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Review Article

Advances in Next-generation Sequencing Technology: A powerful tool for early detection of somatic mutations in Pancreatic Cancer

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ABSTRACT

Pancreatic cancer is a major contributor to the cancer-related mortality rate. The lack of prominent biomarkers is one of the major limitations in the early diagnosis of pancreatic ductal adenocarcinoma (PDAC). Next-generation sequencing (NGS) has led to a major transformation in the field of oncology by easing the process of data collection, pipeline analysis, data interpretation and cost-effectiveness. NGS, high-throughput screening detection and identification of prognostic markers such as KRAS, SMAD4, TP53, CDKN2A and somatic mutations have been the game-changer in the field of Clinical Oncology which can allow early detection, personalised treatment and progressive therapy programs in PDAC. The current review gives an overview of NGS technology and its advancement in understanding the genetics of pancreatic cancer. The unique features of somatic mutation in PDAC, various case studies and different tools to help choose appropriate pipeline for specific analysis, clinical applications, their limitations and future aspects have been highlighted.

Keywords: Next-generation sequencing, early detection, Personalised treatment, Clinical oncology, Prognostic markers, Somatic mutation

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is one of the major causes of death due to cancer, and it has been consistently associated with a poor prognosis[68]. It is the 24thmost common disease in India, with 10860 new cases (1.0per cent) and the 18thhighest fatality rate. In the United States, 60,430 cases were registered in the year 2020 (28,480 females and 31,950 males). The five-year survival rate of pancreatic cancer is as low as 10%. Several factors influence the survival rates, inclusive of time and stage of illness diagnosis.

One of the major constraints at present is the lack of acceptable biomarkers for screening; as a result, most of the patients arrive with advanced disease. Surgical resection is the sole curative option for PDAC; however, only 10% of patients have the resectable disease. The majority of patients with an experience of curative recurrence have a five-year survival rate of 20% to 25%. The emergent requirement for enhanced alternative techniques and methods to enhance the survival rate is crucial as no significant improvement in surgical procedures is reported. Novel methods for screening and diagnosis of PDAC will improve the number of eligible patients for curative resection. PDAC management will be transformed by adjuvant therapy to improve postoperative survival in curative resections

and palliative illness patients. The field of genomics and bioinformatics has grown vastly in the recent decade as a result of technical breakthroughs, resulting in the discovery of a range of biomarkers for both malignant and noncancerous illnesses[64]. Next-generation sequencing (NGS) can sequence genetic segments rapidly and precisely, providing the basis for the identification of many biomarkers. For PDAC, NGS is still in its infancy, with need for the various potential novel targets[66].

BACKGROUND OF NEXT GENERATION SEQUENCING

Next-generation sequencing (NGS) has ushered in a substantial shift in clinical diagnosis. It refers to a group of methods in which at the same point in time several sequencing reactions take place, which results in massive quantities of sequencing data at a cost less than Sanger sequencing. Sequencing at the Base-pair level of the whole genome at a very low cost can be done using NGS methods (Figure 1). Centred on the theory of Sanger sequencing, the Human Genome Project calculated the sequence of 3 billion base pairs and found about 25,000 human genes, resulting in the publication of the human reference genome("Finishing the Euchromatic Sequence of the Human Genome," [21]). The ground-breaking work on DNA sequencing by Paul Berg, Frederick Sanger, and Walter Gilbert allowed for many advancements in the field, including Sanger's "chain-termination" sequencing technology[52, 60].

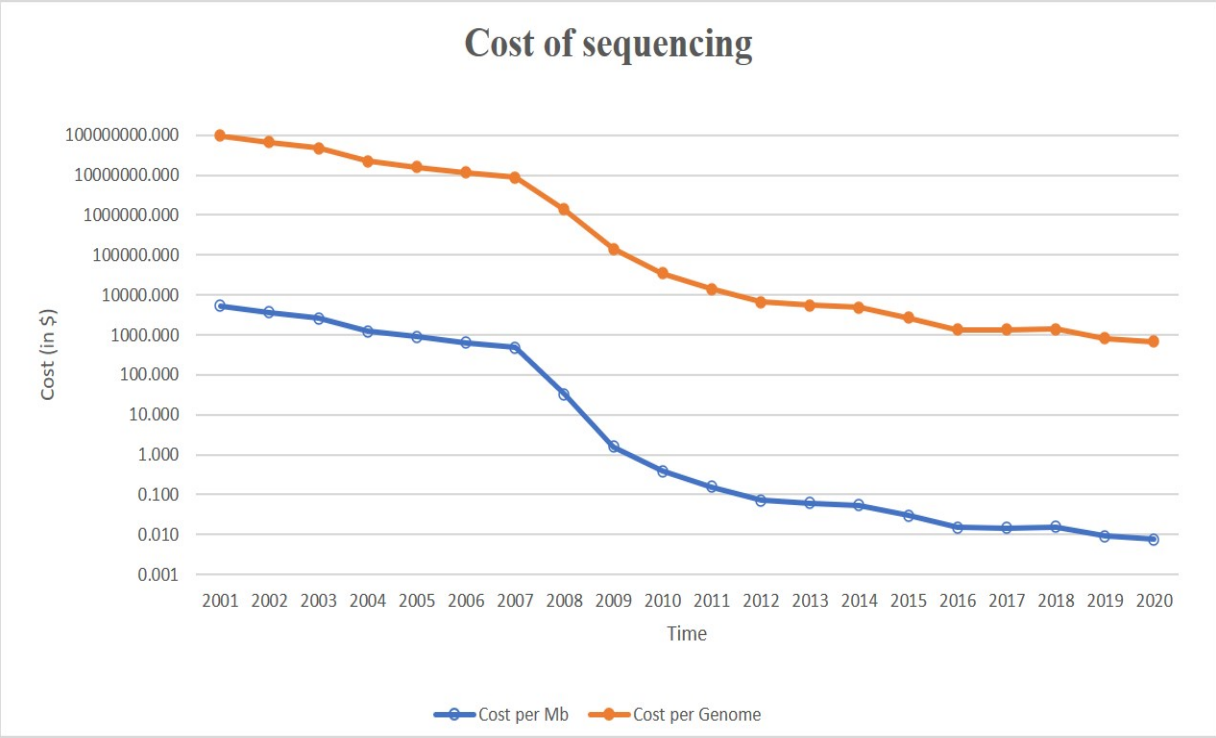

Figure1: Graphical representation of change in the cost of sequencing technology over time.

In the last two decades, next-generation sequencing of tumour and inherited (germline) genomes has revolutionised and refined cancer care, and it is now critical for assessing therapeutic options in many solid and hematologic malignancies. Timeline of sequencing technology and key events in oncology has been shown in figure 2. Currently, the most common form of rapidly detecting sequence variation in cancer patients is to use NGS panels that include sets of genes[23]. A variety of biomarkers in use today, such as mutations in BRAF in melanoma, EGFR mutations, and fusions of ALK in NSCLC, have been clinically validated and identified by NGS research. For example,

biomarkers for immunotherapy responsiveness, such as tumour mutational burden and microsatellite instability; therapeutic range for clinically actionable modifications, such as BRAF V600E in melanoma have been identified. Biomarkers for immunotherapy tolerance, such as loss of B2M [35] mutations in the p53 tumour suppressor gene or either of the RAS proto-oncogenes in lung cancer, cervical cancer, and HRAS [Harvey rat sarcoma viral oncogene homolog], NRAS [neuroblastoma RAS viral (v-Ras) oncogene homolog] and KRAS [Kristen rat sarcoma viral oncogene homolog][27]in breast cancer are reported. A wide range of genes has been studied in a single test using scarce biopsy tissue of patients using NGS[13].

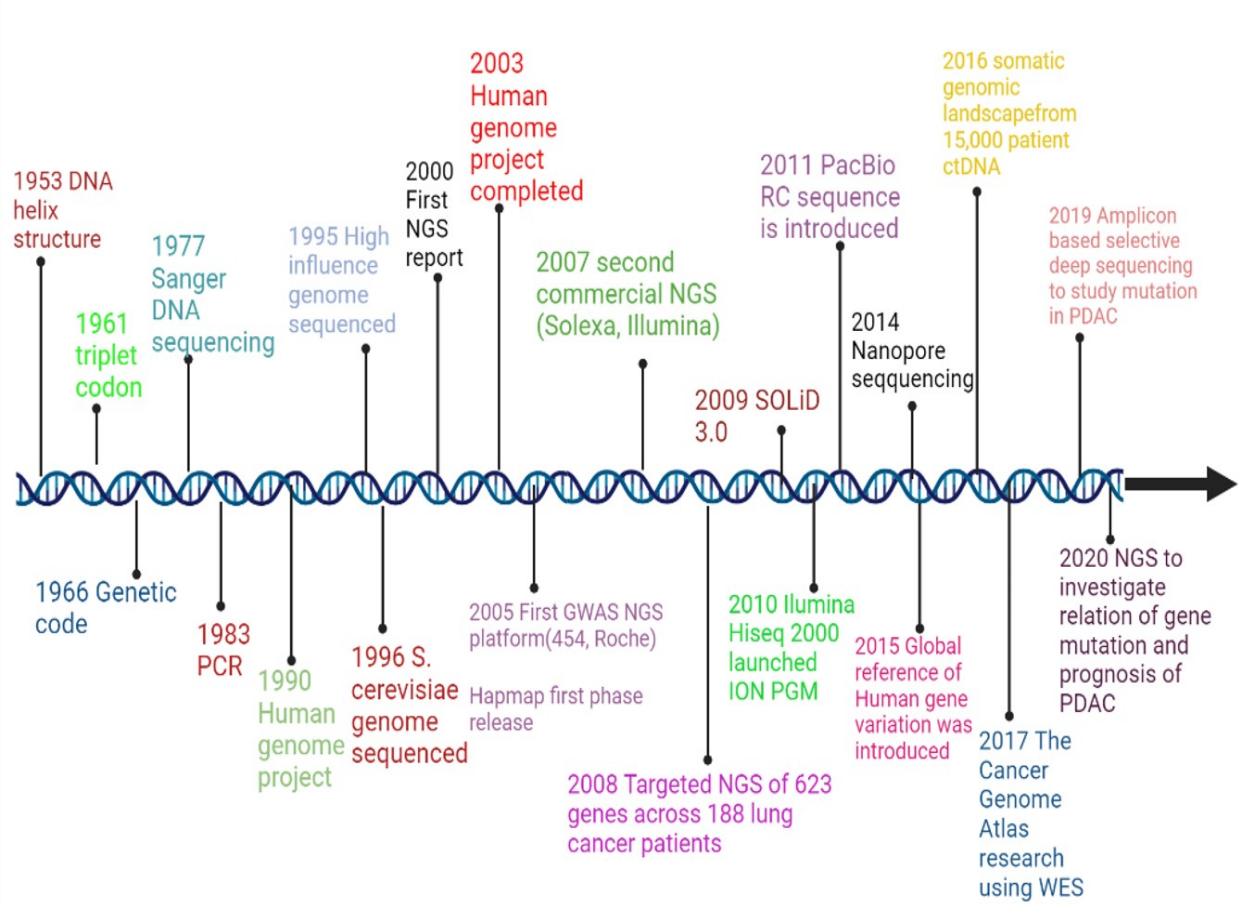

Figure2: Development of NGS technology over the years and key events in the oncology.

In the year 2005 The Cancer Genome Atlas (TCGA) and 2008 the International Cancer Genome Consortium (ICGC) were established to gain a thorough understanding of cancer genetics. TCGA comprises a detailed atlas of cancer genomic profiles of cancer patients containing details of major cancer-causing genome alterations in about 30 different human tumours produced by sequencing a patient's genome[82]. Oxford; Nanopore Technologies recently used the MinION nanopore sequencer to successfully sequence the human reference genome for the GM12878 Utah/Cephcell line. A total of 91.2 GB of sequence data was produced, from which significant structural variations and epigenetic modifications were discovered[31].

The use of paired tumour and normal samples improves variant calling fidelity, increases sensitivity in low-purity tumour samples, and clarifies germline mutation delineation at low costs[33]. This review focuses on the milestone in the research of next-generation sequencing science and its applications in

understanding somatic mutations in pancreatic cancer.

NEXT-GENERATION SEQUENCING

First generation sequencing or Sanger sequencing, was first introduced in 1977and is considered the gold standard for sequencing. It is based on the DNA chain termination theory method, which was later called second-generation sequencing or High throughput sequencing is a high-speed technology, and millions of short sequence reads can be produced with more precision[71], Illumina[3], Roche 454[51], and Bio-technologies/SOLiD[53]developed these. The most commonly used Illumina platform, allows researchers to sequence more than five human genomes at the coverage of 30x or about100 exome samples in a single go, generating a huge number of sequences reads with improved accuracy[48].Third-generation sequencing allows the sequence of a human genome in just a few hours. The nanopore and SMRT sequencing can do the direct examination of base modifications made in various types of DNA[43,62,73,80]. An overview of different steps and process involved in the sequencing is shown in figure 3.

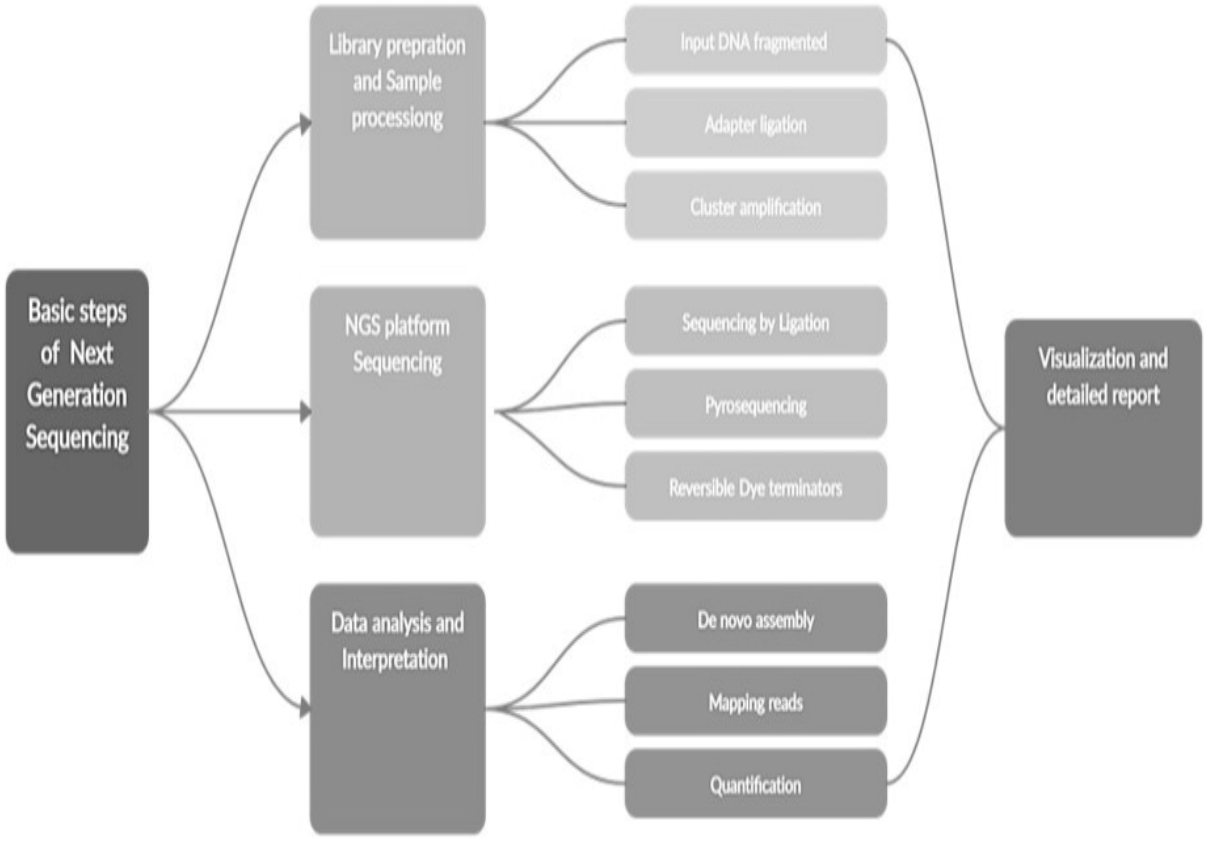

Figure3: Overview of different steps and stages of NGS process.

Samples for the analysis are collected either by clinical or physical assessment. The first step includes the pre-processing of data for the subsequent removal of poor-quality sequences. The second step involves the alignment of the selected sequence with a reference genome (de-novo assembled) followed by sorting and removal of duplicates. The third and last step of analysis involves variant annotation, variant filtering, prioritization and visualisation of data WES- whole exome sequencing, WGS- whole genome sequencing, and VCF- variant calling format (Figure 4).

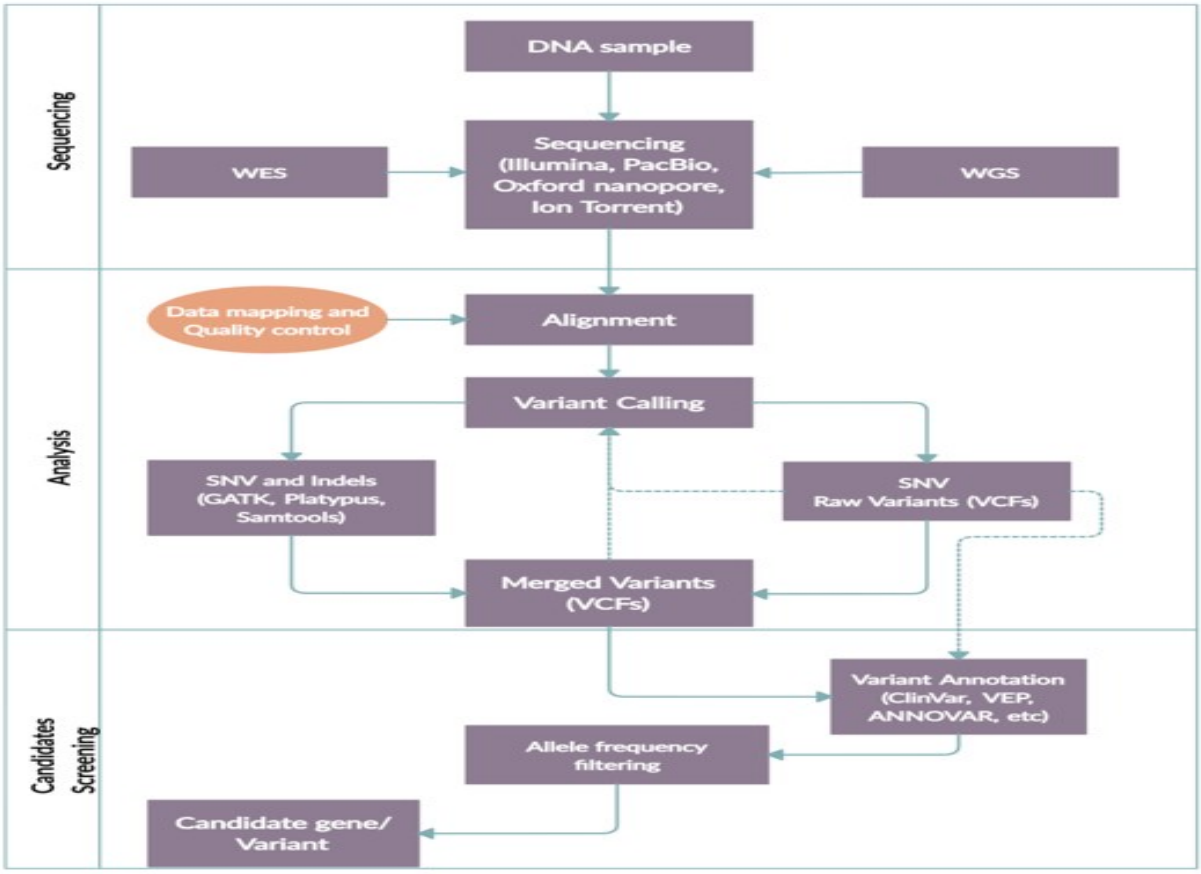

Figure4: Systematic depiction of NGS based workflow in variant calling step.

NGS IN SPORADIC PDAC

The discovery of robust biomarkers is critical for effective diagnosis and therapy in pancreatic cancer patients. However, there has been little improvement in biomarker-based preventive and therapeutic methods, with 5-year survival rates remaining poor, even in early, localised disease stages. Despite national and international organisations' recommendations encouraging PDAC patients to participate in clinical trials, progress in this field has been slow. More precisely, the absence of both successfully targeted drugs and biomarker-directed agents raises concerns about whether a strategy based on traditional clinical trial designs and traditional technology will change the course of the disease. The introduction of next-generation sequencing (NGS) technologies and the advancement of clinical trial designs that incorporate NGS have opened up new avenues for the specific biomarker-based prevention and treatment of pancreatic cancer[2,50]. Large-scale genomic studies have been reported that combine WGS and RNAseq. These studies have resulted in data indicating clinical implications on PDA prognosis and molecular classification[2].

Sporadic PDAC classified into Molecular Subtypes Using NGS

Researchers (Bailey et al., 2016) classified PDAC in a cohort of 456 categorized patients into four subtypes for supporting the presence and distribution of structural variations known by WGS and RNA-Seq, (1) squamous, (2) pancreatic progenitor, (3) immunogenic, and (4) aberrantly differentiated endocrine secretory organ (ADEX), correlating with histopathology features. The research conjointly stated that (1) TP53 and KDM6A mutations were enriched in squamous tumours and has a poor prognosis; and (2) FOXA2/3, PDX1, and MNX1 are among the genes involved in early pancreatic growth that pancreatic progenitor tumours preferentially

express. Immunogenic tumours showed up-regulation of immune networks, as well as pathways implicated in acquired immune suppression; and ADEX tumours had up-regulation of genes that influence KRAS activation networks. Their findings revealed that PDAC subtypes evolved differently in terms of molecular evolution.

Waddell et al[76] examined 100 patients with PDAC for copy number variation and WGS and also included reported PDAC genes (TP53, SMAD4, CDKN2A, ARID1A, and ROBO2). KDM6A and PREX2 were found as new potential driver genes. As per possible clinical utility based on variation chromosomal pattern, they divided PDAC into four subtypes (1) stable: tumour genomes that had structural variation events and extensive frequent aneuploidy less than 50. (2) locally rearranged: subtype that had a significant focal event on one or two chromosomes. (3) scattered: tumours with non-random chromosomal damage in the moderate range and less than 200 structural variation events and (4) unstable: tumours with more than 200 structural variation events. This study suggested that mutations involved in the BRCA pathway measure DNA maintenance defects, germline and somatic uncertainty in the BRCA mutational signature and have therapeutic associations in patients with PDAC. Such findings identify a potential biomarker that must be tested in a clinical trial.

In another research that sequenced 336 pancreatic cancer samples potentially actionable results were identified in 26% of the cases[46]. Eighteen patients (5.5%) had at least one somatic change identified as level 2b an FDA–approved biomarker in cancer. Ninety-five per cent of patients had KRAS mutations, 72 per cent had TP53 mutations, 22 per cent had SMAD4 mutations, and 18 per cent had CDKN2A mutations. Furthermore, 11% of patients had ARID1A mutations, 8% had RNF43 mutations, 4% had BRCA2, 4% had KDM6A mutations, 4% had MLL2 mutations, 4% had PTPRT mutations, and 4% had TGFBR2 mutations.

Brauswetter et al [4], identified molecular subtypes of PDAC by analysing target sequence data from 50 genes in 114 PDAC patients and used it to create molecular profiles of MIA-PaCa2, BxPC3, and PANC-1 cell lines. Only cells with the unusual G12C mutation in KRAS and low EGFR expression are likely to respond to trametinib as a single-agent MEK inhibitor. It is prudent to perform NGS testing in a clinical environment. It is possible to recognise tumours with DNA repair defects, MSI, or another distinct molecular target that predicts response to therapy. NGS testing is the most effective way to gain entry into molecularly driven clinical trials and should be used on a routine basis in these difficult-to-treat cancers with few standard-of-care treatment choices.

Identification of Molecular Biomarkers in Sporadic PDAC: Case studies

In the field of pancreatic cancer, considerable efforts are being put into identifying robust biomarkers and developing a reliable molecular classification with clinical relevance (G. Q. Shen et al., [64]).Xiaofei Zhang et al [15](X. Zhang et al., [83]) studied the genomic landscape using NGS in a cohort of 1080 Chinese pancreatic cancer patients. They identified somatic mutations in KRAS (83.2%), TP53 (70.6%), CDKN2A (28.8%), SMAD4 (23.0%), ARID1A (12.8%) and CDKN2B (8.9%) recurring in the disease. With the most common one being in KRAS G12D in up to 43.6%. Witkiewicz et al [79] discovered new genetic variation within PDAC and shed light on prognostic factors and therapeutic targets. WES was performed on a total of 109 PDAC patients. WES has confirmed the identities of genes that have been reported to promote PDAC as well as discovered various novel genes that were highly mutated in PDAC including IRF6 (4%), AXIN (5%), PIK3CA (4%), GL13 (6%), BCLAF1 (5%), FLG (10%). RBM10 mutations were linked to increased survival, whereas decreased survival rate in the patients was linked to mutations in the chromatin remodelling gene ARID1A.

RNA-Seq was used by[55] Müller and colleagues to identify differentially regulated RNA between normal and diseased patients. Newly discovered PDAC-related transcriptome regions (coding and noncoding) included (1) miR-802, miR-2114, and miR-561 for miRNA; (2) snoHBII-296B and piR-017061 for snoRNA; and (3) LINC00261, LINC00152, HNF1A-AS1, and AFAP1-AS1 for lncRNA.

In another study[49], tissue samples of 14 PDAC patients and a 620 gene panel were sequenced via the NGS method. The outcomes were compared to the patients on chemotherapy, taking into account all side effects. There were no modifications made to the treatment. PDAC driver mutations were confirmed (e.g., KRAS, TP53). Upon analysis using tools some positive biomarkers were observed in patients suggesting expected successful and unsuccessful therapies. A minimum of one biomarker in each patient was reported in relation to elevated toxicity. These findings imply that computational analysis of NGS data offers evidence-based knowledge on efficient, ineffective, and toxic drugs, potentially laying the groundwork for precision cancer medicine in PDAC.

PDAC and intraductal papillary mucinous neoplasms samples were studied, as were tissue samples from 165 patients with intermittent PDAC[75]. It revealed that 31 miRNAs were up-regulated, with has-miR-93, has-miR-16, has-miR-548d-3p, has-miR-320a, has-miR-3120-3p, has-miR-4468, and has-miR-4713-5p being the most important. The endoscopic ultrasound-guided fine-needle aspiration samples identified miRNAs that could make better candidate biomarkers for the early detection of sporadic PDAC and IPMN.

Connor et al[11]studied 160 PDAC cases using whole-genome and RNA sequencing. Integrating both DNA as well RNA analysis results in a better approach for the personalised treatment of PDAC patients. Shindo and colleagues[65] used gene panel sequencing to find germline mutations in 33 of 854 patients with sporadic PDAC. Patients having these reported deleterious germline mutations were younger. PDAC patients commonly hold germline mutations even after having no major history of cancer in their family. Another meta-analysis[7]showed the role of DNA damage response genes causing 14.5 and 16.5% homologous recombination deficiency (HRD) in 21,842 PDAC genomes in the genes such asBRCA1, BRCA2, PALB2, ATM, ATR, CHEK2, RAD51, and FANC.

Sporadic Pancreatic Cancer–Related Susceptibility Genes with Different Functions Confirmed by NGS

There have been several studies of the PDAC exome, genome(Waddell et al., 2015) and transcriptome [11,80] which have concentrated on defining driver somatic mutations and translatable subtypes. KRAS, an oncogene, CDKN2A, SMAD4, and TP53, tumour suppressors, are the most frequently affected driver genes in PDAC[44].

Driver Genes

Early sequencing studies of PDAC and its precursor pancreatic intra-epithelial neoplasms (PanINs) suggested that they were inactivated in a stepwise manner [14,22]. Researchers discovered that two-thirds of PDAC have complex structural rearrangements that resulted in putative simultaneous inactivation of driver tumour suppressor genes [56].

The sequencing of 456 PDAC tumours identified at least 22 substantially mutated genes [2], the majority of which are rare, illustrating substantial inter-tumoral heterogeneity and complicating biomarker-guided therapy. In a high-throughput drug screen using cell lines and PDX, targeted monotherapies had modest efficacy, and no combination therapies were successful for the majority of PDAC, nor were sensitivities easily predictable from exome sequencing. Nevertheless, sequencing-based drug trials were unthinkable a few years ago, and understanding is rapidly expanding.

KRAS

The KRAS gene is part of a gene family (KRAS, NRAS, and HRAS)[24] It is a 21kda

GTPase that activates when it binds to GTP and deactivates upon binding with GDP. When KRAS is activated, the RAF family kinases RAF-1, BRAF and ARAF get activated[17] MEK-1 and MEK-2 are activated after RAF family members are phosphorylated. MEK-1 and MEK-2 then activate the extracellular regulatory kinases ERK-1 and ERK-2, which carry several proteins like cytosolic and nuclear proteins, such as transcription factors, into the cell. Cell proliferation is aided by ELK-1 and c-Jun. Mutations that cause constitutive activation of KRAS to affect a variety of processes, including unregulated proliferation. KRAS is also in charge of controlling several signalling pathways that have been linked to cancer progression, including PI3K-AKT, PLC-PKC, and RAL.

KRAS gene mutations are a key in the development of epithelial-derived cancers, including PDAC Mutations of the codons G12, G13, or Q61 by and large correspond to constitutively active KRAS, activated KRAS and periodic mutations in K117 and A146 appear to be added hotspots. In more than 50% of cases G12D mutation is reported. KRAS is reported undergo frequent mutations in pancreatic cancer ranging between 20% to 100%. Multiple NGS methods have confirmed KRAS gene mutation in pancreatic cancer. Targeted NGS techniques have also been proven useful in detecting KRAS mutation in PDAC.

In a study performed using NGS in 62.5 per cent locally advanced and 87per cent metastatic PDAC patients somatic mutationswere observed in ctDNA. Researchers showed for the first time in multivariable Cox Regression analysis that patients with KRAS mutation showed poorer outcomes as compared to those with KRAS copy number indicating chances of PDAC progression [54].

In another study, Shiwei Guo et al[24]used NGS-based evaluation using a panel of 50ctDNA-screenedd genes on 113 resectable and validation cohorts with 44 resectable patients. This had a high specificity detection rate with KRAS showing the highest mutation (23.0per centnt) among positive ctDNA, while the others were 5%. The mutation in plasma KRAS G12D was found to be strongly associated with early metastasis. Survival analysis in the validation cohort revealed a correlation between plasma KRAS G12D mutation and poor outcomes which suggested that the KRAS G12D mutation is a useful predictive biomarker for the prognosis and detection of resectable PDAC.

Transformation-Related Protein 53 (TP53)

Transformation-Related Protein 53 (TP53) TP53 is known to be vital in PDAC. TP53 located at 17p13 affects DNA repair mechanism[26] and also plays a role in regulating cellular stress inducing cell cycle arrest. TP53 protein encoded is a tumor suppressor[10].

In 70% of pancreatic cancer cases TP53 is most frequently mutated. It adversely effects transcription activation. It has also been reported in enhancing the expression of cyclin-dependent kinase inhibitor CDKN1A, which results in the discontinuation of cell cycle progression. The study used a targeted deep sequencing assay which detected TP53 mutations in of 13% patients[74].

In an Amplicon-based selective deep sequencing performed by Man Hung Choi et al [8] collected samples from 21 patients with pancreatic ductal adenocarcinoma (PDAC), who underwent Whipple's surgery were studied. KRAS mutations were found in 95% of primary tumours and 71% of juice samples. The majority of juice samples contained several KRAS variants that were not present the in primary tumour. TP53 mutations were discovered in 76% of tumours and 29% of juice samples. Just one of the positive juice samples had more than one TP53 mutation. In 33% of cases, both the mutations KRAS and TP53 were found the in primary tumour and the pancreatic juice sample. It revealed that PDAC patients have high KRAS mutations, which are not that often found in the primary tumour and may signify precancerous lesions.

Using Next-generation sequencing Shinchi Takano and colleagues (2017) [70] revealed that the TP53 mutation was linked to malignant IPMNs. The TP53 mutation was also found in pure pancreatic juice, suggesting that it may be used to preoperatively diagnose malignant Intraductal Papillary Mucinous Neoplasms. In another study (Saha et al., [59])prediction analysis was done on mutation annotation using databases such as TCGA and COSMIC. They predicted that amongst the observed 114 somatic mutations, TP53 would be the most frequently mutated (41%) gene, followed bySMAD4, KRAS, CTNNB1, and ERBB3. They identified a new hotspot TP53 mutation (p.A138V, in 17 per cent of all patients). It can also play a role in the selection of treatment regimens. Furthermore, the low prevalence of KRAS hotspot mutation in the Indian PDAC patient suggests the existence of other drivers in early malignant transformation.

Cyclin-Dependent Kinase Inhibitor 2A (CDKN2A)

CDKN2A is a tumour suppressor gene which is responsible for the regulation of cell cycle progression by inhibiting the CDK-4 and CDK-6 complexes. The CDKN2A tumour suppressor region encodes two distinct proteins, P16 and P14. P16, which is made up of three exons, prevents cell growth by stopping the cell cycle at the G1 checkpoint[47]. It prevents the phosphorylation of retinoblastoma protein, which plays an important part in the negative regulation of the cell cycle and tumour development, affecting downstream inhibition of the E2F transcription factor. The other protein, p14ARF, inhibits cell growth by stabilising p53 activation, targeting certain CDKs at the G1 and G2 checkpoints, and inducing apoptosis. The CDKN2A gene sequence was discovered on chromosome 9p21, in an area with a high frequency of loss of Mutations such as heterozygosity, homozygous deletion, or promoter silencing impairs the CDKN2A gene's function. Hosoda et al [30] used WES to perform genetic testing on high- and low-grade pancreatic intraepithelial neoplasia (PanIN). KRAS mutations were observed in both high-grade and low-grade PanIN, and CDKN2A mutations were only found in high-grade PanIN. Such findings indicated CDKN2A to be a potential biomarker for the early detection of PDAC[83].

Allison Doyle et al (2019)[18]using next-generation sequencing demonstrated*CDKN2A* mutations can be a negative prognostic overall survival indicator for patients with PDAC. Their study highlighted the need to select PDAC patients for potential targeted therapies, targeting the cell cycle pathway.

SMAD4 is the uncontrolled growth of a suppressor gene that is inactivated in 30-40% of pancreatic adenocarcinomas, either by the intragenic mutation of one factor in combination with the deprivation of the other factor or by homozygous removal of both alleles [76] The absence of SMAD4 has been studied in association with widespread metastasis and poor prognosis. Even though SMAD4 is important for TGF signalling, no selective therapies are currently being tested in PDAC. Researchers (Yokose et al., [81])used NGS to investigate the link between gene mutation and prognosis, gene mutations including the ones in driver genes in PDAC and other 50 cancer-related gene mutations. Targeted sequencing also revealed the presence of SMAD4 mutation and KRAS mutation in combination to be a prognostic factor in PDAC.

GNAS The GNAS Complex Locus (Guanine Nucleotide Binding Protein, Stimulating) generates multiple transcripts by the use of alternative promoters and alternative splicing(Hosoda et al., [29])GNAS mutations are significant in the transitioning high-grade PanIN to PDAC. According to multivariate analysis, the mutation in GNAS is also common in IPMN[39]

Filaggrin (FLG) is another gene related to advanced diagnosis of sporadic pancreatic

cancer. The FLG gene encodes for pro-filaggrin, a filament protein that binds to keratin fibres in epithelial cells. The absence of FLG expression results in cytoskeletal disorganisation. [79] and colleagues used WGS to sequence 109 PDAC patients and discovered a 10% FLG mutation. Cotterchio and colleagues [12]conducted a genome-wide association analysis of 179 PDAC patients and discovered that FLG was substantially correlated with the risk of PDAC. FLG2 was also shown to be exclusively mutated primary tumours. FLG2 was recently named one of the "untouchable genes" due to functional loss of mutations, implying genes can be used as an alternative therapeutic target by assisting tumor cells with survival advantage [28].

COMPUTATIONAL TOOLS FOR SOMATIC VARIANT ANALYSIS FOR NEXT GENERATION SEQUENCING DATA FOR CANCER

Computational tools and pipelines for variant analysis have been developed by the researchers such as Onco-Pan which helps in identifying molecular markers using NGS. Various tools that can help in analysis are listed in Table 1such as VarScan[38] a platform-independent software that calls variant in NGS. It can detect Somatic mutation and Somatic copy number alterations.

Virmid[37] can detect SNPs with low allele frequencies. It calculates accurate level of impurity amongst control and disease sample, it takes input as short reads sequence which are aligned with the help of reference genome to generate better alignment.Strelka2 [61]optimizes analysis in somatic variation in control and diseased datasets. Strelka2 improves liquid tumour analysis by introducing a model that calculates errors such as insertion, and deletion for individual samples. Somatic Sniper[41]finds single nucleotide locations that differ in diseased and normal patients. SomVarIUS[68]identifies somatic variants in exome-seq data with at least 67.7 per cent precision rates.

S.NO	TOOL NAME	TYPE OF VARIANT	ALGORITHM	LINKS	REFERENCES
1.	Virmid	SNV	Joint genotype analysis	http://sourceforge.net/projects/virmid/.	(Kim et al., 2013) [37]
2.	VarScan/VarScan2	SNV, indel	Heuristic threshold	http://dkoboldt.github.io/varscan/using-varscan.html	(Koboldt et al., 2012) [38]
3.	VarDict	SNV, indel, SV	Heuristic threshold	https://github.com/AstraZeneca-NGS/VarDict	(Lai et al., 2016) [40]
4.	TVC	SNV, indel, SV	Ion Torrent specific		(Deshpande et al., 2018) [15]
5.	Strelka/Strelka2	SNV, indel	Allele frequency analysis	ftp://strelka@ftp.illumina.com/. https://github.com/Illumina/strelka	(Saunders et al., 2012) [61]
6.	SomaticSniper	SNV	Joint genotype analysis	http://gmt.genome.wustl.edu/packages/somatic-sniper/	(Larson et al., 2012) [41]
7.	SomaticSeq	SNV	Machine learning (Ensemble caller)	https://bioinform.github.io/somaticseq/	(Fang et al., 2015) [20]
8.	SOAPsnv	SNV	Heuristic threshold	http://soap.genomics.org.cn/SOAPsnv.html	(Zhu et al., 2015) [84]
9.	qSNP	SNV	Heuristic threshold	http://www.qcmg.org/bioinformatics/qsnp/	(Kassahn et al., 2013) [36]
10.	RADIA	SNV	Heuristic threshold	https://github.com/aradenbaugh/radia/.	(Radenbaugh et al., 2014) [57]
11.	SNVSniffer	SNV, indel	Joint genotype analysis	http://snvsniffer.sourceforge.net/homepage.htm#latest	(Y. Liu et al., 2016) [45]
12.	SNooPer	SNV, indel	Machine learning	http://www.somaticsnooper.com/	(Spinella et al., 2016) [69]

13.	Shimmer	SNV, indel	Heuristic threshold	http://www.github.com/nhansen/Shimmer	(Hansen et al., 2013) [25]
14.	Seurat	SNV, indel, SV	Joint genotype analysis	https://satijalab.org/seurat/	(Christoforides et al., 2013) [9]
15.	Platypus	SNV, indel, SV	Haplotype analysis	https://www.well.ox.ac.uk/research/research-groups/lunter-group/platypus-a-haplotype-based-variant-caller-for-next-generation-sequence-data	(Rimmer et al., 2014) [58]
16.	SAMtools	SNV, indel	Joint genotype analysis	https://sourceforge.net/projects/samtools/	(Li, 2011) [42]
17.	MuTect2	SNV	Allele frequency analysis	https://www.broadinstitute.org/cancer/cga/mutect	(do Valle et al., 2016) [16]
18.	LoLoPicker	SNV	Allele frequency analysis	https://github.com/jcarrotzhang/LoLoPicker.	(Carrot-Zhang & Majewski, 2017) [6]
19.	LoFreq	SNV, indel	Allele frequency analysis	https://csb5.github.io/lofreq/	(Wilm et al., 2012) [78]
20.	LocHap	SNV, indel	Haplotype analysis	http://www.compgenome.org/lochap.	(Sengupta et al., 2016) [63]
21.	HapMuC	SNV, indel	Haplotype analysis	http://github.com/usuyama/hapmuc.	(Usuyama et al., 2014) [72]
22.	FaSD-somatic	SNV	Joint genotype analysis	http://jjwanglab.org/FaSD-somatic/.	(Wang et al., 2014) [77]
23.	BAYSIC	SNV	Machine learning (Ensemble caller)	http://genformatic.com/baysic	(Cantarel et al., 2014) [5]
24.	CaVEMan	SNV	Joint genotype analysis	https://github.com/cancerit/CaVEMan	(D. Jones et al., 2016) [32]
25.	ISOWN	SNV	Supervised learning	https://github.com/ikalatskaya/ISOWN	(Kalatskaya et al., 2017) [34]
26.	Pisces	SNV, indel	Poisson model on read count	http://www.fccc.edu/research/labs/dunbrack/pisces	(Dunn et al., 2019) [19]
27.	SomVarIUS	SNV, indel	Noise level estimation	https://github.com/kylessmith/SomVarIUS	(Smith et al., 2016) [68]
28.	Galaxy Platform	SNV, indel	Somatic Tumor Mutations Detection	https://galaxyproject.org/	(Afgan et al., 2018) [1]

Table 1: List of Somatic Variant analysis tools for Next generation sequencing data

CONCLUSION AND DISCUSSION

The incorporation of next-generation sequencing (NGS) into properly designed studies paves a new path towards the development of valid biomarkers for predictive, prognostic and therapeutic purposes. Through the combination of RNAseq analysis and Whole genome sequencing, the classification of pancreatic cancer has been done in an excellent manner, which calls for conducting additional clinical trials to investigate the therapeutic and preventive effectiveness of genome-based analysis.

In the past five years, an increasing amount of genomic and transcriptomic data on many paediatric and adult cancers was accumulated. This resulted in the discovery of several prognostic and predictive biomarkersseveral molecular alterations have been translated into clinical practice for metastatic cancers, including lung adenocarcinoma, colorectal cancer, prostate cancer, ovarian carcinoma, and cholangiocarcinoma. With declining costs and more accessible technology, the next five years will likely see an increase in the accumulation of cancer sequencing data, as well as its convergence with epigenomic, proteomic, and genomic data as well as protein structural information. In terms of treatment, various clinical genomic studies that incorporate, RNA seq, WGS, MR-NGS and serial CGs detection raise high hopes for personalised and precise treatment of

individual pancreatic cancer patients. Creation of biologic and small molecule therapeutics, paralleling the previous year's rise in sequencing data we hope by the means of these researches from the last five-year, Future research would be able to attract a larger number of patients using biomarker-driven trials and a better understanding of genomic variation as biomarker-driven trials become more effective. Restricted, selective sequencing using tumor-specific gene panels that have been rationally engineered will prove beneficial.

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