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REVIEW
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DNA Damage Repair Deficiency in Pancreatic Ductal Adenocarcinoma: Preclinical Models and Clinical Perspectives

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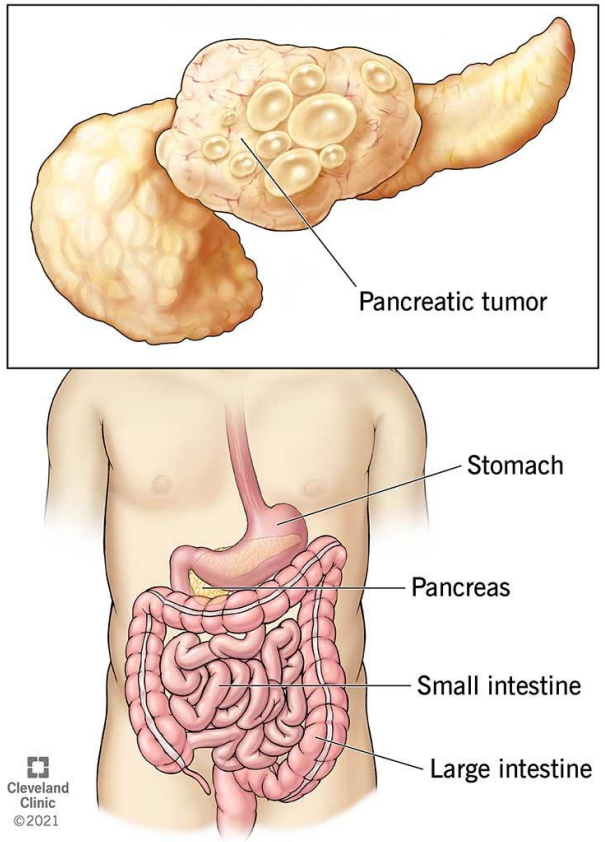
Pancreatic cancer

- Pancreatic cancer is one of the most lethal cancers worldwide, accounting for 2.6% of all new cancer cases but causing 4.8% of all cancer deaths (Ferlay et al., 2019).

- Despite recent advances in personalized and targeted therapy, little progress has been made to improve overall survival (OS) and the **5-year survival rate is estimated at 9%** (Siegel et al., 2020).

- Currently, curative treatment is limited to low-stage, resectable disease but over 80% of patients present with advanced or metastatic disease.



Current standard of care treatment for PDAC

Current standard of care treatment for advanced pancreatic ductal adenocarcinoma (PDAC):

- Combination of nab-paclitaxel with gemcitabine
- 5-fluorouracil, leucovorin, irinotecan, oxaliplatin (FOLFIRINOX)

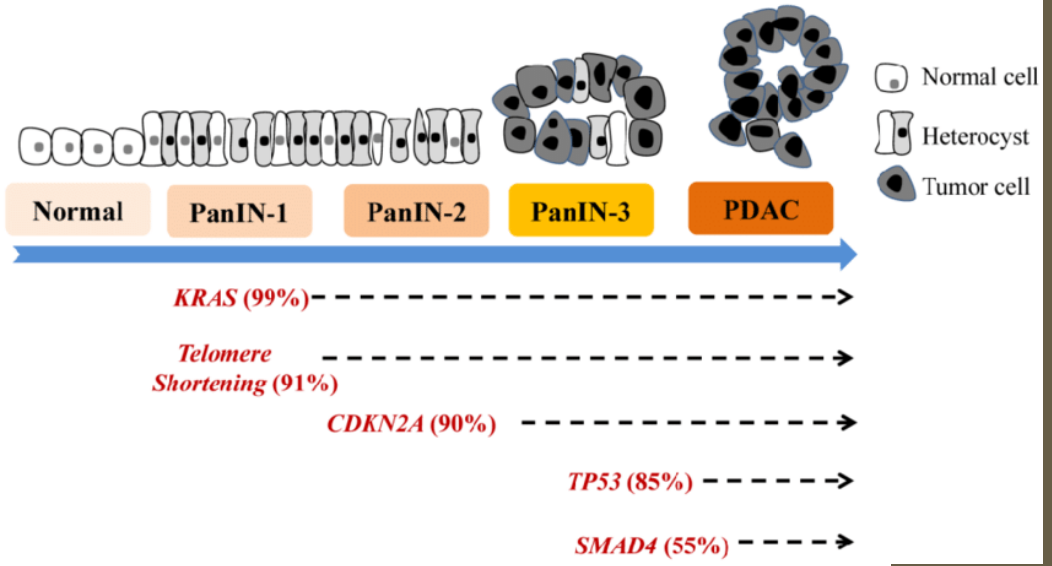
- The **PRODIGE trial** found that **FOLFIRINOX** improved OS by 4.3 months compared to gemcitabine alone (11.1 vs. 6.8 months, $p < 0.001$) (Conroy et al., 2011). However, FOLFIRINOX is associated with **higher toxicity** profiles and is therefore generally reserved for patients with a **good performance status**.

- In 2013, the **MPACT trial** showed that **nab-paclitaxel with gemcitabine** improved OS by 1.8 months compared to gemcitabine alone (8.5 vs. 6.7 months, $p < 0.001$) (Von Hoff et al., 2013).

Other treatment options

- Gemcitabine
- Gemcitabine with erlotinib
- Nanoliposomal irinotecan with 5-FU
- Pembrolizumab (patients with microsatellite instability)
- Larotrectinib/entrectinib (patients with NTRK-fusion)
- Olaparib (patients with gBRCA mutation)

Genomic analyses of PDAC



- Genomic analyses have revealed a complex mutational landscape that is predominated by mutations in **TP53, KRAS, SMAD4, and CDKN2A**.
- Despite extensive research, **targeted therapies for these mutations have not reached clinical practice**.
- In addition, PDAC is characterized by **genome instability**.
- Genome instability has been described as one of the enabling hallmarks of cancer by Hanahan and Weinberg (2011) and can be attributed to multiple sources: (Increased sensitivity to mutagenic agents, Defects in the genomic maintenance machinery, Loss of telomeric DNA, Aberrant surveillance mechanisms)
- While these aberrations can partly be contributed to these four commonly mutated genes, additional pathway deficiencies are also involved.

- Genomic instability:
- The increased tendency for DNA mutations (changes) and other genetic changes to occur during cell division. Genomic instability is caused by **defects in certain processes that control cell division**. These defects may include **mutations in certain genes involved in repairing damaged DNA** or mistakes that don't get corrected when DNA is copied in a cell. They may also include defects such as **broken, missing, rearranged, or extra chromosomes**.

- Microstaellite:
- **A short sequence of DNA, usually 1 to 4 basepairs** (a unit of DNA), that is repeated together in a row along the DNA molecule. There is variation from person to person in the number of repeats. There are hundreds of places in human DNA that contain microsatellites.

- Microstaellite instability:
- A change that occurs in certain cells (such as cancer cells) in which the number of **repeated DNA bases in a microsatellite** is different from what it was when the **microsatellite was inherited**. Microsatellite instability may be caused by **mistakes that don't get corrected** when DNA is copied in a cell. It is found most often in colorectal cancer, gastric cancer, and endometrial cancer, but it may also be found in many other types of cancer. Knowing whether a cancer has microsatellite instability may help plan the best treatment.

DDR deficiency in PDAC

- The DNA damage response (DDR) pathway plays a central role in genome maintenance and repair.
- In contrast to TP53 and KRAS, etc., DDR deficiency is targetable, with multiple drugs already available in the clinic for non-PDAC cancer types, such as breast and prostate cancer (Wengner et al., 2020).
- This review briefly covers the role and definition of DDR deficiency in PDAC and provides an overview of clinical trials that investigate DDR targeting drugs.
- The main focus is on how **cell lines, organoids, and mouse models** are used to study DDR deficient pathways in PDAC.

DNA Damage Repair Pathways in Pancreatic Ductal Adenocarcinoma

DDR pathways:

- Base excision repair (BER)
- Nucleotide excision repair (NER)
- Mismatch repair (MMR)
- Interstrand crosslink repair (ICL repair)
- Double strand break repair [both homologous recombination (HR) and non-homologous end-joining (NHEJ)].

Each pathway can roughly be divided into three phases or steps:

- Recognition of the damage
- Excision or processing of the damaged strand(s)
- Actual repair

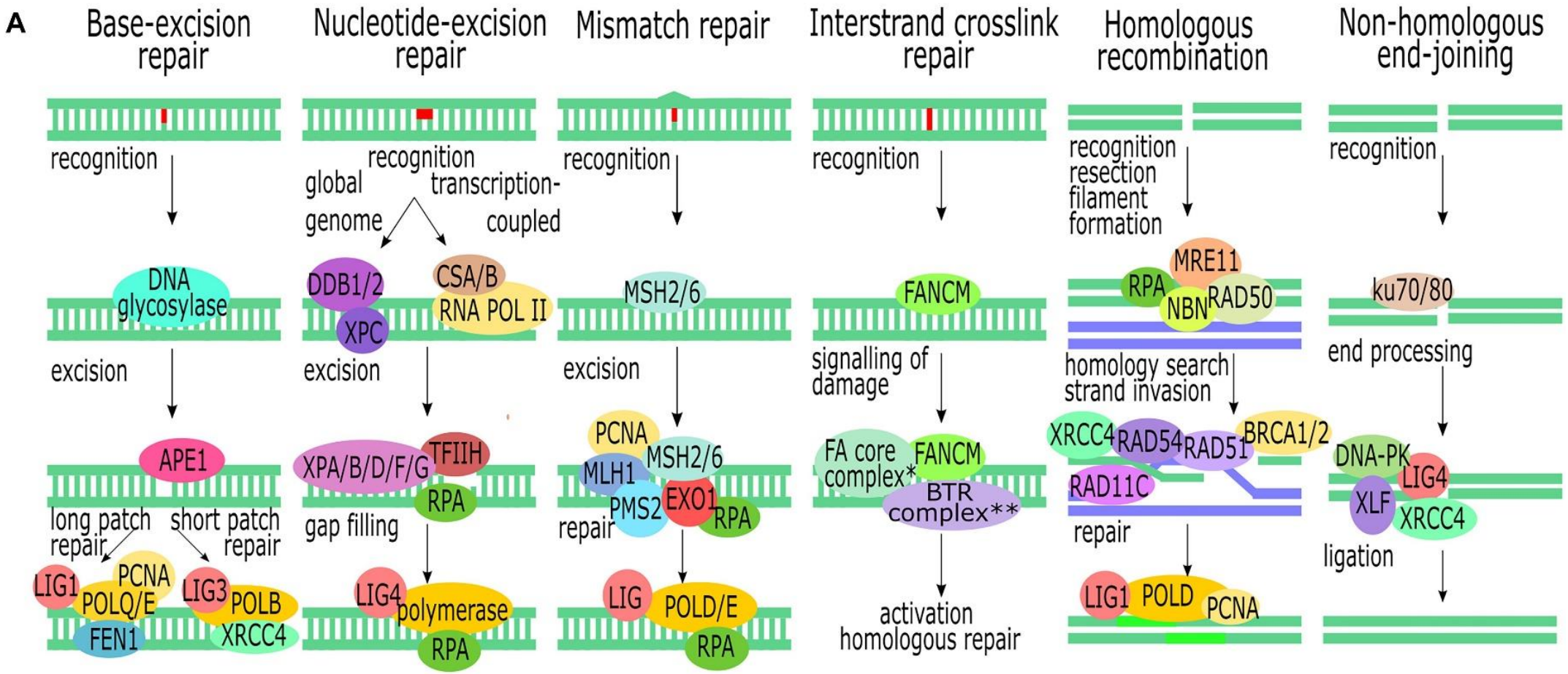
Base excision repair

- Base excision repair removes non-bulky single-base lesions such as oxidation or deamination damage.

- The damaged base is recognized and removed by one of multiple specific DNA **glycosylases** (such as UNG, SMUG1, or NEIL1), depending on the type of lesion.

- Next, the newly created abasic site is excised and processed by **APE1** to generate a 3’-hydroxyl site. This 3’-hydroxyl is then used by DNA polymerase to fill the gap using the opposing strand as template.

FIGURE 1. DNA damage repair



- (A) Overview of the major DNA repair pathways. The Fanconi anemia core complex consists of FANCA, FANCB, FANCC, FANCD, FANCE, FANCF, FANCG, and FAAP100. The BTR complex consists of BLM, TOPOIII, RMI1, and RMI2. (B) Molecular targets within the DDR pathways and available inhibitors.

Nucleotide excision repair

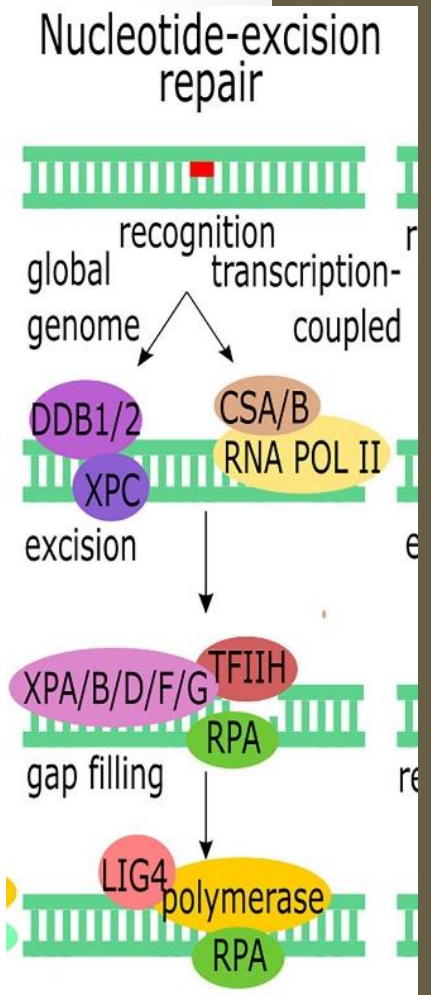
- Nucleotide excision repair is the main pathway for the removal of bulky lesions but can also remove **intrastrand crosslinks and pyrimidine dimers** that are produced by UV radiation.

Two subpathways can be distinguished:

- Global genome NER (GGNER) for the whole genome
- Transcription-coupled NER (TC-NER) for the transcribing strand of active genes

- GG-NER recognizes distortions of the DNA helix through DDB1, DDB2, and XPC,
- In TCNER, CSA and CSB recognize blockage of the RNA polymerase.

- TFIIH opens up the DNA to enable XPD to verify the lesion upon which several other XP endonucleases and RPA are recruited to excise the lesion. Finally, the resulting **22–32 nt long gap** is filled and ligated to the original DNA strand by DNA polymerases and ligases.



MMR pathway

- The MMR pathway removes single nucleotide mismatches and small insertions or deletions created by DNA polymerase during DNA synthesis.
- The lesions are recognized by the heterodimer MSH2/MSH6. The dimer recruits another heterodimer, MLH1–PMS2, and together they recruit several other proteins including Exo1 to excise the damage.
- Finally, polymerase eta or delta fills the newly created gap.

Interstrand crosslinks

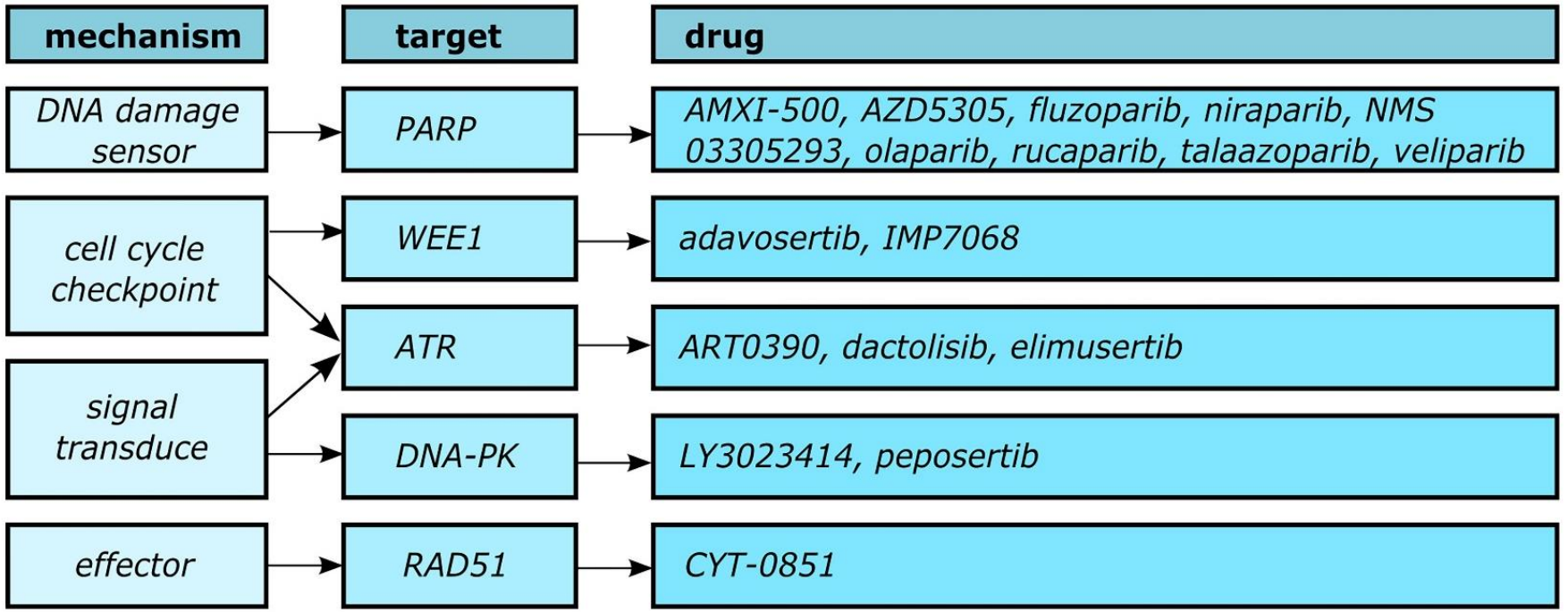
- Interstrand crosslinks (ICLs) are caused by bifunctional alkylating agents that form covalent bonds between the two DNA strands.
- In **quiescent cells** the lesion is recognized and **repaired by the NER** pathway, but during the **S phase several steps take place to activate the HR pathway.**
- When a DNA replication fork encounters an ICL the fork stalls and, through a complex containing FANCM, the lesion is recognized and the Fanconi anemia complex and BTR complex are recruited.
- These complex create a **double-strand break (DSB**) which is subsequently recognized and repaired by the HR pathway.

Double-strand breaks are repaired through two main pathways: HR and NHEJ

- HR can take place during the **S- and G2-phase of the cell cycle** when it can use the homologous sequence of a sister chromatid to accurately repair the break.
- The DSB is recognized by the **MRN complex (consisting of MRE11, RAD50, and NBS1**), the ends of the break are resected.
- Next, **BRCA2** recruits **RAD51** to replace the RPA-filament and, assisted by several other proteins, homology search and **strand invasion of the sister chromatid** takes place. Using the sister chromatid as template, polymerase delta synthesizes the missing nucleotides of the broken strand and the ends are ligated.
- **NHEJ**, in contrast, can take place during **every phase of the cell cycle** and is quicker than HR but is also error-prone and commonly results in small deletions.
- The break is recognized by the ku70/80 heterodimer which subsequently recruits DNA-PKcs, XLF, XRCC4, and Lig4 to process and ligate the broken ends of the DNA strands.

Molecular targets within the DDR pathways and available inhibitors.

B



- In recent years, important progress has been made in deciphering the molecular underpinnings of PDAC due to the unparalleled power of **next generation sequencing (NGS)** technologies.

Germline Mutations

- Approximately 10% of all PDAC cases are considered familial; defined as a family with at least two first-degree relatives with PDAC.
- While several germline pathogenic alterations that increase an individual's lifetime risk of PDAC (e.g., hereditary pancreatitis and Lynch syndrome) have been characterized, the **causative germline mutation of most familial cases remains unclear** (Klein, 2012).
- The most commonly mutated genes in **familial pancreatic cancer** are BRCA2, CDKN2A, BRCA1, and PALB2 (Perkhofer et al., 2020).
- **Pathogenic germline alterations** have also been identified in **patients who do not meet criteria for familial PDAC**, and may involve genes beyond those previously associated with hereditary pancreatic cancer.
- These pathogenic germline alterations are therapeutically considered actionable in 5–10% of patients, and clinical guidelines now support routinely offering **germline genetic testing with a broad panel of known hereditary cancer predisposition genes** to all PDAC patients.

Somatic Mutations in **GENIE cohort**

- The presence of DDR gene mutations has been reported in **17–43%** of all sporadic PDAC patients (Waddell et al., 2015; Aguirre et al., 2018).
- However, these papers focused on a limited selection of well-characterized DDR genes and potentially actionable DDR mutations may be more prevalent.
- We queried the **GENIE cohort (The AACR Project GENIE Consortium, 2017)** containing 3706 PDAC patients with somatic mutation profiling for the presence of mutations in **any of the genes of the six major DDR pathways (BER, NER, HR, NHEJ, ICL repair, and MMR).**
- A comprehensive list **of 352 genes** was collected based on the gene lists of the respective pathways in the Gene Ontology database. Mutations were reported in 117 (33%) of the genes, with 46 (13%) and 14 (4.0%) genes being mutated in more than 1 and 2% of the patients, respectively.
- The most commonly mutated genes were TP53 (68.9%), BRCA2 (4.4%), ATM (4%), and PRKDC (3.9%).

AACR PROJECT GENIE: POWERING PRECISION MEDICINE

Precision medicine requires an end-to-end learning health care system, wherein the treatment decisions for patients are informed by the prior experiences of similar patients. Oncology is currently leading the way in precision medicine because the genomic and other molecular characteristics of patients and their tumors are routinely collected at scale. A major challenge to realizing the promise of precision medicine is that no single institution is able to sequence and treat sufficient numbers of patients to improve clinical-decision making independently. To overcome this challenge, the AACR launched Project GENIE (Genomics Evidence Neoplasia Information Exchange).

AACR Project GENIE is a publicly accessible international cancer registry of real-world data assembled through data sharing between 19 of the leading cancer centers in the world. Through the efforts of strategic partners Sage Bionetworks and cBioPortal, the registry aggregates, harmonizes, and links clinical-grade, next-generation cancer genomic sequencing data with clinical outcomes obtained during routine medical practice from nearly every cancer patient treated at these institutions. The consortium and its activities are driven by openness, transparency, and inclusion, ensuring that the project output remains accessible to the global cancer research community for the benefit of all patients. AACR Project GENIE fulfills an unmet need in oncology by providing the statistical power necessary to improve clinical decision-making, particularly in the case of rare

TABLE 1 | Prevalence of the top 25 most frequently found somatic DDR gene mutations and associated pathways in a cohort of 3706 PDAC patients.

Gene	Mutation frequency (%)	Affected pathways (respective GO term)					
		HR (GO:724)	NHEJ (GO:6303)	BER (GO:6284)	NER (GO:6289)	ICL repair (GO:36297)	MMR (GO:6298)
TP53	68.90				x		
BRCA2	4.40	x			x		
ATM	4.00		x				
PRKDC	3.90		x				
MCM4	3.50	x					
NIPBL	3.20	x					
POLQ	3.20	x	x	x			
RIF1	3.10	x	x				
WRN	2.40	x		x			
FAAP100	2.40					x	
FANCD2	2.40					x	
ERCC6	2.20	x	x	x	x		
EP300	2.10				x		
RECQL4	2.00	x					
HELQ	1.90	x					
CUL4A	1.80				x		
ARID2	1.80	x					
FANCM	1.80	x				x	
FANCA	1.80					x	
PAXIP1	1.70		x				
FAN1	1.60	x			x	x	
BRCA1	1.60	x	x				
MUS81	1.60	x				x	
SETD2	1.60	x					x
ATR	1.60					x	

HR, homologous recombination; NHEJ, non-homologous end-joining; BER, base-excision repair; NER, nucleotide-excision repair; ICL repair, interstrand crosslink repair; MMR, mismatch repair.

- Currently only gBRCA is used in the clinic as biomarker for sensitivity to PARP inhibition (PARPi) olaparib.

- Our query of somatic DDR mutations found that BRCA2 is mutated in 4% of all PDAC patients indicating that a larger group of patients may benefit from targeted therapy.

- In addition, multiple clinical trials are recruiting patients for treatment with DDR inhibitors based on a larger selection of DDR mutations, including but not limited to **PALB2, CHEK2, ATM, and RAD51**.

Synthetic lethality

- The relatively high prevalence of DDR gene mutations opens up opportunities for targeted therapies based on the **synthetic lethality principle**: tumors with a **DDR pathway deficiency** are more dependent on **alternative DNA repair pathways** to repair double-stranded DNA breaks.

- Synthetic lethality has been applied successfully in cancers harboring BRCA1/2 mutations (homologous repair pathway) by treating them with PARP inhibitors (PARP is involved in the single-strand break repair pathway by BER).

- **Unrepaired single-strand breaks will turn into DSBs** during DNA replication which will accumulate to the point of cell death due to the HR deficiency.

DNA Damage Repair Pathways Genomic Profiling/Biomarkers

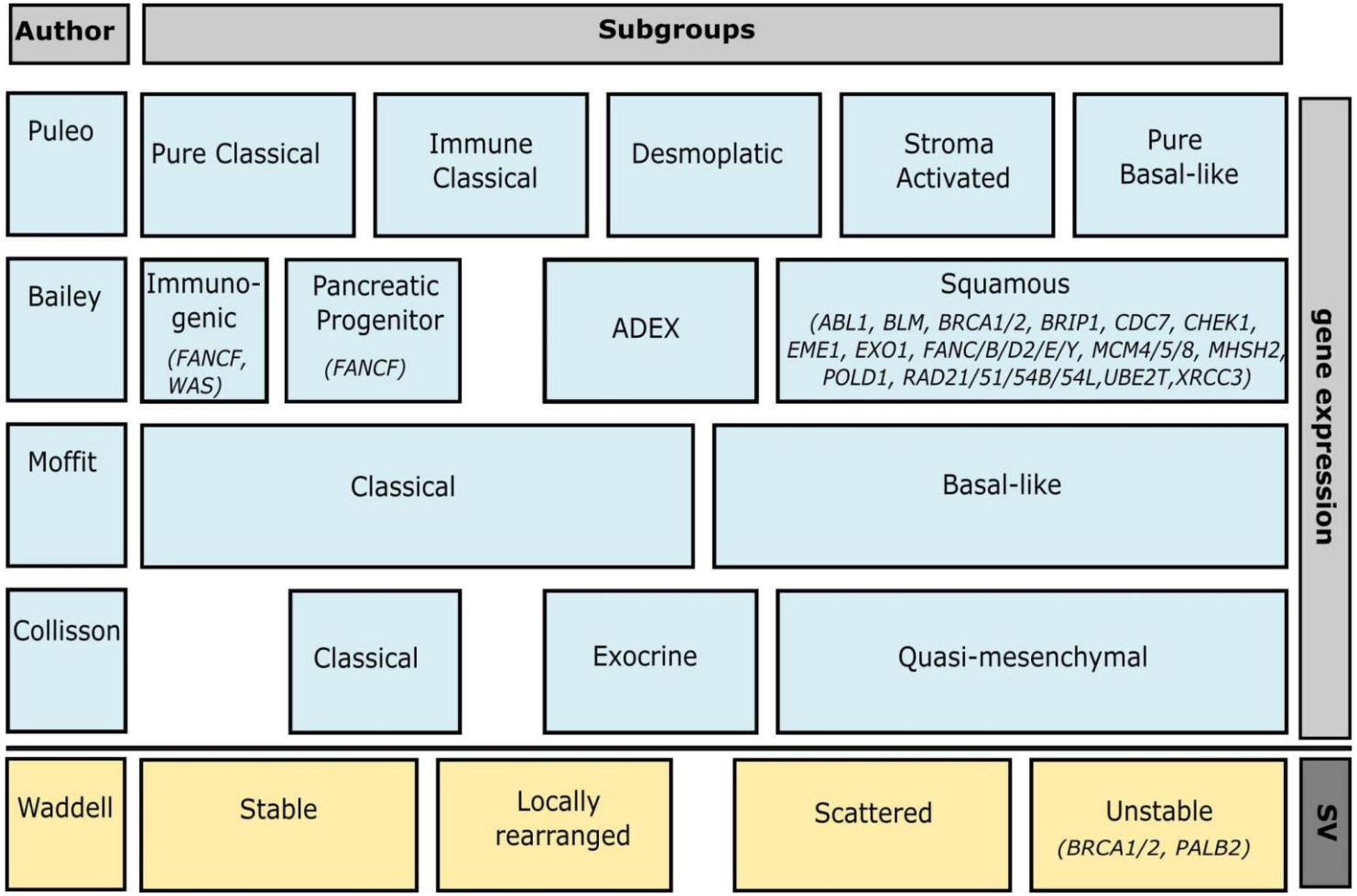
- Multiple research groups have performed **next-generation sequencing and expression profiling** to classify molecular PDAC subtypes that can be used to tailor therapies and guide clinical decision making (Collisson et al., 2011; Moffitt et al., 2015; Bailey et al., 2016; Puleo et al., 2018).

- Bailey et al. (2016) defined four PDAC subtypes (immunogenic, pancreatic progenitor, aberrantly differentiated endocrine exocrine (ADEX), and squamous) based on 10 discriminatory gene programs found by transcriptional network analysis.

- Over 50 DDR genes were included in the gene program “proliferation” which is associated with the squamous subtype. Functionally, the squamous subtype is associated with histological adenosquamous carcinoma and a poor survival.

- The classifications by Collisson et al. (2011), Moffitt et al. (2015), and Puleo et al. (2018) found no associations with DDR deficiency.

Figure 2. Overview of genomic pancreatic subtypes and how they overlap.



- Associated DDR genes of which mutations have been found in PDAC patients are included under their respective subtypes.
- Associated DDR genes not mutated in patients include CDC45, FEN1, GINS2/4, MAD2L2, MCM2/3/6/7, RMI2, RPA3, TIMELESS, HMGB2, POLA1, LIG1, DNA2, RDC2/3/4/5, PCNA, COPS5, BRIP1, HMGA2M, CETN2, UBC, TP73, PSMD14, POLR2D, and CDK7 for Bailey's squamous subtype, and USP7 for Bailey's Pancreatic Progenitor subtype.

DDR deficiency and prognosis

- Already, DDR deficiency has been associated with a **significantly better patient survival** compared to DDR proficiency independently of tumor subtype classification (Zimmermann et al., 2021).

- A small retrospective study in 36 patients treated with first-line FOLFIRINOX in a metastatic setting found that **DDR deficiency**, as based on a 14-gene panel, was significantly associated with **improved survival** ($p = 0.04$) (Sehdev et al., 2018).

- A similar retrospective study in 40 patients with metastatic PDAC treated with first-line platinum chemotherapy in combination with FOLFIRINOX was published a year later (Palacio et al., 2019). Based on a 35-gene panel, the patients with DDR deficiency had a significantly **longer progression-free survival (PFS)** (18.5 vs. 6.9 months, $p = 0.003$), with a trend toward superior median OS as well.

- Further research is needed to confirm these findings in a larger cohort and to investigate whether DDR deficiency is associated with response to FOLFIRINOX treatment or OS in general.

Preclinical Models

- Despite the promising results for many targeted therapies in other solid tumors such as breast, lung, and colon, the use of targeted therapies in PDAC has had limited survival benefit in the clinic.
- Target discovery and successful development of targeted therapies is highly dependent on the relevance of the preclinical models used and therapies frequently fail at the transition to clinical trials.
- This review will extend upon published literature by focusing on the application of these models to further target DDR pathways.

Cell Lines

Cell lines remain the most commonly used preclinical model for cancer research:

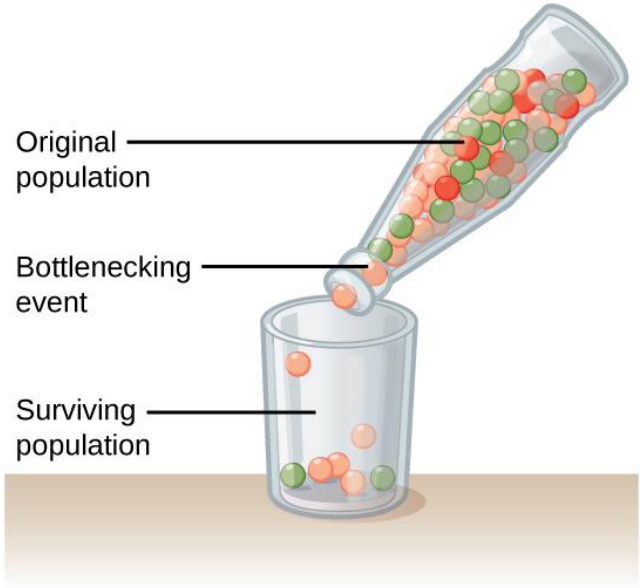
- They are readily available
- Most commercial cell lines are well characterized.

The main advantages of cell lines:

- Cheap
- Require little maintenance
- Are easy to manipulate
- More homogenous than other preclinical models, thus contributing to a better reproducibility (well-suited for high throughput drug screening)

- However, this also means that cell lines lack the complexity and heterogeneity typical of tumors.

Disadvantages of 2D culture



- Clonality and adaptation to 2D culturing conditions as well as immortalization and repeated passaging can all contribute to **genomic drift** which can significantly affect drug responses.

Other disadvantages of 2D culture:

- Loss of part of the normal 3D morphology, cell polarity, and cell–cell or cell–stroma interactions, especially the interaction with cancer-associated fibroblasts (CAFs) and immune cells.

Some of these disadvantages can be resolved or diminished by adapting the culture methods (other disadvantages are inherent to the model system itself):

- Using early passage patient-derived cell lines (PDCLs) instead of established cell lines
- Co-culturing with fibroblasts

BRCAness and sensitivity to PARPi in PDAC cell lines

- DNA damage repair pathway deficiency has been shown to confer sensitivity to PARPi.
- Multiple studies found that the BRCA2-deficient cell line Capan-1 is **significantly more sensitive to several PARP inhibitors and cisplatin**, but not to gemcitabine, compared to the BRCA2-proficient cell lines MiaPaCa-2 and Panc-1 (Porcelli et al., 2013; Andrei et al., 2015; de Soto, 2020).
- **Restoration of BRCA2 expression in Capan-1** cell lines: was shown to reduce sensitivity to olaparib and HYDAMTIQ (Mini et al., 2017; Sullivan-Reed et al., 2018).
- **shRNA-mediated knockdown of BRCA2** in **Panc-1 cells:** impaired homology-directed repair and conferred sensitivity to BMN-673 (but not to veliparib) (Andrei et al., 2015).
- Increased sensitivity to PARPi (olaparib, BMN-673, and rucaparib) and cisplatin has been found in **DDR deficient PDCLs** (Dreyer et al., 2021).

- Acquired resistance is a problem in many cancer treatments. Likewise, long-term treatment of Capan-1 cells with low dose PARPi can induce resistance, including cross-resistance to other PARPi and cisplatin. Several mechanisms have been suggested for the development of resistance in Capan-1 BRCA2- deficient cell line, the simplest being the restoration of BRCA2 expression.

How DDR status correlates with sensitivity to other DDR genes (ATR, WEE1, etc)?

Sensitivity to the WEE1 inhibitor and DDR status

- Sensitivity to the WEE1 inhibitor AZD-1775 has been evaluated in multiple studies, but due to contradicting findings its role in **DDR deficiency remains unclear**.

- Two studies found **that Capan-1 is more sensitive to AZD-1775** than other (PDAC) cell lines, suggesting that BRCA2 deficiency might play a role (Dréan et al., 2017; Parsels et al., 2018).

- On the other hand, Lal et al. (2016) investigated sensitivity to AZD-1775 in a panel of nine PDAC cell lines and reported a medium sensitivity for Capan-1.
- In addition, they found that knockdown of BRCA2 by siRNA in MiaPaCa-2 and PL5 induced **resistance** to AZD-1775.

- These contradicting findings highlight the need for further investigation.

ATR inhibitors and DDR status

- The application of ATR inhibitors in PDAC has been investigated in multiple in vitro studies in both human PDAC cell lines and mouse KPC and KPCB cell lines but so far drugs have shown limited potential and **sensitivity to treatment does not correlate with the DDR status** (Wallez et al., 2018; Elliott et al., 2019; Dreyer et al., 2021).

- However, multiple studies have found that ATRi (VE-821 and VE-822) **sensitizes to gemcitabine and radiotherapy** through impairment of the DNA repair (Fokas et al., 2012; Wallez et al., 2018).

- siRNA knockdown of another major signal transducer, ATM, in combination with ATRi in MiaPaCa-2 was able to prevent gemcitabine-induced activation of ATR completely (Wallez et al., 2018), suggesting that **ATM mutant tumors may be especially sensitive to this combination treatment.**

- Combination treatment with chloroquine, an autophagy inhibitor that is used in the treatment of malaria, significantly reduced proliferation in 24 or 17 out of 26 tested PDAC cell lines compared to VE-822 (ATRi) or chloroquine alone (Elliott et al., 2019).

DDR and sensitivity to gemcitabine

- Azorsa et al. (2009) used an RNAi screen to identify which genes, when silenced, sensitized pancreatic cancer cells to gemcitabine:

- Silencing of CHK1 was found to be most effective and was further validated with additional siRNAs and two small molecule inhibitors (SB218078 and PD407824) in MiaPaCa-2 and BxPC3 cell lines.

DDR targeted therapy is not inherently cytotoxic

- A disadvantage of DDR targeted therapy is that it **is not inherently cytotoxic.**

- By **inhibiting multiple DNA repair genes** the **cancer cells will accrue DNA damage**, but whether this results in cell **death or senescence depends on additional factors**, such as the proliferation rate and how well the cells tolerate replicative stress.

- **Combination treatment** with chemo- or radiotherapy can increase the anti-tumor effect by inducing additional DNA damage (Porcelli et al., 2013).

- Perkhofer et al. (2017) generated stable mouse cell lines from tumors with pancreas-specific loss of Kras (KC), and Kras and Atm (AKC). Atm-deficient AKC cells showed a significant increase in DNA damage markers 53BP1 and gammaH2AFX upon treatment with 5 Gy of ionizing radiation compared to KC cells ($p < 0.03$), indicating impaired DSB repair, and had decreased proliferation.
- No significant differences were observed in sensitivity to cisplatin, 5-FU, or gemcitabine.
- Treatment with olaparib or niraparib reduced viability in an Atm dependent manner and was potentiated by combination with gemcitabine or radiation ($p < 0.01$).

- The cell lines used for DDR pathway studies in PDAC are mainly limited to Capan-1 as model for DDR/BRCA2-deficiency, and MiaPaCa-2, BxPC-3, and Panc-1 for DDR-proficiency.

- Studies in additional cell line models are required to analyze the role of the DDR pathways in more depth.

- **Table 2** provides an overview of the mutations found in the 10 most frequently mutated DDR genes (excluding TP53) for all PDAC cell lines found in the **Cancer Cell Line Encyclopedia.**

- **Twenty of the 46 cell lines** had a mutation in one or more of the investigated genes, of which three (**Capan-1, PL18, and SNU-324) had a mutation annotated as pathogenic or likely pathogenic** in the **ClinVar or COSMIC database**.

Table 2. Mutation status of DDR genes in PDAC cell lines.

Cell line	Gene	Nucleotide change	Protein change	ClinVar	COSMIC FATHMM
BXPC3	*POLQ*		p.ILL1421fs	n/a	n/a
Capan-1	*BRCA2*	c.5946del	p.S1982fs	Pathogenic	n/a
	ATM	c.4755A>C	p.R1585S	n/a	n/a
CFPAC1	*PRKDC*	c.1945T>C	p.F649L	Uncertain significance	n/a
HPAC	*NIPBL*		p.T735I	n/a	n/a
HuP-T3	*BRCA2*	c.6131G>T	p.G2044V	Benign/likely benign	n/a
	NIPBL		p.1532_1532E>DK	n/a	n/a
KP2	*PRKDC*		p.G2261S	n/a	n/a
	FAAP100		p.K333R	n/a	n/a
MZ1PC	*PRKDC*		p.W1355C, p.W1355I, p.F1028V	n/a	n/a
Panc-02.03	*POLQ*		p.L1430fs	n/a	n/a
Panc-03.27	*ATM*	c.7052A>G	p.E2351G	Uncertain significance	n/a
Panc-04.03	*NIPBL*		p.S2389I	n/a	n/a
Panc-08.13	*ATM*		p.F1234S	n/a	n/a
PATU8988S/T	*ATM*		p.R919M	n/a	n/a
PK-45H	*POLQ*		p.G2225R	n/a	n/a
PK-59	*BRCA2*	c.6131G>T	p.G2044V	Benign/likely benign	n/a
PL18	*NIPBL*	c.3G>T	p.M1I	Pathogenic	n/a
			p.S1517*	n/a	n/a
PL4	*RIF1*	c.1331C>T	p.A444V	n/a	Neutral (0.12)
PSN1	*NIPBL*		p.K601fs	n/a	n/a
SNU-324	*BRCA2*	c.7480C>T	p.R2494*	Pathogenic	n/a
	ATM		p.Q2809fs	n/a	n/a
	MCM4	c.1579G>A	p.V527I	n/a	Pathogenic (0.96)
SW-1990	*NIPBL*		p.K1180*	n/a	n/a
TCCPAN2	*POLQ*		p.R6P	n/a	n/a

*Representation of the top 10 (excluding TP53) most frequently mutated DDR genes in PDAC cell lines. The genes WRN and FANCD2 were not found to be mutated in any PDAC cell line. When available the pathogenicity status/score is included in the table. The * indicates that the mutation results in an early terminated gene product. n/a, not available.*

CCLE Cancer Cell Line Encyclopedia

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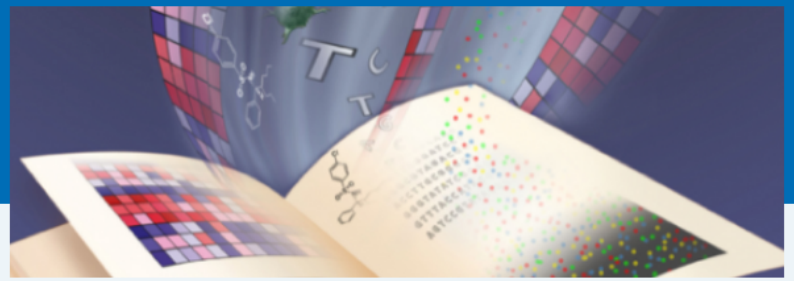


Motivations for the Cancer Cell Line Encyclopedia (CCLE)

Cancer cell lines are the most commonly used models for studying cancer biology, validating cancer targets and for defining drug efficacy. Prior to the CCLE, cell line investigations were limited to a few commonly used cell lines or at most the 60 cell lines of the NCI60 panel. For example, at the time of the discovery of EGFR mutations in lung cancer, EGFR inhibitors had been developed using a single cell line, A549 as the EGFR-inhibitor sensitive model. This starkly contrasts with the number of patients (n=952) treated on the initial phase III trials of EGFR inhibitors. Hence, the profound sensitivity of cancers bearing activating EGFR mutations was initially missed, at least in part due to the lack of large-scale, robust well-defined cancer cell line models. As The Cancer Genome Anatomy (TCGA) project embarked on the efforts to define the genetic basis of human cancers it was clear that a similar effort would be required to characterize the cancer cell lines.

Initial forays into the large-scale genetic and chemical characterization cancer cell lines

With the advent of high-density SNP arrays, the Sellers lab undertook the genetic characterization of NCI60 cell lines using high density SNP arrays. Intersecting the SNP-array derived copy-number and LOH data with mRNA expression date generated by the NCI60 cell line team led to the discovery of novel amplification events in melanoma targeting the MITF transcription factor.

Following this work NCI60 cell line genomic DNA was subjected to mutation specific genotyping to identify known oncogenic mutations in K-RAS and other oncogenes. This data along with the published BRAF mutation data was used to search for selective compound sensitivities among the 42,796 compounds for which the −log10(GI50)) was available from the NCI60 profiling efforts. Here, several MEK inhibitors were found to have markedly increased anti-proliferative activity in BRAF mutant melanoma cells. In short, BRAF mutation predicted sensitivity to MEK inhibition a finding later confirmed in phase III trials. In aggregate, these data suggested that larger-scale genetic characterization of the cancer cell lines coupled to compound or other cell perturbations might unveil predictive drug sensitivities in cancer.

Integrative genomic analyses identify MITF as a lineage survival oncogene amplified in malignant melanoma
Nature 2005;436(7047):117-122. DOI:10.1038/nature03664

BRAF mutation predicts sensitivity to MEK inhibition
Nature 2006;439(7074):358-362. DOI:10.1038/nature04304

The Cancer Cell Line Encyclopedia Project - A collaboration between the Broad Institute and the Novartis Institutes for Biomedical Research

In 2006 Sellers (Novartis), Garraway (Broad Institute) and Schlegel (Novartis) crafted the initial project plan for large-scale genetic characterization of ~1000 cancer cell lines. This project was subsequently renewed on two occasions and hence we think of these as the three phases of the CCLE project.

Phase I of the Cancer Cell Line Encyclopedia project

Initiated in January 2008, the **overarching goals** of this collaboration were: 1) to conduct a detailed genetic and pharmacologic characterization of a large panel of human cancer models; 2) to develop integrated computational analyses that link distinct pharmacologic vulnerabilities to characteristic genetic, gene expression, and cell lineage patterns; and, 3) to translate cell line integrative genomics into cancer patient stratification.

Accordingly, the team set out to generate the following datasets from comprehensive genetic characterization of 1000 human cancer models. In phase I, the collective teams acquired 1000 cell lines directly from the relevant publicly accessible cell line repositories including ATCC (American Type Culture Collection), DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen) and the KCLB (Korean Cell Line Bank). Thus, the genomics data generated are as close to the repository cell line derivatives as we could achieve.

After expansion of each cell line, DNA and RNA was extracted and used to generate Affymetrix SNP 6.0 data, Affymetrix U133 2.0+ expression array data

depmap portal

Search for a gene, cell line, compound, lineage, or file

Tools Downloads About Forum

CCLE data is hosted on the Cancer Dependency Portal (DepMap) where it has been harmonized with our genomics and sequencing data. You can access the latest and all past datasets here.

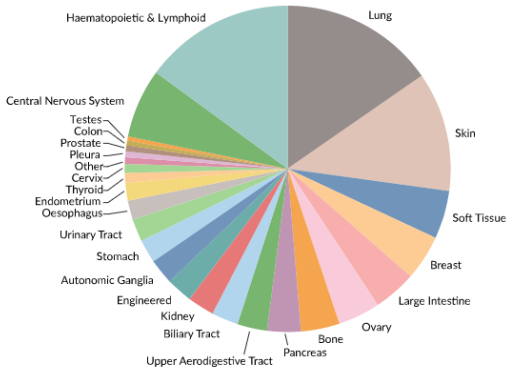
CCLE Cancer Cell Line Encyclopedia

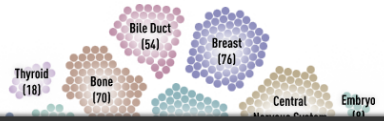
View CCLE data by searching for gene, cell line or lineage on the DepMap portal

Examples: BRAF, afatinib, lung

Cancer Cell Line Encyclopedia (CCLE)

The Cancer Cell Line Encyclopedia (CCLE) project started in 2008 as a collaboration between the Broad Institute, and the Novartis Institutes for Biomedical Research and its Genomics Institute of the Novartis Research Foundation. The goal is to conduct a detailed genetic and pharmacologic characterization of a large panel of human cancer models, to develop integrated computational analyses that link distinct pharmacologic vulnerabilities to genomic patterns and to translate cell line integrative genomics into cancer patient stratification. Later the MD Anderson and Harvard Medical school joined the project. As of summer of 2018 CCLE continues its efforts as part of the Broad Cancer Dependency Map Project.





Select Publications

Mahmoud Ghandi, Franklin W. Huang, Judit Jané-Valbuena, Gregory V. Kryukov, ... Todd R. Golub, Levi A. Garraway & William R. Sellers. 2019. Next-generation characterization of the Cancer Cell Line Encyclopedia. *Nature* 569, 503–508 (2019). https://doi.org/10.1038/s41586-019-1186-3

Cancer Cell Line Encyclopedia Consortium, and Genomics of Drug Sensitivity in Cancer Consortium. 2015. Pharmacogenomic Agreement between Two Cancer Cell Line Data Sets. *Nature* 528 (7580):84–87. https://doi.org/10.1038/nature15736.

Jordi Barretina, Giordano Caponigro, Nicolas Stransky, Kavitha Venkatesan, ... William R. Sellers, Robert Schlegel, & Levi A. Garraway. 2012. The Cancer Cell Line Encyclopedia Enables Predictive Modelling of Anticancer Drug Sensitivity. *Nature* 483 (7391):603–7. https://doi.org/10.1038/nature11003

Processed data downloads are available in the DepMap portal data page. Raw sequencing data for the 2019 publication is available through the Sequence Read Archive under accession number PRJNA523380.

Contact us

For questions about the CCLE project and data please use the DepMap forum.

- Apart from Capan-1, SNU-324 is the only other available cell line with a suspected deleterious BRCA2 mutation (Ku et al., 2002).

- SNU-324, established in 2001, is derived from a poorly differentiated primary pancreatic tumor of a 50-year-old male. SNU-324 does not contain mutations in KRAS or TP53, but is microsatellite instable (Ku et al., 2002). Despite its usefulness for BRCA2-deficiency studies no other publications are available which have used this cell line.
- Therefore there is a need for additional well-defined BRCA2-deficient PDAC cell lines.