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Original Article

Real-World Experiences of Next-Generation Sequencing in Oncology: From an Indian Multicenter Registry and Collaborative Centers

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Abstract

Background The integration of next-generation sequencing (NGS) in guiding personalized therapy for oncology faces the challenges, primarily, of cost and drug accessibility. Limited data from Indian academic centers accentuate the need for comprehensive insights into the real-world applications of NGS in oncology.

Methods The Network of Oncology Clinical Trials in India (NOCI), accessible at www.noci-india.com, compiled data on patients who underwent NGS for solid organ cancers from January 1, 2018, to December 31, 2021. This study aimed to elucidate the testing indications, sample types analyzed, and the resultant impact on patient care.

Results Analysis of data from six centers included 278 subjects, with 24 specimens (9%) excluded due to quality test failure. Tissue constituted 59.7% of specimens, blood 38.5%, and both 1.8%. Predominantly, NGS was employed for identifying *BRCA*1/2 mutations (56%) and for targeted therapy in non-small-cell lung cancer (NSCLC; 28%). Only 41 (16%) patients with other cancers underwent multigene NGS panels in pursuit of targetable mutations. Among them, 13 exhibited targetable mutations, and 3 received treatment based on NGS findings.

Conclusion This study underscores that the majority of NGS applications focused on screening for *BRCA*1/2 mutations and identifying targetable mutations in NSCLC. However, among those undergoing NGS for advanced cancers, only a limited number received personalized therapy. The findings underscore the challenges of utilizing NGS in off-label indications within resource-constrained settings.

Keywords
- hereditary cancer syndromes
- next-generation sequencing
- oncology
- targeted therapy

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Introduction

The Human Genome Project in 2003 gave us a better understanding of the molecular basis of diseases, including cancer. Further, the development of molecular oncology has ushered in the era of precision oncology.[1] The success of trastuzumab (approved in 1998 for metastatic breast cancer) and imatinib (approved in 2001 for chronic myeloid leukemia) showed that a more effective and less toxic way of treating cancer was possible, paving the way to personalized medicine (PM) and targeted therapy.[2] The Sangers method[3] and Maxwell–Gilbert method were the initial methods used to sequence genes. However, they were time-consuming, labor intensive, and financially demanding. Currently, multiple parallel testing using (NGS) has reduced the time and cost of doing these tests. NGS is being used in oncology practice for various purposes. In oncology, NGS helps detect somatic driver mutations, resistance mechanisms, quantification of mutational burden, and evaluation of hereditary cancers.[4,5] Since the last decade, use of NGS has become standard practice in the evaluation and management of several cancers. However, its use is limited in India, primarily due to cost, but also due to limited availability and access to the newer targeted agents.[6,7] The European Society for Medical Oncology (ESMO) guidelines 2020 recommend using NGS on tumor samples in advanced nonsquamous NSCLC, prostate cancers, ovarian cancers, and cholangiocarcinoma.[8] However, the practical uses of NGS may be limited in real-world settings and data from low- and middle-income countries are limited.[9,10]

Materials and Methodology

Study Design

We conducted a retrospective, multicenter descriptive study under the aegis of the Network of Oncology Clinical Trials India (NOCI) and other collaborative centers. NOCI is a cooperative research network developed with a grant from the Department of Biotechnology, Government of India, having six member institutes from all parts of the country. Electronic and paper databases from these institutes were checked from January 1, 2018 to December 31, 2021, and data on the NGS test done were collected. Anonymized data were obtained after ethical approval from each institute and the data were collected in a spreadsheet. Details on patient demographics, biopsy type, prior lines of systemic therapy, type and number of mutations, NGS platform used, genome-directed therapy received, and rationale behind the rejection of sequencing-directed therapies were extracted from data capture forms. The proportion of profiled patients receiving sequencing-directed therapies and the reasons for declining targeted therapies were then evaluated using descriptive statistics.

Inclusion and Sample Size

For this study, we included all adult patients (>18 years) with nonhematological cancers who had undergone NGS testing for any indication and for whom a verifiable report was available. As it was an observational retrospective descriptive study, it was planned to include all patients from electronic and paper databases for whom NGS reports were available from January 1, 2018 to December 31, 2021, and no separate sample size calculation was applicable.

Primary and Secondary Outcome

To assess the utilization and impact of NGS in the management of solid organ cancers in resource-constrained settings in India, specifically focusing on indications for testing, types of samples tested, and the presence of druggable mutations.

Statistical Analysis

Data were anonymized and recorded in MS Excel spreadsheet program (2019) and used for analysis. Before interpreting the results, the data were cleaned, checked for accuracy, missing values noted, labeled appropriately, sorted, and recoded as required.

Descriptive statistics were elaborated in the form of means/standard deviations and medians/interquartile ranges (IQRs) for continuous variables, and frequencies and percentages for categorical variables.

Ethics

The study was done after ethics committee approval, vide Ref: BMHR-IECCMCL/0322-115/Apprvl/NEXT-G/Med-Onco, dated March 19, 2022. Waiver of consent was taken as it was a noninterventional retrospective descriptive study and patients' details were anonymized. The study was done in accordance with the ethical standards of the ICH GCP ICMR guidelines. The study was registered in Clinical Trials Registry-India (CTRI), vide number: CTRI/2022/01/039233

Results

A total of 278 patients had undergone NGS analysis during the study period. The median age of the patients was 55 years (range: 27–82 years) and females ($N = 183$) accounted for 65.3% of the patients (►**Table 1**). Specimens used for testing were tissue ($N = 166$; 59.7%), blood ($N = 107$; 38.5%), and a combination of blood with tissue ($N = 5$; 1.8%). Of the 278 samples sent for testing, 9% ($N = 24$) were rejected as they failed the quality testing and only 254 samples were analyzed (►**Fig. 1**). The majority of the patients had advanced stage ($N = 185$, 66.5%) and test was ordered upfront at diagnosis in 178 patients (64%). One hundred and ninety-nine patients (78.6%) had tested for limited gene panels of less than 50 genes. The most common indications for testing were lung cancer ($N = 72$, 28.3%), ovarian cancer ($N = 65$, 25.6%), and breast cancer ($N = 54$, 21.3%). For patients with lung cancer ($N = 72$), the use of NGS identified 56.9% ($N = 41$) specimens with targetable mutations leading to the use of targeted therapy in 36.1% ($N = 26$) patients (►**Table 2**). Among the patients in which NGS was tested for off-label indications ($N = 41$, 16.1%), 13 patients were found to have targetable mutations and 3 patients were receiving targeted therapy based on the NGS reports (►**Table 3**).

Table 1 Baseline characteristics

Variable	Number	Frequency (%)
Gender ($N=278$)		
Male	95	34
Female	183	66
Type of tissue used ($N=278$)		
Tissue	166	59.7
Blood	107	38.5
Blood and tissue	5	1.8
Stage ($N=278$)		
1	8	2.9
2	19	6.8
3	66	23.8
4	185	66.5
Clinical scenarios of NGS testing ($N=278$)		
Upfront at diagnosis	178	64
Post 1st line	41	14.7
Post 2nd line	59	21.3
No. of genes tested ($N=254$)		
<50 gene assays	199	78.6
50–150 gene assays	16	6.32
150–350 gene assays	33	13
>350 gene assays	6	2
Types of cancer ($N=254$)		
Lung cancer	72	28.3
Ovarian cancer	65	25.6
Breast cancer	54	21.3
Pancreaticobiliary cancers	22	8.6
Prostate cancer	16	6.3
Gastrointestinal cancer	5	2.0
Gynecological cancer excluding ovary	4	1.6
Genitourinary malignancies	4	1.6
Others[a]	12	4.7

[a]Other cancers included dual primary ($N=2$), carcinoma unknown primary ($N=2$), head and neck squamous cell carcinoma ($N=2$), sarcomas ($N=3$), skin carcinoma ($N=1$), and thyroid cancer ($N=2$).

However, with regard to testing for hereditary cancer syndromes, the most common indications were for breast and ovarian cancers ($N=119$, 84.3%), followed by prostate and pancreatic cancers ($N=22$, 15.6%). Out of the 141 blood and tissue samples analyzed for hereditary cancer syndromes, 25% ($N=35$) samples were positive for *BRCA1* and *BRCA2*, which led to the use of poly-ADP ribose polymerase (PARP) inhibitors in 43% ($N=15$) patients (►**Table 4**). The 106 samples considered negative included 5 samples with variants of unknown significance (VUS).

Discussions

The results of this study provide valuable insights into the real-world experiences of the use of NGS in the field of oncology from government and academic centers that deal with patients having resource constraints. Compared to other studies where about 8 to 20% samples failed the NGS test,[9,11] our study also showed that in 8.6% samples the test could not be done as the tissue did not have sufficient deoxyribonucleic acid (DNA) to run the test. This is an important factor in ordering these tests as the quality and quantity of tissue play a role in obtaining optimal results. In our observation, the most common indication for ordering NGS-based assay is for hereditary cancer syndromes and for lung cancer.[6,9] This is in accordance with the general practice and worldwide data, as they have high chance of being positive and also have easily accessible targeted agents. Moreover, identification of hereditary *BRCA* mutations has significant implications for the patient and the family. A point to note while testing NGS in hereditary cancer syndromes is that in case the physician is using limited gene test for BRCA1 and BRCA2 only, about 5% patients may be missed if they have large deletions and duplications, which can be tested by multiplex ligand-dependent probe amplification (MLPA). Also, it is important to offer pre- and posttest counseling as these tests have varied implications with regard to treatment, outcomes, cascade testing, psychological issues, and confidentiality. Unfortunately, there are no Indian guidelines for these; also there are limited quality control practices.

The use of limited gene panels is common in India as the testing is restricted to genes that are druggable. This could also account for the fact that the majority of the patients in our study underwent limited gene panel in lung cancer and for hereditary cancer syndromes. Our series showed prevalence of 25% for *BRCA1* and *BRCA2* genes using limited panel testing; it needs to be seen if the use of multigene panels covering for other genes involved in hereditary cancer syndromes would have made any significant impact to our population of patients. For those identified with *BRCA*1 and BRCA2 mutation, 43% ($N=15$) received PARP inhibitors during their course of treatment. This number is bound to increase as the indications for using PARP inhibitors are increasing and the cost of PARP inhibitors are coming down with more generics being available. With regard to the use of NGS for lung cancer, our study shows the feasibility of using NGS platforms as it may be convenient to test multiple targets with limited tissue available. Most of the patients in our study had used limited gene testing for lung cancer, and it is common practice in the NOCI centers to use alternative methods for testing targetable mutations in lung cancer as it is cheaper. With regard to non-BRCA and non-lung-cancer subjects, our series shows limited number of patients. This could be due to the limited options for treating these patients outside of clinical trials in resource-constraint settings like ours. The NOCI network comprises six centers, of which two are central government institutes, two are charitable minority institutes, and two private academic

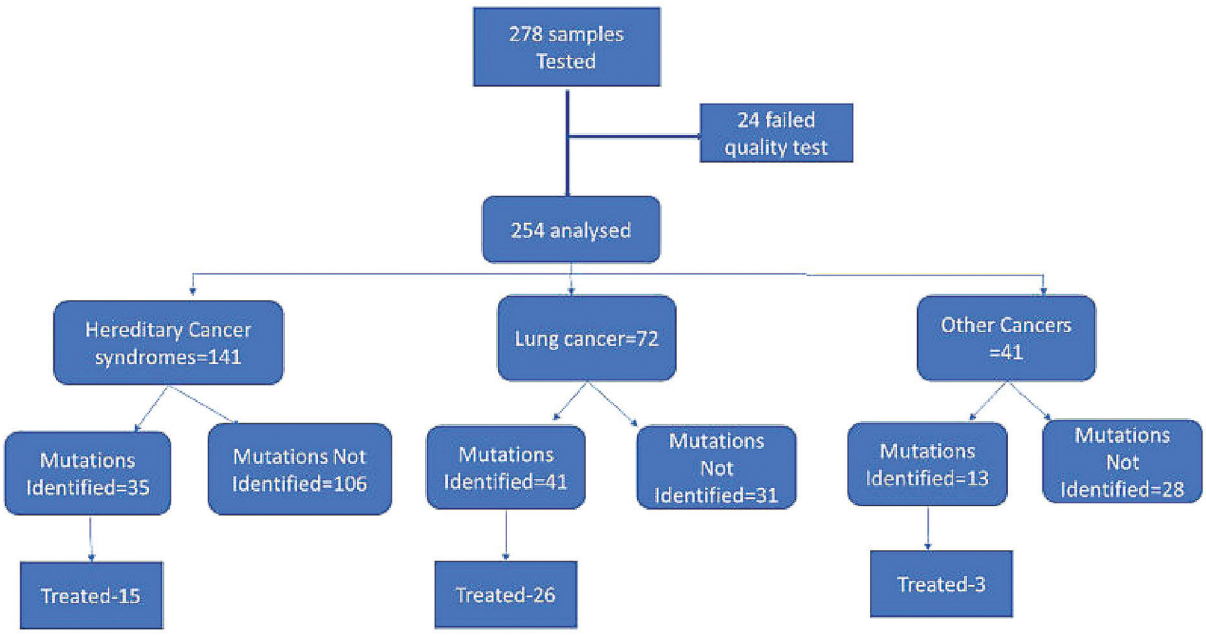

Fig. 1 Consort diagram.

Table 2 Targetable Mutations identified in lung cancer ($N = 72$)

Diagnosis	Mutation	Incidence	Percentage
Adenocarcinoma	*EGFR*	21	29
Adenocarcinoma	*ALK*	7	9.72
Adenocarcinoma	*KRAS*	4	5.5
Adenocarcinoma	*BRAF*	3	4.1
Adenocarcinoma/ squamous[a]	*RET*	3	4.1
Adenocarcinoma	*HER2*	2	2.8
Adenocarcinoma	*MET*	1	1.4

Abbreviations: *ALK*, anaplastic lymphoma kinase translocation; *EGFR*, epidermal growth factor receptor; *HER2*, human epidermal growth factor receptor 2; *KRAS*, Kirsten rat sarcoma; *MET*, mesenchymal epithelial transition factor receptor; *RET*, rearranged during transfection.

[a]*RET* was identified in one case of squamous cell carcinoma. 72 cases were analyzed, of which 66 were adenocarcinomas, 3 undifferentiated carcinomas, 2 squamous cell carcinomas, and 1 epithelioid hemangioendothelioma.

institutes. None of these centers had in-house facilities for performing NGS at the time of the study and samples had to be outsourced. Only one academic private institute had government funding for testing *BRCA1* and *BRCA2* mutations and this could have been the reason for having more patients testing for *BRCA1* and *BRCA 2*. With regard to off-label use of targeted therapy using NGS, durable clinical benefit was seen in only one patient. This patient had *Her2/Neu* driver mutation in a metastatic gallbladder carcinoma (►**Table 3**). A similar study from India showed the limitations of performing NGS testing in off-label indications.[6] That study had a different cohort of subjects and had more representation from the corporate settings. Our cohort dealt with patients presenting in resource-constraint settings and highlights the limitations of asking for these tests in the first place. Even in prospective studies using NGS for patients in advanced cancer, the benefit of using NGS for off-label indication ranges from 5 to 7%.[12–14] Some studies show that when NGS was done for identifying treatment options, most

Table 3 Off-label targets identified by NGS and patterns of treatment

Cancer type	Mutations identified	Targeted agents used as per NGS report (yes/no)	If yes, name of agent used	Summary
Carcinoma gall bladder	*TP53*, *ATR*, *JAK2*, *AIRD1A*, *ERBB4*	No		12# FOLFOX-metabolic CR for 2 y and disease relapsed and passed away before treatment
Carcinoma gall bladder	*HER2*	Yes	Trastuzumab	Post 6# gemcitabine + cisplatin 1st line PFS 6 mo, 2nd line FOLFOX + trastuzumab. PFS 1.2 y in the 2nd line with chemotherapy, progression

Table 3 (*Continued*)

Cancer type	Mutations identified	Targeted agents used as per NGS report (yes/no)	If yes, name of agent used	Summary
Carcinoma stomach	*BRAF V600E and TMB > 40 TMB >20 mutation/Mb*	Yes	Trametinib + vemurafenib, followed by pembrolizumab	Post 6# FOLFOX, progression, post 2nd line docetaxel 2# progression, then 2 mo trametinib + vemurafenib progression and then 2# pembrolizumab and passed away. No response to both agents in 2nd and 3rd lines
Bladder carcinoma	*TMB > 33 mutation/Mb*	Yes	Atezolizumab	No response in the first-line single agent 3# and succumbed to disease
Mediastinal germ cell tumor	*TMB >20 mutation/Mb, KRAS, TP53, ATR, ARID1A*	No	–	NA[a]
Carcinoma of unknown primary	*BRAF*	No	–	NA[a]
Pancreas	*TMB >20 mutation/Mb, KRAS*	No	–	NA[a]
Carcinoma stomach	*MET* amplification	No	–	NA[a]
Suspected GIST/ carcinoma unknown primary	*CKIT*	Yes	Imatinib	On imatinib for 2 y with partial response
Carcinoma endometrium	*FGFR2*	No	–	NA[a]
Carcinoma of unknown primary	*TMB > 15 mutation/Mb*	No	–	NA[a]
Cholangiocarcinoma	*TMB*-30 *mutation/Mb* and *KRAS*	No	–	NA[a]
Gingivobuccal sulcus	*TP53 and ERBB2*	No	–	NA[a]

Abbreviations: *ATR*, ataxia telangiectasia and Rad3; *AIRD1A*, AT-rich interactive domain-containing protein 1A; *ERBB4*, receptor tyrosine-protein kinase erbB-4; *FGFR2*, fibroblast growth factor receptor 2; GIST, gastrointestinal stromal tumor; *HER2*, human epidermal growth factor receptor 2; *JAK2*, Janus kinase 2; *KRAS*, *Kirsten rat sarcoma*; *MET*, mesenchymal epithelial transition; NGS, next-generation sequencing; *TP53*, tumor protein 53; *TMB*, tumor mutational burden.
[a]Not applicable -these subjects did not receive treatment based on NGS and received standard of care.
[#]cycles of chemotherapy

Table 4 Patterns of hereditary syndromes testing ($N = 141$)

Type of cancer	Incidence	Frequency
Ovary and breast	119	84.3%
Prostate cancer	11	7.8%
Pancreaticobiliary	11	7.8%

patients could be enrolled in clinical trials.[15] That is an important point to note as we may have limited options in our setting and it is important to develop good research facilities doing ethical work. Our study has major limitations as it was a retrospective study and had a heterogenous population of patients undergoing various types of NGS testing. Also, an important point to note is the role of developing molecular tumor boards and see if the inputs would lead to better outcomes and results. It is important for the oncologist to understand and select the right patients based on the clinical profile and general condition and select the right panel when planning these tests in resource-constraint settings.

Conclusion

Our study offers practical insights into the real-world impact of using NGS in oncology in resource-constraint settings. The predominant use of NGS in resource-constrained Indian hospitals is mainly in lung and ovarian cancers where some access to targeted agents may be possible with the use of generics and support programs.

Patient Consent

Waiver of consent was taken as it was a noninterventional retrospective descriptive study and patients' details were anonymized.

Funding
None declared.

Conflict of Interest
None declared.

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References

1 Green ED, Watson JD, Collins FS. Human Genome Project: twenty-five years of big biology. Nature 2015;526(7571):29–31
2 Fischer OM, Streit S, Hart S, Ullrich A. Beyond Herceptin and Gleevec. Curr Opin Chem Biol 2003;7(04):490–495 In
3 Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci U S A 1977;74(12): 5463–5467
4 Morganti S, Tarantino P, Ferraro E, D'Amico P, Duso BA, Curigliano G. Next Generation Sequencing (NGS): a revolutionary technology in pharmacogenomics and personalized medicine in cancer. Adv Exp Med Biol 2019;1168:9–30
5 Sabour L, Sabour M, Ghorbian S. Clinical applications of next-generation sequencing in cancer diagnosis. Pathol Oncol Res 2017;23(02):225–234
6 Mathew A, Joseph S, Boby J, et al. Clinical benefit of comprehensive genomic profiling for advanced cancers in India. JCO Glob Oncol 2022;8:e2100421
7 Ghosh J, Lopes G, Chopra S. Are we right on target? Is comprehensive genomic profiling ready for prime time in resource-constrained settings?. JCO Glob Oncol 2022;8:e2200135
8 Mosele F, Remon J, Mateo J, et al. Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO Precision Medicine Working Group. Ann Oncol 2020;31(11):1491–1505
9 Sunami K, Ichikawa H, Kubo T, et al. Feasibility and utility of a panel testing for 114 cancer-associated genes in a clinical setting: a hospital-based study. Cancer Sci 2019;110(04):1480–1490
10 Tan AC, Lai GGY, Tan GS, et al. Utility of incorporating next-generation sequencing (NGS) in an Asian non-small cell lung cancer (NSCLC) population: Incremental yield of actionable alterations and cost-effectiveness analysis. Lung Cancer 2020;139:207–215
11 Colomer R, Miranda J, Romero-Laorden N, et al. Usefulness and real-world outcomes of next generation sequencing testing in patients with cancer: an observational study on the impact of selection based on clinical judgement. EClinicalMedicine 2023;60:102029
12 Suh KJ, Kim SH, Kim YJ, et al. Clinical application of next-generation sequencing in patients with breast cancer: real-world data. J Breast Cancer 2022;25(05):366–378
13 Massard C, Michiels S, Ferté C, et al. High-throughput genomics and clinical outcome in hard-to-treat advanced cancers: results of the MOSCATO 01 trial. Cancer Discov 2017;7(06):586–595
14 Le Tourneau C, Delord JP, Gonçalves A, et al; SHIVA investigators. Molecularly targeted therapy based on tumour molecular profiling versus conventional therapy for advanced cancer (SHIVA): a multicentre, open-label, proof-of-concept, randomised, controlled phase 2 trial. Lancet Oncol 2015;16(13):1324–1334
15 De Falco V, Poliero L, Vitello PP, et al. Feasibility of next-generation sequencing in clinical practice: results of a pilot study in the Department of Precision Medicine at the University of Campania "Luigi Vanvitelli.". ESMO Open 2020;5(02):e000675

