



Profiling Molecular Changes in Radiation Resistant Triple Negative Breast Cancer through RNA-Seq Analysis

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Medical Sciences

Disclosure of Affiliations, Financial and In-Kind Support:

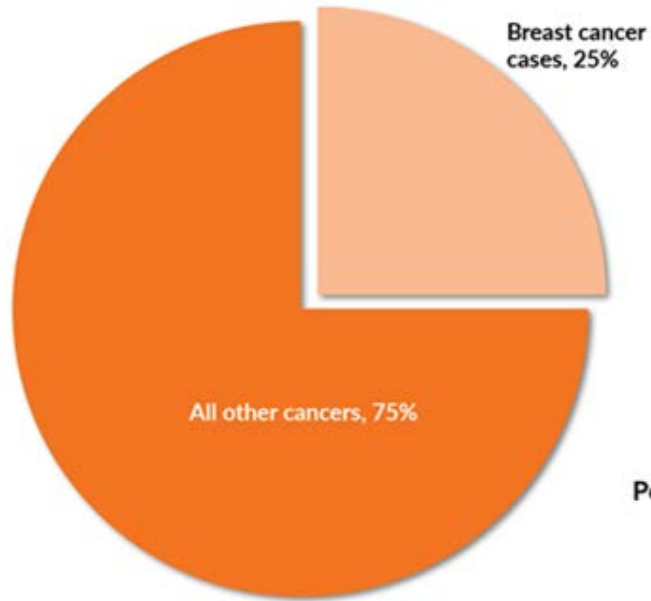
Acknowledgement Statement:

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The following steps have been taken to mitigate bias: (examples below)

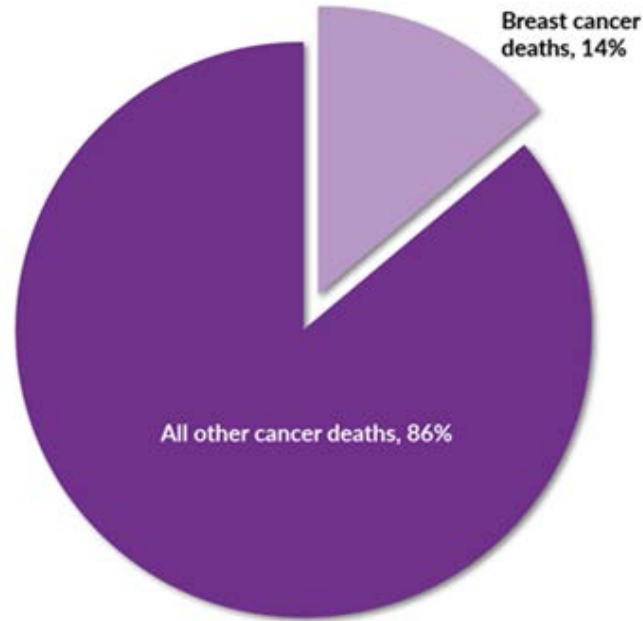
- All speakers have been provided with a speaker letter outlining the certification/accreditation requirements for their presentation.
- The SPC or designate has reviewed the presentation(s) prior to their delivery.
- If a breach is detected the SPC will approach the speaker to discuss the concern and update the presentation as required.

Percentage of All Estimated New Cancer Cases
in Women in 2022



© Canadian Cancer Society

Percentage of All Estimated Cancer Deaths
in Women in 2022



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Breast cancer in Canada



Breast cancer develops in the cells of the breast tissue. When these cells change or no longer behave normally, they may lead to benign tumours (non-cancerous). In some cases, the changes may cause malignant breast tumours (cancerous).

The data^{1,2}

It is the **2nd MOST COMMON** cancer in Canada

1 IN 8 WOMEN
will be diagnosed with breast cancer in their lifetime

About **27,400 women** will have been diagnosed with breast cancer in 2020

In **WOMEN**, breast cancer is the **#1 CANCER**

83% of cases occur in **WOMEN AGED 50+**

Risk factors include*



AGING



FAMILY HISTORY



BRCA GENE MUTATION



REPRODUCTIVE STATUS (E.G. LATE MENOPAUSE)



HORMONE EXPOSURES (E.G. ESTROGEN)



ALCOHOL INTAKE



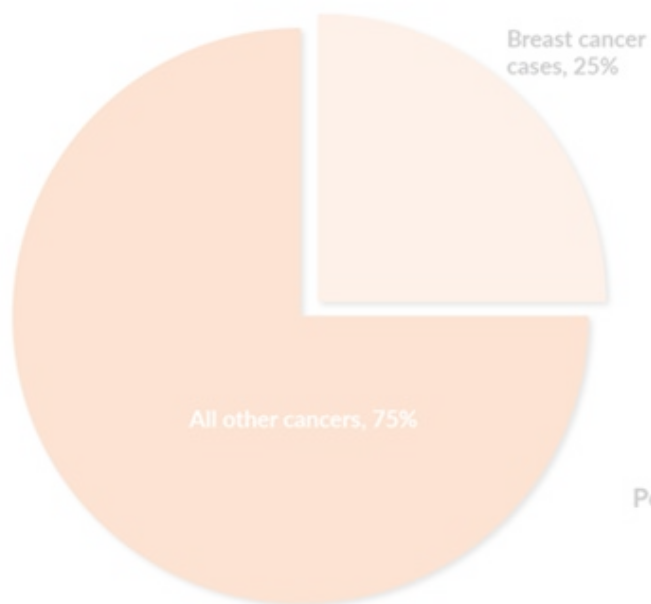
OVERWEIGHT OR OBESITY (AFTER MENOPAUSE)



PHYSICAL INACTIVITY

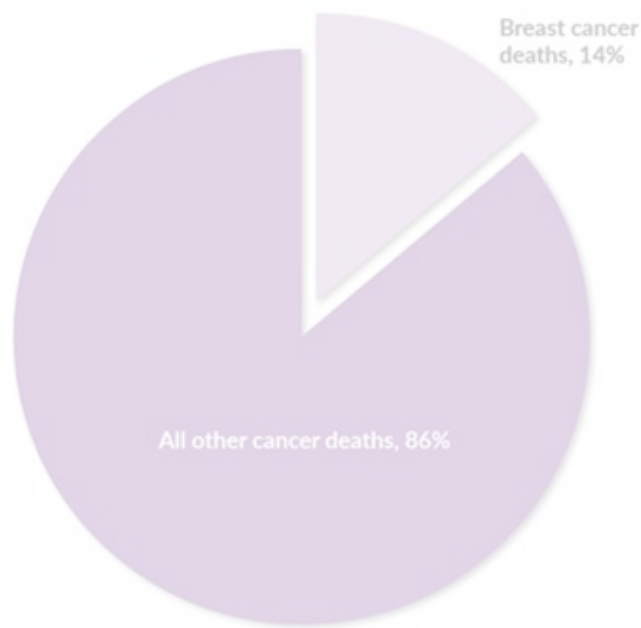
* For more information, please visit: <https://data.prevent.cancer.ca/current>

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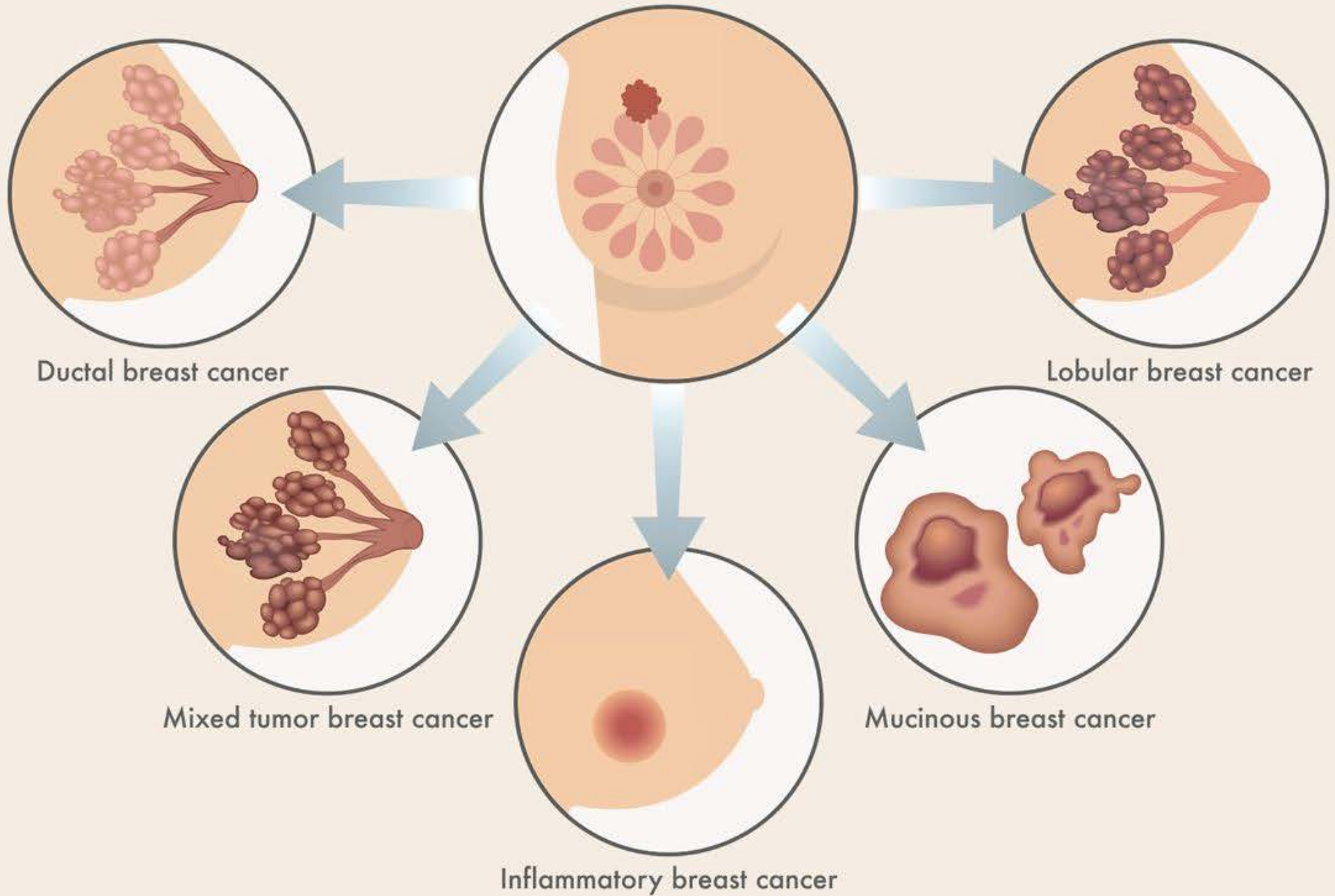
OVERWEIGHT OR OBESITY (AFTER MENOPAUSE)



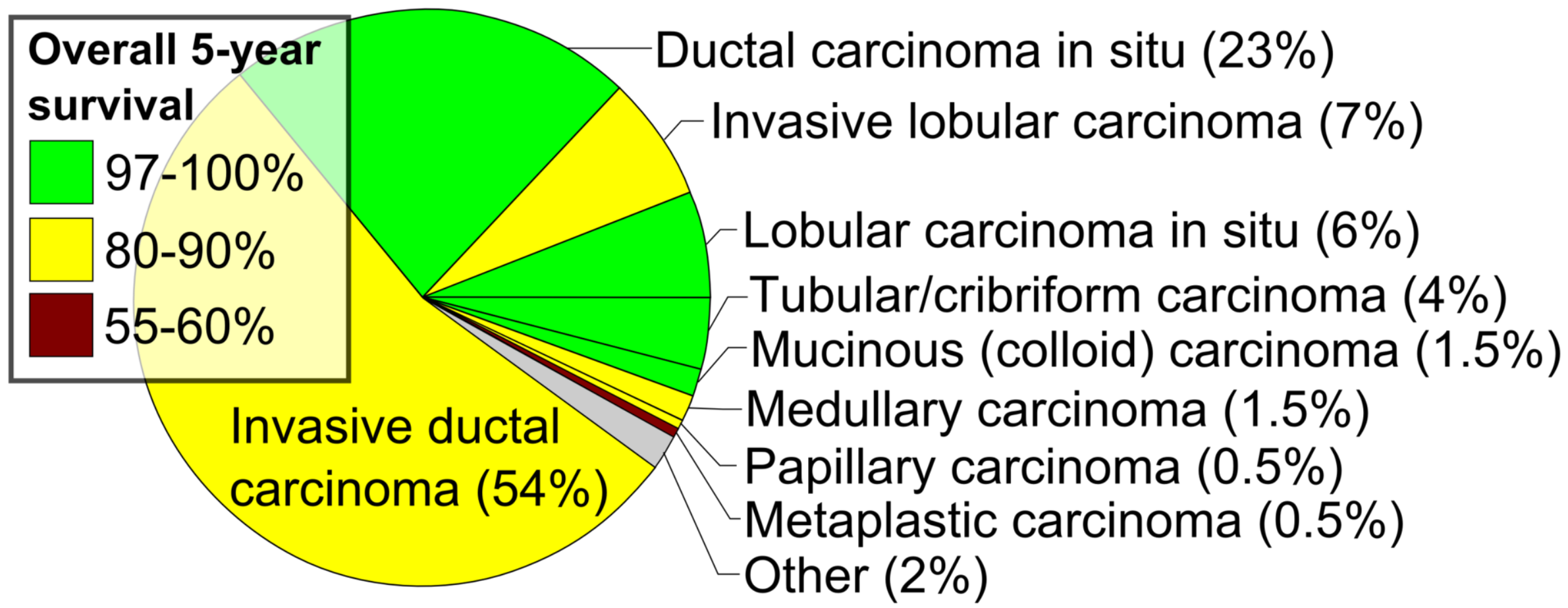
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Types of Breast Cancer

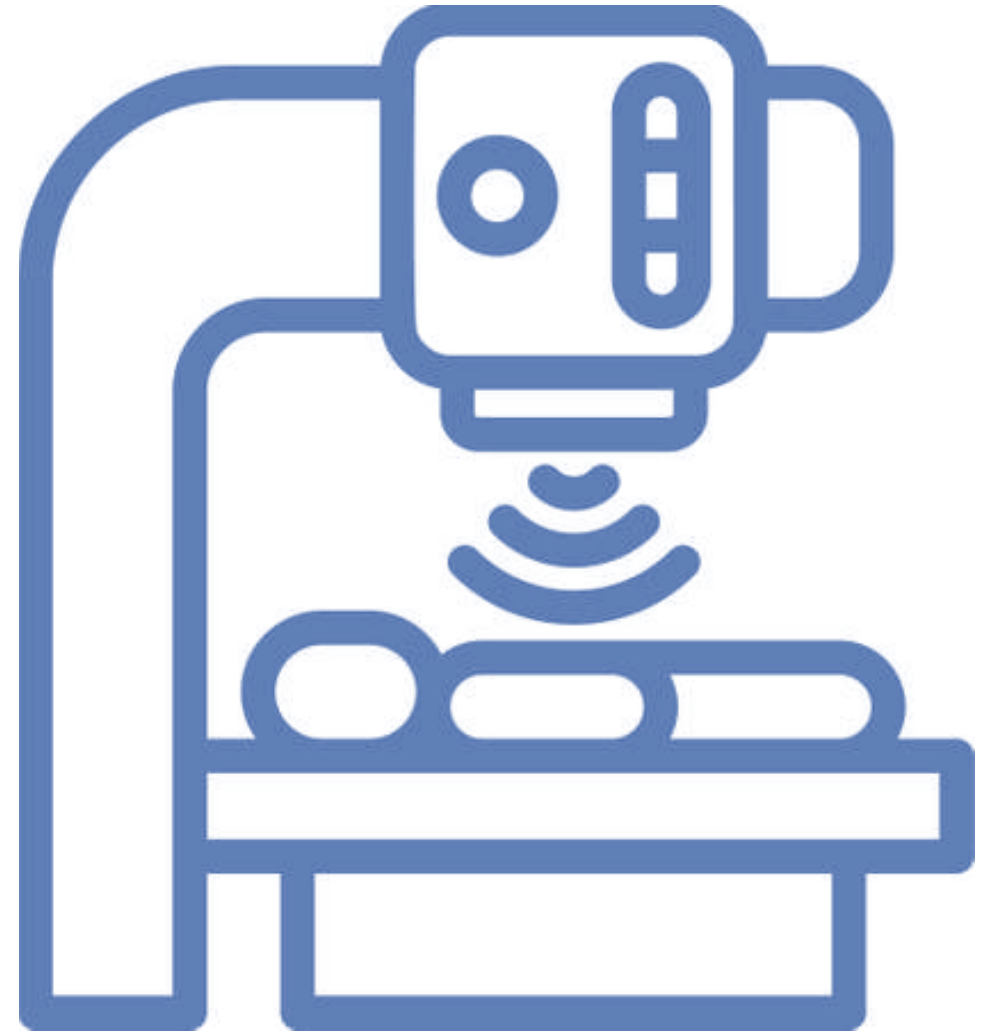


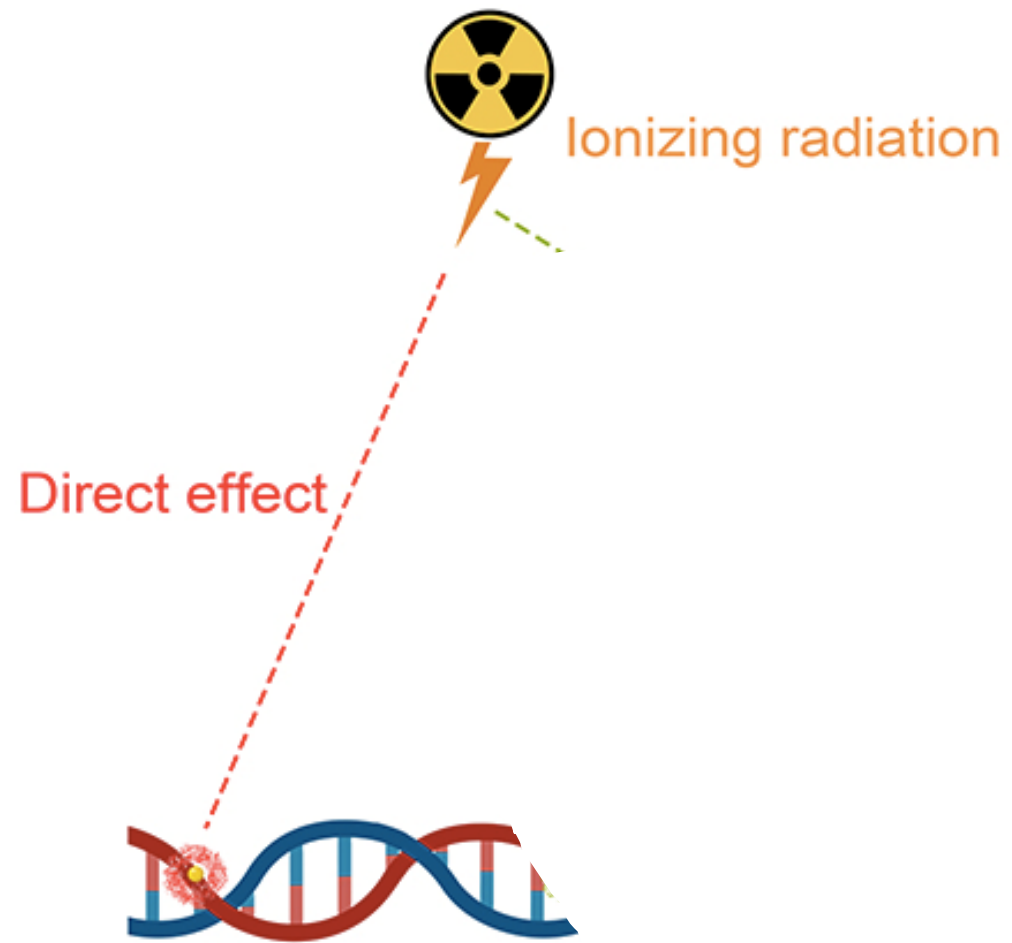
Breast Cancer Types and Relative Incidence (%)



Radiation Resistant Breast Cancer

- Despite technological advances in irradiation methodologies, certain breast cancer cells remain resistant to radiation-induced cell killing resulting in radioresistant cancer variants
- In many instances, these radioresistant cancer cells are also resistant to other chemotherapeutic agents





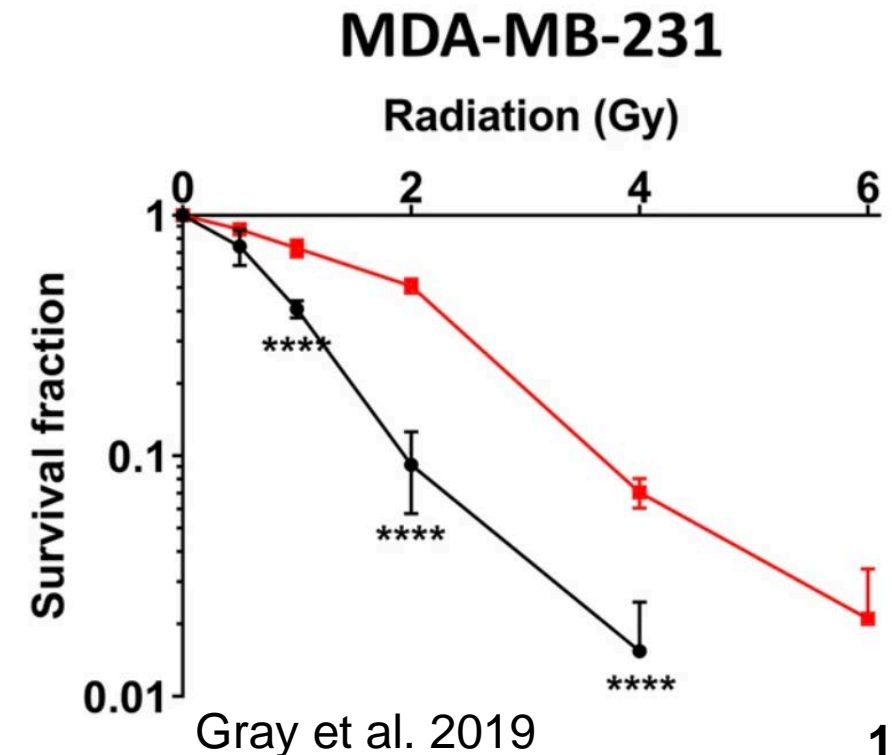
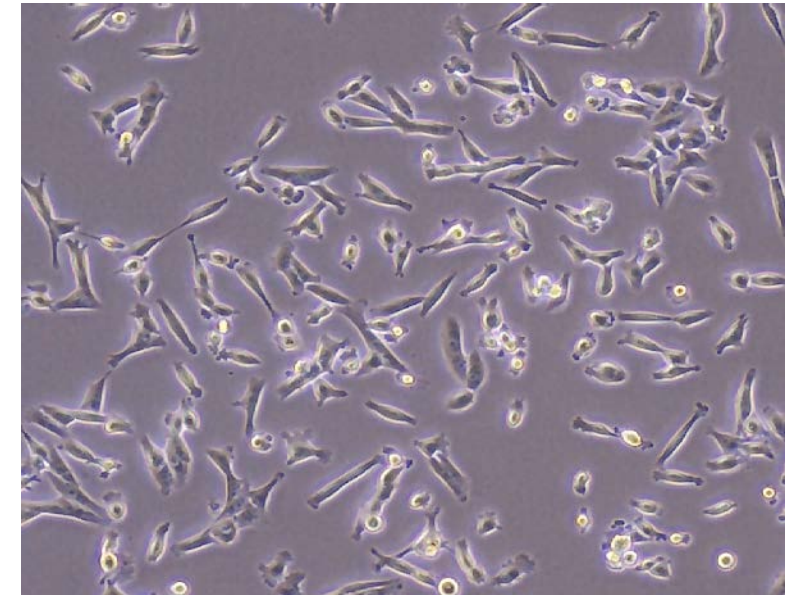
Overall Project Goals

- The **overall goal** of this **research program** is to unravel the molecular mechanisms that contribute to the development of **radiation resistant breast cancer**
- The **specific goals** of this current project are to:
 - Profile genetic differences between breast cancer cells (MDA-MB-231) and a radiation resistant subtype
 - See what genetic differences occur when each of these cell types are subjected to a subsequent radiation challenge



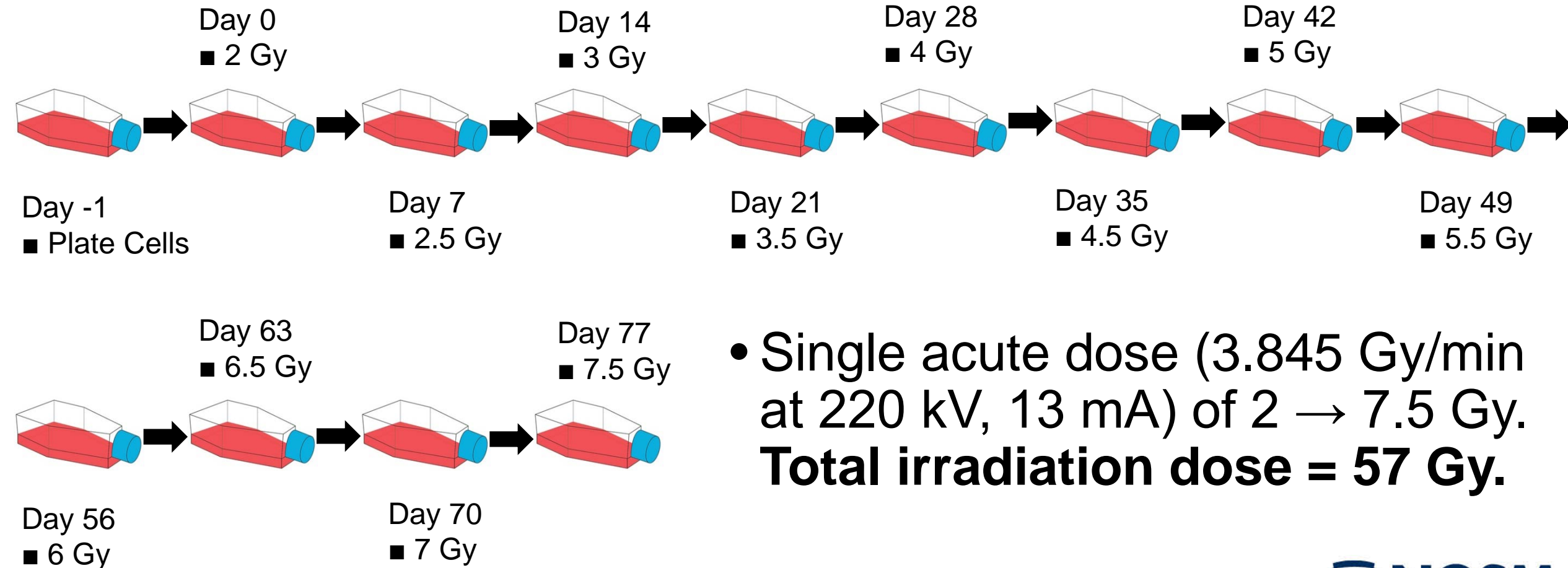
Model: MDA-MB-231 cells

- Isolated at the *M. D. Anderson Cancer Center* from a pleural effusion of a patient with **invasive ductal carcinoma**
- Commonly used model of late-stage breast cancer
- **Triple Negative Breast Cancer** (TNBC; lacking ER, PR and HER-2)
- Difficult to treat – patients have poorer outcome compared to other subtypes
- Relatively resistant to chemo and radiation therapy regimens
- Repeated radiation exposure results in radioresistant (RR) variants

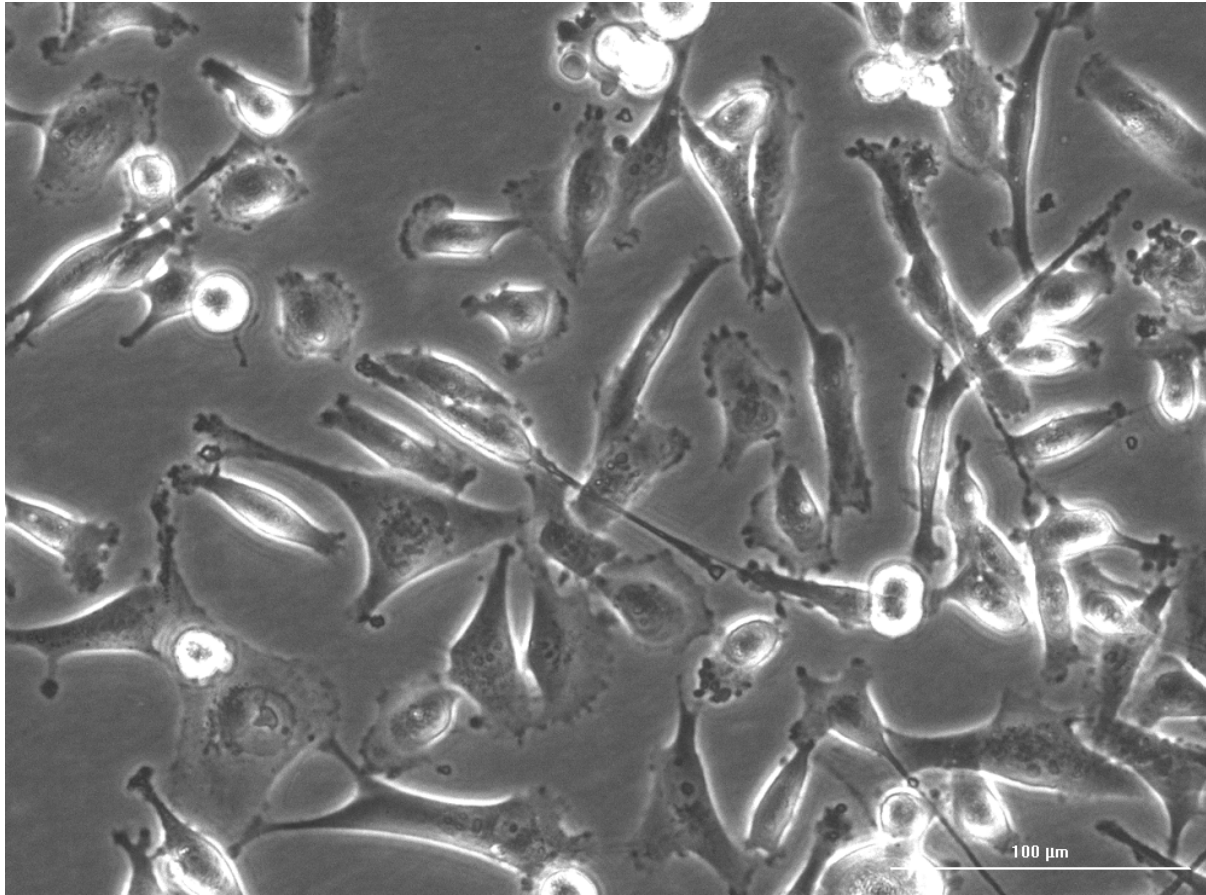


PROTOCOL:

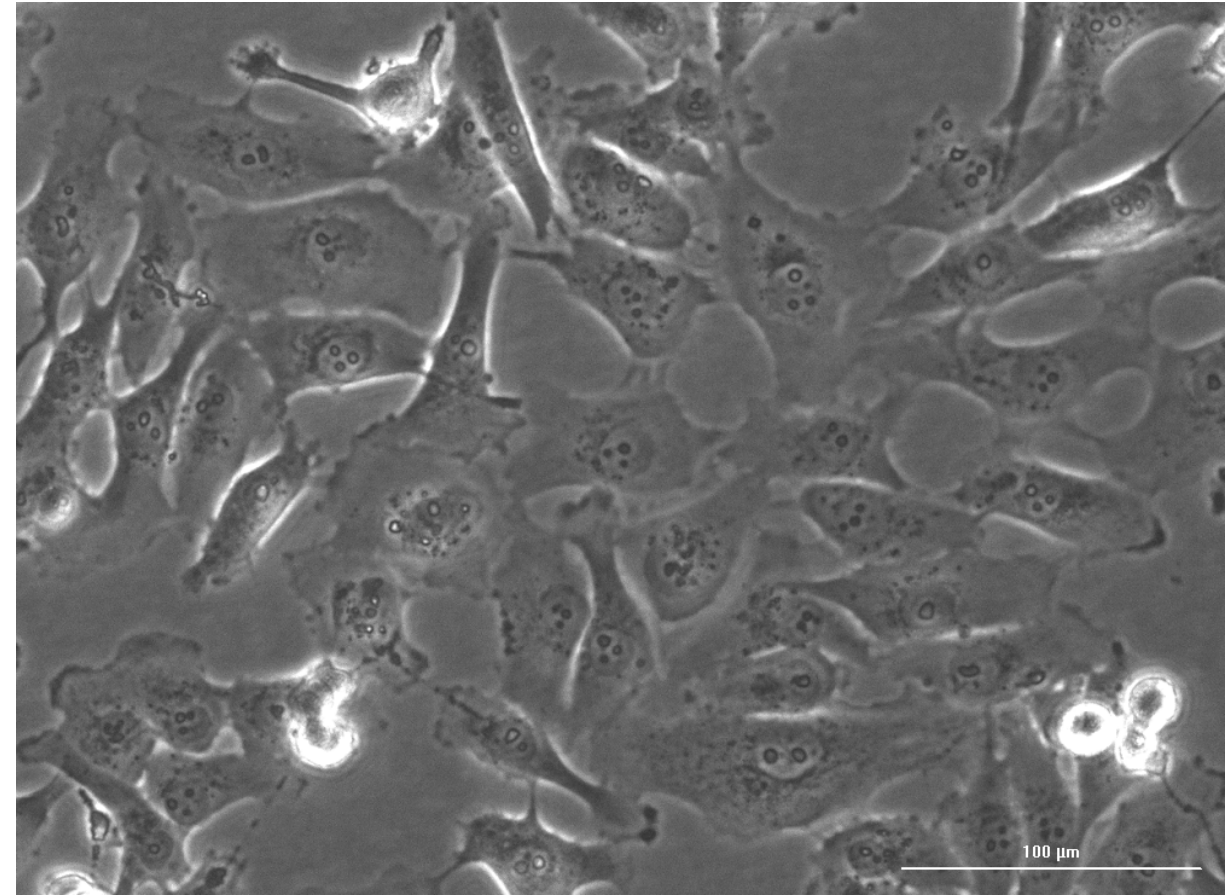
Generation of Radiation Resistant Cells



- Single acute dose (3.845 Gy/min at 220 kV, 13 mA) of 2 → 7.5 Gy. Total irradiation dose = 57 Gy.



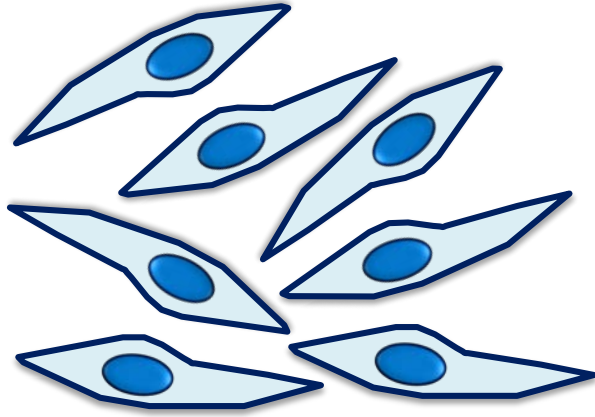
MDA-MB-231 Control Cells (20X)



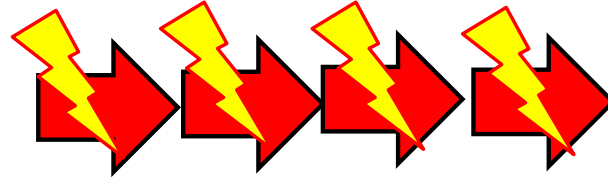
Radiation Resistant (RR) Cells (20X)

Transcriptome Analysis of Clonal RR Cells

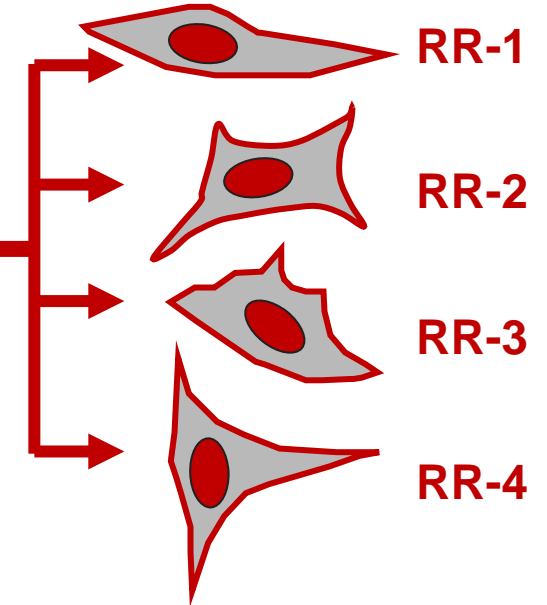
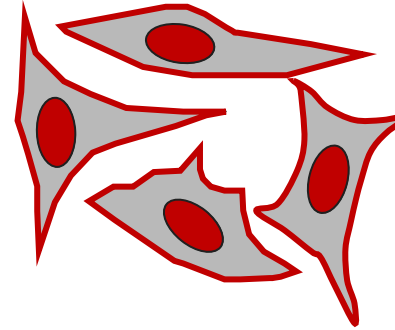
MDA-MB-231 Control Cells



Repeated
High Dose Radiation
(57 Gy Total)



Radiation Resistant
(RR) Cells



Transcriptomics (RNA-Seq)

**Differential Gene Expression in TNBC Cells with a
Radiation Resistant Phenotype**



Summary



Genes



GO Terms ▼



Pathways



Upstream Regulators ▼



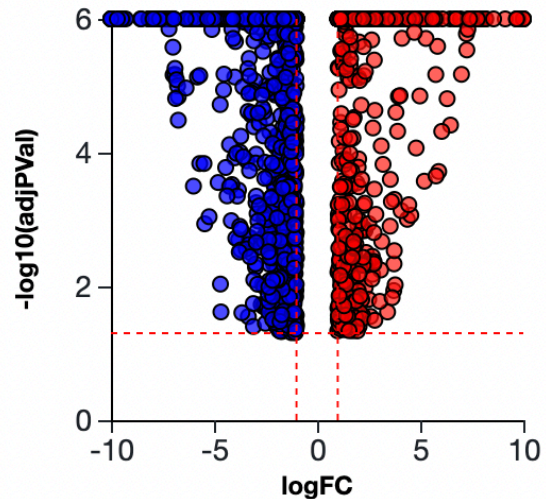
Diseases



Networks



Volcano Plot



(c) Advaita Corporation 2022

Organism: Homo sapiens

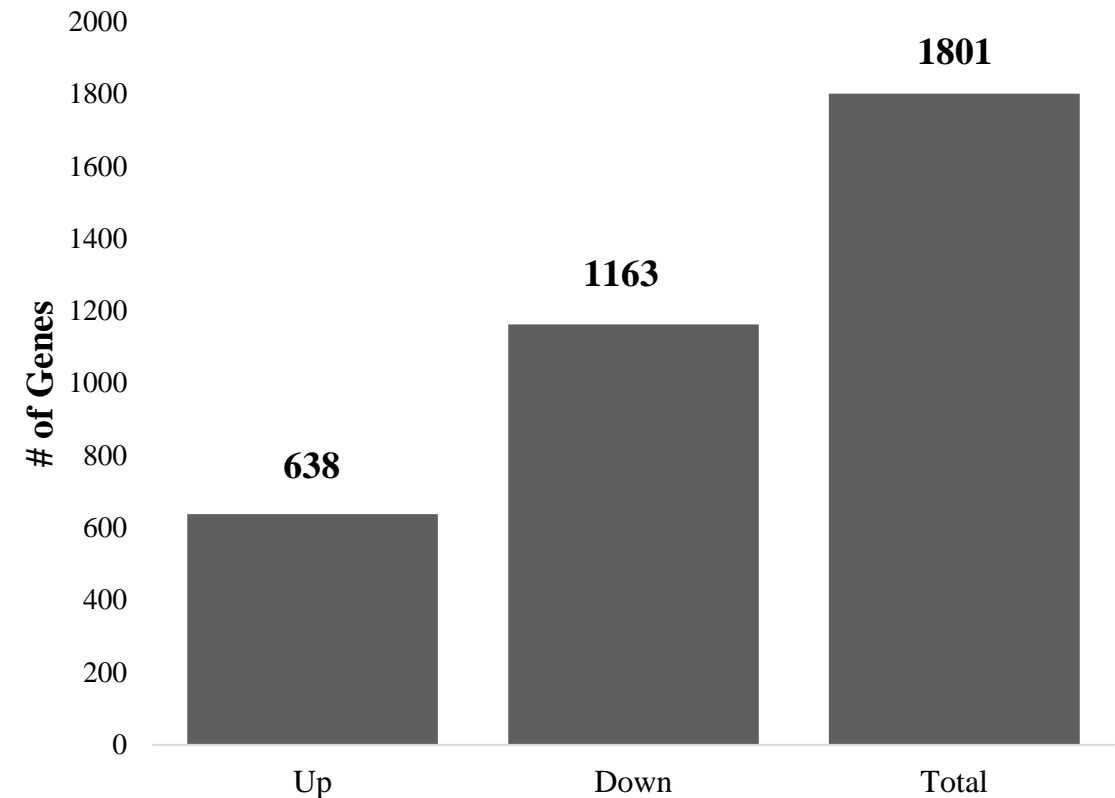
Differentially Expressed (DE) genes: 1801

All genes with measured expression: 12827

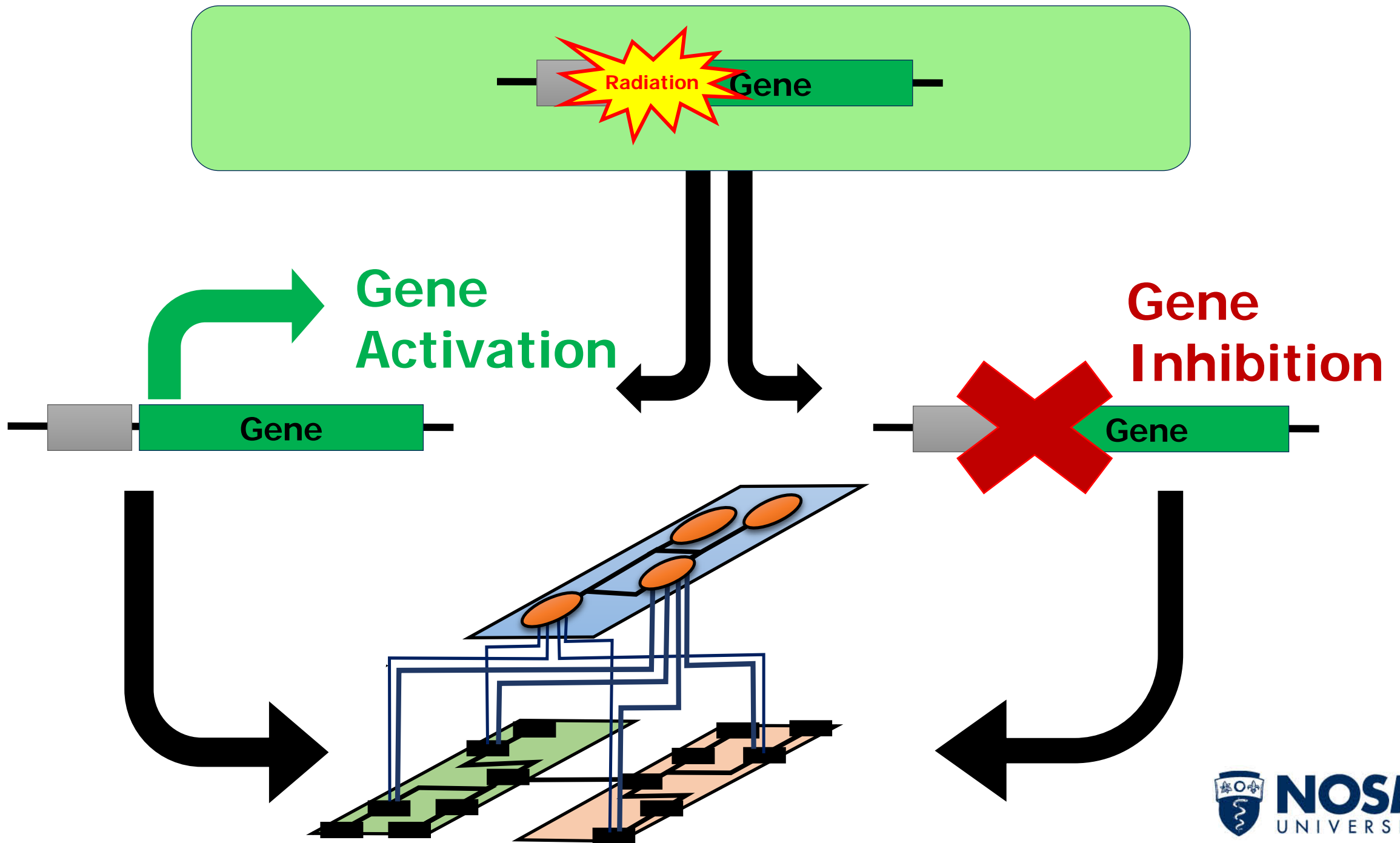
DE thresholds:

- fold change: 1
- p-value: 0.05

Differentially Expressed Genes



Control vs RR Lines SHAM



Top 10 Significant Molecular Functions

Description	DEGs	Total Genes	p (adj) for FDR
Transmembrane Signaling Receptor Activity	92	322	1.92E-08
Signaling Receptor Binding	173	761	4.12E-08
Antigen Binding	18	26	1.66E-07
Signaling Receptor Activity	108	431	3.60E-07
Molecular Transducer Activity	108	431	3.60E-07
Calcium Ion Binding	86	329	1.95E-06
Glycosaminoglycan Binding	36	101	1.53E-05
Peptide Antigen Binding	12	16	1.68E-05
G Protein-Coupled Receptor Activity	35	101	4.04E-05
Extracellular Matrix Structural Constituent	26	65	5.55E-05

Top 10 Significant Biological Processes

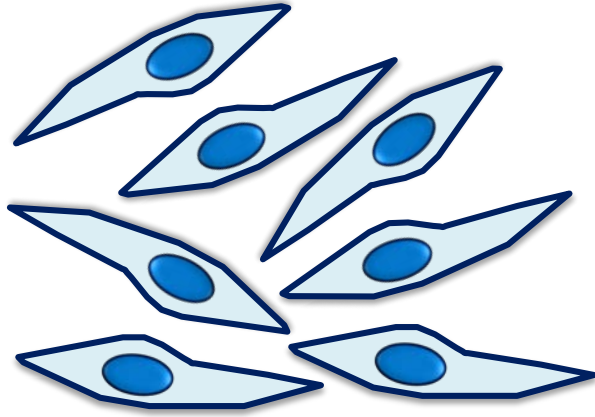
Description	DEGs	Total Genes	p (adj) for FDR
Biological Adhesion	214	805	3.81E-18
Cell Adhesion	212	802	6.50E-18
Multicellular Organismal Process	722	3968	4.62E-16
Regulation of Multicellular Organismal Process	323	1515	4.11E-13
Anatomical Structure Morphogenesis	336	1621	4.91E-12
Cell-Cell Adhesion	130	468	4.91E-12
Signaling	627	3509	5.32E-11
Cell Communication	632	3552	8.66E-11
Signal Transduction	580	5258	2.02E-09
Response to Stimulus	830	4973	3.12E-09

Top 10 Significant Signaling Pathways

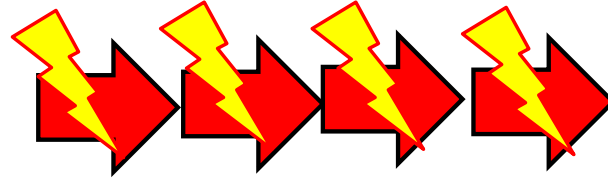
Pathway (ID)	p (adj) for FDR	Upregulated DEGs	Downregulated DEGs
Complement and Coagulation Cascades (04610)	0.008	FGB, F2RL2, SERPINB2, F3, CD55, ITGB2, PROS1	SERPINA1, CFD, C1R, PLAUI, C4BPB, F12
Neuroactive Ligand-Receptor Interaction (04080)	0.01	HTR2C, GABRA3, P2RY10, GABBR2, F2RL2, ADM, S1PR3, GRPR, PRLR, KISS1, PTGER4, HRH1	VIPR1, SSTR2, NMB, GRIK4, ADORA2A, S1PR5, PTGER2, HTR7, P2RY2, LPAR2
Cell Adhesion Molecules (04514)	0.027	VCAN, NEO1, CD22, CD274, ITGB2, CDH4	CD40, L1CAM, CLDN2, ICAM1, HLA-DRB1, ITGA6, NRCAM, HLA-A, HLA-DRA, CLDN3, NCAM2, VSIR, HLA-F, ICOSLG, HLA-C, HLA-B
ABC Transporters (02010)	0.033	ABCB7, ABCA3	ABCC5, ABCB7, ABCG2, TMEM16, ABCA2, ABCD1, TAP2, ABCA1, ABCC4
Pathways in Cancer (05200)	0.033	CCND2, MMP1, PTGS2, LAMA1, HEYL, FN1, PLCB1, IL7R, FGF5, COL4A5, FGF1, ESR2, PTGER4, IL13RA1, CAMK2D, HHIP, MGST3, WNT7B, MITF, EGF, GADD45B, GNAS, GADD45A, CXCL8	PDGFRB, EGLN3, FGFR4, JAG2, FHH, GSTM2, SUFU, JUP, PTGER2, PGF, FLT3LG, ITGA6, LAMA5, TERT, IL15RA, CDKN1A, ADCY6, FRAT1, NCOA1, TGFB2, CCND3, IL15, ADCY7, CCNA1, PLCG1, DDB2, PLCG2, NCOA3, ITGA2, GSTM4, HES1, COL4A1, COL4A2, GNG11, RALB, PLD2, DVL2, LPAR2, LRP5, FZD1, TRAF5, EML4, TRAF3, AKT1, LAMB1, EPOR, STAT1
Protein Digestion and Absorption (04974)	0.033	CPA3, ATP1A3, COL8A1, COL4A5	COL5A1, COL6A2, COL6A3, COL27A1, COL7A1, COL13A1, COL4A1, COL4A2, KCNN4
ECM-Receptor Interaction (04512)	0.033	LAMA1, FN1, TNC, COL4A5	COL6A2, COL6A3, ITGB4, ITGA6, LAMA5, FREM2, ITGA2, COL4A1, COL4A2, ITGA10, DAG1, LAMB1
AGE-RAGE Signaling Pathway in Diabetic Complications (04933)	0.033	FN1, PLCB1, COL4A5, F3, PRKCZ, EGR1, CXCL8	NFATC1, ICAM1, PLCD1, MAPK13, TGFB2, PLCG1, PLCG2, COL4A2, PRKCE, PLCD3, AKT1, STAT1
Cytokine-Cytokine Receptor Interaction (04060)	0.034	IL7R, TNFRSF10D, PRLR, IL13RA1, IL1RL2, CXCL8	CD40, INHBB, TNFRSF1B, NGFR, CX3CL1, IL11, GDF5, IFNLR1, TNFSF12, IL24, TNFRSF11B, IL15RA, CRLF2, TGFB2, IL15, IL17RE, TNFRSF19, EPOR
Insulin Secretion (04911)	0.04	RYR2, ATP1A3, SNAP25, PLCB1, CAMK2D, KCNMB4, RIMS2, GNAS	KCNMB3, ADCY6, ADCY7, PCLO, ATF6B, KCNN4

Transcriptome Analysis of Clonal RR Cells

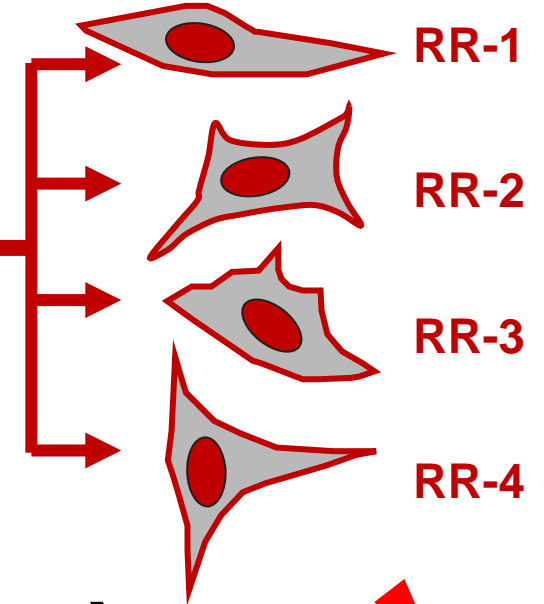
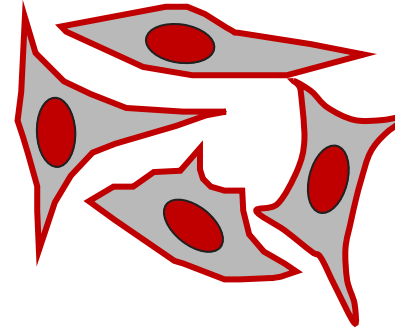
MDA-MB-231 Control Cells



Repeated
High Dose Radiation



Radiation Resistant
(RR) Cells



Transcriptomics (RNA-Seq)

6 Gy

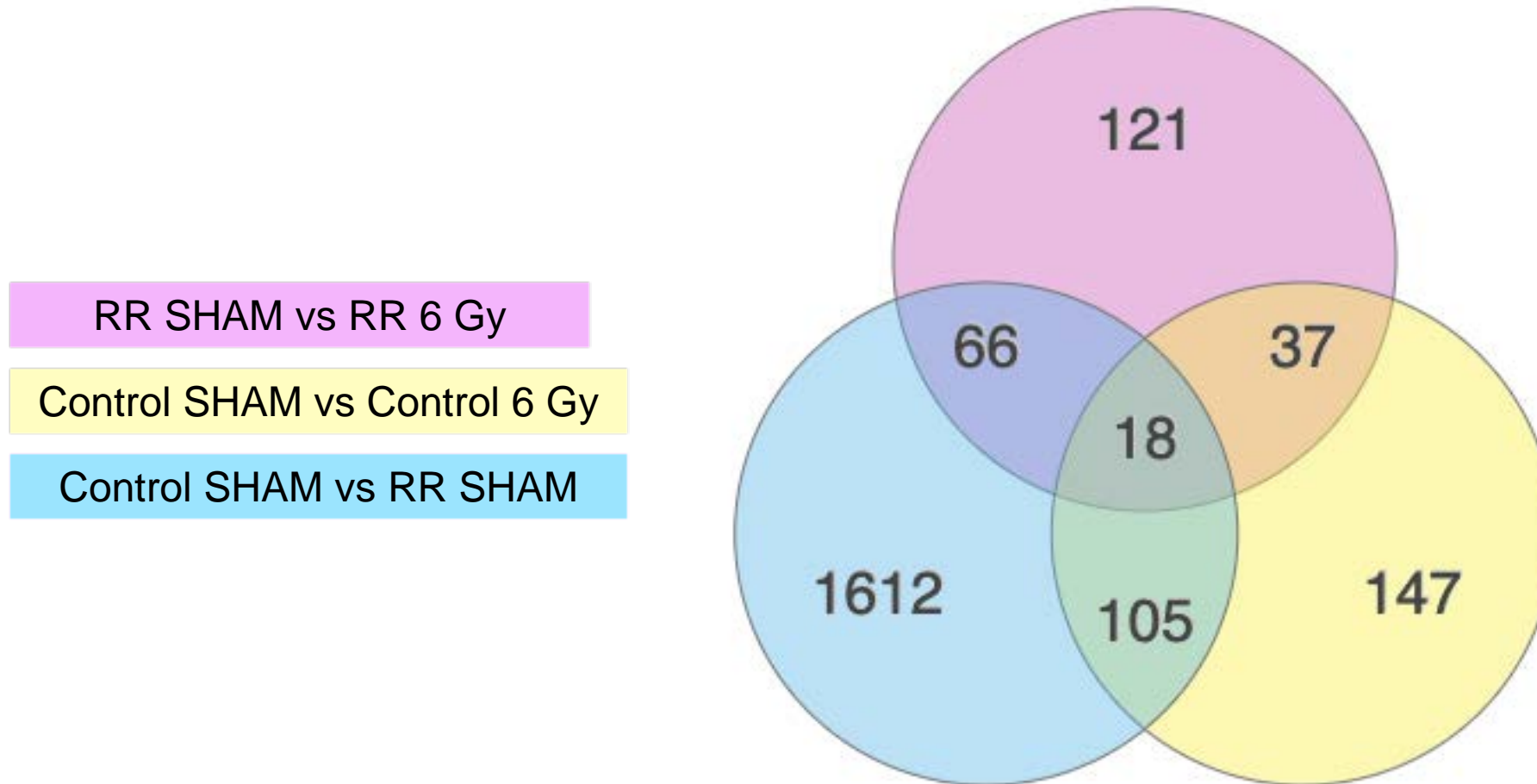
6 Gy

**Differential Gene Expression in TNBC
Cells with a Radiation Resistant
Phenotype Post Radiation Challenge**

Differential Expression Reports

Differential Expression Analysis	Differentially Expressed Gene Count
4 hr Control SHAM vs 6 Gy	14
4 hr RR SHAM vs 6 Gy	133
48 hr Control SHAM vs 6 Gy	1059
48 hr RR SHAM vs 6 Gy	2562

Meta-Analysis Results – Gene Summary



Next Steps

- Validate the transcriptome results to ensure findings are true and accurate
- Perform cell adhesion assay to further understand the differences between the control cells and radiation resistant cells
- Target some of the top genes affected in the transcriptome results to try to revert these radiation resistant cells back to a non-RR phenotype (using CRISPR gene editing)



Acknowledgements



Team Members:

- **Alyssa Murray**
- Jessica Dougherty
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- Dr. Ramya Narendrula

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- Dr. Douglas Boreham
- All members of NOSM Radiobiology Team

