REPORT

Report on participation of the ICMR International Fellow (ICMR-IF) in Training/Research abroad.

1.	Name and designation of ICMR- IF	**Dr. Vivek Gupta** **Associate Professor,** **Department of Pathology**
2.	Address	**Department of Pathology, Government Institute of Medical Sciences, Greater Noida, Uttar Pradesh, India 201310**
	Frontline area of research in which training/research was carried out	**Molecular Pathology**
3.	Name & address of Professor and host institute	**Dr. Ravindra Kolhe,** **(M.D. Ph.D. FCAP.)** **Professor and Interim Chair,** **Department of Pathology, Medical College of Georgia** **Mail: BF-212, 1120 15th Street| Augusta, GA 30912**
4.	Duration of fellowship with exact date	**Six months** **March 25th, 2023 to September 24th 2023**
5.	Highlights of work conducted	
	(i) Technique/expertise acquired	**Molecular Pathology** **(Details in Annexure 1)**
	(ii) Research results, including any papers, prepared/submitted for publication	**2 manuscripts were prepared, and the Abstract was accepted in the AMP Annual meeting and expo Nov, 14-18, 2023, UT, USA** **(Details in Annexure 2)**
	(iii) Proposed utilization of the experience in India	**(Details in Annexure 3)**

Vivek Gupta

Signature of ICMR-IF

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Highlights of research work conducted

Annexure 1

(i) Technique/expertise acquired: in Molecular Pathology

The ICMR-DHR sponsored fellowship under my mentor Dr. Ravindra Kolhe at Georgia Esoteric and Molecular Laboratory, Department of Pathology, Medical College of Georgia, Augusta University, in Molecular Pathology has provided me with extensive technical expertise in various molecular technologies and equipment used in both patient care and research. Here's a summary of technology and expertise I have acquired:

1. Optical Genome Mapping: (OGM) I have acquired training over this cutting-edge genomic technology that allows for the high-resolution analysis of the structure and organization of an individual's genome. This technology as in cytogenomics has lot of potential and I have learnt the wet lab as well as data analysis of OGM. Working on this technology I have observed the interpretation of translocation and structural variants in Acute Myleoid Leukemia cases.
2. The NGS TSO 500 (TruSight Oncology 500 developed by Illumina): an 523 gene panel for targeted sequencing in cancer genomic profiling. I learnt about it and used it though Next Seq NGS platform in solid tumors and in hematological malignancies. I have had hand on training in wet lab as well, data analysis and interpretation and preparing the reports for sign out. I have learnt the applied aspect of this technology in research and clinical applications. It is particularly valuable in identifying mutations, copy number variations that can inform cancer diagnosis and guide treatment decisions. The TSO 500 523 gene panel run of Next Seq platform has been used by me in both cancer research and clinical diagnostics. In research, it contributes to a deeper understanding of the genetic basis of cancer. In the clinical setting, it assists oncologists in selecting appropriate therapies based on the specific genomic profile of a patient's tumor.
3. Chromosomal microarray: Oncoscan is based on microarray technology, which involves hybridizing DNA from the sample to a chip containing a known set of DNA probes. This allows for the simultaneous analysis of thousands of genomic loci. It is designed for the comprehensive analysis of genomic alterations in cancer, including copy number variations (CNVs) and loss of heterozygosity (LOH) events. Oncoscan provides valuable information

for cancer research and clinical diagnostics. I have done the wet lab part and analyzed 107 cases (57 gliomas and 50 solid tumor) through this technology.

4. NxClincal 6.0 (Biodiscovery): Through this genomic software based on bioinformatics analysis involving Hidden Markov's Model, I have done validation studies for copy number alterations in solid tumors using BAM files derived from NGS TSO500 (a 523 gene panel) and OSCHP files of chromosomal microarray and compared them.
5. PGDx: The PGDx elio™ tissue complete kit used through NGS. I have learnt about the technology with partly in wet lab with hands on for research work. It is used for the analysis of genomic alterations in cancer cells. It helps identify mutations, copy number variations, and other genomic changes in solid tumors.

Annexure 2

ii) Research results, including any papers, prepared/submitted for publication

I have handled multiple data sets and could come up with:

A. Two papers (Manuscripts prepared due for submission) in Journal of Molecular Diagnostics and Cancer Genetics

- Manuscript 1: Clinical validation of NxClinical for evaluating copy number alterations (CNAs) on 523-Gene Next Generation Sequencing panel in solid tumors.

Abstract

The study analyzed BAM files derived from the Next Generation Sequencing (NGS) TSO500 523 gene panel using the NxClinical platform to detect CNAs in solid tumors and validates its results with chromosomal microarray analysis. This study included 50 independent de-identified samples representing 50 unique individuals having cancer proven on previous genomic testing and having a pathogenic/ likely pathogenic structural variant. The study included a variety of copy number alterations (eg, loss, gain, amplification) in 24 regions across different chromosomes. The BAM file and OSCHP file (generated by Onco-Scan) were analyzed in NxClinical 6.2 software by (Bionano Genomics Inc.). The samples included Breast cancer, Ovarian carcinoma, Pancreatic cancer, Melanoma, and Prostate carcinoma in 10, 8, 8, 4, and 20 cases each. Copy number gains, amplifications, and loss were detected by analysis in NxClinical for TSO500 in 35, 11, and 8 regions whereas OncoScan detected them in 34, 12, and 8 regions, respectively. Sensitivity, Specificity, Positive predictive value, Negative predictive value, and diagnostic accuracy of NxClinical NGS TSO500 were 96.29%, 100%, 100%, 99.82% and 99.98% respectively. Overall complete agreement in the whole genome was observed in 97.92% of cases. The integrated workflow of NxClinical, with high diagnostics parameters, is a highly reliable method for accurately identifying CNA derived from targeted panels of NGS data.

- Manuscript 2: Genetic profiling and copy number analysis of Grade II–III and Grade IV gliomas from archival FFPE samples using OncoScan assay.

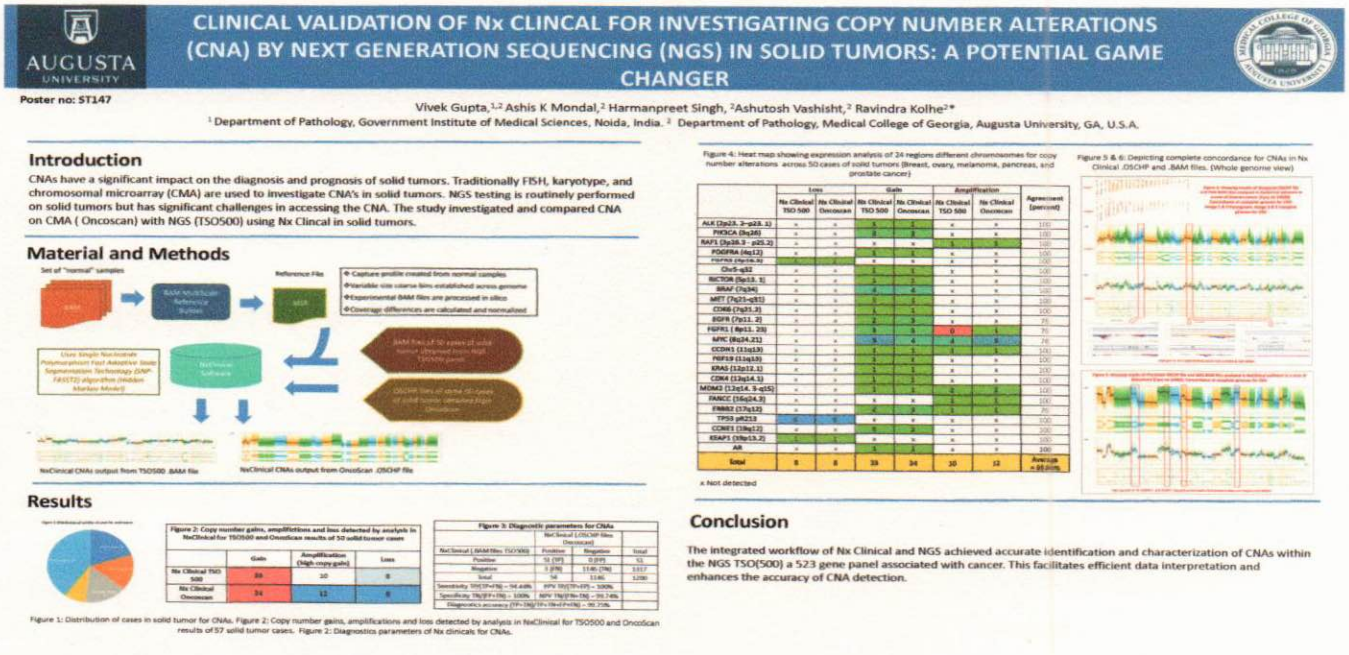
Abstract

Gliomas are the most prevalent primary malignant brain tumors. The study investigates copy number variations (CNVs) within distinct glioma subgroups. 57 archival FFPE high-grade

glial tumor specimens from different patients were studied using the OocoScan. The data was analyzed in conjunction with pathological and clinical findings. Age at diagnosis ranged from 17 - 84 years, with a median age of 58 years. Analysis revealed 12(21%) in category II; 11(20%) in category III and 34(59%) in category IV of WHO. The most common genomic aberrations were noted in PTEN, FGFR2, MET, EGFR, BRAF, and CDK6. 1p deletion was observed in 26(45%) cases, 19q deletion in 20(35%) cases and IDH1:p.R132H:c.395G>A in 14(24%) cases. 1p and 19q co-deletion was found in 10(17%) cases. Chromosomal copy number abnormalities were gain of 7p and 7q; loss of 1p, 9p, 10q/10p, 13q, 15q, 22p/22q, Xp (6 of 28 females); and 17p LOH. Chromothripsis was observed in two cases. The CNVs compared in the IDH wild and IDH mutant group showed a significant difference. These findings indicate the potential of CNVs as a valuable prognostic indicator in the context of clinical oversight.

B. An abstract and (Accepted in AMP Annual Meeting and Expo 2023, USA

Abstract no ST147: Title: Clinical validation of NxClincal for investigating copy number alterations (CNAs) by next generation sequencing (NGS) in solid tumors: a potential game changer. It will be subsequently published in conference proceedings in Journal of Molecular Diagnostics



AUGUSTA UNIVERSITY

CLINICAL VALIDATION OF Nx CLINCAL FOR INVESTIGATING COPY NUMBER ALTERATIONS (CNA) BY NEXT GENERATION SEQUENCING (NGS) IN SOLID TUMORS: A POTENTIAL GAME CHANGER

Poster no: ST147

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Introduction

CNAs have a significant impact on the diagnosis and prognosis of solid tumors. Traditionally FISH, karyotype, and chromosomal microarray (CMA) are used to investigate CNA's in solid tumors. NGS testing is routinely performed on solid tumors but has significant challenges in accessing the CNA. The study investigated and compared CNA on CMA (Oncoscan) with NGS (TSO500) using Nx Clincal in solid tumors.

Material and Methods

Results

Conclusion

The integrated workflow of Nx Clinical and NGS achieved accurate identification and characterization of CNAs within the NGS TSO(500) a 523 gene panel associated with cancer. This facilitates efficient data interpretation and enhances the accuracy of CNA detection.

C. A report prepared and submitted on topic: "Identification of tumor mutation burden, its clinical interpretation, and role in precision oncology"

Tumor Mutation Burden (TMB), defined as number of non-inherited/somatic mutations per megabase can be identified using next generation sequencing (NGS) platforms involving whole exome sequencing or targeted panel sequencing. In this particular study we used targeted panel sequencing.

The following three objectives were set in (1) to identify the TMB using laboratory test that uses NGS platform. (2) to analyze the TMB in different tumor subsets. (3) to understand the clinical relevance of TMB in the different subset of tumors

Objective 1 was fulfilled described herein as: TMB was identified using next generation sequencing platform with TSO500 523 gene panel with procedure described below.

Library preparation for sequencing: The experimental procedure adhered strictly to the manufacturer's guidelines for library preparation, employing the TruSight Oncology 500 Library Preparation Kit (Illumina, San Diego, CA) based on hybrid capture principles. The fragmentation of DNA was executed using an ultrasonicator (Covaris, Woburn, MA), yielding DNA fragments ranging from 90 to 250 base pairs (bp) with a specific target peak at approximately 130 bp. Subsequent steps included end repair, A-tailing, and adapter ligation. The DNA fragments, now linked to adapters, were subjected to amplification through index PCR utilizing primers tailored to the UP-index. Further refinement was achieved through sample enrichment, employing probe-based hybridization (OPD2). The enriched samples underwent a series of procedures encompassing capture, PCR-driven enrichment, purification, and quantification of double-stranded DNA using the high Sensitivity Qubit kit (#Q32854 Invitrogen, USA). Following library normalization, the DNA libraries were combined to create a pooled sample, primed for ultimate loading onto the sequencer for downstream analysis.

Post-sequencing variant analysis: The TSO500 sequencing procedure encompassed a 101 base pair paired-end sequencing methodology, employing 202 cycles. The library specimens were subjected to sequencing on the NextSeq 550 high-capacity sequencer, utilizing the V2 flow cell kit (Illumina, San Diego, CA, USA). The data analysis was performed using the BaseSpace TSO500 Assessment App (Illumina). In brief, the output of the sequencing procedure produced

FASTQ files, which were then converted into Binary Base Call (BCL) files. These BCL files were subsequently processed through Qiagen's QCI Cloud Connect software, converting them into Variant Calling Format (VCF) files. These VCF files were then uploaded to the QCI Variant Interpreter platform, where advanced computational algorithms were applied to classify the variants. The classification process adhered to the collaborative guidelines set forth by the American College of Medical Genetics and the Association for Molecular Pathology, ensuring a standardized and rigorous assessment of the identified genetic variants. TMB score is calculated as somatic mutation burden per mega base (mut/Mb)

Objective 2 was achieved by analyzing TMB in different tumor subsets as described:

TMB was observed prospectively and retrospectively in hematological malignancies and solid tumors.

There were 188 cases of hematological malignancies and 50 cases of solid tumor in which TMB analysis was done.

Distribution of 188 cases of hematological malignancies and 50 cases of solid tumor for TMB analysis

Figure 1: Distribution of 188 cases of Hematological Malignancies

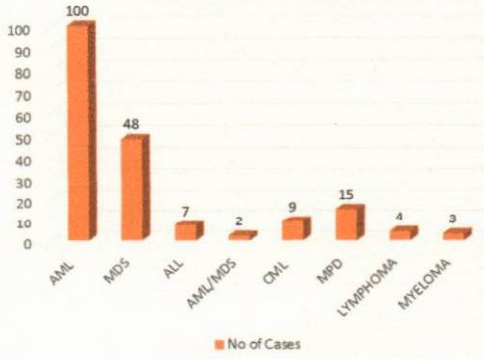
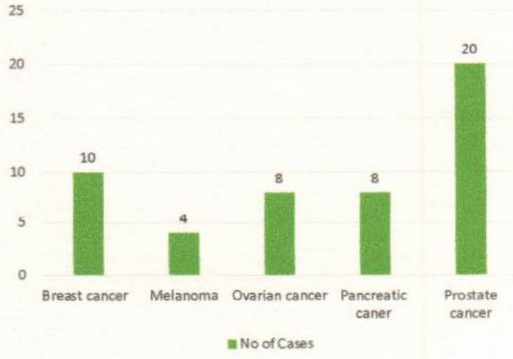


Figure 2: Distribution of 50 cases of Solid tumors



TMB score ≤ 5/Mb was considered low; 5-10/Mb was considered intermediate, >10 and ≤50/Mb was considered high and >50/Mb was considered very high.

The analysis suggested:

i) Among the 193 cases of hematological malignancies TMB score was low ≤ 5/Mb

ii) Among the 50 cases of solid tumors TMB score was high (>10 and ≤ 50/Mb) in (three out of four i.e., 75 % cases of melanoma), 1/10 i.e., 10% cases of breast cancer, and 1/10 i.e., 10% cases of ovarian carcinoma. TMB score was intermediate (5-10/Mb) in 1 case of melanoma, breast cancer, ovarian cancer and pancreatic cancer. In the remaining cases it was low.

Objective 3 was achieved by understanding the clinical relevance TMB score in different subsets of tumor.

The TMB is known to be associated with an increased number of neoantigens, which are tumour specific markers displayed by cells. Therefore, an increase in these antigens may then lead to increased detection of cancer cells by the immune system and more robust activation of cytotoxic T-lymphocytes. Activation of T-cells is further regulated by immune checkpoints that can be displayed by cancer cells. Thus, treatment with Immune checkpoint inhibitors (ICI) can lead to improved patient survival. Immune checkpoint inhibitor (ICI) using antibodies like anti-CTLA-4, anti-PD-1, and anti-PD-L1, that disrupt PD1 can lead to durable responses in a wide variety of human cancers.

The results signify that tumors with high TMB score may have benefits form immune check point inhibitors drugs.

Annexure 3

iii) Proposed utilization of the experience in India

This experience and expertise gained during this long-term International fellowships by ICMR at the Georgia Esoteric & Molecular Laboratory, at Augusta University, Georgia in Molecular Pathology will help to improve technical, interpretive, clinical applications, and quality control/quality assurance aspects of molecular genetic testing for infectious diseases, hematopathology and oncology.

The implementation of experience and expertise in following areas is proposed:

1. Improvement of molecular testing in clinically relevant areas and independently interpreting the results from qualitative, quantitative, and cancer mutation profile test, including appropriate reporting and medical significance of testing results at Institute.
2. Establishing and implementing technology such as Optical genome mapping for structural variants in diseases including cancer
3. Practicing the knowledge of quality control and quality assurance for molecular testing to improve the standard of patient care
4. Utilization of experience of gained in molecular testing in clinical interpretation of results for Optical genome mapping, Next Generation Sequencing mutation profiling and genotyping assays, including appropriate reporting and medical significance of testing results.
5. Delivering guest lectures and disseminating the knowledge and expertise at various platforms (conference, educational societies) across India and training the faculty and staff in latest technology
6. Working on related research projects and getting extramural funds for further research in the Molecular Pathology