Omics in modern oncology:

Methods and general applications

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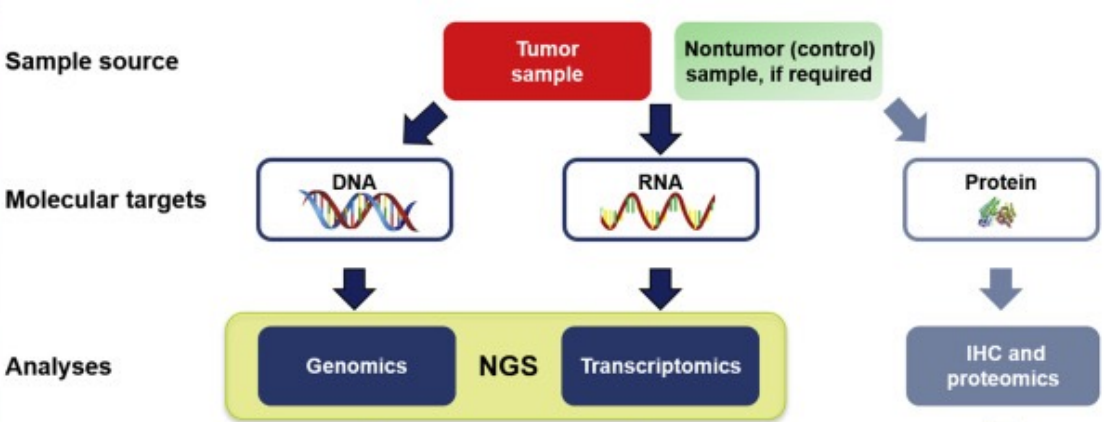
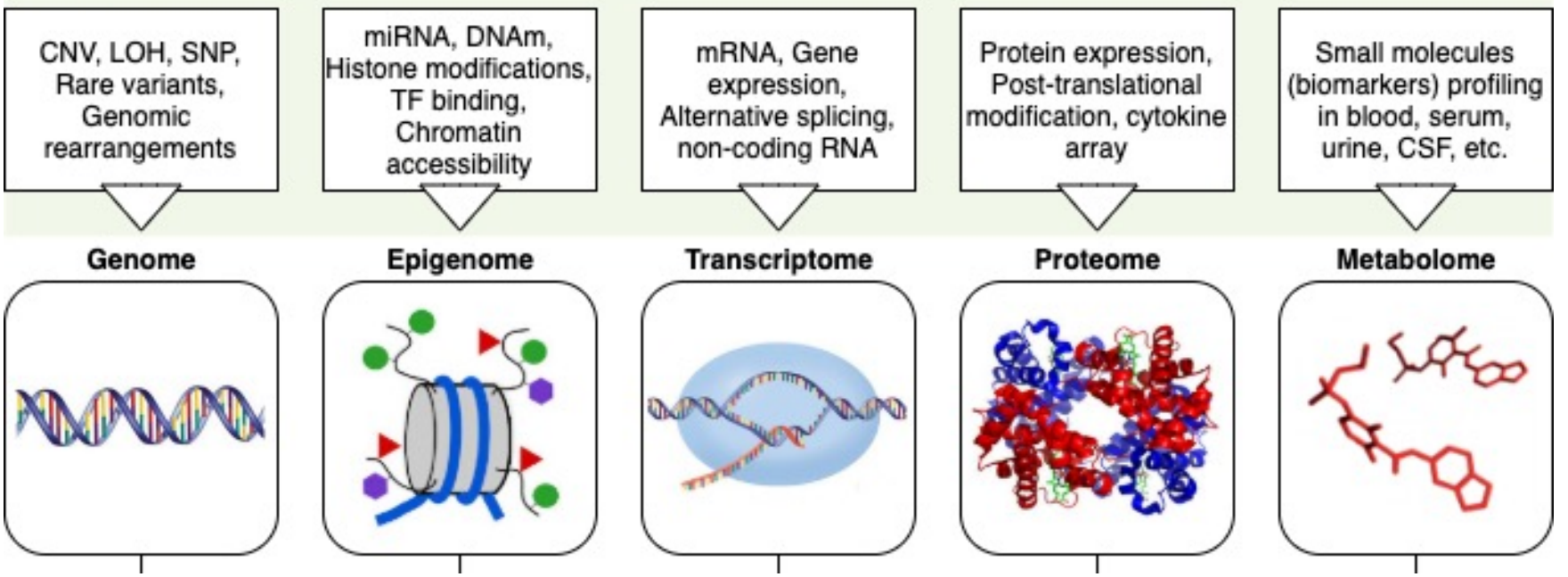
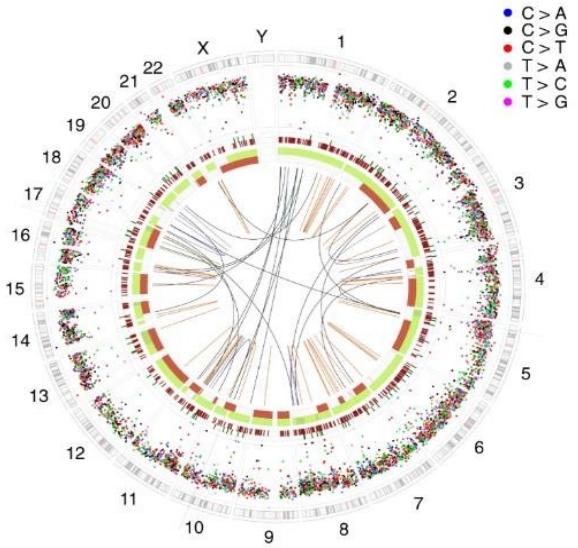
Omics in Cancer



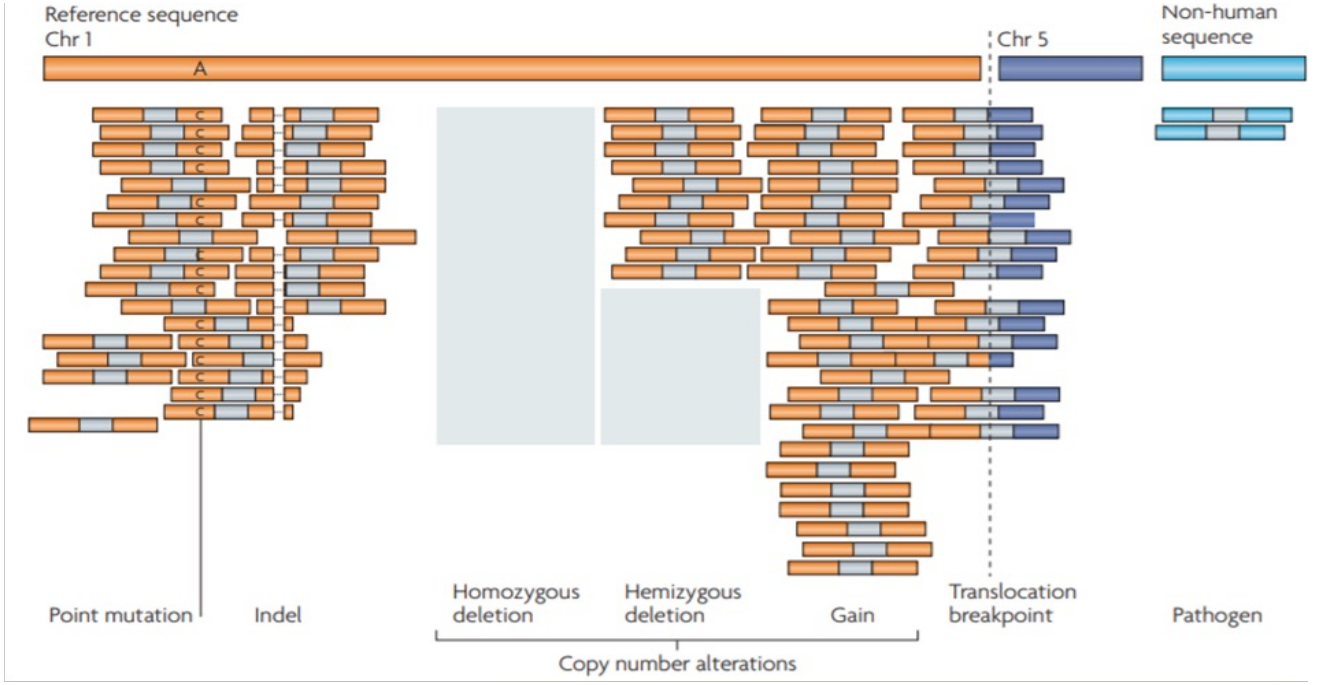


Advantages of Whole Genome Sequencing

Re-arrangements and Copy number variations



Sequencing data can be mapped and reanalysed at a later stage



SNV
Single Nucleotide Variant

CNV
Copy Number Variant

SV
Structural Variant

Staaf et al. Nature Medicine 2019; 25:1526

Meyerson, Nature Reviews Genetics 2010; 11:685

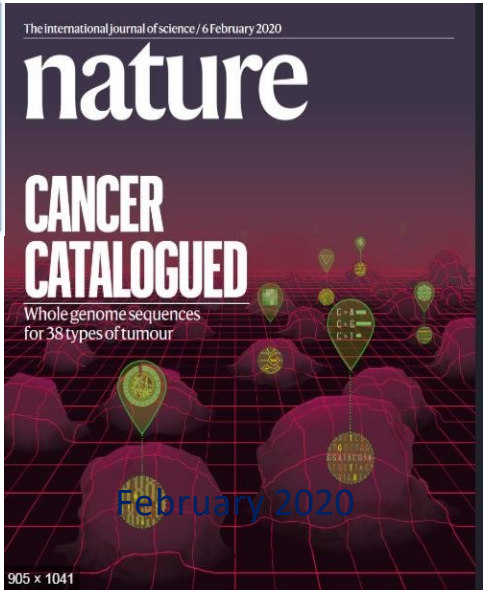
WGS initiatives Worldwide

PCAWG
PanCancer Analysis of WHOLE GENOMES

International Cancer Genome Consortium

TCGA

N=2,658 cancers
38 types of cancer



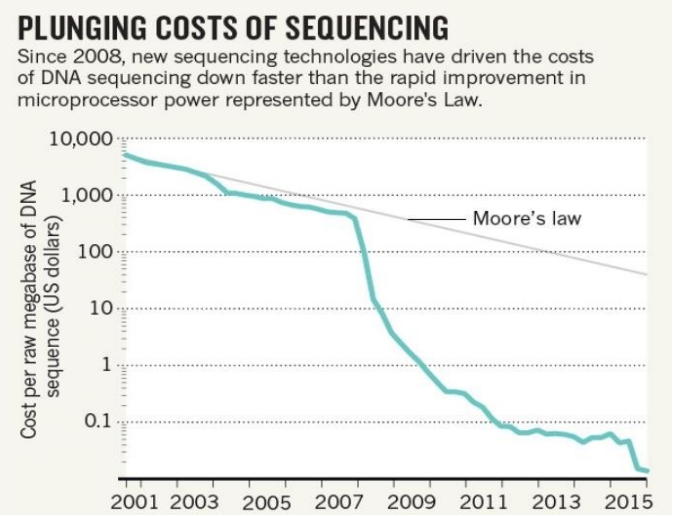
Sequence 100,000 genomes from 85,000 NHS patients affected by a rare disease, or cancer

The 100,000 Genomes Project

Cancer arm launched in 2014

Genomics england

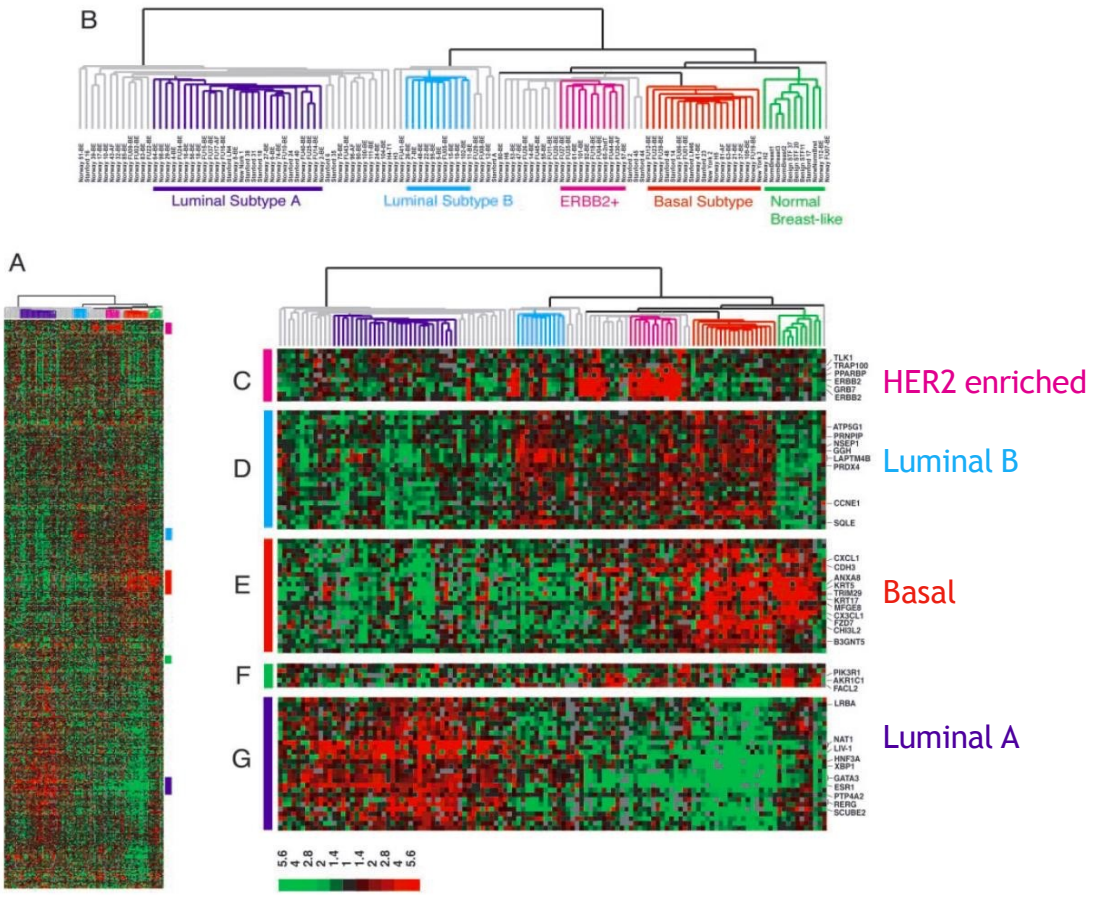
Berner et al. Current Genetic Medicine Reports 2019; 7:136
Turnbull C. Ann Oncol 2018; 29:784

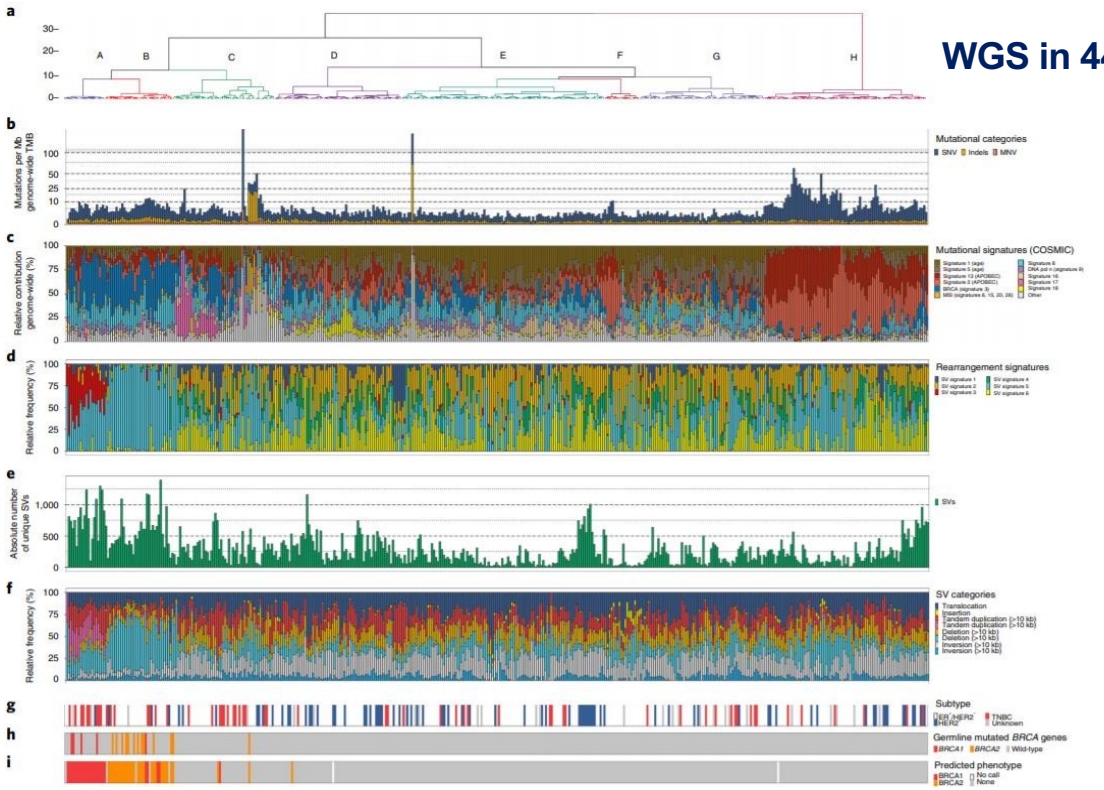


Nature 534, 462–463 (23 June 2016)

Breast Cancer *Intrinsic Subtypes* : Diagnosis and Prognosis

- ***luminal A-like*** : ER+, low proliferat. (Ki67 <20%)
- ***luminal B-like*** : ER+, high proliferat. (Ki67 >30%)
  - HER2 negative
  - HER2 positive
- ***HER2 enriched*** : ER/PR- ; HER2+
- ***Basal-like*** : ER/PR/HER2 – (TNBC)





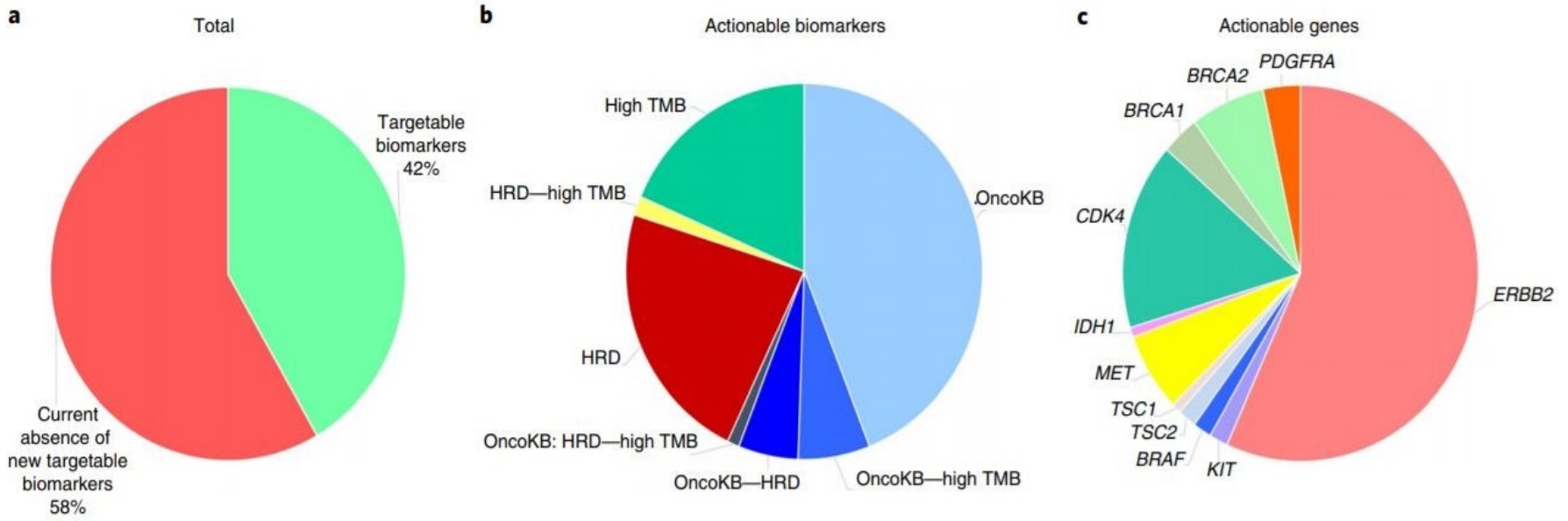
WGS in 442 metastatic BC

Mutational Signatures in BC

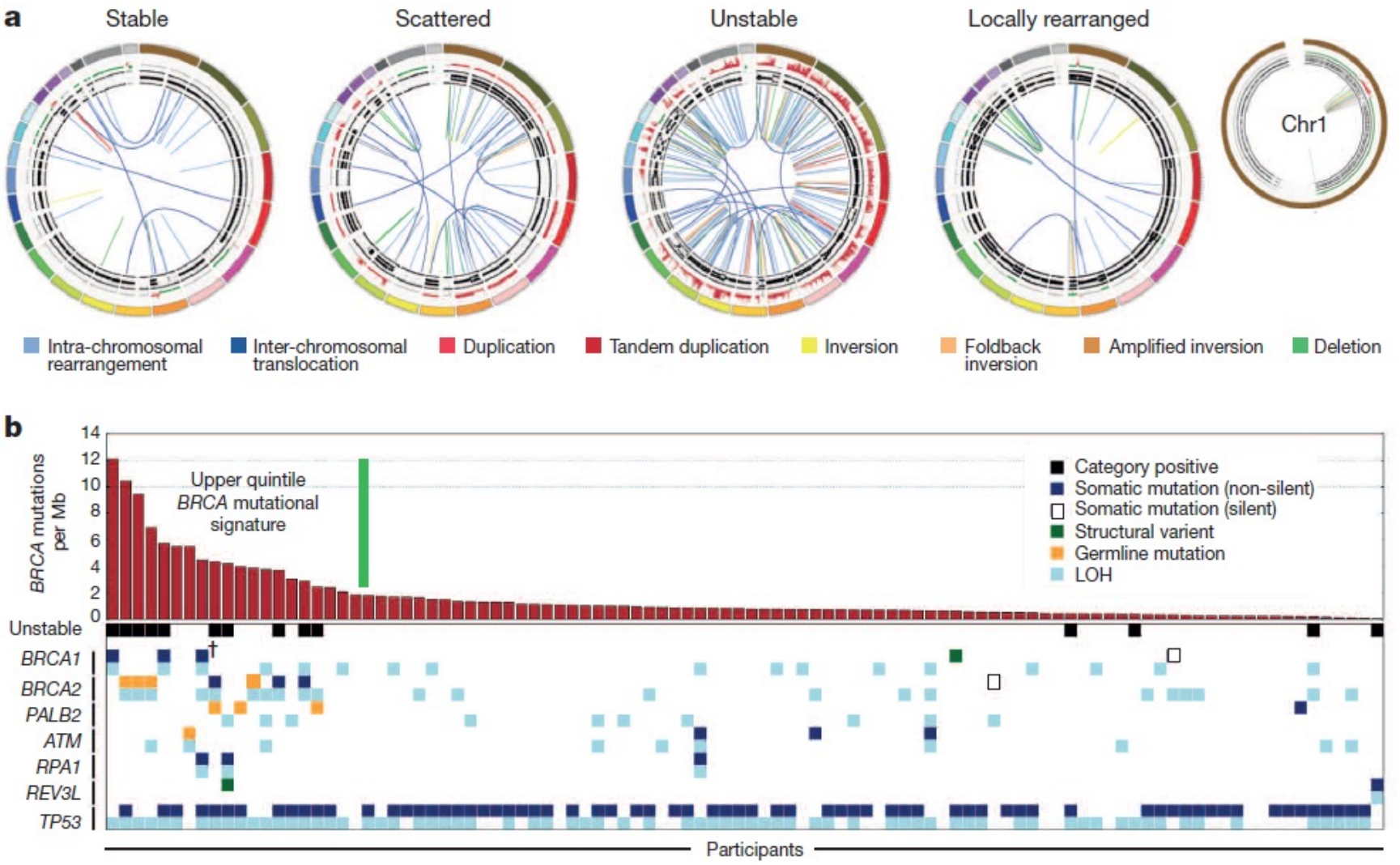
Angus et al. Nature Genetics 2019; 51:1450

WGS to identify patients for targeted therapy

N=155

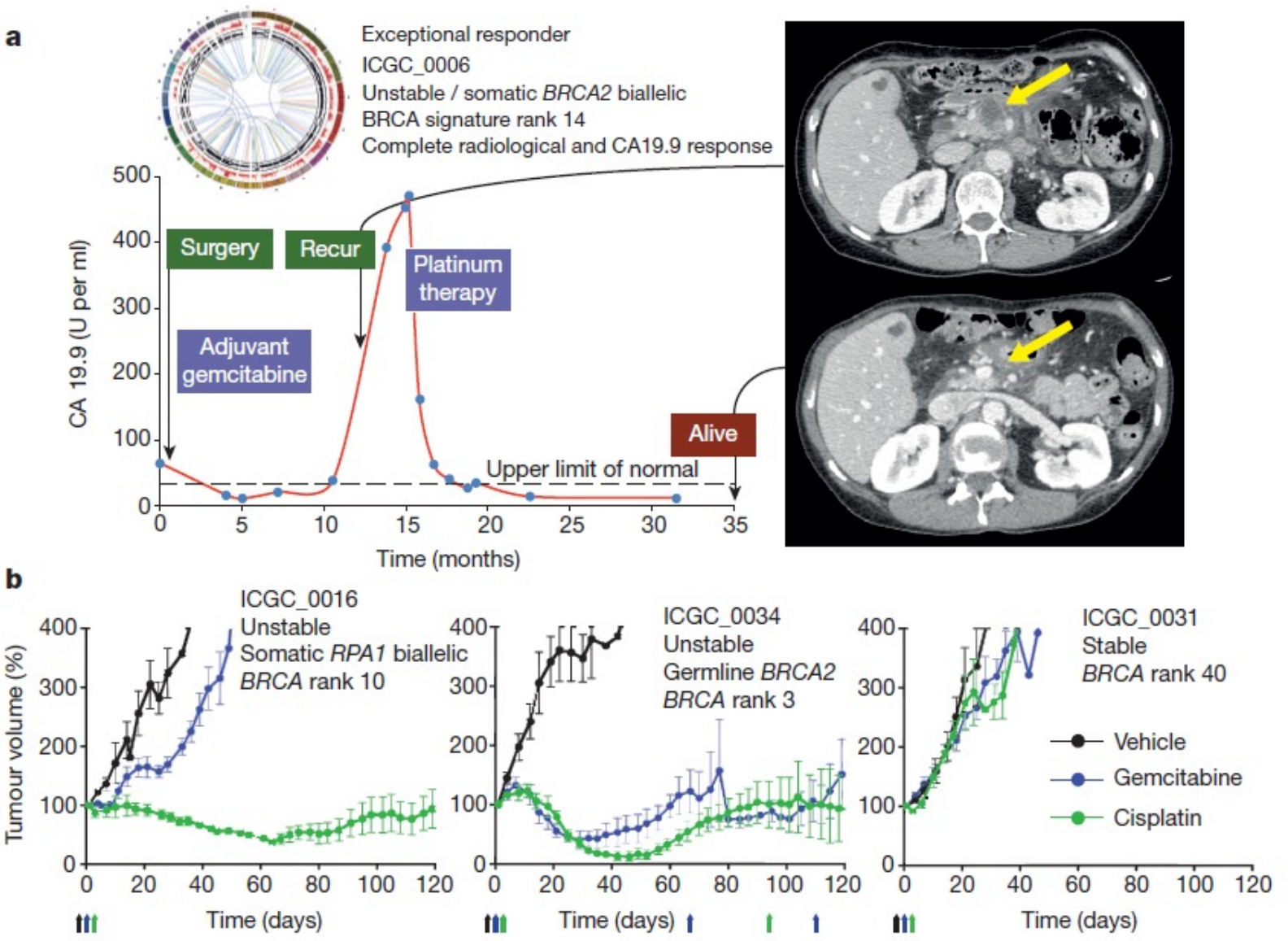


Subtypes of pancreatic cancer: Classifying by structural variation



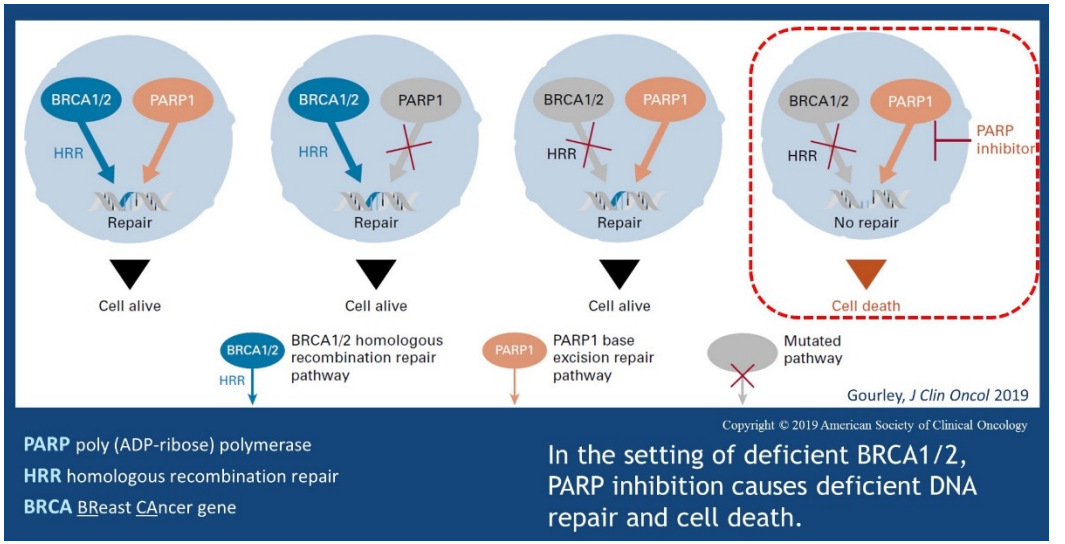
Waddell N et al. ***Nature*** 518:495-504, 2015

Responses to platinum therapy



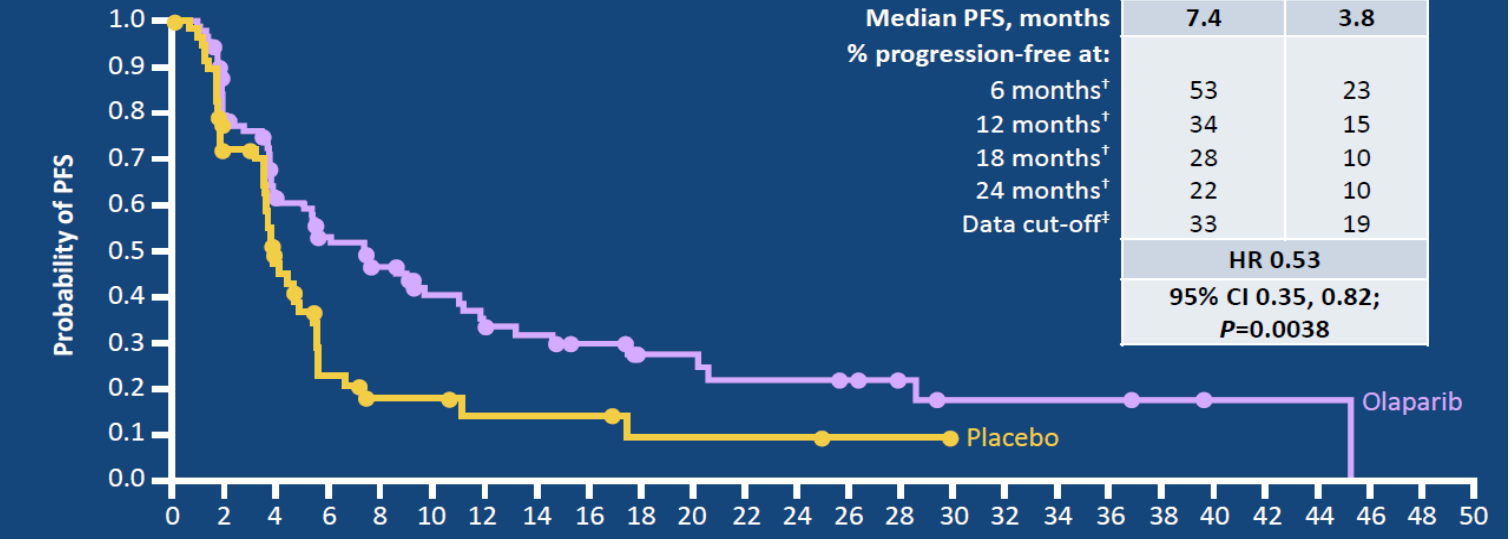
Waddell N et al. ***Nature*** 518:495-504, 2015

PARP inhibition is effective in germline BRCA 1/2 mutant tumors



Phase III POLO study: Olaparib after platinum therapy in BRCA mutant patients

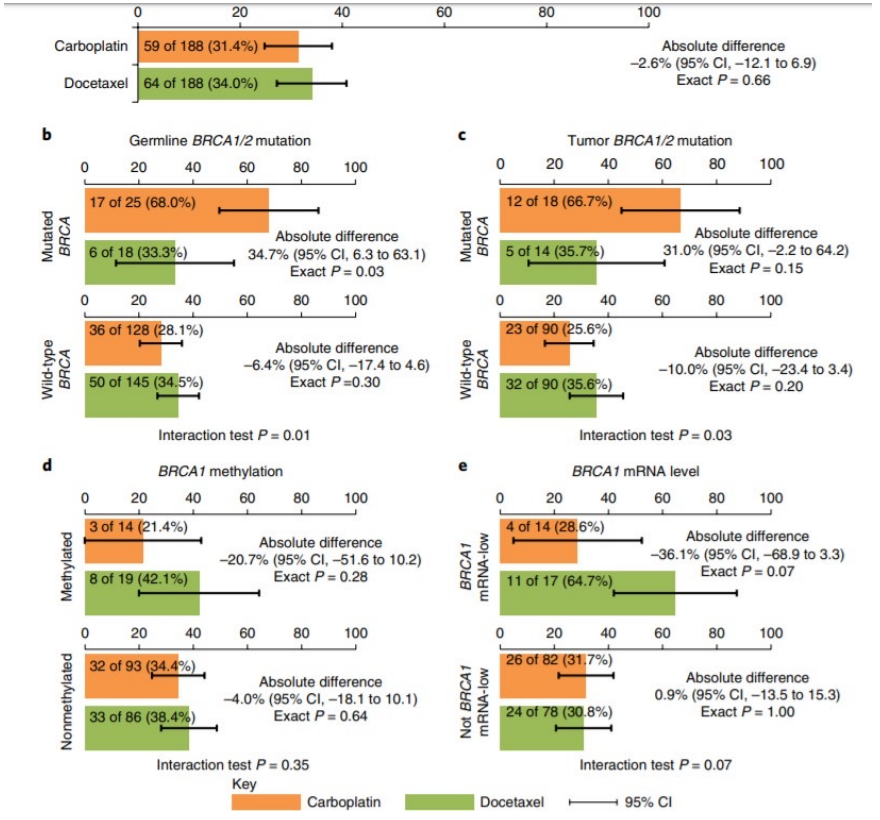
	Olaparib (N=92)	Placebo (N=62)
Median PFS, months	7.4	3.8
% progression-free at:		
6 months†	53	23
12 months†	34	15
18 months†	28	10
24 months†	22	10
Data cut-off‡	33	19
HR 0.53		
95% CI 0.35, 0.82; *P*=0.0038		



Golan T. et al., ***N Engl J Med*** 2019;381:317-27

Clinical utility HRD deficiency in BC

Platinum Salts



Tutt et al. Nature Medicine 2018; 24:628

Talazoparib in deleterious mutation in HR pathway gene (somatic and germline) other than *BRCA1* or *BRCA2*

Best Response	Response Rate, n (%) Efficacy Evaluable (N=13)
Complete Response (CR)	0 (0%)
Partial Response (PR)	4 (31%)
Stable Disease (SD)	6 (46%)
Progressive Disease (PD)	3 (23%)
ORR (CR+PR)	4 (31%)
CBR (CR+PR+SD ≥ 6 months)	7 (54%)

Gruber et al. J Clin Oncol.2019;37(suppl 15; abstr 3006)

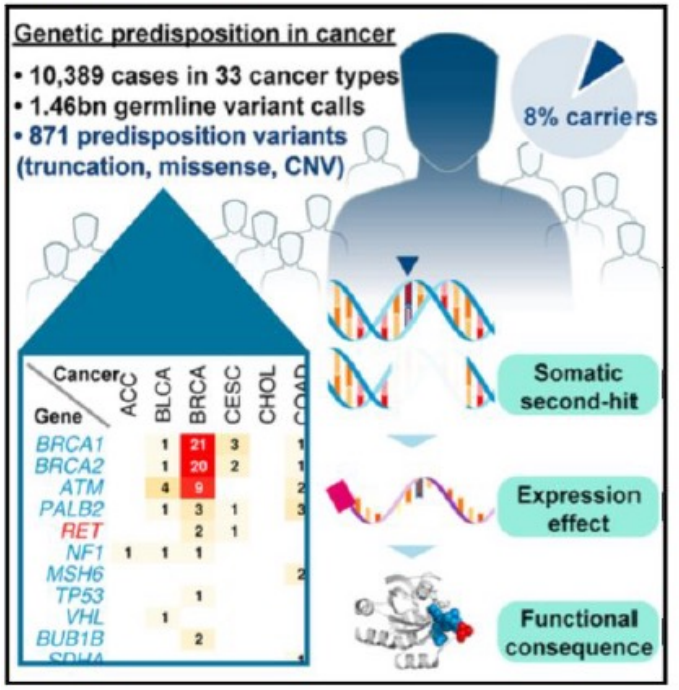
PanCancer analysis identifies predisposing germline variants

Article

Cell

Pathogenic Germline Variants in 10,389 Adult Cancers

Graphical Abstract



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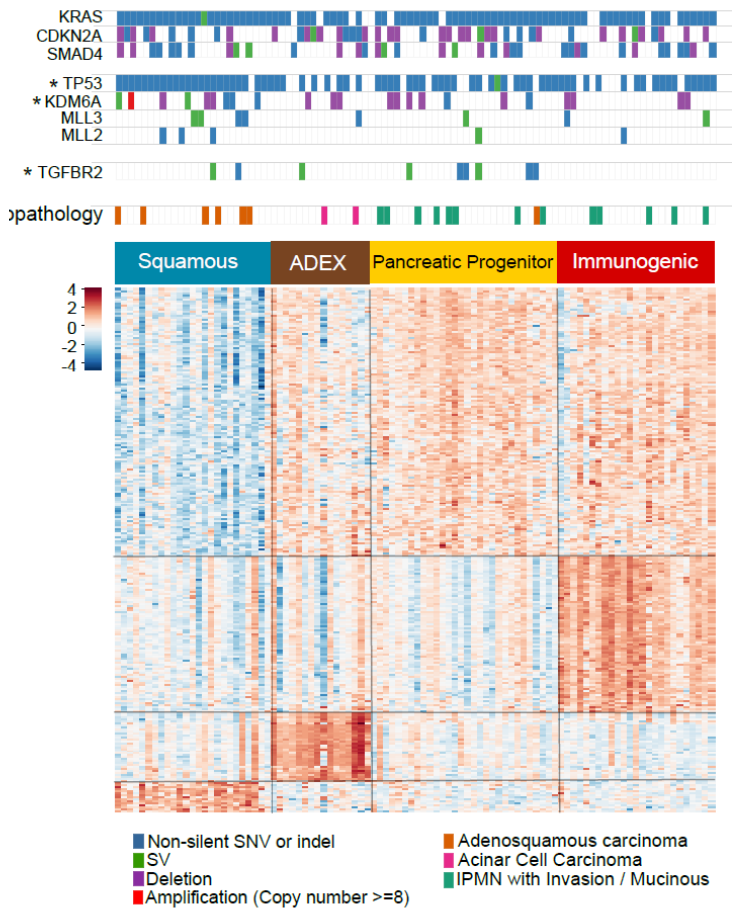
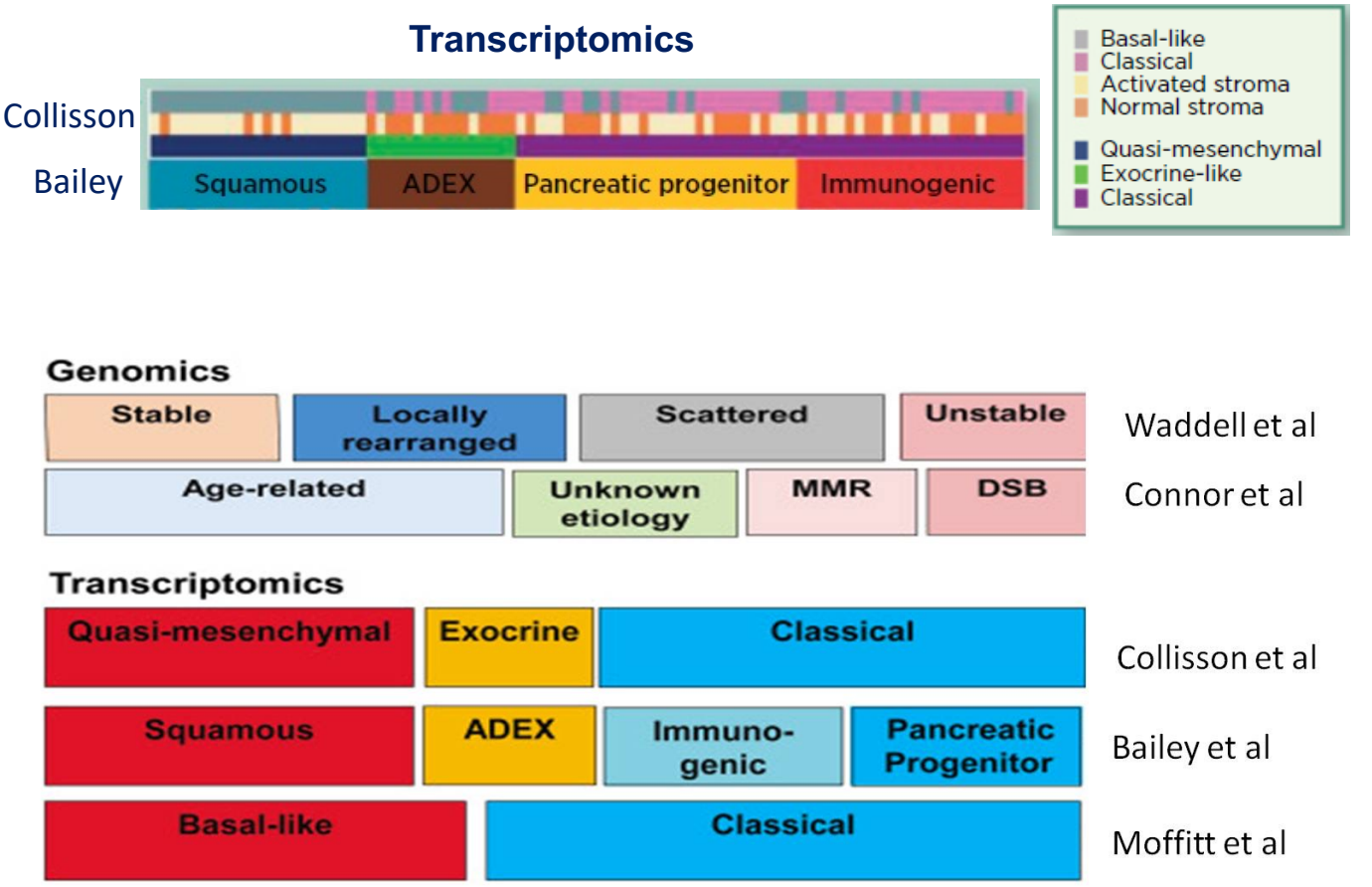
lding@wustl.edu (L.D.)

In Brief

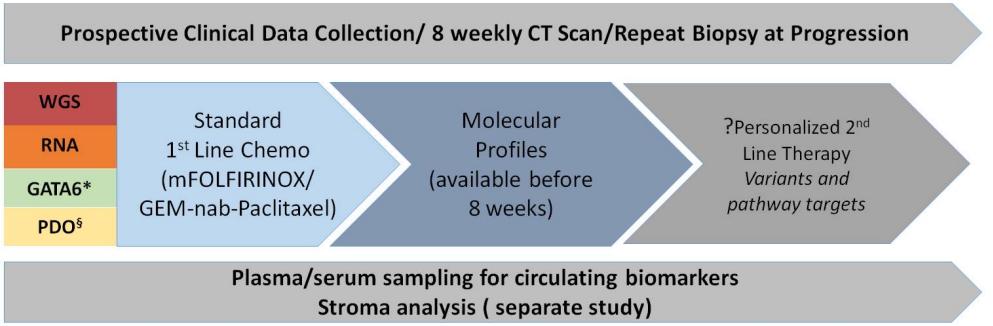
A pan-cancer analysis identifies hundreds of predisposing germline variants.

Compared classification from PDAC profiling studies





Dreyer S.B. et al. *Clin Cancer Res*; 23:1638-46, 2017; Le Large et al., Seminars in Cancer Biology, 2017

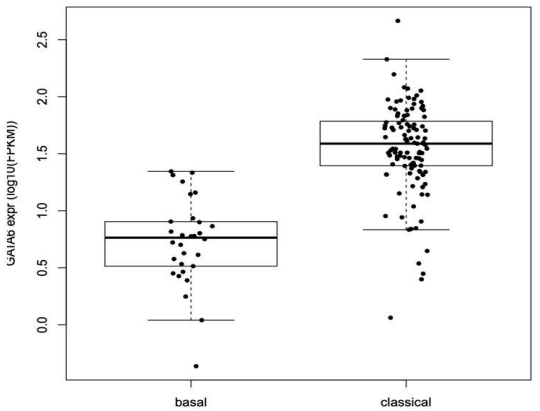


The COMPASS study design

- **Secondary endpoints:**
  - Correlate genomic subgroups including COSMIC signatures/novel genomic subgroups (eg unstable >200 SV) - with survival
  - **RNA classifiers and GATA6 with survival**

Lack of GATA6 correlates with basal-like subtype and worse response to mFOLFIRINOX

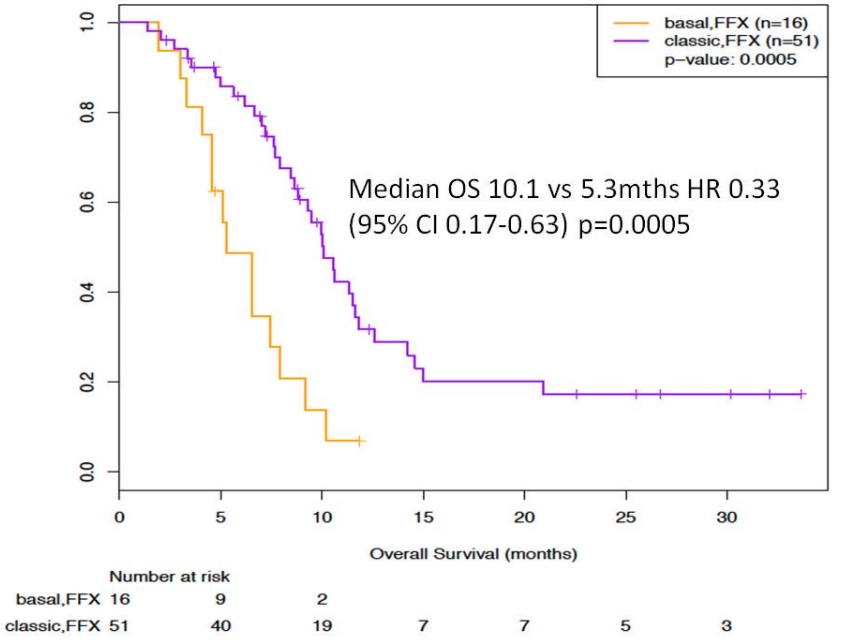
- GATA6 expression (RNA) is highly related to Moffitt subtype



Moffitt basal-like vs classical

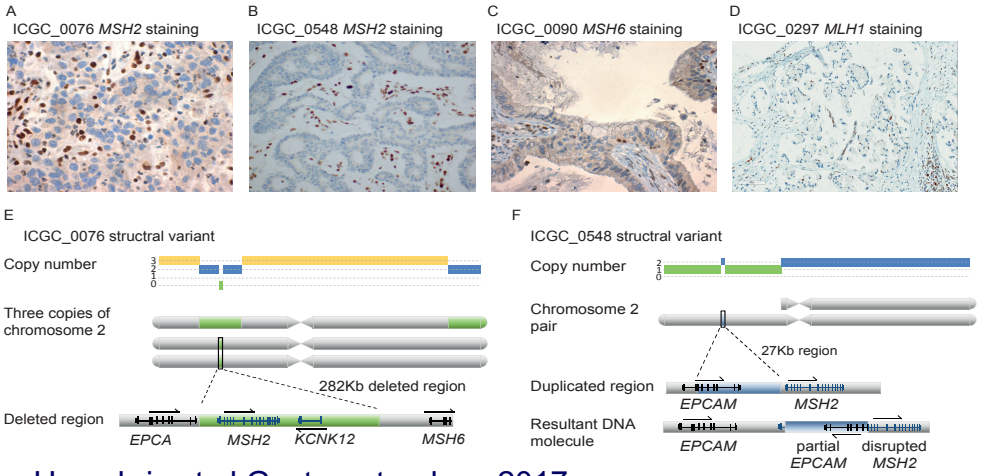
P <0.0001

OS mFFX only n=67



O'Kane ASCO GI 2019;
Clin Cancer Res 2020

MMR and HDR in PDAC

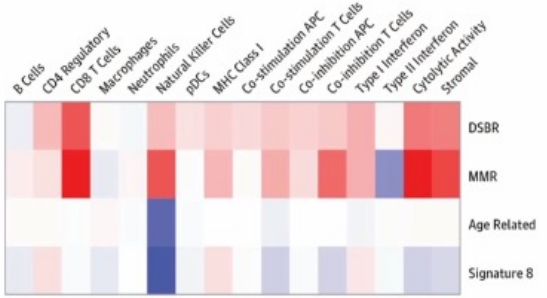


- Humphris et al Gastroenterology 2017;

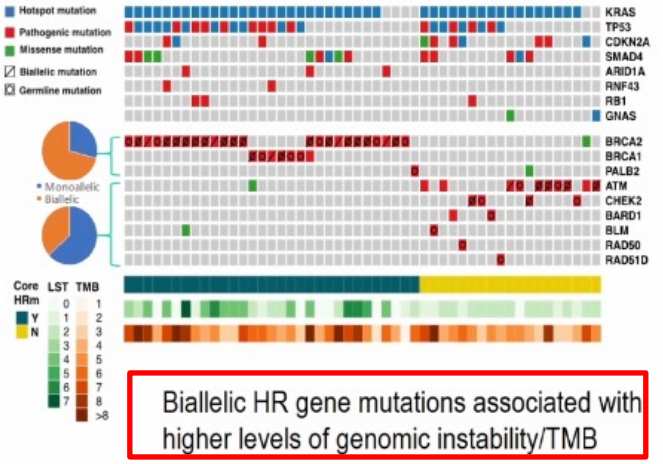
1% PDAC with MMR
IHC was the most accurate method to define MMR
ORR to pembrolizumab was 62% (5/8)

Lee al Science 2017

Mutational Signatures & Increased Immune Activity in PDAC



Heat map median expression immune function gene sets by Signature
DSBR: Double strand break repair



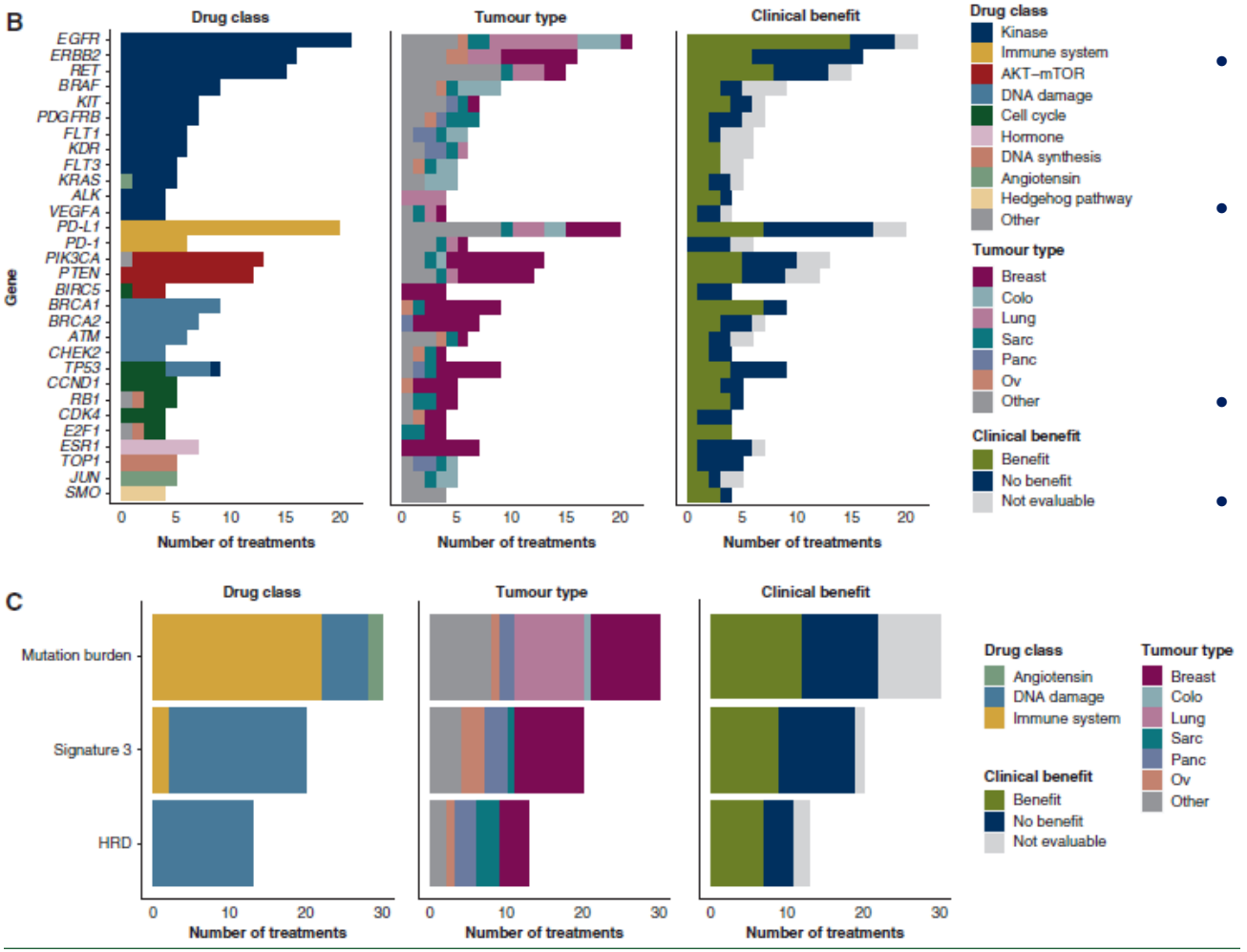
Biallelic HR gene mutations associated with higher levels of genomic instability/TMB

Homologous Recombination and DDR mutations increase immunogenicity

Connor, AA… Gallinger, S. JAMA Onc, 2017. Park, W…O'Reilly, EM. Clin Can Res, 2020

Whole-genome and transcriptome analysis enhances precision cancer treatment options

Frequently informative genes and genome signatures



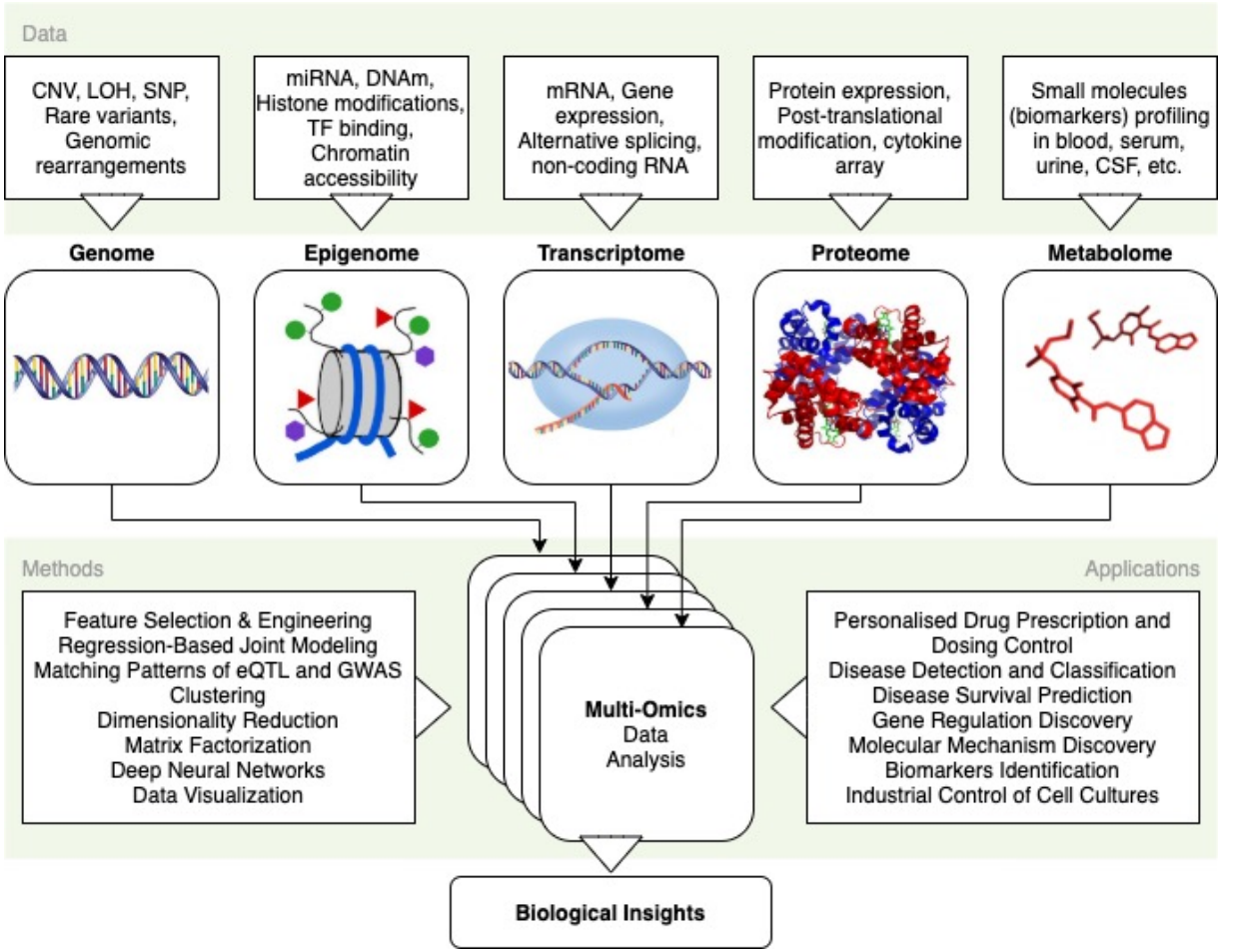
- Samples from 570 patients with advanced or metastatic cancer of diverse types underwent WGTA.
- Clinically actionable targets were identified for 83% of patients; 37% of them received WGTA informed treatments.
- RNA expression data were particularly informative
- 46% of treated patients experienced positive clinical benefit.

Pleasance et al., Annals of Oncology 2022

Multiomics in Cancer

- While a single type of *omics* can provide a significant amount of information at a specific level, the complexity of intra and extracellular mechanisms can only be addressed by combining several *omics* approaches to provide a complete picture of cancer pathogenesis and progression

- With Multiomics (the combined use of genomics, transcriptomics, proteomics, metabolomics, and other technologies yet to fully unfold) we can obtain a **complete dynamic vision of cancer**.



Multiomics data are increasingly being combined with spatial information.

SINGLE-CELL ANALYSIS ENTERS THE MULTIOMICS AGE

A rapidly growing collection of software tools is helping researchers to analyse multiple huge '-omics' data sets. **By Jeffrey M. Perkel**

In-bulk multiomics provide a deep insight into cancer biology, but there are still **some limitations** and bias with this technology, such as **tumor heterogeneity, tumor stroma contamination**.

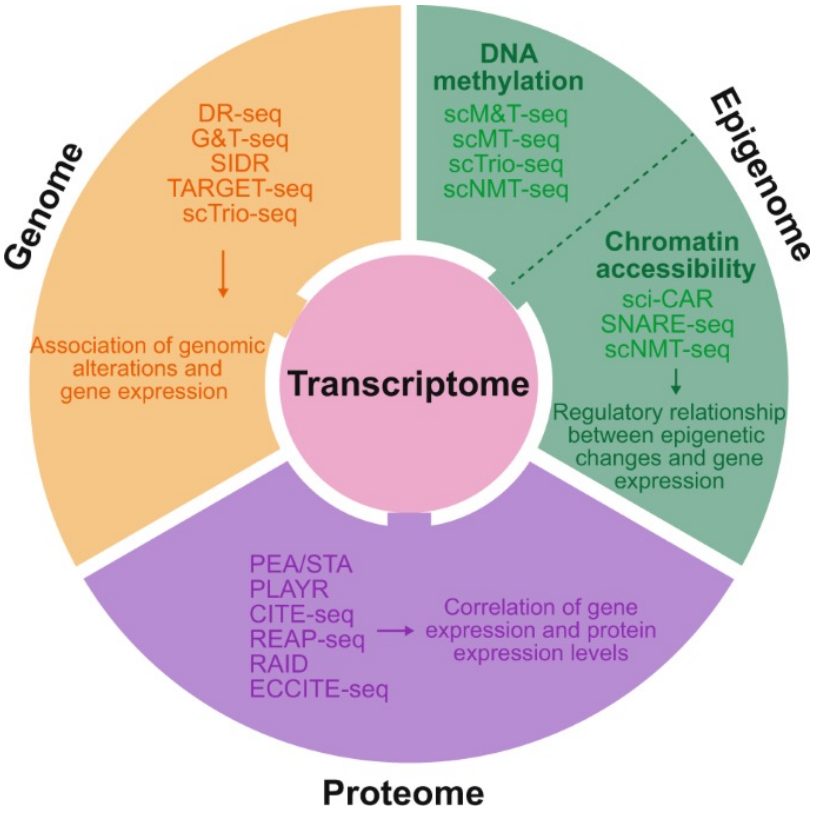
These problems may be overcome with **new multiomics single-cell methods,** able to study the different tumor cell populations

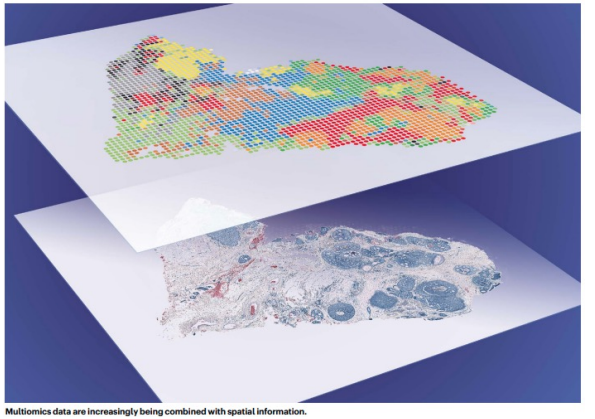
Today is possible to combine single-cell gene expression with single-cell:

- Genomics
- Surface Proteomics
- DNA methylation
- ATAC-seq
- TCR profiling
- Antigen Specificity

Recently, also **spatial transcriptomics** methods were developed to map cancer transcriptome on the tissue

Single Cell DNA sequencing: Fresh tissue or frozen and FFPE*
Single Cell RNA sequencing: Fresh tissue





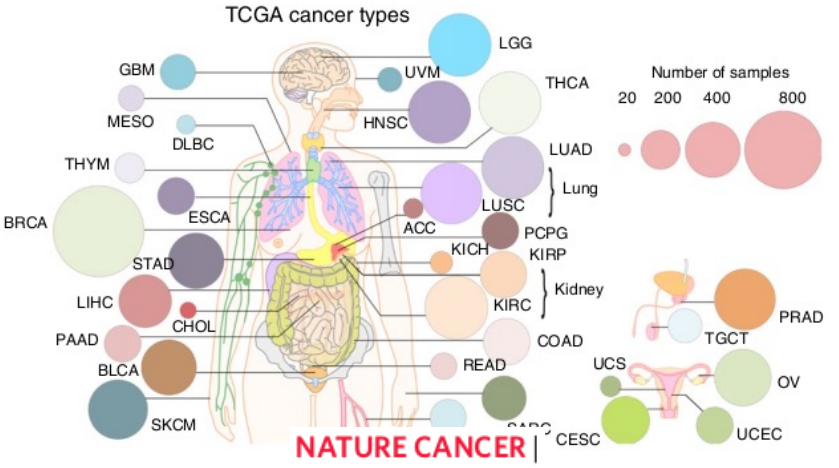
spatial transcriptomics

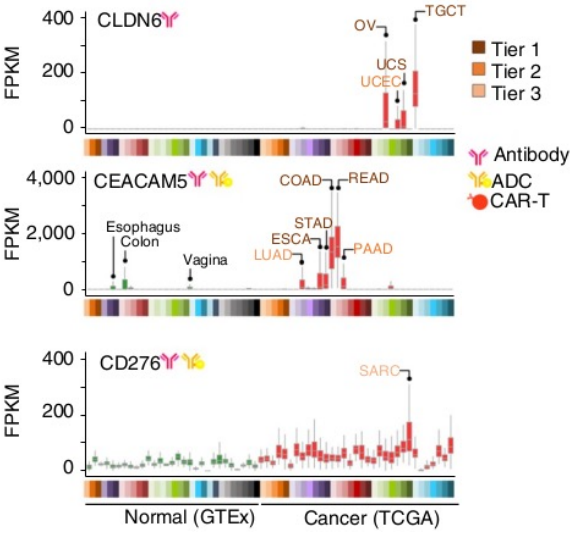
The Cancer Surfaceome Atlas integrates genomic, functional and drug response data to identify actionable targets

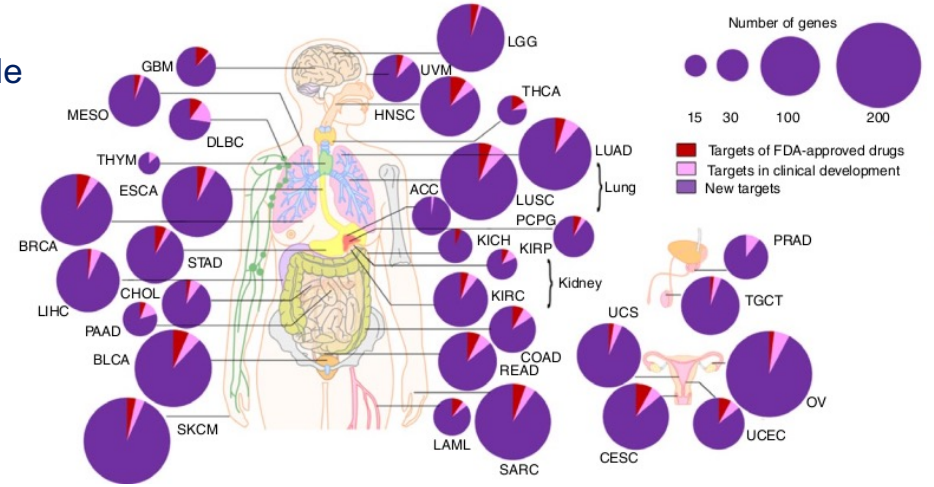
Cell-surface proteins are a rich source of immune and targeted therapies

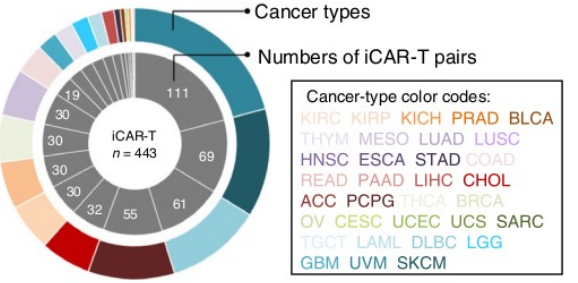
The Cancer Surfaceome Initiative integrating data **from single-cell and bulk genomics and transcriptomics,and target actionability created a compendium of the surface proteome (surfaceome)**

With this compendium they were able to identify novel target for personalized medicine









ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

Annals of Oncology 0: 1–8, 2018
doi:10.1093/annonc/mdy263

SPECIAL ARTICLE

A framework to rank genomic alterations as targets for cancer precision medicine: the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT)

J. Mateo[1], D. Chakravarty[2], R. Dienstmann[1], S. Jezdic[3], A. Gonzalez-Perez[4], N. Lopez-Bigas[4,5], C. K. Y. Ng[6], P. L. Bedard[7], G. Tortora[8,9], J. -Y. Douillard[3], E. M. Van Allen[10], N. Schultz[2], C. Swanton[11], F. André[12*] & L. Pusztai[13]

	Readiness for use in clinical practice	**Current examples of genomic alterations**
Tier I (I-A, I-B, I-C)	Targets ready for implementation in routine clinical decisions	HER2 in breast cancer BRCA1/2 in ovarian and breast cancer EGFR, ROS1/ALK in NSCLC TRK, PD1 in multiple cancers BRAF in metastatic melanoma
Tier II (II-A, II-B)	Investigational targets likely to define patients who benefit from a targeted drug, but additional data needed	PTEN pathway (PIK3CA, AKT1)
Tier III (III-A, III-B)	Clinical benefit previously demonstrated in other tumour type or for similar molecular targets	BRAF in non-melanoma cancers PALB2 and other non-BRCA DNA repair mutations
Tier IV (IVA, IVB)	Preclinical evidence of actionability	Hypothetical targets for future clinical testing
Tier V	Evidence supporting co-targeting approaches	PIK3CA in ER+, HER- breast cancer
Tier X	Lack of evidence for actionability	

REVIEW ARTICLE

Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO Precision Medicine Working Group

F. Mosele[1], J. Remon[2], J. Mateo[3], C. B. Westphalen[4], F. Barlesi[1], M. P. Lolkema[5], N. Normanno[6], A. Scarpa[7], M. Robson[8], F. Meric-Bernstam[9], N. Wagle[10], A. Stenzinger[11], J. Bonastre[12,13], A. Bayle[1,12,13], S. Michiels[12,13], I. Bièche[14], E. Rouleau[15], S. Jezdic[16], J-Y. Douillard[16], J. S. Reis-Filho[17], R. Dienstmann[18] & F. André[1,19,20*]

Table 10. List of genomic alterations level I/II/III according to ESCAT in advanced cholangiocarcinoma (CC)

Gene	Alteration	Prevalence	ESCAT	References
IDH1	Mutations	20%	IA	Abou-Alfa G. K, et al. *Ann Oncol.* 2019[129]
FGFR2	Fusions	15%	IB	Vogel A, et al. *Ann Oncol.* 2019[130]
	MSI-H	2%	IC	Marabelle A, et al. *J Clin Oncol.* 2020[131]
NTRK	Fusions	2%	IC	Doebele RC, et al. *Lancet Oncol.* 2020[50]
BRAF^{V600E}	Mutations	5%	IIB	Wainberg Z, et al. *J Clin Oncol.* 2019[132]
ERBB2	Amplifications Mutations	10% 2%	IIIA	Javle MM, et al. *J Clin Oncol.* 2017[133]
PIK3CA	Hotspot mutations	7%	IIIA	André F, et al. *N Engl J Med.* 2019[72]
BRCA 1/2	Mutations	3%	IIIA	De Bono J, et al. *N Engl J Med.* 2020[93]
MET	Amplifications	2%	IIIA	Camidge D, et al. *J Clin Oncol.* 2018[52]

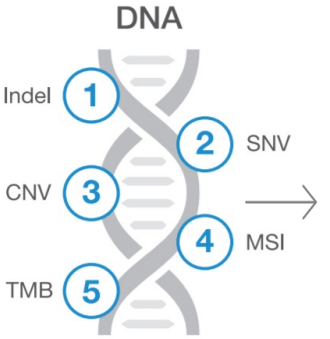
ESCAT, ESMO Scale for Clinical Actionability of molecular Targets (I-IV levels)

The **ESMO** Precision Medicine WG recommended for CCA a targeted multigene NGS-based genomic profiling for the detection of *ESCAT level I* actionable alterations (improved outcomes in clinical trial), such as:
IDH1 mutations, FGFR2 and NTRK fusions and MSI-H.

In US: FoundationOne CDx, an assay targeting up to 324 genes, was recently approved as a companion diagnostic test for pemigatinib therapy in patients with CCA with FGFR2 fusions or other rearrangements.

Mosele et al, *Ann Oncology* 2020

The FPG500 Project : Tecnologies and criteria



- Microlab STAR-Hamilton for automated library preparation and Illumina ™ Novaseq6000 for sequencing.
- Profiling performed by TruSight Oncology 500 high throughput (TSO500HT, Illumina) (DNA or RNA ≥ 40 ng).
- Samples below the required quantity for TSO500HT, undergo Oncomine Focus assay (Thermofisher) for DNA and Archer's FusionPlex Lung panel for RNA evaluation.

ABL1	BRD4	CUX1	FAM175A	GATA6	IGF1	MAP3K13	NOTCH4	POLE	RPTOR	TAF1
ABL2	BRIP1	CXCR4	FAM46C	GEN1	IGF1R	MAP3K14	NPM1	PPARG	RUNX1	TBX3
ACVR1	BTG1	CYLD	FANCA	GID4	IGF2	MAP3K4	NRAS	PPM1D	RUNX1T1	TCEB1
ACVR1B	BTK	DAXX	FANCC	GLI1	IKBKE	MAPK1	NRG1	PPP2R1A	RYBP	TCF3
AKT1	C11orf30	DCUN1D1	FANCD2	GNA11	IKZF1	MAPK3	NSD1	PPP2R2A	SDHA	TCF7L2
AKT2	CALR	DDR2	FANCE	GNA13	IL10	MAX	NTRK1	PPP6C	SDHAF2	TERC
AKT3	CARD11	DDX41	FANCF	GNAQ	IL7R	MCL1	NTRK2	PRDM1	SDHB	TERT
ALK	CASP8	DHX15	FANCG	GNAS	INHA	MDC1	NTRK3	PREX2	SDHC	TET1
ALOX12B	CBFB	DICER1	FANCI	GPR124	INHBA	MDM2	NUP93	PRKAR1A	SDHD	TET2
ANKRD11	CBL	DIS3	FANCL	GPS2	INPP4A	MDM4	NUTM1	PRKCI	SETBP1	TFE3
ANKRD26	CCND1	DNAJB1	FAS	GREM1	INPP4B	MED12	PAK1	PRKDC	SETD2	TFRC
APC	CCND2	DNMT1	FAT1	GRIN2A	INSR	MEF2B	PAK3	PRSS8	SF3B1	TGFBR1
AR	CCND3	DNMT3A	FBXW7	GRM3	IRF2	MEN1	PAK7	PTCH1	SH2B3	TGFBR2
ARAF	CCNE1	DNMT3B	FGF1	GSK3B	IRF4	MET	PALB2	PTEN	SH2D1A	TMEM127
ARFRP1	CD274	DOT1L	FGF10	H3F3A	IRS1	MGA	PARK2	PTPN11	SHQ1	TMPRSS2
ARID1A	CD276	E2F3	FGF14	H3F3B	IRS2	MITF	PARP1	PTPRD	SLIT2	TNFAIP3
ARID1B	CD74	EED	FGF19	H3F3C	JAK1	MLH1	PAX3	PTPRS	SLX4	TNFRSF14
ARID2	CD79A	EGFL7	FGF2	HGF	JAK2	MLL	PAX5	PTPRT	SMAD2	TOP1
ARID5B	CD79B	EGFR	FGF23	HIST1H1C	JAK3	MLLT3	PAX7	QKI	SMAD3	TOP2A
ASXL1	CDC73	EIF1AX	FGF3	HIST1H2BD	JUN	MPL	PAX8	RAB35	SMAD4	TP53
ASXL2	CDH1	EIF4A2	FGF4	HIST1H3A	KAT6A	MRE11A	PBRM1	RAC1	SMARCA4	TP63
ATM	CDK12	EIF4E	FGF5	HIST1H3B	KDM5A	MSH2	PDCD1	RAD21	SMARCB1	TRAF2
ATR	CDK4	EML4	FGF6	HIST1H3C	KDM5C	MSH3	PDCD1LG2	RAD50	SMARCD1	TRAF7
ATRX	CDK6	EP300	FGF7	HIST1H3D	KDM6A	MSH6	PDGFRA	RAD51	SMC1A	TSC1
AURKA	CDK8	EPCAM	FGF8	HIST1H3E	KDR	MST1	PDGFRB	RAD51B	SMC3	TSC2
AURKB	CDKN1A	EPHA3	FGF9	HIST1H3F	KEAP1	MST1R	PDK1	RAD51C	SMO	TSHR
AXIN1	CDKN1B	EPHA5	FGFR1	HIST1H3G	KEL	MTOR	PDPK1	RAD51D	SNCAIP	U2AF1
AXIN2	CDKN2A	EPHA7	FGFR2	HIST1H3H	KIF5B	MUTYH	PGR	RAD52	SOCS1	VEGFA
AXL	CDKN2B	EPHB1	FGFR3	HIST1H3I	KIT	MYB	PHF6	RAD54L	SOX10	VHL
B2M	CDKN2C	ERBB2	FGFR4	HIST1H3J	KLF4	MYC	PHOX2B	RAF1	SOX17	VTCN1
BAP1	CEBPA	ERBB3	FH	HIST2H3A	KLHL6	MYCL1	PIK3C2B	RANBP2	SOX2	WISP3
BARD1	CENPA	ERBB4	FLCN	HIST2H3C	KMT2B	MYCN	PIK3C2G	RARA	SOX9	WT1
BBC3	CHD2	ERCC1	FLI1	HIST2H3D	KMT2C	MYD88	PIK3C3	RASA1	SPEN	XIAP
BCL10	CHD4	ERCC2	FLT1	HIST3H3	KMT2D	MYOD1	PIK3CA	RB1	SPOP	XPO1
BCL2	CHEK1	ERCC3	FLT3	HLA-A	KRAS	NAB2	PIK3CB	RBM10	SPTA1	XRCC2
BCL2L1	CHEK2	ERCC4	FLT4	HLA-B	LAMP1	NBN	PIK3CD	RECQL4	SRC	YAP1
BCL2L11	CIC	ERCC5	FOXA1	HLA-C	LATS1	NCOA3	PIK3CG	REL	SRSF2	YES1
BCL2L2	CREBBP	ERG	FOXL2	HNF1A	LATS2	NCOR1	PIK3R1	RET	STAG1	ZBTB2
BCL6	CRKL	ERRFI1	FOXO1	HNRNPK	LMO1	NEGR1	PIK3R2	RFWD2	STAG2	ZBTB7A
BCOR	CRLF2	ESR1	FOXP1	HOXB13	LRP1B	NF1	PIK3R3	RHEB	STAT3	ZFHX3
BCORL1	CSF1R	ETS1	FRS2	HRAS	LYN	NF2	PIM1	RHOA	STAT4	ZNF217
BCR	CSF3R	ETV1	FUBP1	HSD3B1	LZTR1	NFE2L2	PLCG2	RICTOR	STAT5A	ZNF703
BIRC3	CSNK1A1	ETV4	FYN	HSP90AA1	MAGI2	NFKBIA	PLK2	RIT1	STAT5B	ZRSR2
BLM	CTCF	ETV5	GABRA6	ICOSLG	MALT1	NKX2-1	PMAIP1	RNF43	STK11	
BMPR1A	CTLA4	ETV6	GATA1	ID3	MAP2K1	NKX3-1	PMS1	ROS1	STK40	
BRAF	CTNNA1	EWSR1	GATA2	IDH1	MAP2K2	NOTCH1	PMS2	RPS6KA4	SUFU	
BRCA1	CTNNB1	EZH2	GATA3	IDH2	MAP2K4	NOTCH2	PNRC1	RPS6KB1	SUZ12	
BRCA2	CUL3	FAM123B	GATA4	IFNGR1	MAP3K1	NOTCH3	POLD1	RPS6KB2	SYK	

ABL1	BCL2	CSF1R	ESR1	EWSR1	FLI1	KIF5B	MSH2	NRG1	PAX7	RAF1
AKT3	BRAF	EGFR	ETS1	FGFR1	FLT1	KIT	MYC	NTRK1	PDGFRA	RET
ALK	BRCA1	EML4	ETV1	FGFR2	FLT3	MET	NOTCH1	NTRK2	PDGFRB	ROS1
AR	BRCA2	ERBB2	ETV4	FGFR3	JAK2	MLL	NOTCH2	NTRK3	PIK3CA	RPS6KB1
AXL	CDK4	ERG	ETV5	FGFR4	KDR	MLLT3	NOTCH3	PAX3	PPARG	TMPRSS2

Tumor type	Target
Breast	PIK3CA
Lung	EGFR
	ALK
	ROS1
	BRAF
	NTRK
	RET
Ovary	BRCA 1/2
Pancreas	BRCA 1/2
	NTRK
Prostate	BRCA 1/2
Melanoma	BRAF
GIST	c-kit
	PDGFRα
Colorectal	KRAS
	NRAS
	BRAF
	NTRK
Thyroid	RET
Endometrium	POLE

FPG500 profiling of 1057 pts at Oct 14, 2022