SABCS
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Classification of triple negative breast cancer (TNBC) by DNA damage immune response (DDIR) signature and homologous recombination deficiency (HRD) status:

Analysis of SWOG S9313 adjuvant trial

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Presented by: Shane Stecklein, MD, PhD

SWOG CANCER RESEARCH NETWORK
NCI National Clinical Trials Network a National Cancer Institute program
NCI Community Oncology Research Program A program of the National Cancer Institute of the National Institutes of Health

Introduction

- Triple negative breast cancer (TNBC) is heterogeneous.
- Systemic chemotherapy is recommended for most patients with early-stage TNBC.
- Chemotherapy response biomarkers:
  - Number of stromal tumor infiltrating lymphocytes (sTILs)[1,2,5]
  - Homologous recombination deficiency (HRD)[3,7]
  - Gene expression signatures
    - TNBC molecular subtype[4]
    - Proliferation associated gene expression signatures[5]
    - Immune signatures, including DDIR[6]
  - PD-L1 expression[8]
- The overlap and prognostic interaction between HRD, sTILs, immune signatures, and molecular subtype in early-stage TNBC has not been examined.

[1]Denkert et al., *Lancet Oncology*, 2018; [2]Loi et al., *JCO*, 2019; [3]Telli et al., ASCO (2018); [4]Masuda et al., *Clin Cancer Res*, 2013; [5]Stover et al., *Clin Cancer Res*, 2016; [6]Sharma et al., *JCO*, 2019 [7]Sharma et al., *Ann Oncol*, 2018; [8]Schmid, *NEJM*, 2020

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SWOG S9313

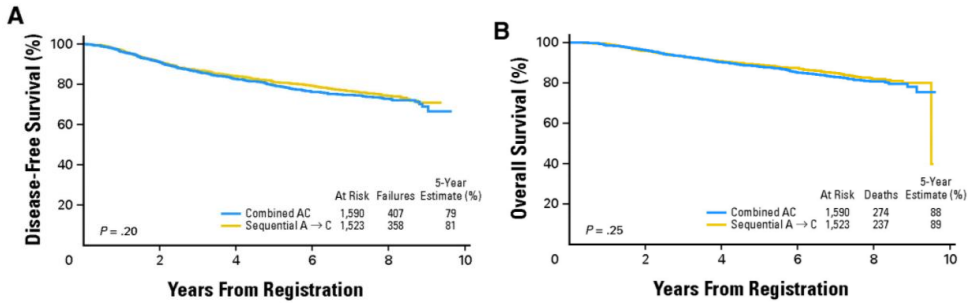
Design

High-Risk* Stage I-II Breast Cancer

*>1 cm and ER/PR-negative, >2 cm (regardless of ER/PR expression), or 1-3 positive axillary nodes

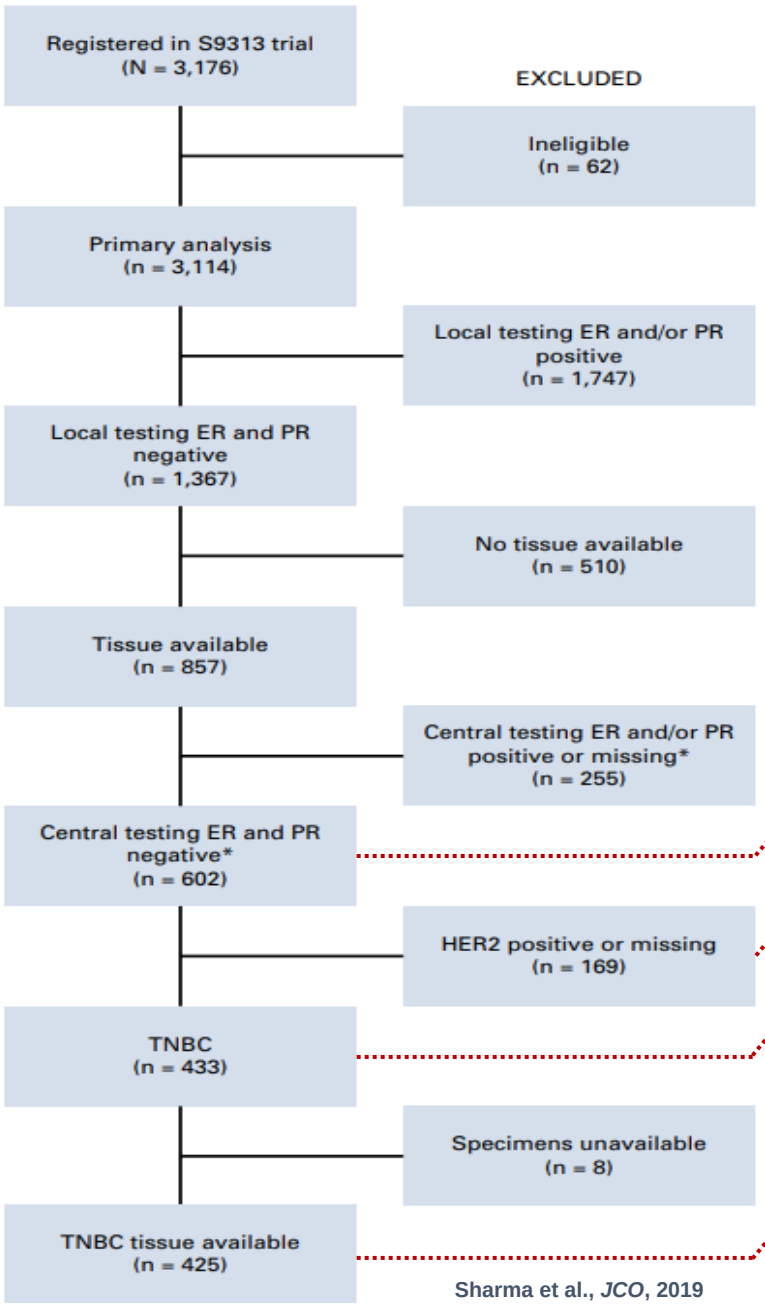
Arm I (Concurrent)
Doxorubicin: 54 mg/m² + Cyclophosphamide: 1.2 g/m² D1 Q3weeks x 6 cycles

Arm II (Sequential)
Doxorubicin: 40.5 mg/m² D1-2 Q3weeks x4 cycles
Cyclophosphamide: 1.2 g/m² D1 Q2weeks x3 cycles



Linden et al., *JCO*, 2007

Patients enrolled on 9313 from 1994 -1997



Sharma et al., *JCO*, 2019

S9313

TNBC Cohort Selection

Defined as Allred = 0

ASCO-CAP Criteria

13.6% of S9313 cohort

Median Age: 45 years
pN+: 33%
5-Year DFS: 74%
5-Year OS: 82%

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Assessing impact of *BRCA*ness, Immune Markers, and Subtype on outcomes in TNBC Patients Treated with Adjuvant AC on S9313

- DNA Damage Immune Response (DDIR) signature (ALMAC Diagnostic Services)
  - FFPE samples analyzed by Xcel™ Array
  - 44 gene signature
  - DDIR+ = Score of ≥ 0.3681[1,2]
- myChoice® HRD (Myriad Genetics)
  - Measurement of LOH[3*], TAI[4*], LST[5*]
  - Tumor *BRCA1/2* mutation
  - HRD^{+} = HRD score of ≥ 42[6] or mutation in *BRCA1* or *BRCA2* detected in tumor
- sTIL quantification
  - Scored blindly by two breast pathologists
- TNBC Subtype[7] and CIBERSORTx[8]
  - Imputed from bulk tissue microarray gene expression analysis
- gDNA NGS (pending)

Assay	Available (%)
DDIR	381/425 (89.6)
HRD	363/425 (85.4)
sTIL	423/425 (99.5)

All three markers available for 328/425 (77%)

[1]Sharma et al., *JCO*, 2019; [2]Mulligan et al., *JNCI*, 2014; [3]Abkevich et al., *Br. J. Cancer*, 2012; [4]Birbak et al., *Cancer Discov*, 2012; [5]Popova et al., *Cancer Res*, 2012; [6]Telli et al., *Clin Cancer Res*, 2016; [7]Lehmann et al., *J Clin Invest*, 2011; [8]Newman et al., *Nature Biotech*, 2019
*LOH = Loss of heterozygosity; TAI = Telomeric allelic instability; LST = Large-scale state transitions

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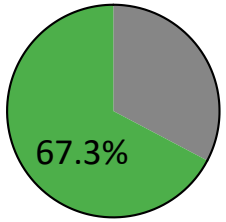
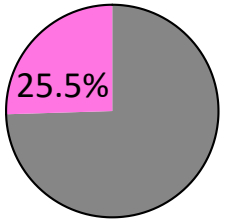
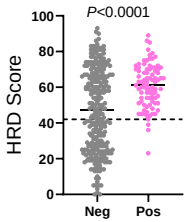
HRD, DDIR, sTIL, and Subtype are Prognostic in TNBC

HRD

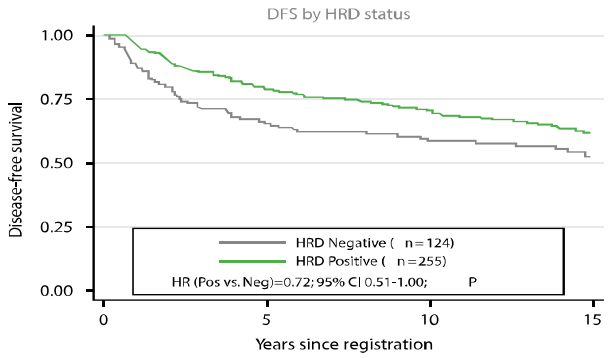


t*BRCA1/2* Mutation



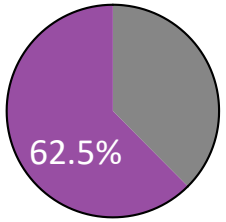


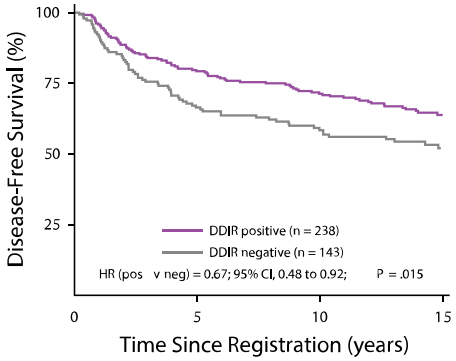
97% of t*BRCA1/2* mutation with HRD score > 42



Sharma et al., *Ann Oncol*, 2018

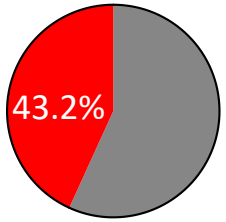
DDIR Signature

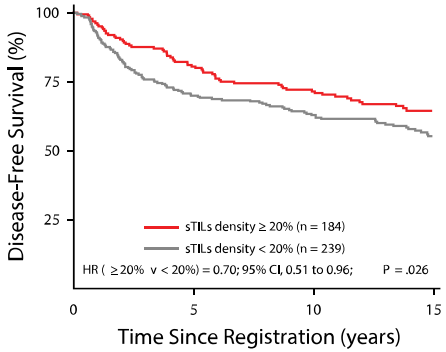




Sharma et al., *JCO*, 2019

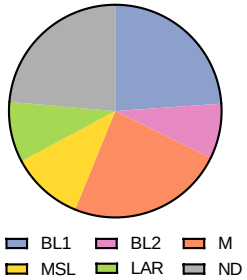
sTIL ≥ 20%

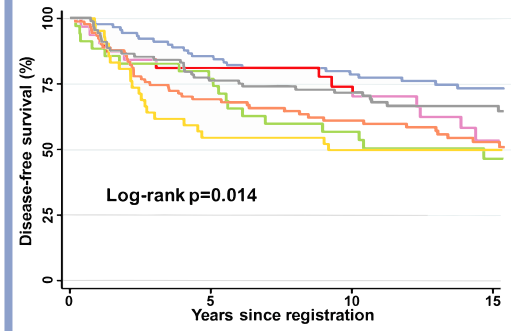




Sharma et al., *JCO*, 2019

Subtype





Sharma, SABCS (2018)

DFS defined as time from registration to first invasive recurrence (local/regional/distant), new primary contralateral invasive cancer, or death from any cause.

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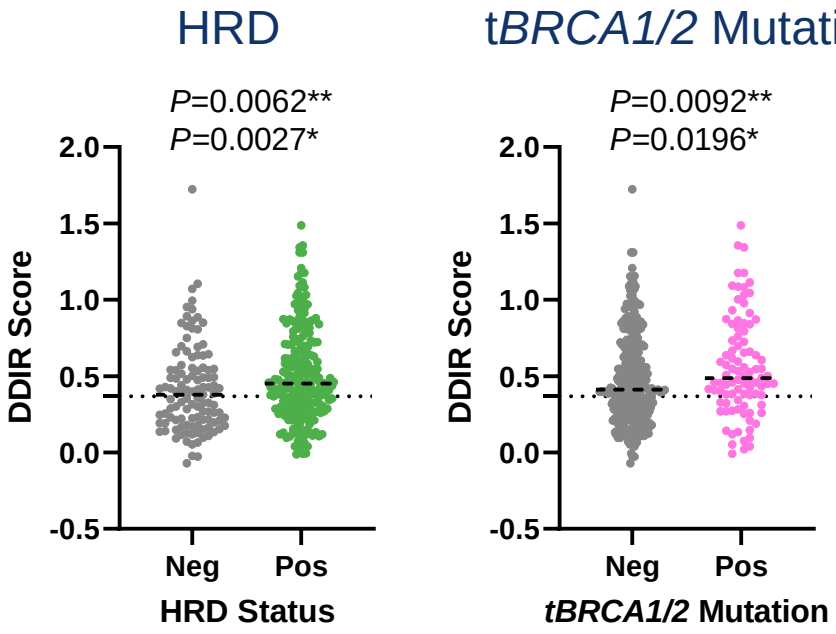
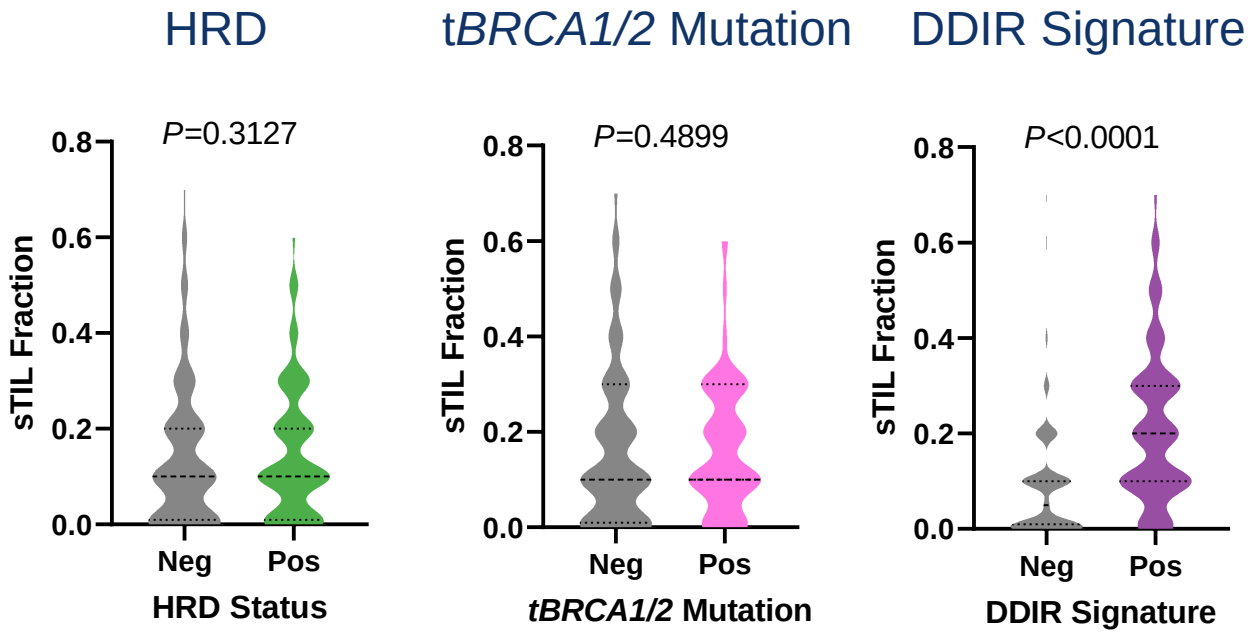
HRD and t*BRCA1/2* Mutation are Associated with Induction of DDIR but not with sTIL Infiltration





*Continuous comparison
**Categorical comparison (by threshold)

What is the prognostic utility of dual classification of TNBC by DDIR and HRD status?

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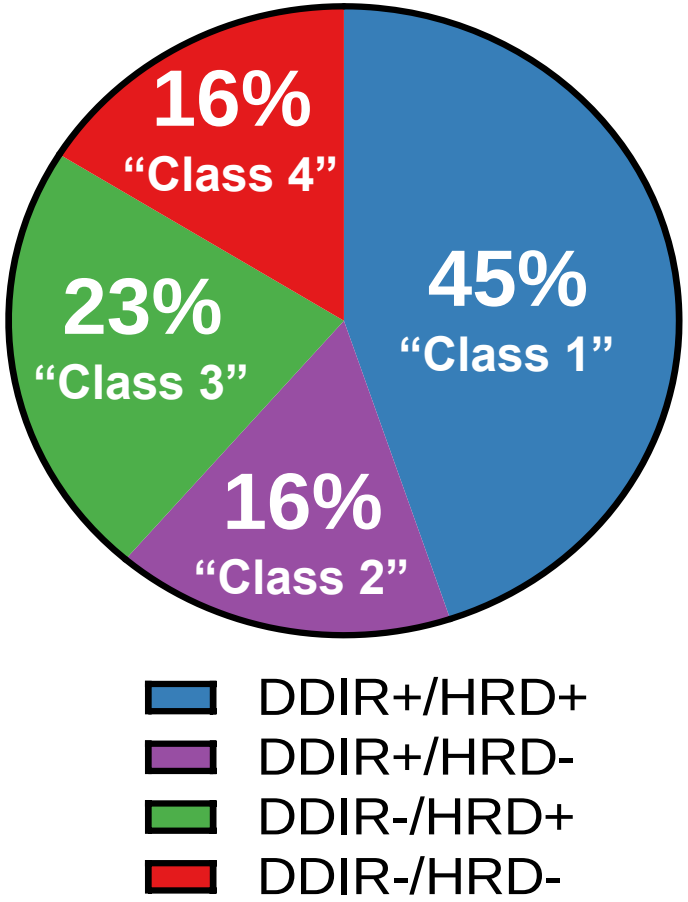
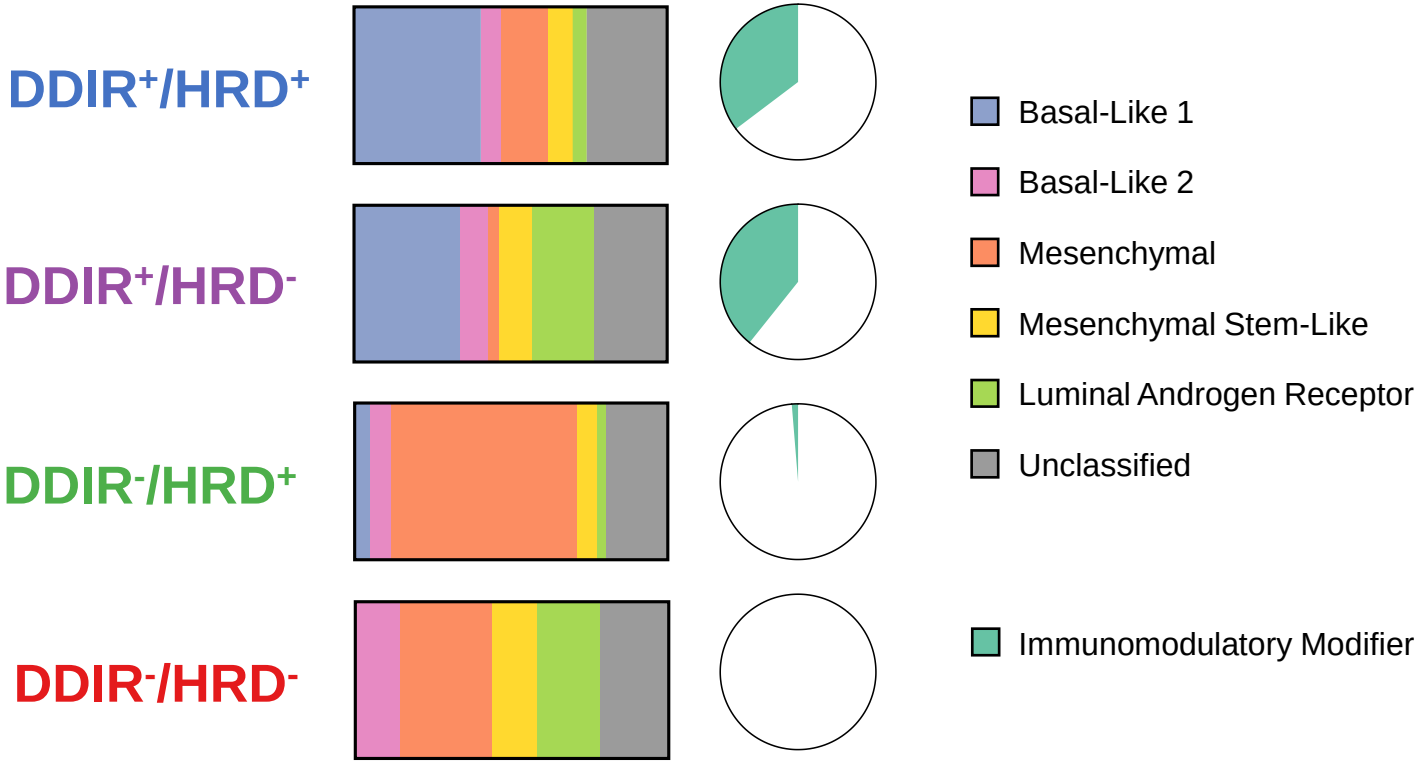
Classification by DDIR and HRD Status and Distribution of Molecular Subtypes within DDIR/HRD Classes





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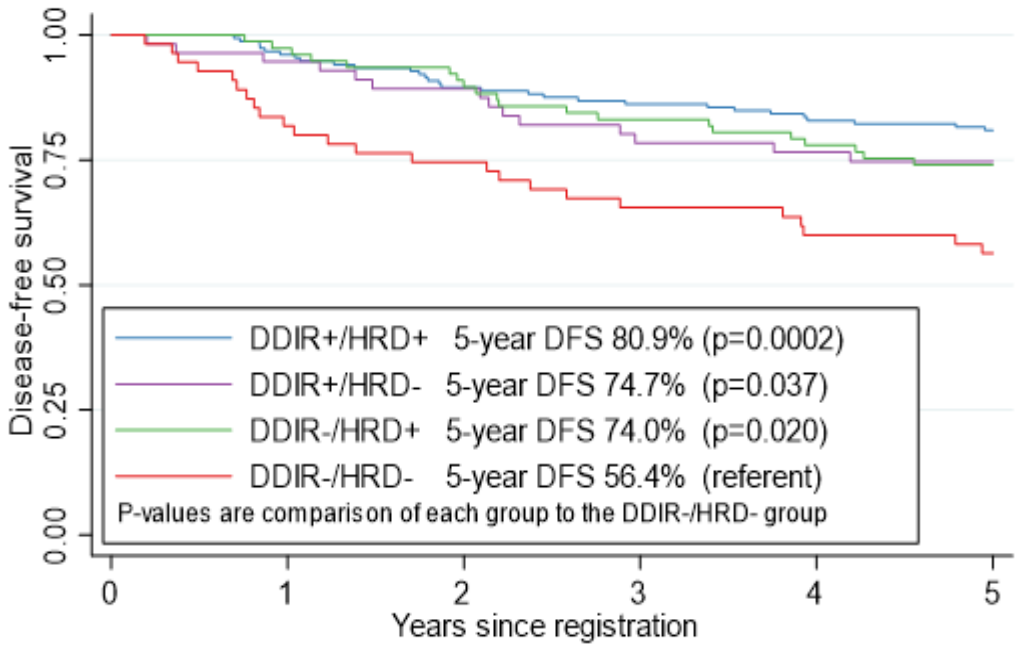
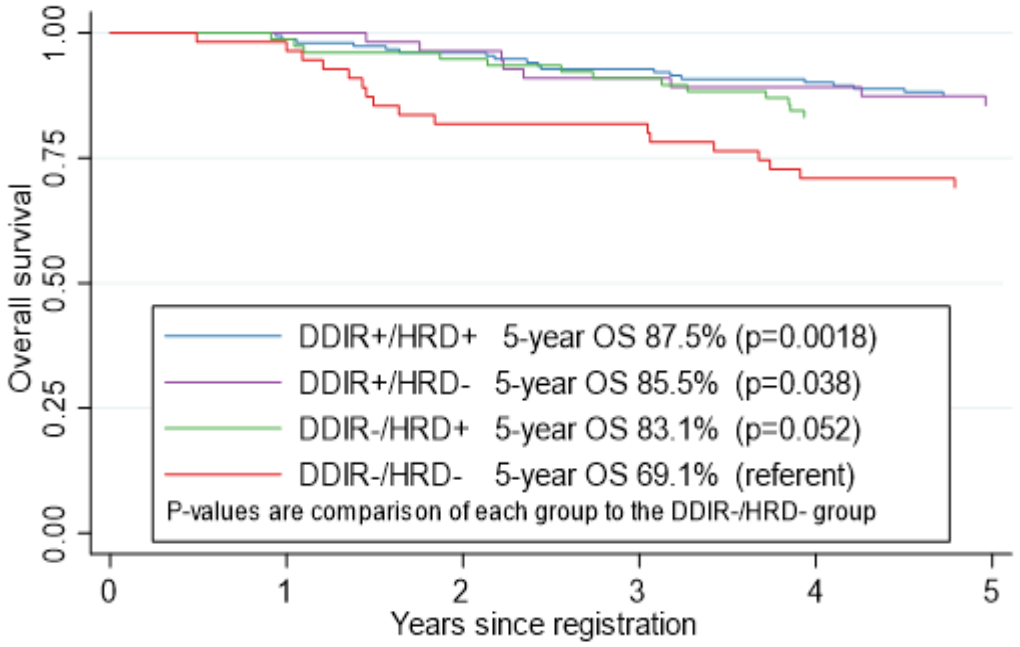
Combined DDIR/HRD Classes are Prognostic



N at risk						
DDIR+/HRD+	153	146	136	131	126	123
DDIR+/HRD-	56	53	49	43	42	41
DDIR-/HRD+	77	75	69	64	60	57
DDIR-/HRD-	55	45	41	36	33	31



N at risk						
DDIR+/HRD+	153	150	146	141	137	133
DDIR+/HRD-	56	56	53	50	49	47
DDIR-/HRD+	77	76	73	70	64	64
DDIR-/HRD-	55	53	45	45	39	38

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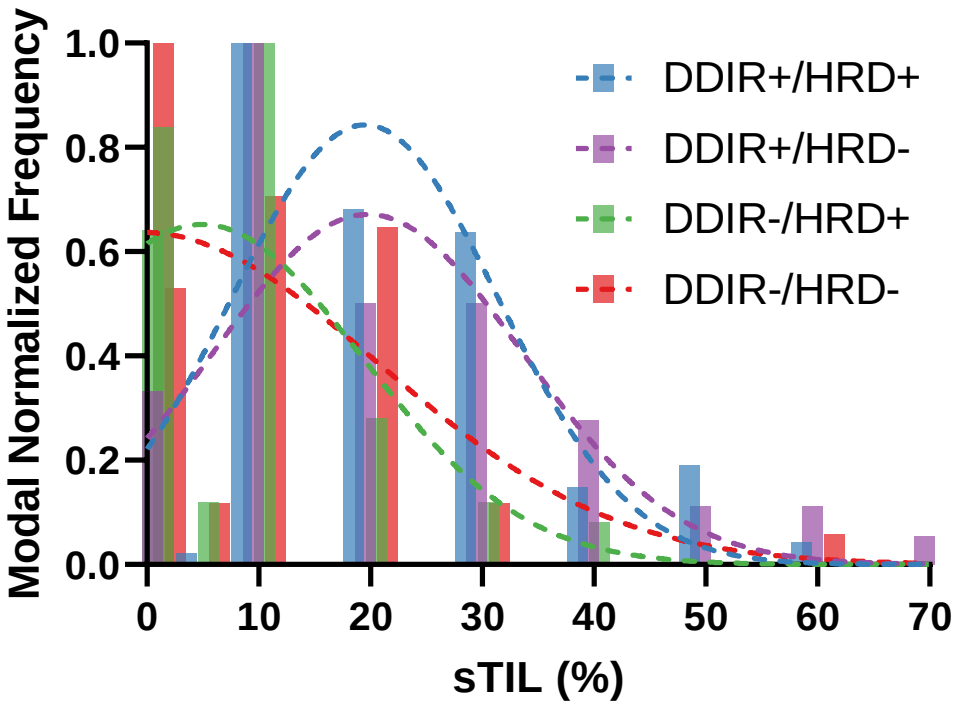
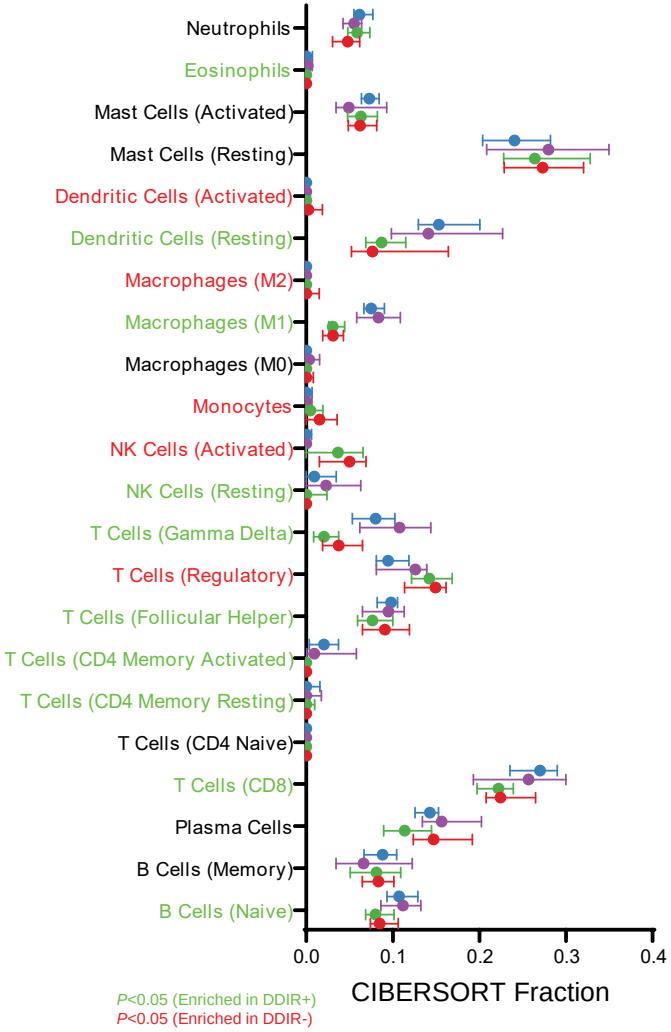
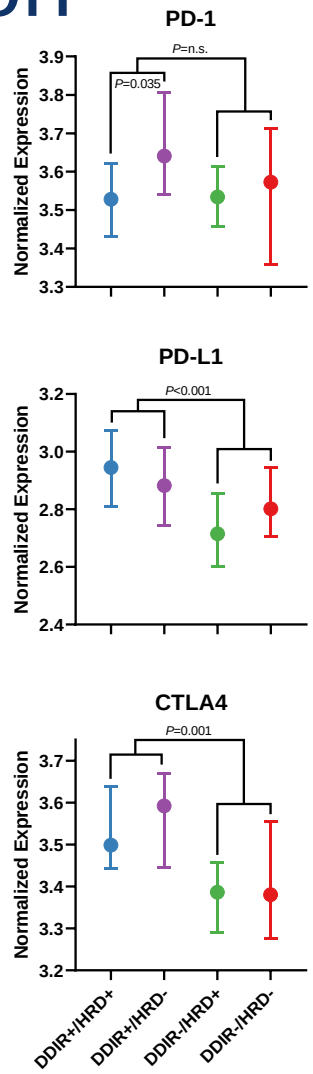
DDIR/HRD Class, Immune Cell Infiltration, Imputed Immune Cell Fractions, and ICI Target Expression



DDIR/HRD Class	Median sTIL
DDIR^{+}/HRD^{+}	20%
DDIR^{+}/HRD^{-}	20%
DDIR^{-}/HRD^{+}	5%
DDIR^{-}/HRD^{-}	5%





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Gene Expression Signature Cluster Analysis Based on DDIR and HRD Status

Image adapted from The Hallmarks of Cancer. Originally published in Cell 144, Hanahan D & Weinberg RA, Hallmarks of Cancer: The Next Generation, 646-674, © 2011. With permission from Elsevier.

10	92	100	7,337
Key biologies	Gene expression signatures	Drug targets	Single genes relevant to content

clara™ Total mRNA Report
ALMAC Partnering to Advance Human Health

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Top Gene Expression Signatures Discriminating DDIR^{+} and DDIR^{-} TNBC

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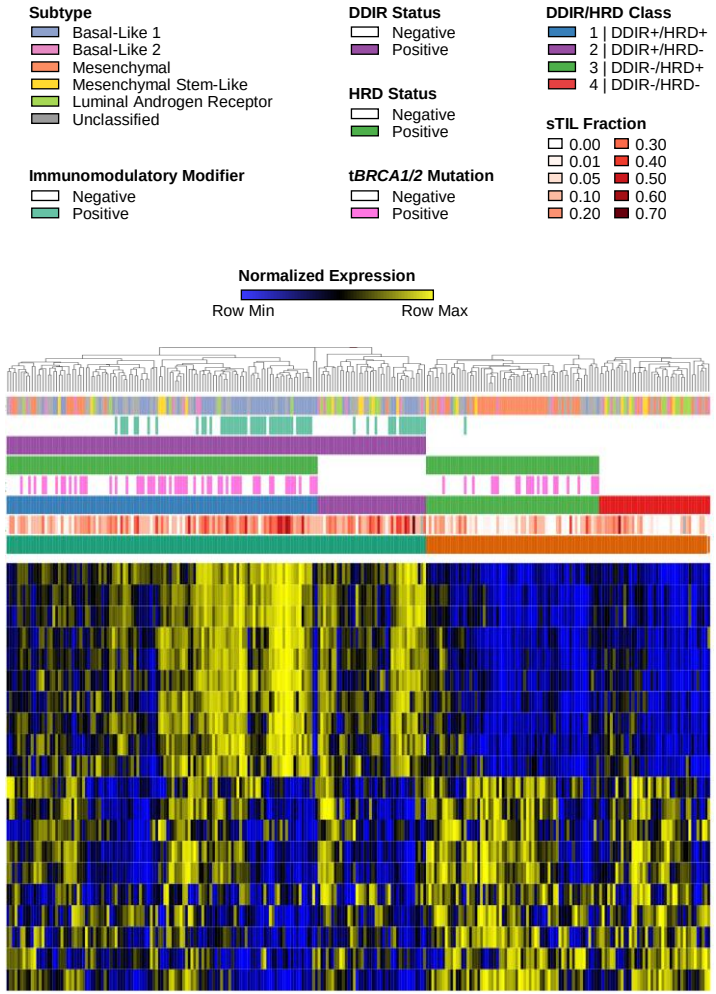

Signature scores generated by clara^{T} analysis (ALMAC)

Hallmark	Signature (Reference)	Description	Signal-to-Noise Ratio (*P* value)
IO	Almac IO Assay [DDIR] (Mulligan et al., 2014)	cGAS-STING pathway	1.34 (<0.001)
IO	IFNγ Response (Almac Hallmark)	--	0.97 (<0.001)
IO	IFNα Response (Almac Hallmark)	--	0.93 (<0.001)
IO	CTLA4 Response Signature (Ji et al., 2011)	IFNγ and Th1-associated genes	0.89 (<0.001)
IO	Allograft Rejection (Almac Hallmark)	--	0.85 (<0.001)
Inflammation	NFκB Activity Signature (Hopewell, et al., 2013)	NFκB signaling and immune surveillance	0.81 (<0.001)
Inflammation	TCGA CSF1 Response (Beck et al., 2009)	Macrophage CSF-1 response genes	0.79 (<0.001)
IO	T-cell inflamed GEP (Ayers et al., 2017)	T-cell inflamed IFNγ response genes	0.79 (<0.001)
IO	Immune Response (Prat et al., 2017)	Granzyme-mediated apoptosis	0.75 (<0.001)
IO	Immune Response (Prat et al., 2017)	Cell adhesion and toll-like receptors	0.71 (<0.001)
Angiogenesis	Global Angiogenesis Signature (Anders et al., 2013)	Correlated aniogenic genes	-0.24 (<0.001)
Angiogenesis	Angiogenesis (Almac Hallmark)	--	-0.24 (<0.001)
Energetics	Metabolic progression signature (Nath et al., 2016)	Lipogenesis and fatty acid metabolism genes	-0.26 (<0.001)
IO	TCGA TGFβ Signature (Teschendorff et al., 2010)	TGFβ signaling genes	-0.27 (<0.001)
EMT	MCPCounter-Fibroblasts (Becht et al., 2016)	Stromal genes and cell counterparts	-0.28 (<0.001)
EMT	MAPK Activity Score (Wagle et al., 2018)	MAPK signaling pathway	-0.28 (<0.001)
Cell Death	Autophagy-related Risk Score (An et al., 2018)	Autophagy-related phenotype expression	-0.30 (<0.001)
Energetics	Myogenesis (Almac Hallmark)	--	-0.38 (<0.001)
Inflammation	M1/M2 GE Signature (Yuan et al., 2015)	Classification of M1/M2 macrophages	-0.41 (<0.001)
Angiogenesis	Almac Angiogenesis Assay (Gourley et al., 2014)	Anti-angiogenic signature	-0.59 (<0.001)

Up in DDIR^{+}

Up in DDIR^{-}

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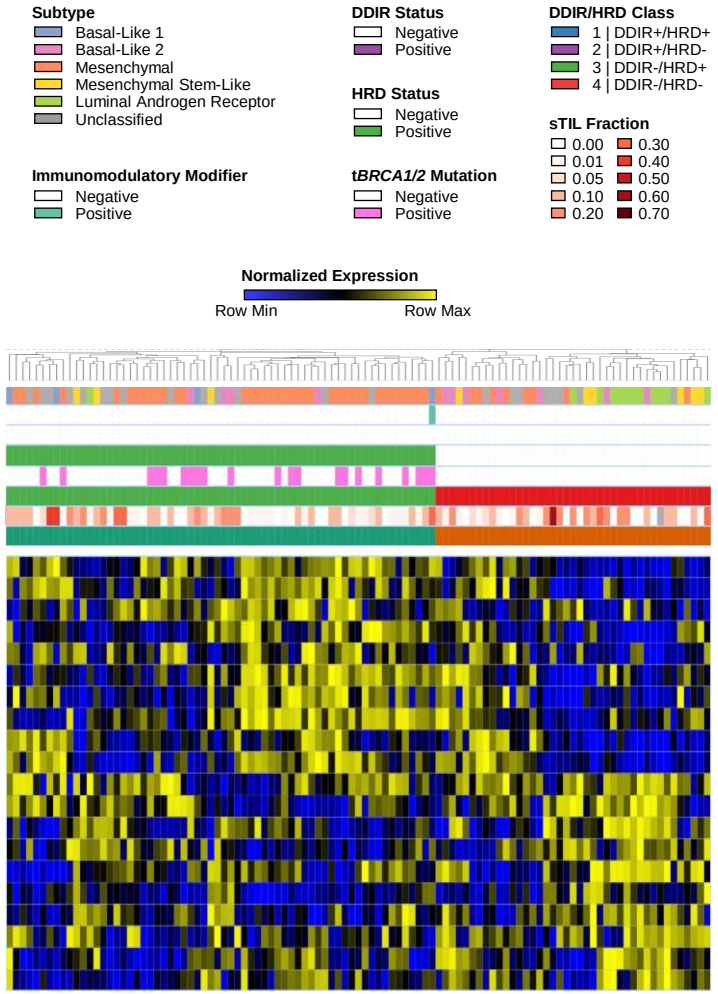
Top Gene Expression Signatures Discriminating Class 3 (DDIR$^-$/HRD$^+$) and Class 4 (DDIR$^-$/HRD$^-$)



Signature scores generated by clara^T analysis (ALMAC)

Hallmark	Signature (Reference)	Description	Signal-to-Noise Ratio (*P* value)
Genome Instab	*BRCA1*ness Signature (Severson et al., 2017)	DNA replication, recombination & repair	0.54 (<0.001)
Cell Death	TP53 Classifier (Knijenburg et al., 2018)	TP53 target genes and deficiency	0.45 (<0.001)
Angiogenesis	Vascular Proliferation (Stefansson et al., 2015)	Microvessel proliferative signaling	0.31 (0.004)
Proliferation	pSTAT3-GS Score (Sonnenblick et al., 2015)	Constitutive activation of STAT3	0.27 (0.007)
Genome Instab	DDR Pathway Focused Score (Kang et al., 2012)	Platinum-induced DNA damage repair	0.27 (0.008)
IO	TGFβ Signaling (Almac Hallmark)	--	0.25 (0.015)
EMT	Notch Signaling (Almac Hallmark)	--	0.25 (0.011)
IO	TGFβ Response Signature (Mariathasan et al., 2018)	TGFβ signaling genes	0.25 (0.011)
Ev Grow Supp	E2F Target Expression Classifier (Lan et al., 2018)	E2F highly and lowly dependent genes	0.25 (0.009)
Genome Instab	Mitotic Spindle (Almac Hallmark)	--	0.24 (0.021)
Inflammation	M1/M2 GE Signature (Yuan et al., 2015)	Classification of M1/M2 macrophages	-0.18 (0.168)
EMT	EMT Signature Estimate (Tan et al., 2014)	--	-0.20 (0.041)
EMT	MCPCounter-Neutrophils (Becht et al., 2016)	Stromal genes and cell counterparts	-0.20 (0.039)
EMT	EMT Signature (Byers et al., 2013)	EGFR and PI3K/AKT response genes	-0.21 (0.047)
Immortality	Candidate Senescence Signature (Wu et al., 2019)	Regulators of p21-induced senescence	-0.21 (0.019)
EMT	MCPCounter-B-lineage (Becht et al., 2016)	Stromal genes and cell counterparts	-0.22 (0.044)
IO	TILs Mast Cells (Danaher et al., 2017)	Immune cell populations	-0.25 (0.012)
Genome Instab	DNA Damage Sensitivity (McGrail et al., 2017)	PARPi responsive and sensitive genes	-0.27 (0.009)
Energetics	Xenobiotic Metabolism (Almac Hallmark)	--	-0.27 (0.008)
Energetics	Bile Acid Metabolism (Almac Hallmark)	--	-0.29 (0.005)

Up in Class 3

Up in Class 4

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Therapeutic Targets within DDIR-/HRD- TNBC Hallmarks

EMT	Immortality	IO	Genome Instability	Energetics
AXL	TERT	CTLA4	BRCA1	TXNIP
FAK1	RB1	PDL1	BRCA2	GLUT1
NOTCH1	p21	PD1	ERCC1	MYC
EGFR	p16	LAG3	ATR	IDH1
BRAF	TP53	TIM3	ATM	FASN
KRAS	Ki67	OX40	PARP1	AMPK
MET	MDM2	ICOS	WEE1	HIF1A
TGFB1	E2F1	CD27	CHK1	mTOR
FGFR1	TERF1	CD40	CHK2	PRDX1
FGFR2	POT1	IDO1	DNAPK	SOD1

α1
α2
β1
β2
γ1
γ2
γ3

Upregulated Downregulated

in DDIR-/HRD- compared to DDIR-/HRD+

Targets by hallmark in clara^{T} analysis (ALMAC)

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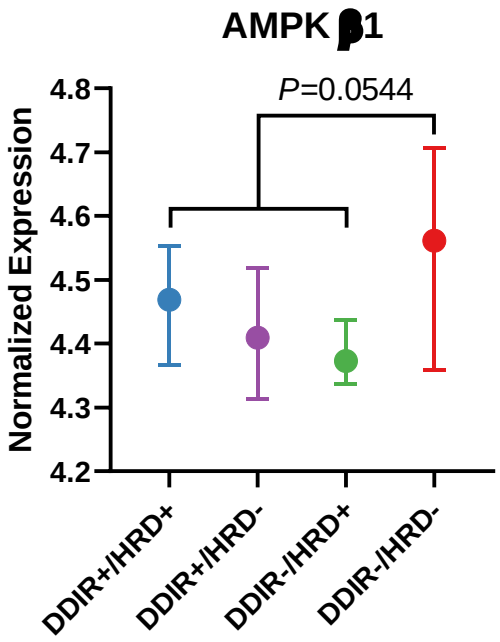
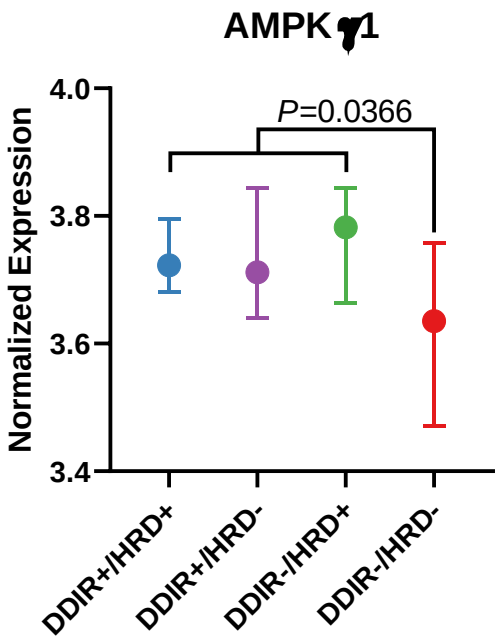
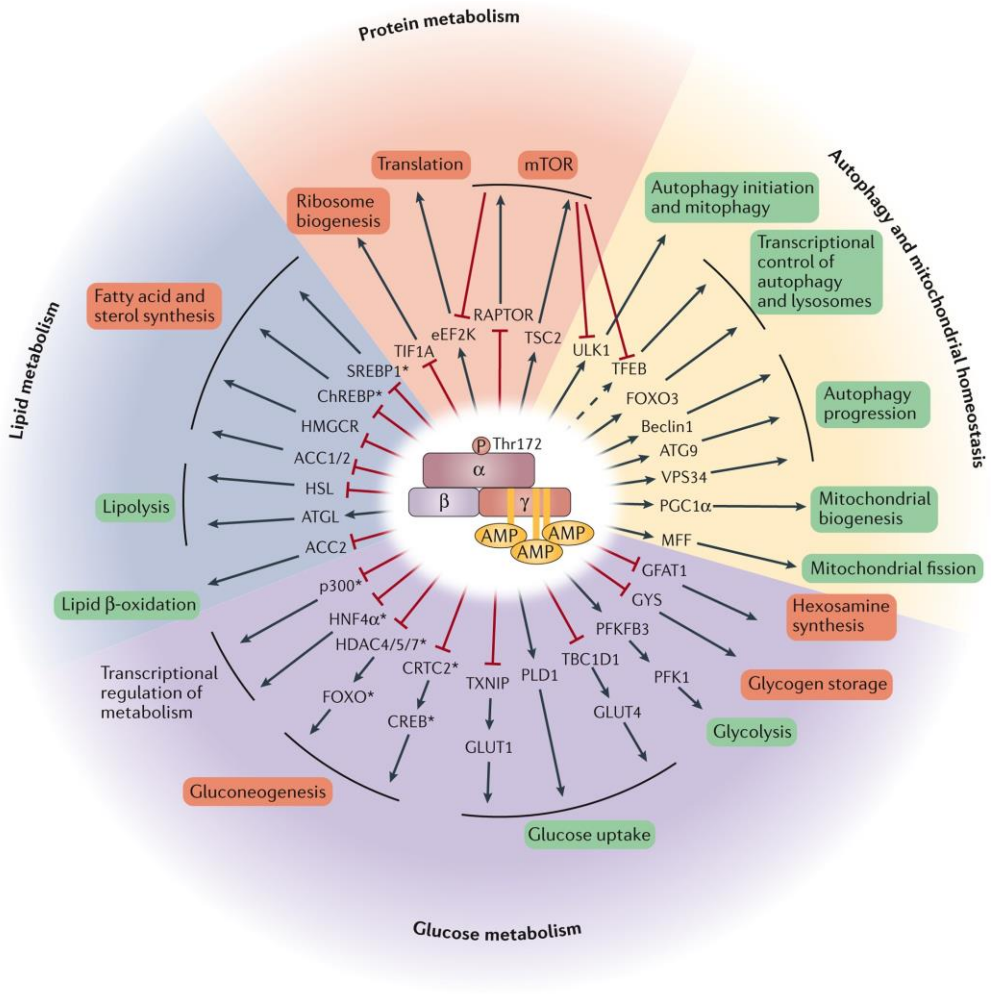
Dysregulation of AMPK β/γ Regulatory Subunits in DDIR-/HRD- TNBC







Herzig and Shaw., *Nat Rev Mol Cell Biol*, 2017

Nature Reviews | Molecular Cell Biology

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Conclusions

- Immune activation and DNA repair deficiency have incomplete overlap in TNBC
- Classification of TNBC by DDIR and HRD status identifies biology-driven prognostic categories in patients treated with adjuvant AC
- Immunologic and DNA repair-mediated therapeutic vulnerabilities may be independent
  - Both contribute to favorable outcomes noted in Class I (DDIR+/HRD+)
- Immunologically “cold” Class 3 (DDIR-/HRD+) does not have poor outcome
  - Rescued by sensitivity to DNA damaging chemotherapy
- Unfavorable Class 4 ($DDIR^-/HRD^-$) TNBC may have metabolic vulnerabilities associated with dysregulation of AMPK-mediated energy sensing
- These findings should be validated in other cohorts of patients treated with contemporary chemotherapy regimens
  - Adjuvant treatment on S9313 did not include a taxane

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Conclusions

- Classification of TNBC by DDIR and HRD has potential therapeutic implications:

DDIR/HRD Category	Prognosis	Features		Therapeutic Opportunities
		sTIL Infiltration Immune Activation ICI Target Expression	*BRCA*ness & DNA Repair Deficiency	
$DDIR^+/HRD^+$	Favorable	✓	✓	(?) De-intensified chemotherapy Immunotherapy DNA damaging agents
$DDIR^+/HRD^-$	Favorable Intermediate	✓	✗	Immunotherapy Strategies to induce DNA repair deficiency (?) Intensified chemotherapy (ADC)
$DDIR^-/HRD^+$	Favorable Intermediate	✗	✓	DNA damaging agents and PARPi Strategies to induce anti-tumor immunity
$DDIR^-/HRD^-$	Unfavorable	✗	✗	Novel agents Strategies to induce anti-tumor immunity

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- Patients and their families
- Participating sites
  - Physicians, nurses, and research coordinators
- Advocate community

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