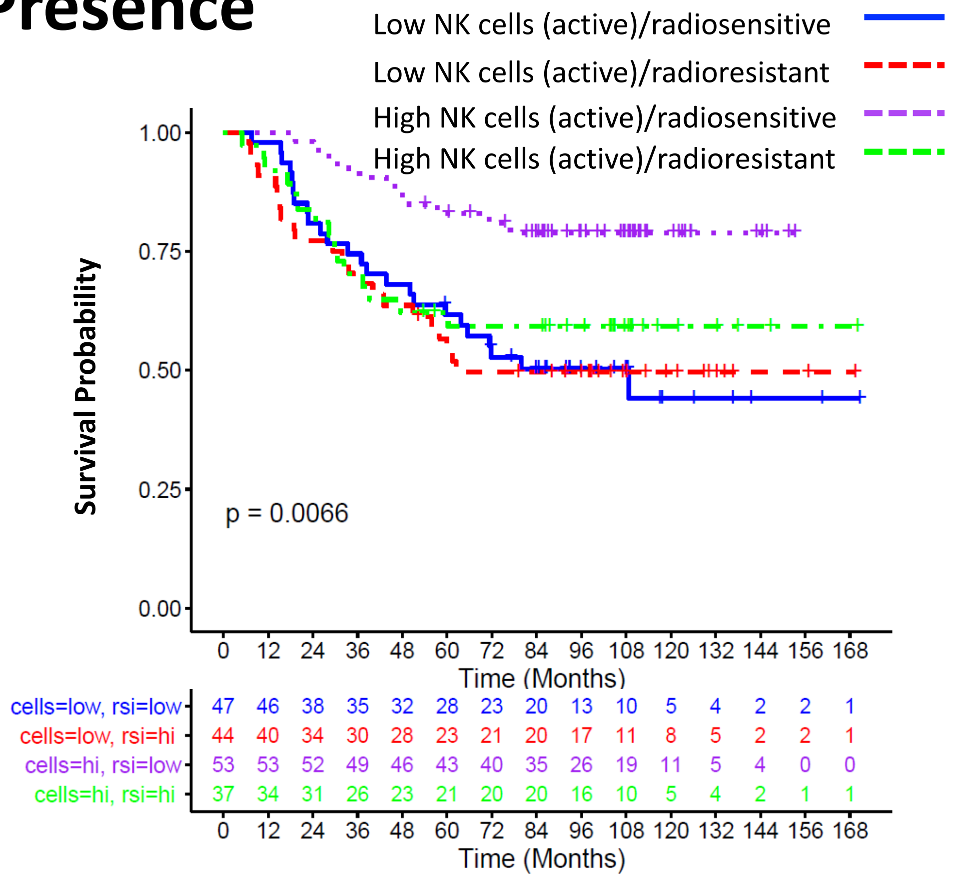
Integration of Intrinsic Radiosensitivity and TIL Presence





Integration of Intrinsic Radiosensitivity and TIL Presence





A New Framework for Radiation Oncology

- Conclusions
  - We propose a new framework for radiation oncology
    - Genomic-based
    - Non-uniform benefit
    - Biologically optimized, individualized RT dose
    - Integrates interaction with immune system
    - Opens the door to precision genomic radiation therapy

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