PERSONALIZATION OF HADRON THERAPY FOR RADIORESISTANT CANCERS THROUGH BIOINDICATORS OF RADIORESISTANCE OR CONDITIONAL VULNERABILITY

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HITRI+ WORKSHOP
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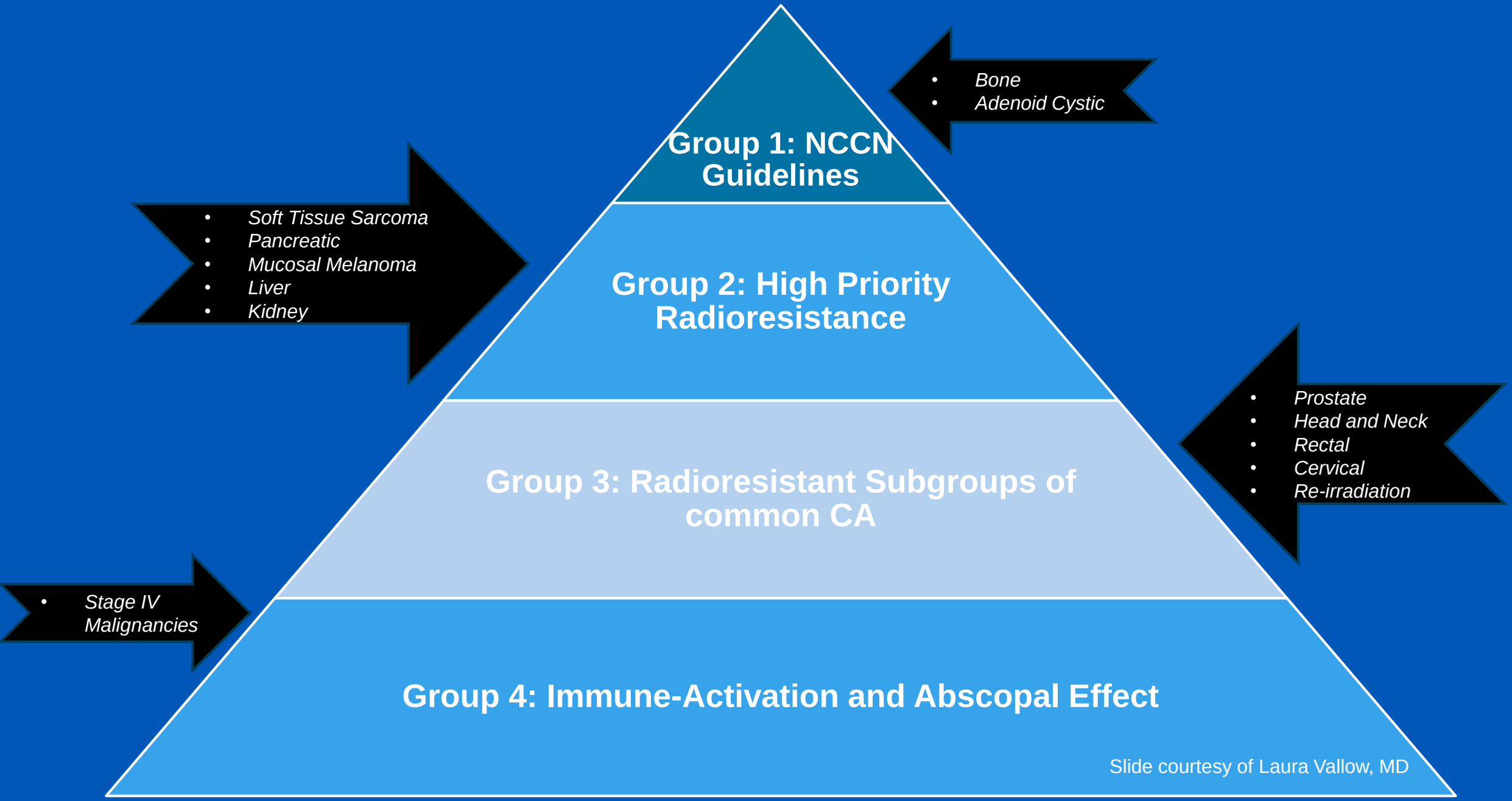
THESSALONIKI, GREECE

BUILDING ON THE CLINICAL EXPERIENCE WITH CARBON ION THERAPY

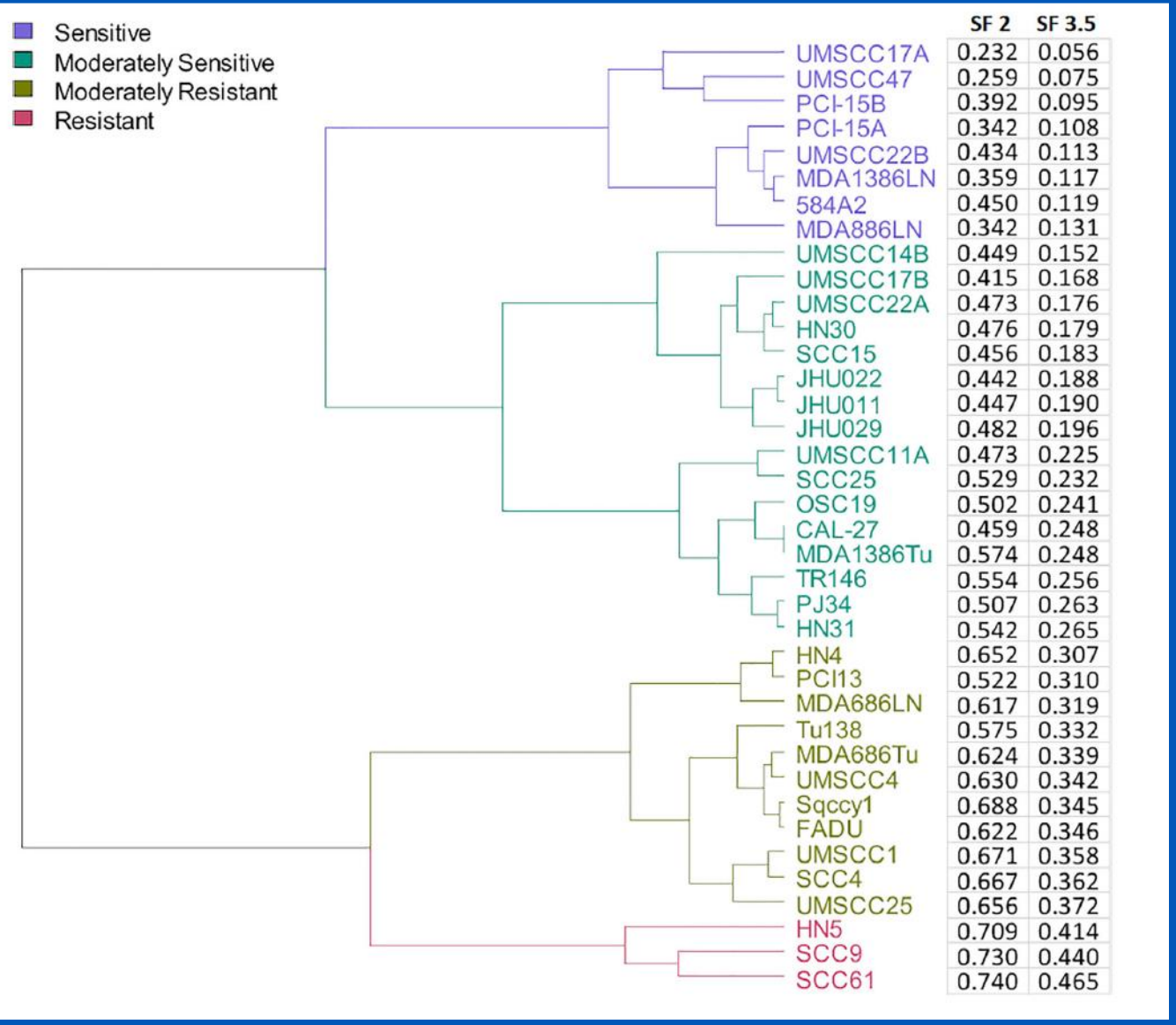


Slide courtesy of Laura Vallow, MD

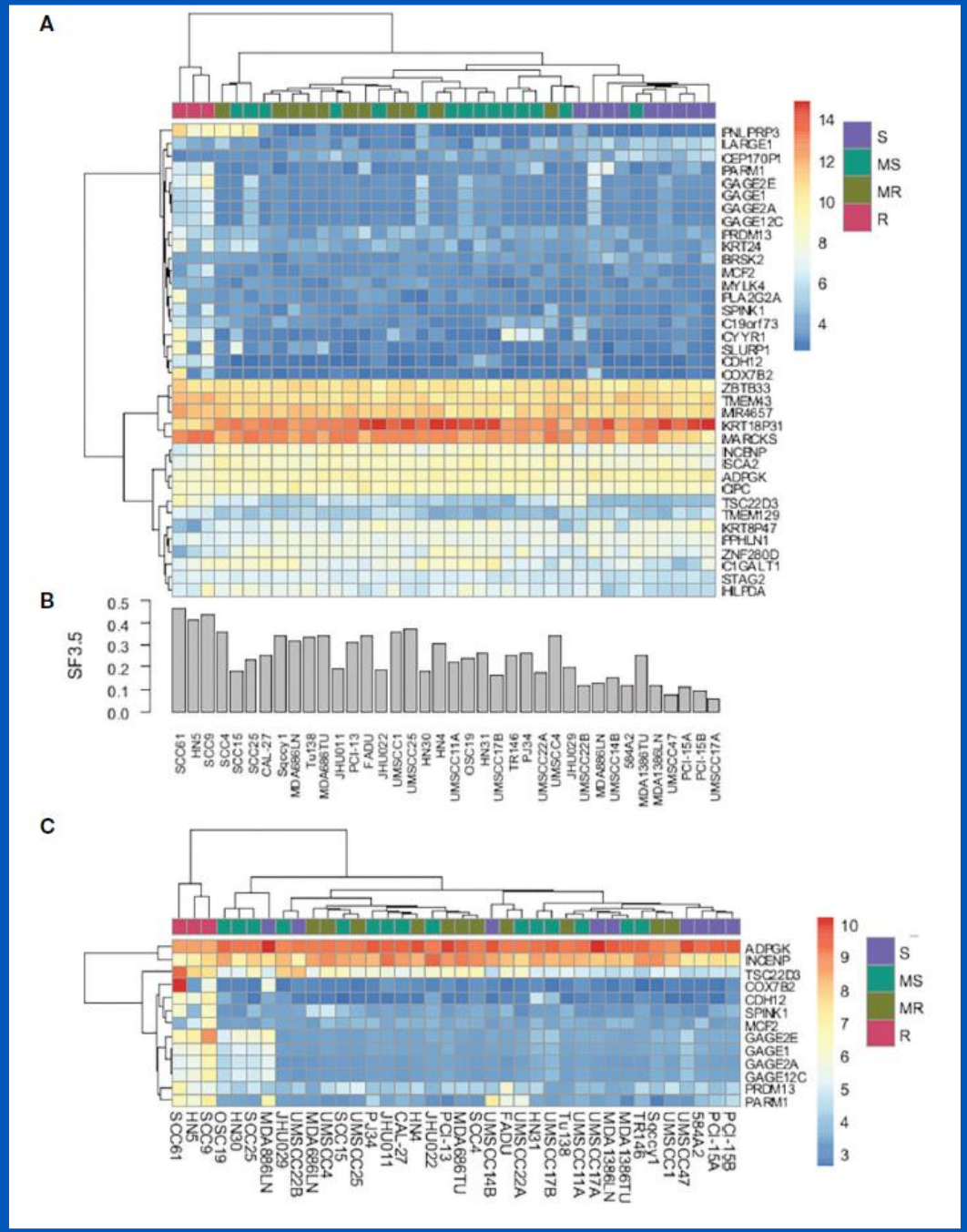
TWO APPROACHES TO PERSONALIZATION

- Approach taken may be specific to a given tumor site
  - Tumor sample availability
    - Bioindicators of radiation response
    - Bioindicators for targeted therapy combined with high LET hadrons
- Head and Neck cancer
  - Tumor availability via surgery or biopsy
    - Omics approach to define radiation sensitivity
      - RNA sequencing
      - Isoform analysis
- Pancreatic cancer
  - 25-30% of pancreatic tumors have mutations in DDR genes
    - Conditional vulnerability to heavy particles
    - Targeted agents against specific DNA repair pathways

HEAD AND NECK CANCER



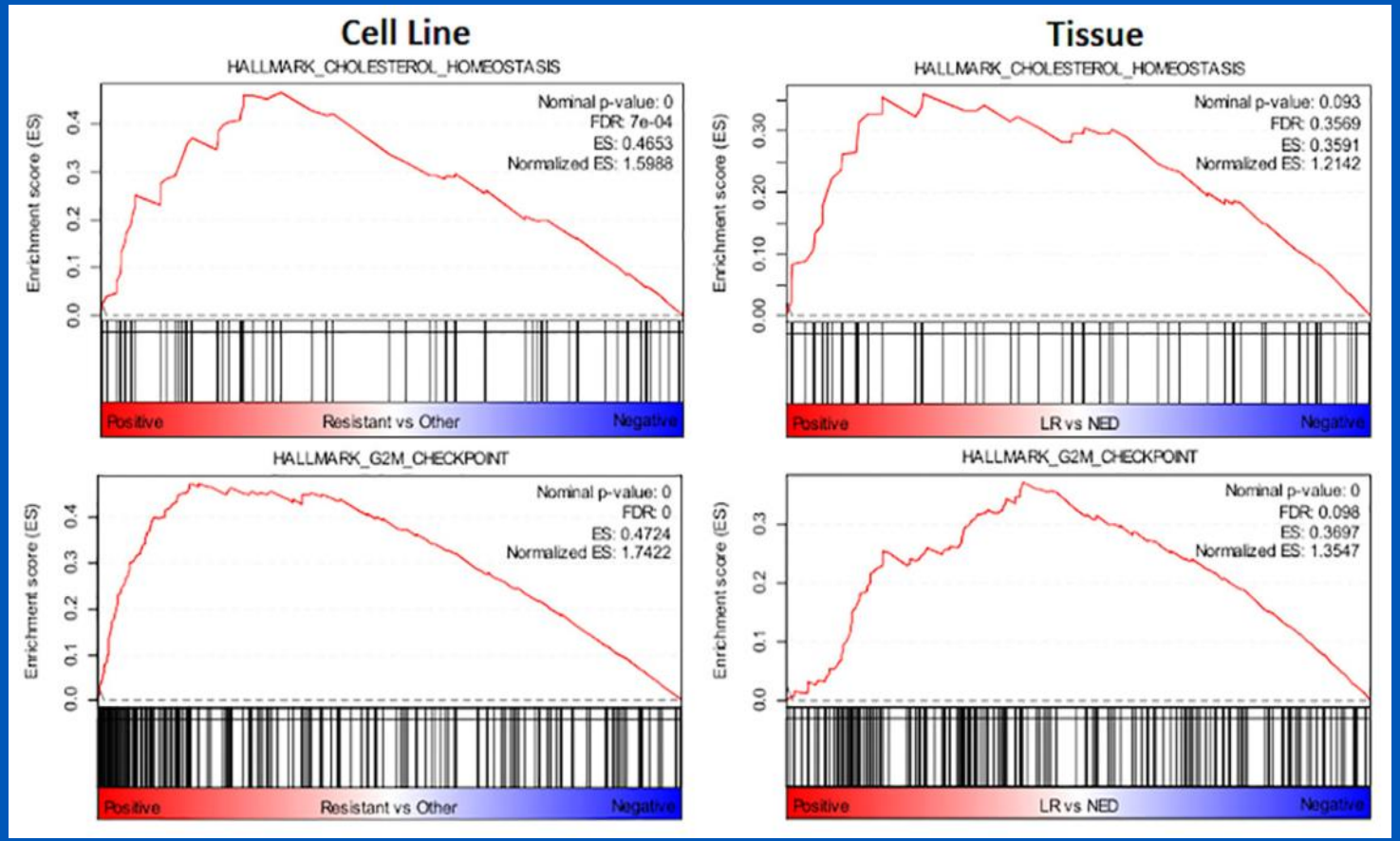
	SF 2	SF 3.5
UMSCC17A	0.232	0.056
UMSCC47	0.259	0.075
PCI-15B	0.392	0.095
PCI-15A	0.342	0.108
UMSCC22B	0.434	0.113
MDA1386LN	0.359	0.117
584A2	0.450	0.119
MDA886LN	0.342	0.131
UMSCC14B	0.449	0.152
UMSCC17B	0.415	0.168
UMSCC22A	0.473	0.176
HN30	0.476	0.179
SCC15	0.456	0.183
JHU022	0.442	0.188
JHU011	0.447	0.190
JHU029	0.482	0.196
UMSCC11A	0.473	0.225
SCC25	0.529	0.232
OSC19	0.502	0.241
CAL-27	0.459	0.248
MDA1386Tu	0.574	0.248
TR146	0.554	0.256
PJ34	0.507	0.263
HN31	0.542	0.265
HN4	0.652	0.307
PCI13	0.522	0.310
MDA686LN	0.617	0.319
Tu138	0.575	0.332
MDA686Tu	0.624	0.339
UMSCC4	0.630	0.342
Sqccy1	0.688	0.345
FADU	0.622	0.346
UMSCC1	0.671	0.358
SCC4	0.667	0.362
UMSCC25	0.656	0.372
HN5	0.709	0.414
SCC9	0.730	0.440
SCC61	0.740	0.465



GENE EXPRESSION SUGGESTS COMMON AND NOVEL SIGNALING IN RADIORESISTANT GROUP

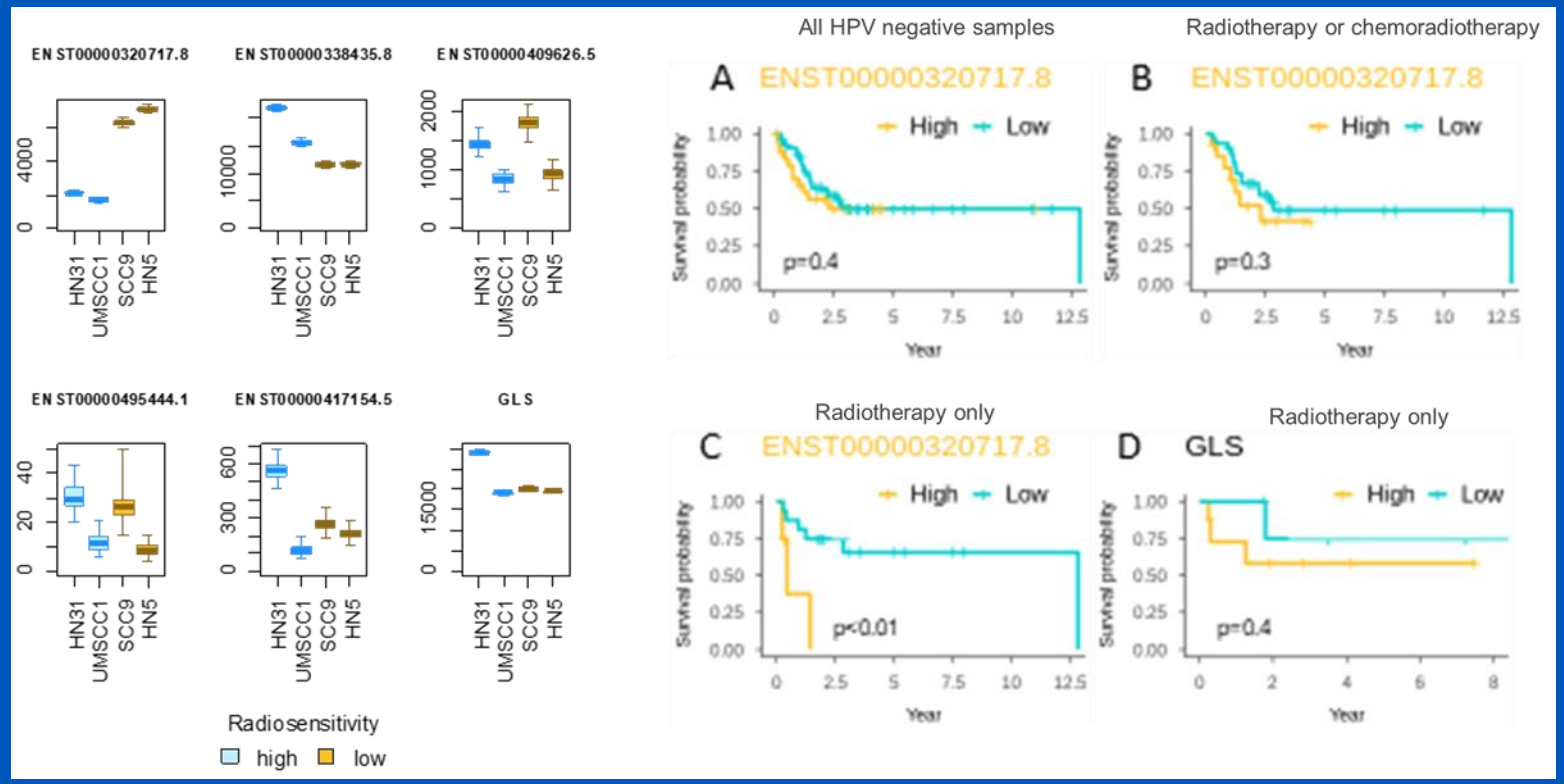
- GSEA and Ingenuity Pathways Analysis
- Leading Edge significant Enrichment Scores
  - Cholesterol biosynthesis
  - G2/M checkpoint
  - PI3K_AKT_MTOR
  - MTORC

	LR vs NED	R vs All Other
HIF1α Signaling	1.63	1.61
ERK/MAPK Signaling	1.51	1.62
Tumor Microenvironment Pathway	1.67	1.2
Production of Nitric Oxide and Reactive Oxygen Species in Macrophages	0.71	1.43
mTOR Signaling	1	1.02
NF-κB Activation by Viruses	1	0.48
ILK Signaling	1.34	0.71
Role of CHK Proteins in Cell Cycle Checkpoint Control	-2	-0.45
AMPK Signaling	2	0.41
CDK5 Signaling	-1.41	-0.22
LPS-stimulated MAPK Signaling	0.45	0.42



ISOFORM ANALYSIS

- High depth of coverage RNAseq
  - Interrogate for the abundance of specific gene isoforms
    - Gene isoforms can be tissue or context specific
    - Changes in gene function (or not) based upon isoform expressed



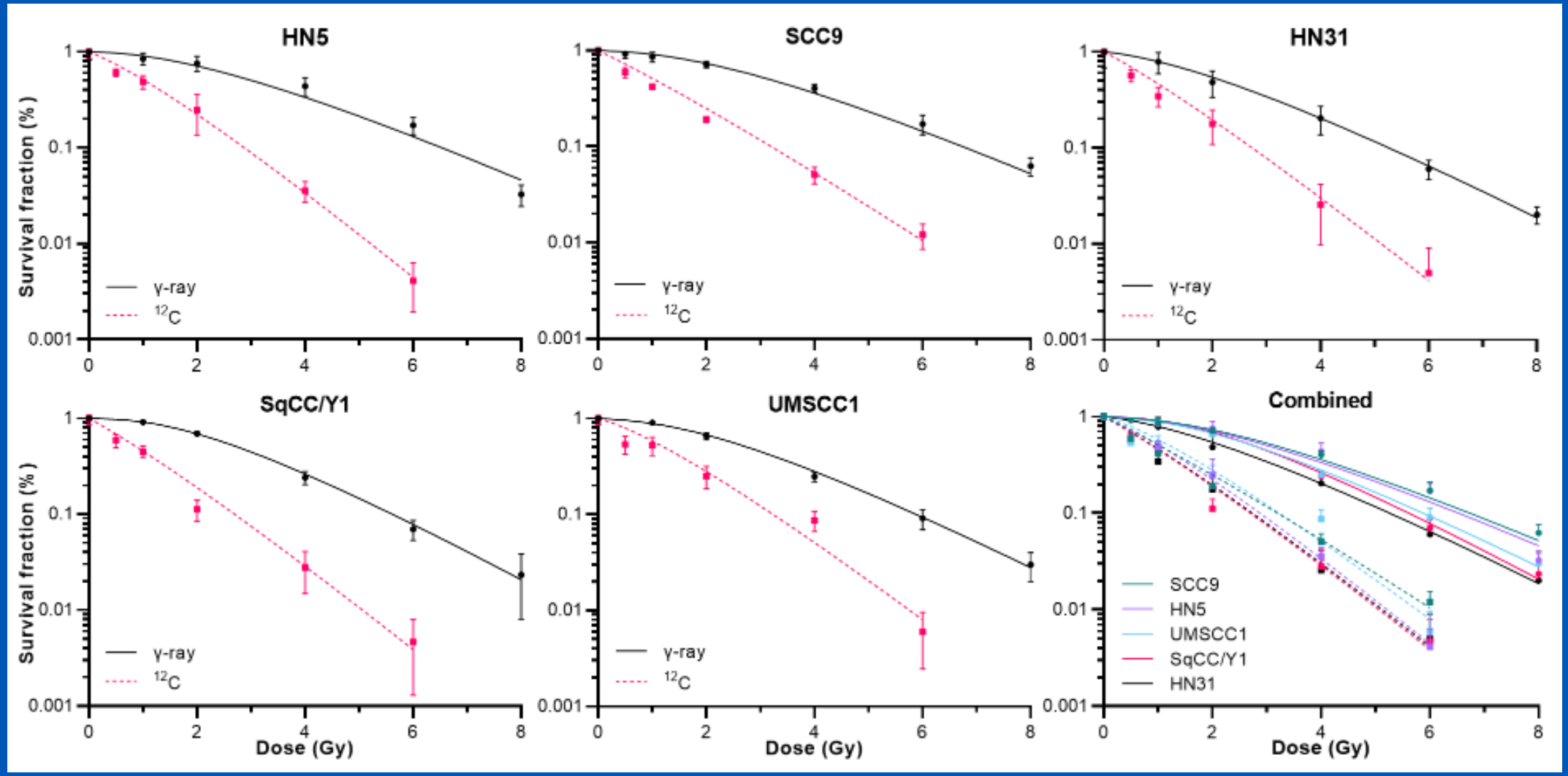
NOVEL GENES

- Genes segregating the R cohort include:
  - Radio/chemoresistance (GAGE12C, GAGE2E, SPINK1)
  - Metabolic processes (PNLIPRP3)
  - Proliferation, migration, invasion and metastasis (PARM1, CDH12, CYYR1, GAGE12C)
  - Inhibition of apoptosis and chemoresistance (SPINK1).

- GAGE genes not expressed in normal tissue with the exception of testes.
  - Found on X chromosome
  - Activation in tumors may be through demethylation
  - In tumors GAGE1 and 2 are CD4+ T cell antigens

Ding et al Frontiers in Radiation Oncology 2021

Γ-RAY AND ^{12}C SURVIVAL IN 5 HNSCC CELL LINES



RBE VALUES VARY BASED UPON BIOLOGICAL ENDPOINT

Cell line	$RBE_{SF10\%}$	$RBE_{Dbar\ parm}$	$RBE_{Dbar\ AUC}$	RBE_{D0}	*Average*	*std dev*	*CV*
SCC9	2.11	2.55	2.55	1.51	2.18	0.49	0.23
HN5	2.27	2.61	2.56	1.93	2.34	0.31	0.13
SqCC/Y1	2.08	2.58	2.57	1.51	2.19	0.51	0.23
HN31	1.92	2.14	2.12	1.61	1.95	0.25	0.13
Average	*2.095*	*2.47*	*2.45*	*1.64*			
std dev	*0.14341*	*0.22136*	*0.22015*	*0.199*			
CV	*6.84539*	*8.96192*	*8.98577*	*12.134*			

$RBE_{SF10\%}$	*RBE calculated using 10% survival*
$RBE_{Dbar\ parm}$	*RBE calculated using mean inactivation dose derived from RCR parameters*
$RBE_{Dbar\ AUC}$	*RBE calculated using mean inactivation dose derived from Reimann sum*
RBE_{D0}	*RBE calculated as ratio of limiting slopes*

RBE DETERMINATIONS IN CURRENT TPS

- How applicable is a generalized RBE if the intrinsic radiosensitivity of tumors of a given type are highly variable?
- RBE says more about the low LET response than the effect of hadron exposure
- Heterogeneous dose distributions
- Fractionation regimens are moving to limited fraction numbers
- Input parameters for LEM include:
  - radius of cell nucleus
  - radial energy deposition
  - photon survival curve*** based upon α/β ratios
    - At some point (D_T) the survival curve is linearized
    - Biphasic survival curve

$$S(D) = \begin{cases} e^{-(\alpha_X d + \beta_X d^2)}; & d < D_t \\ e^{-(\alpha_X D_t + \beta_X D_t^2 + s_{max}(d - D_t))}; & d \geqslant D_t \end{cases}$$

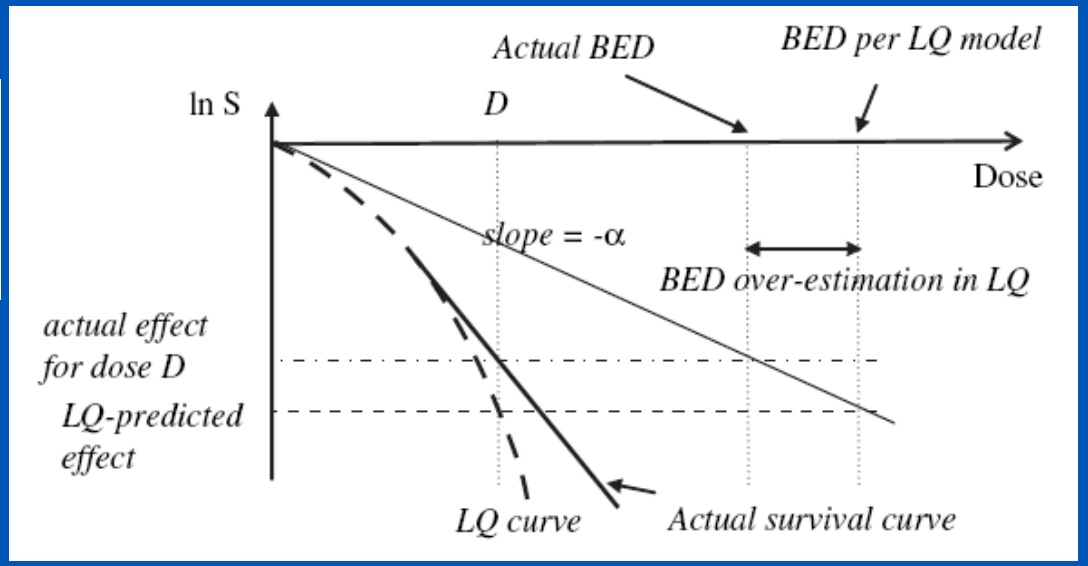
- **Why not use a model that does not require the determination of D_T**

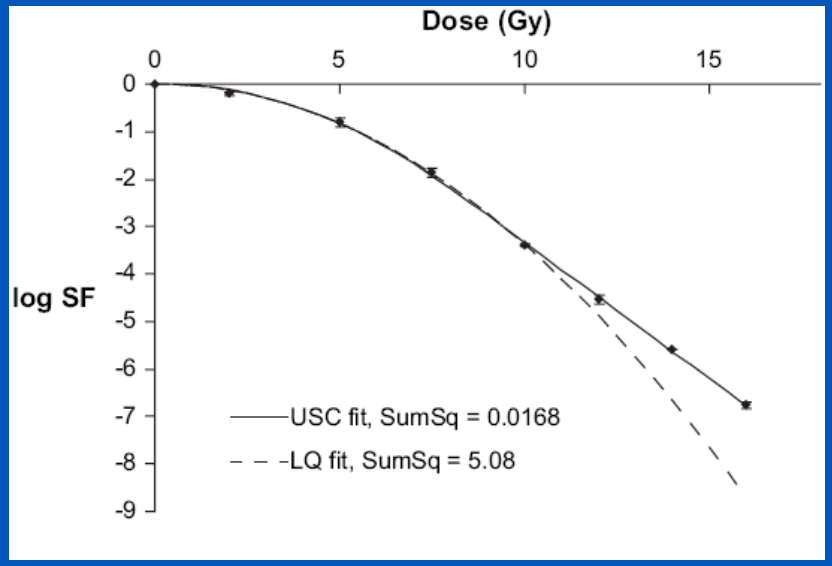
OVERESTIMATION OF CELL KILLING

- Biologically **Effective** dose calculations
  - Allows comparisons between different dose fractionation schemes
  - The doses used for the 2nd order polynomial are generally below the ablative doses used for SAbR

$$BED = D \cdot \left(1 + \frac{d}{\alpha/\beta}\right)$$

D = nd
n= # of fractions
d= dose per fraction





UNIVERSAL SURVIVAL CURVE AND SINGLE FRACTION EQUIVALENT DOSE: USEFUL TOOLS IN UNDERSTANDING POTENCY OF ABLATIVE RADIOTHERAPY

CLINT PARK, M.D. M.S., LECH PAPIEZ, PH.D., SHICHUAN ZHANG, M.D., PH.D.,
MICHAEL STORY, PH.D., AND ROBERT D. TIMMERMAN, M.D.

IN SILICO MODELING OF TUMOR CONTROL PROBABILITY

- Repair Conditionally Repairable Damage (Lind et al., 2003)

  (* bi-exponential approximation)

$$S(d) = e^{-ad} + bde^{-cd}$$

- Transpose cell survival data to tumor response (Antonovic et al., 2015)
- N_{vox} is the number of voxels in an *in silico* tumor
- N_i is the number of cells in voxel i, (1 cm tumor contains 10^8 tumor cells)
- $S_{i,j}$ (d,L,pO2) is the surviving fraction in voxel i at fraction j with dose d, oxygen partial pressure pO2, and LET L.

$$\mathrm{TCP} = \exp\left\{-\sum_{i=1}^{N_{\mathrm{vox}}} N_i \prod_{j=1}^{n} S_{i,j}(d, L, pO_2)\right\}$$

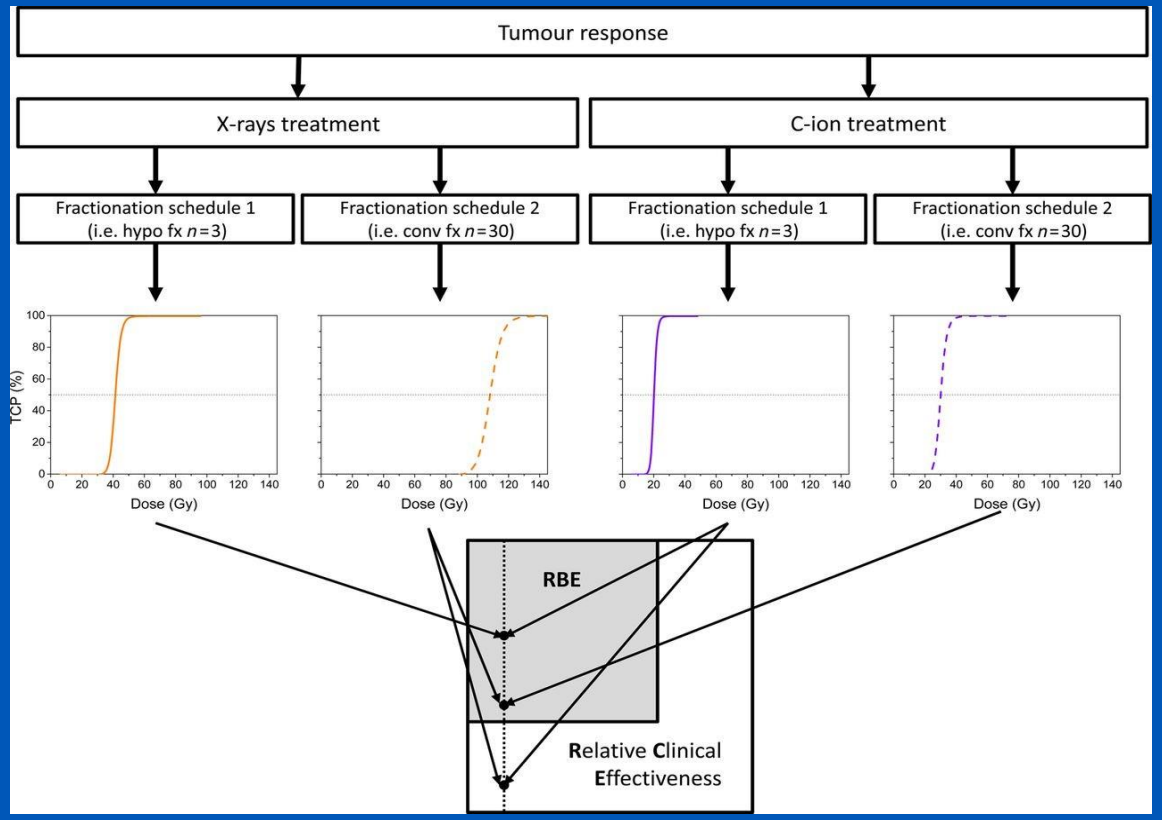
- Added tumor kickoff time and regrowth rates

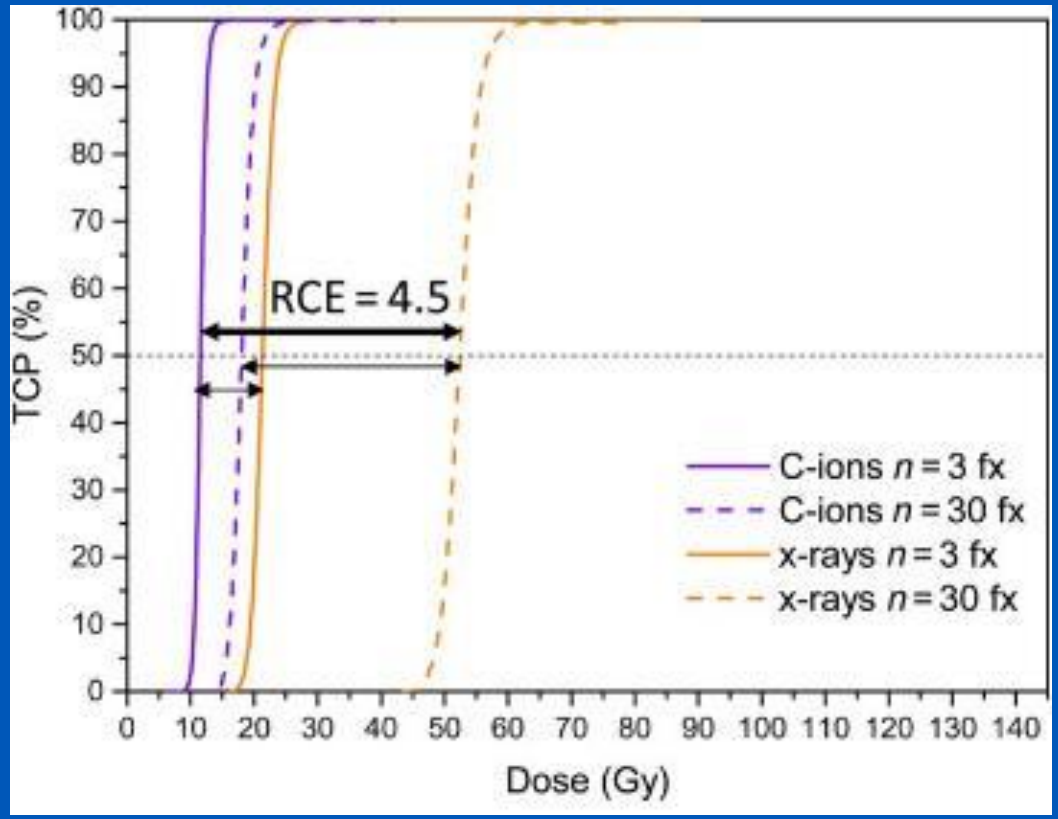
RELATIVE CLINICAL EFFECTIVENESS (RCE)

Antonovic, et al., 2015

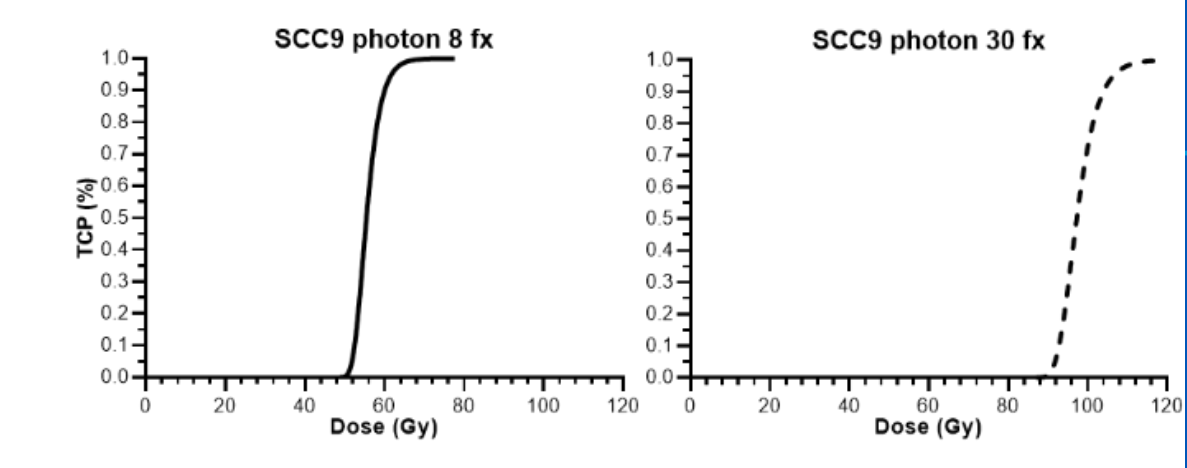
Model H&N across range of radiosensitivities

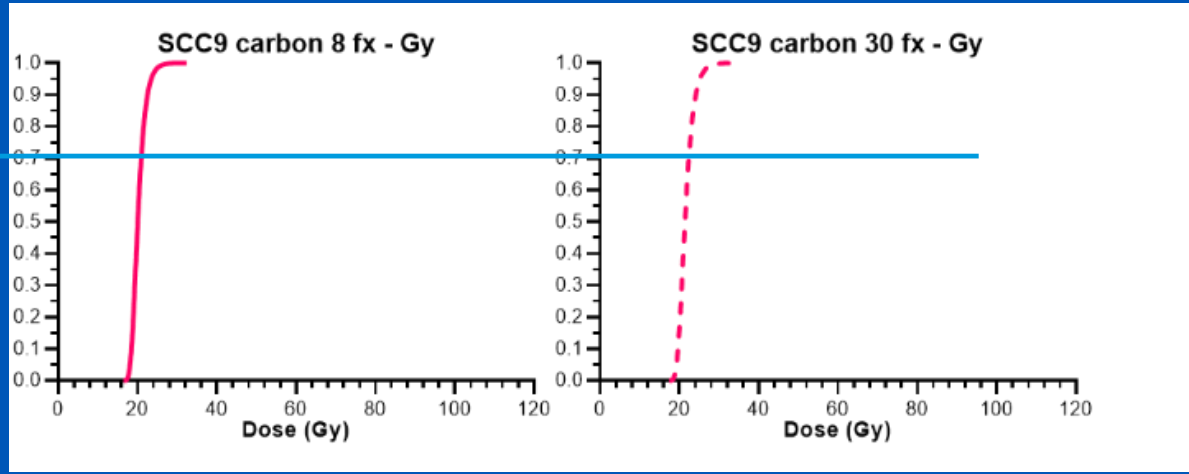
Conduct in vivo experiments for biological validation of the use of RCE





RELATIVE CLINICAL EFFECTIVENESS SSC9 CELLS

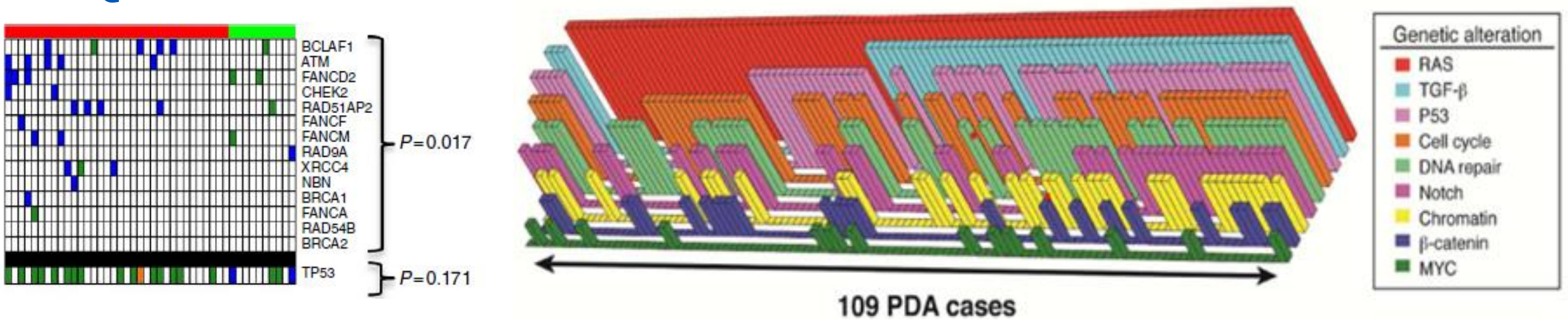




RCE 8 fx ^{12}C vs 30 fx photon: 4.75
RCE 8 fx ^{12}C vs 8 fx photon: 2.75

- All models are bad but some are useful.
- *In vivo validation required*
- If RBE cannot be abandoned addition of RCE may be an invaluable addition

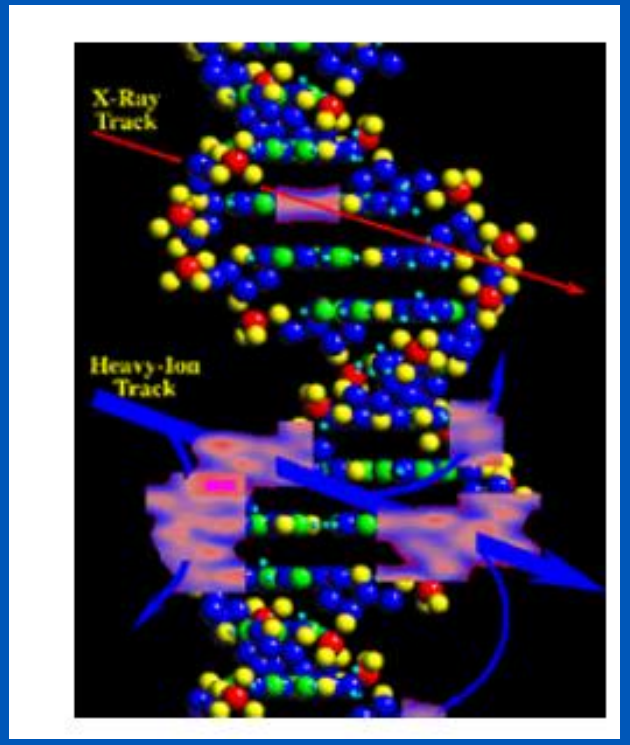
PANCREATIC CANCER: CONDITIONAL VULNERABILITIES UNIQUE TO CHARGED PARTICLES

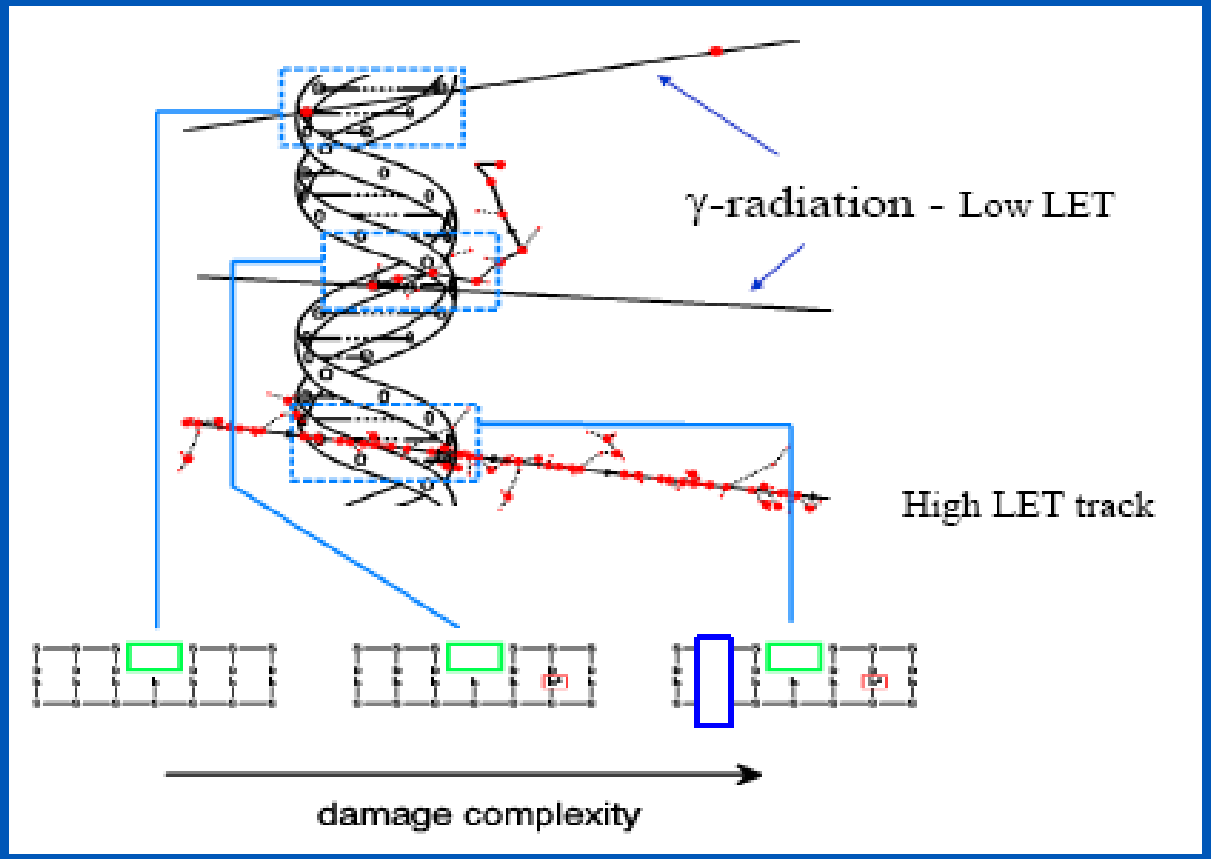


Witkiewicz et al, Whole-exome sequencing of pancreatic cancer defines genetic diversity and therapeutic targets Nature Communications 2015
Knudsen, E.S., et al., *Genetic Diversity of Pancreatic Ductal Adenocarcinoma and Opportunities for Precision Medicine.* Gastroenterology 2016

- Can ^{12}C ion therapy be enhanced by targeting mutations associated with DNA repair and DNA replication stress?
- Would charged particles hold a particular advantage over X-rays for defects in specific DNA repair pathways?
- Could increased DNA damage be exploited to elicit an anti-tumor immune response?

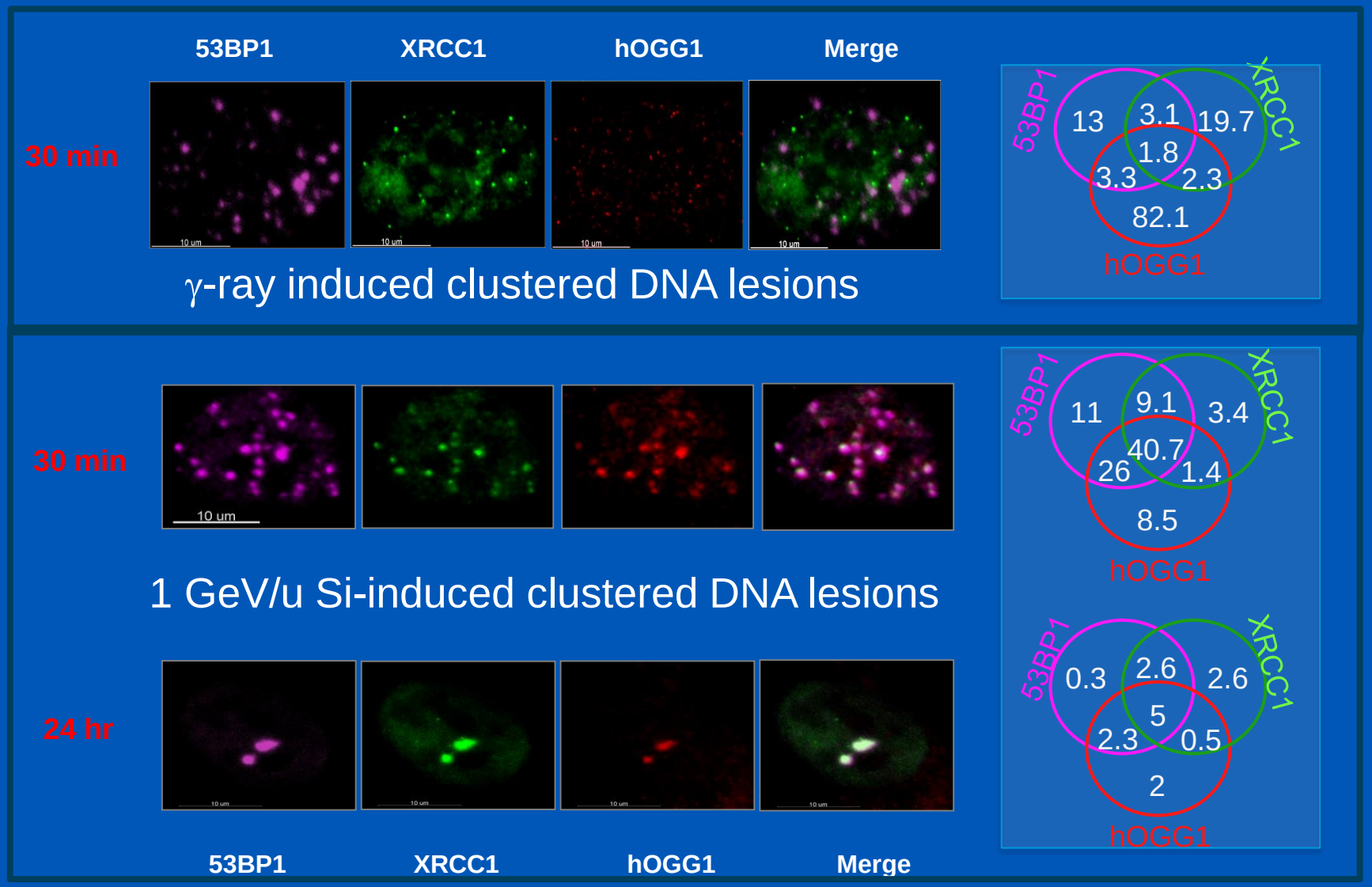
THE DENSER THE ENERGY DEPOSITION PATTERNS THE MORE COMPLEX THE DNA DAMAGE





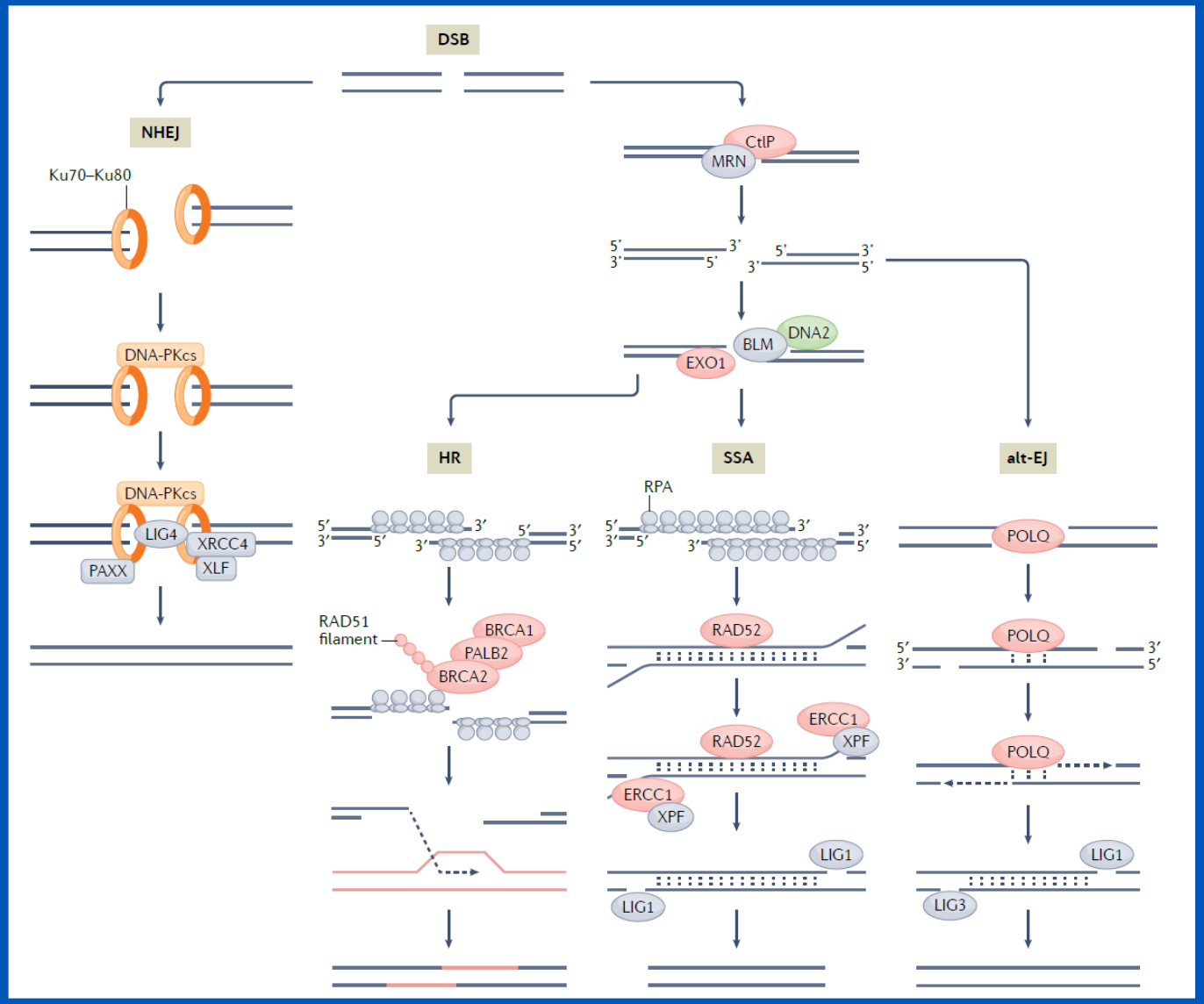
Simple Complex Clustered

DNA DAMAGE: LESION COMPLEXITY



Asaithamby et al, PNAS 2011

THE MAJOR DNA REPAIR PATHWAYS



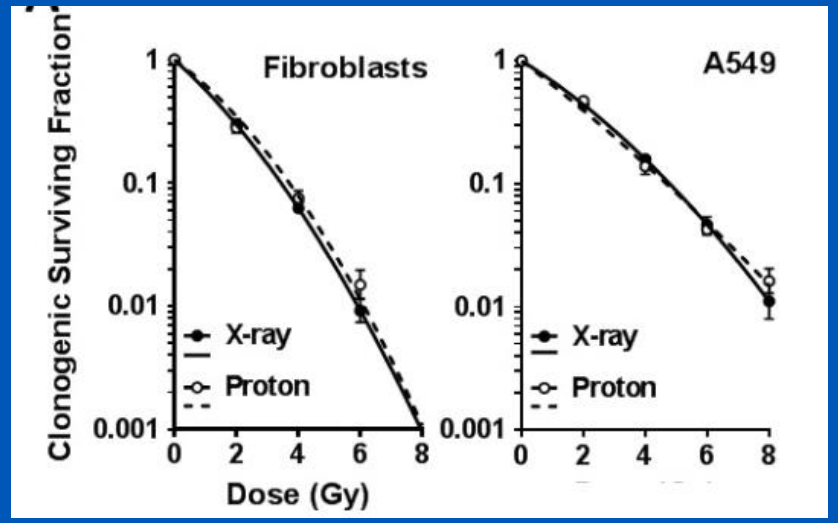
nature reviews cancer
https://doi.org/10.1038/s41568-022-00535-5
Review article
Check for updates

Targeting DNA damage response pathways in cancer

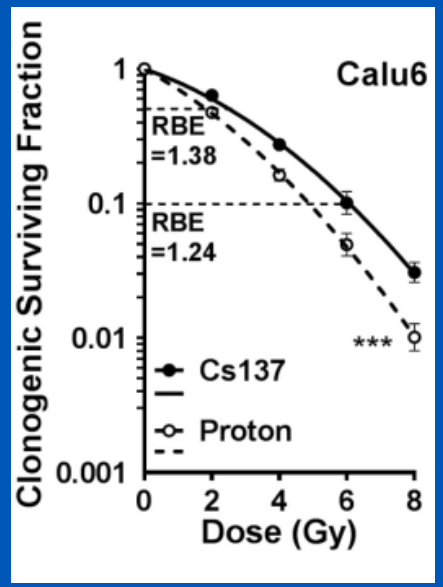
Florian J. Groelly[1], Matthew Fawkes[2], Rebecca A. Dagg[1], Andrew N. Blackford[2] & Madalena Tarsounas[1]

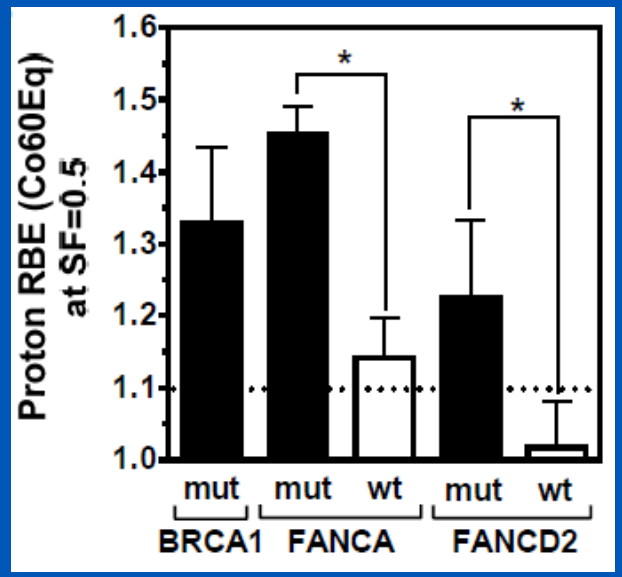
LESION COMPLEXITY INFLUENCES BIOLOGICAL RESPONSE

- 225 kVp X-ray: 2 keV/u
- Proton LET: 2.3 keV/u
- The RBE's (^{60}Co) are the same
  - (1.1-1.15)



- HR repair deficient cell line Calu6
- H^+ radioresponse tied to HR gene defects
- Higher LET particles?



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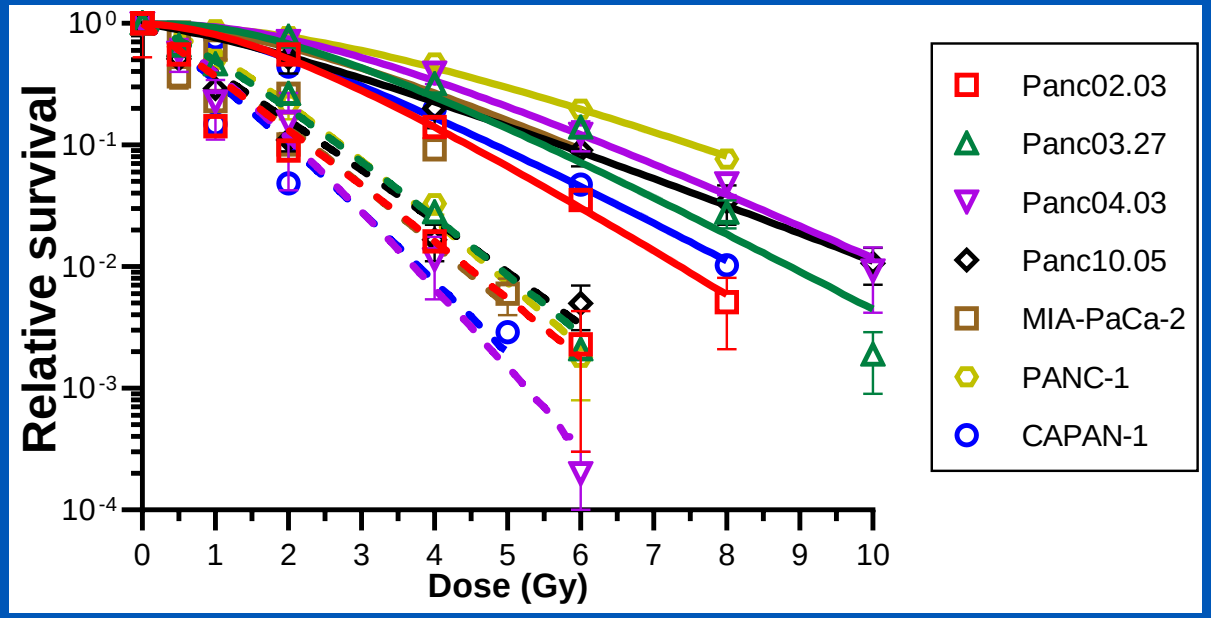
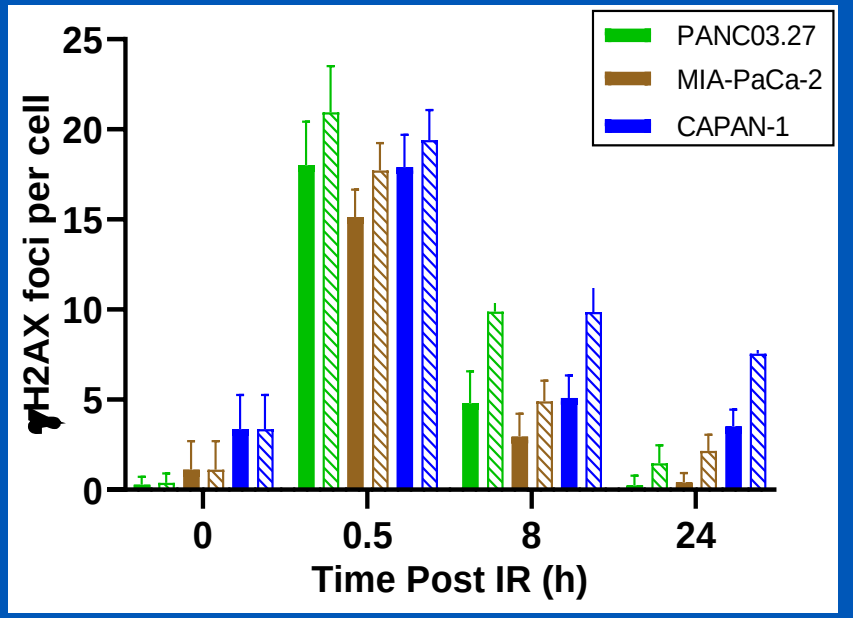
Biology Contribution

Lung Cancer Cell Line Screen Links Fanconi Anemia/BRCA Pathway Defects to Increased Relative Biological Effectiveness of Proton Radiation

Qi Liu PhD *, Priyanjali Ghosh BA *, Nicole Magpayo BS *, Mauro Testa PhD †, Shikui Tang PhD †, Liliana Gheorghiu MS *, Peter Biggs PhD †, Harald Paganetti PhD †, Jason A. Efstathiou MD, DPhil *, Hsiao-Ming Lu PhD †, Kathryn D. Held PhD *, Henning Willers MD *

INCREASED RESIDUAL DNA DAMAGE AFTER ^{12}C IRRADIATION





DNA REPAIR GENES AS TARGETS OF OPPORTUNITY

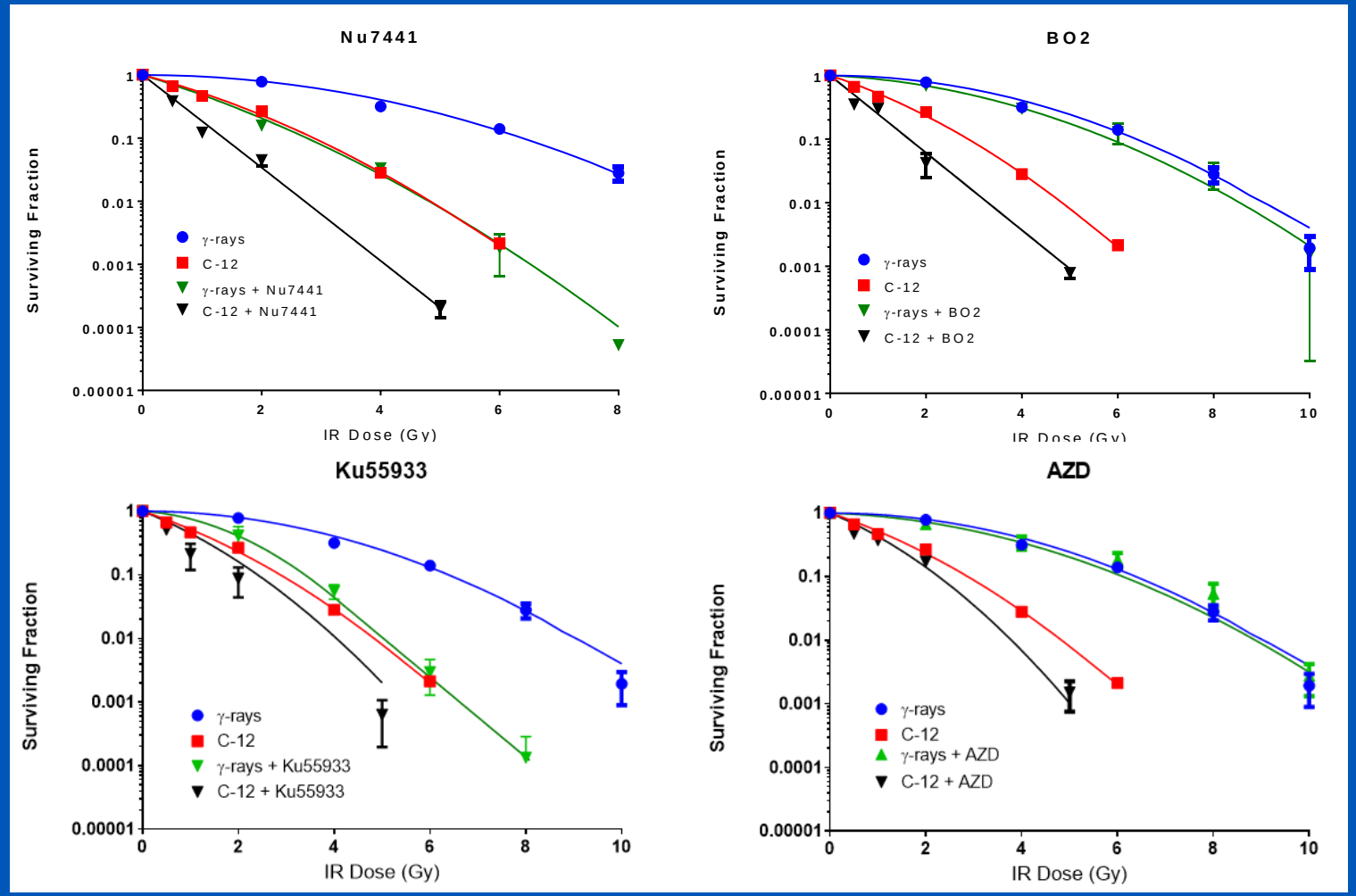
Response of a γ-ray resistant cell line 03.27 to combined irradiation with targeted DNA repair inhibitors.

Nu7441 (DNA-PKcs/NHEJ)

BO2 (Rad51/HR)

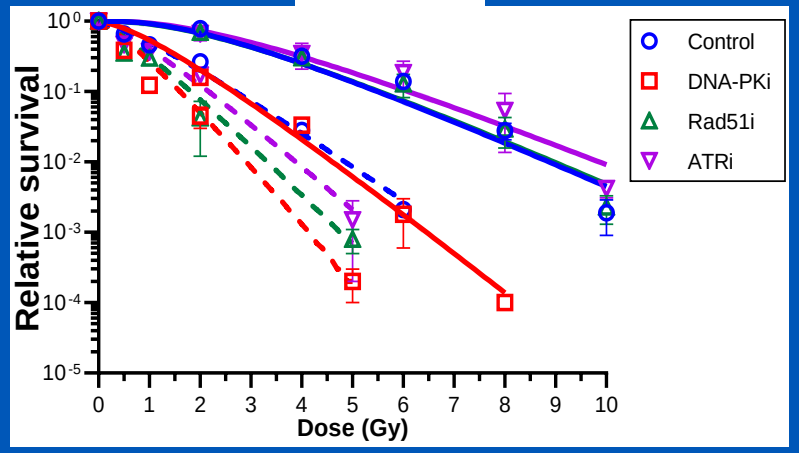
Ku55933 (ATM)

AZD6738 (ATR)

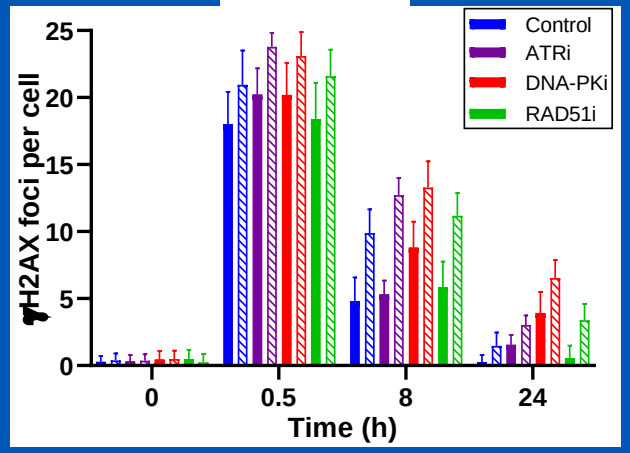


PDAC CANCER CELLS ARE SUSCEPTIBLE TO DDR INHIBITORS

PANC.03.27

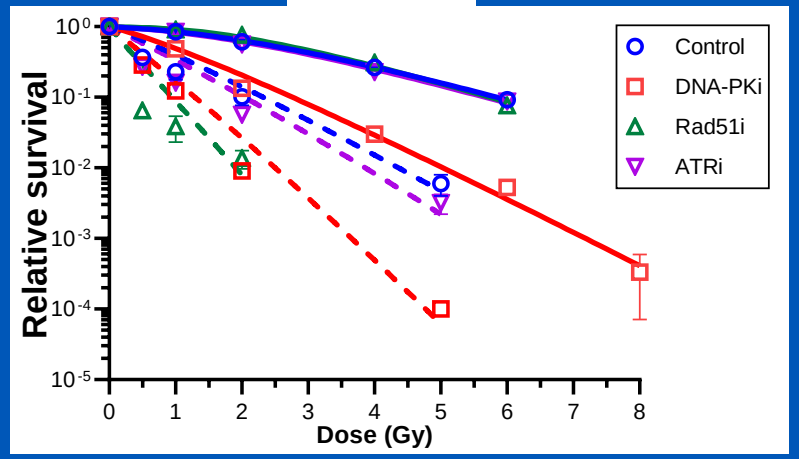


PANC.03.27

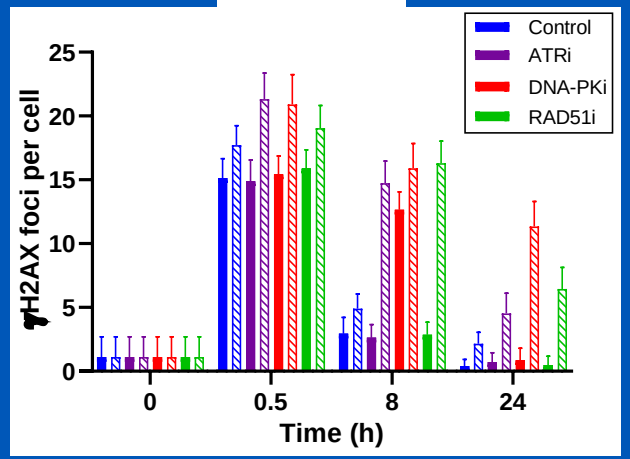


Group	MID(Gy)	SER	VAR	StDEV	p value
γ-rays + DMSO	3.61	1.00	0.02	0.22	
γ-rays + DNA-PKi	1.24	2.92	2.18	0.88	0.0041
γ-rays + DNA-RAD51i	3.57	1.01	3.36	1.83	0.9649
γ-rays + DNA-ATRi	3.89	0.93	0.07	0.31	0.0739
^{12}C + DMSO	1.04	1.00	0.03	0.23	
^{12}C + DNA-PKi	0.59	1.77	0.00	0.17	0.0100
^{12}C + DNA-RAD51i	0.69	1.51	0.00	0.17	0.0264
^{12}C + DNA-ATRi	0.81	1.29	0.65	0.73	0.1565

MIA-PaCa-2



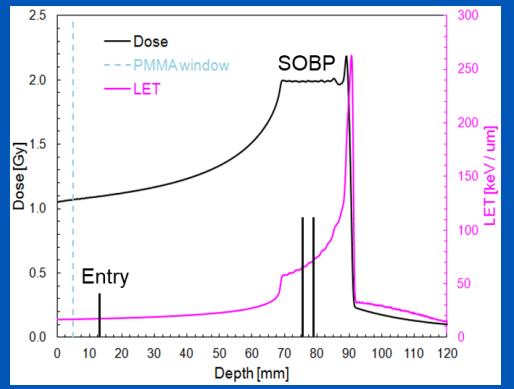
MIA-PaCa-2



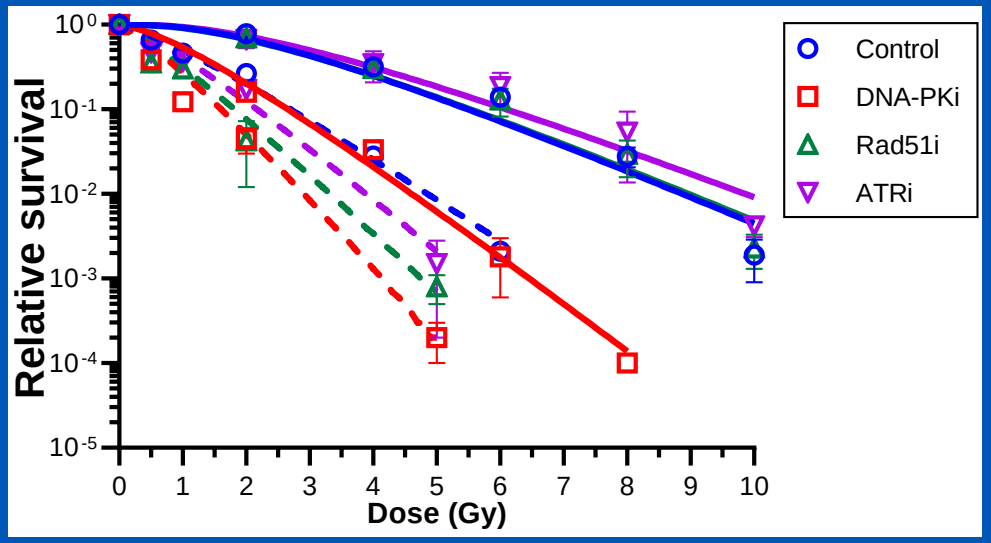
Group	MID(Gy)	SER	VAR	StDEV	p value
γ-rays + DMSO	2.26	1.00	1.22	1.56	
γ-rays + DNA-PKi	1.06	2.14	0.01	1.11	0.0241
γ-rays + DNA-RAD51i	3.28	0.69	0.11	1.17	0.1772
γ-rays + DNA-ATRi	2.77	0.82	2.87	2.18	0.5961
^{12}C + DMSO	0.94	1.00	0.03	0.24	
^{12}C + DNA-PKi	0.52	1.81	0.01	0.18	0.0056
^{12}C + DNA-RAD51i	0.40	2.37	0.05	0.23	0.0051
^{12}C + DNA-ATRi	0.82	1.15	0.04	0.26	0.3753

DIFFERENTIAL RESPONSE OF 03.27 CELLS TO DDR INHIBITORS BASED UPON POSITION IN BRAGG CURVE

- ^{12}C Spread Out Bragg Peak vs Entry
  - 78 keV/u vs 13 keV/u

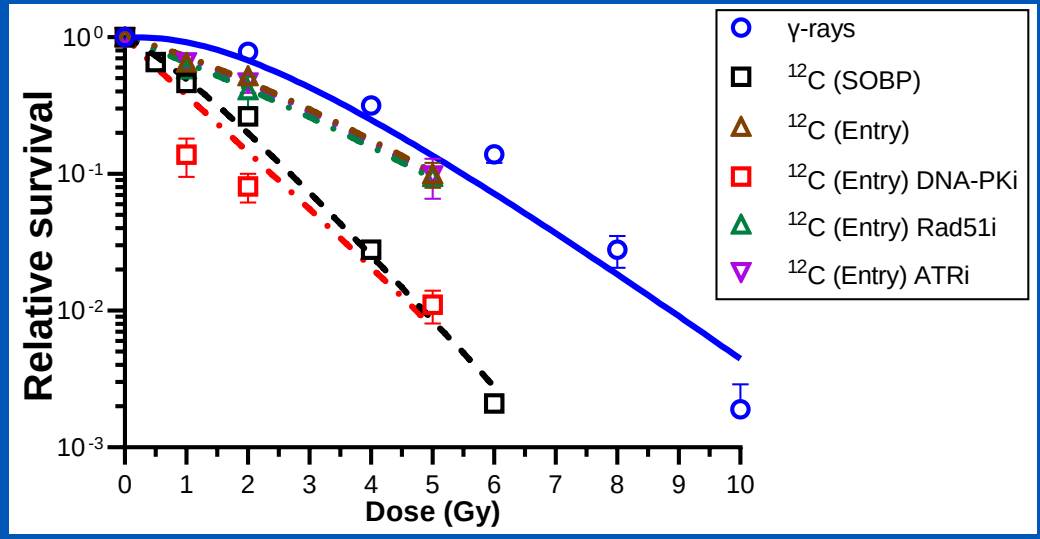


Group	MID(Gy)	SER	VAR	StDEV	p value
^{12}C + DNA-PKi	0.59	1.77	0.00	0.17	0.0100
^{12}C + DNA-RAD51i	0.69	1.51	0.00	0.17	0.0264
^{12}C + DNA-ATRi	0.81	1.29	0.65	0.73	0.1565



Spread Out Bragg Peak

Group	MID(Gy)	SER	VAR	StDEV	p value
^{12}C (Entry)	2.39	1.00	0.24	0.81	0.0004
^{12}C (Entry) + DNA-PKi	1.02	2.35	0.18	0.57	0.0033
^{12}C (Entry) + DNA-RAD51i	2.13	1.12	0.02	0.51	0.4293
^{12}C (Entry) + DNA-ATRi	2.21	1.08	0.07	0.69	0.5987



Entry

TREATMENT SELECTION VIA MUTATIONS IN DNA REPAIR GENES

- Patients undergo genetic tests at higher frequency
- Genetic tests are scaling to include larger and larger gene sets
- Would charged particles hold a particular advantage over X-rays for defects in specific DNA repair pathways?
- Could increased DNA damage be exploited to elicit an anti-tumor immune response?

PDAC Cell Line	DNA Damage Response	Other Genes of Interest
CAPAN-1	*BRCA2 V1532Sfs*2*, *FANCA 1196**, *RAD50*, *PRKDC*	*CDK6*, *MYC*
MIA-PaCa-2	*KMT2C K724**	*ARID1A Q321**, *ARID1B*
PANC-1		*KRAS*, *AKT2*
PANC.02.03	*BRCA2*, *BAP1*, *XPC*, *FANCD2*, *SETD2*, *ATRIP*, *FANCG*	*MYC*, *RB1*, *MAPK1*, *ARID1B*
PANC.03.27	*FANCC*	*CDK4*, *RAF1*, *MAPK1*, *FBXW7*
PANC.04.03	*RAD21 X33_splice*, *KMT2C D372Yfs*15*	*ATRX-PGK1*, *TOP1*
PANC.10.05	*BRCA2*, *CHEK2*, *RAD51C*, *FANCD2*, *PRKDC*, *XPC*	*MYC*, *EP300 K1488**, *FBXW7*, *RB1*

Black	Point mutations
Blue	Deletions
Purple	Amplifications
Orange	Structural variants

- ** Once caveat is the determination of mutation status being somatic vs. germinal
  - Under analysis

BIOLOGY WILL DRIVE ADVANCES IN CHARGED PARTICLE RADIOTHERAPY

- Physics: the accuracy of dose delivery and imaging will continue to improve outcomes, but do so incrementally.
  - The problem is now more engineering than physics.
- The greatest benefit for protons over X-rays is conformality.
  - Limiting intermediate doses to normal tissues.
- The benefit for heavier charged particles over protons is biology.
  - The biological uncertainties are greater for charged particle therapy.
  - There are potentially distinct advantages due to novel biology with charged particle exposure that need better defining –and exploiting.
  - Exploitation requires moving from population-based advances to individualizing therapies based upon the vulnerability identified for a given individual.

THANK YOU FOR YOUR ATTENTION

- Questions?

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