Best selected papers of the applicant highlighting important discoveries

1. Dharavath B, Butle A, Chaudhary A, Mangade S, Pal A, Desai S, Thorat R, Upadhyay P, Nair S, **Dutt A***. UBEC3-LRP5 Fusion is a Novel Oncogenic Driver in Head and Neck Cancer with Therapeutic Implications. ***NPJ Precision Oncology***. 2024: Mar 4;8(1):63. ***IF: 10.1***

About: We identified a previously unidentified fusion transcript *UBE3C-LRP5* in 5.3% and 1.2% of cases, respectively using whole genome sequencing. This *UBE3C-LRP5* fusion activates *in vitro* and *in vivo*, and promotes the proliferation, migration, and invasion of head and neck cancer cells. It activates the Wnt/β-catenin pathway by promoting the nuclear accumulation of β-catenin. Pyrvinium pamoate, an anti-helminthic FDA-approved drug, which inhibits the Wnt/β-catenin pathway by degrading β-catenin, significantly reduces the transforming ability of cells expressing the fusion protein and improves survival in mice bearing tumors of fusion-overexpressing cells. Furthermore, patients with the *UBE3C-LRP5* fusion showed a tendency for poor survival in survival analysis. These findings suggest that the *UBE3C-LRP5* fusion could be a promising target for treating head and neck cancer.

2. Dharavath B, Butle A, Pal A, Desai S, Upadhyay P, Rane A, Khandelwal R, Manavalan S, Thorat R, Sonawane K, Vaish R, Gera P, Bal M, D'Cruz AK, Nair S, **Dutt A***. Role of miR-944/MMP10/AXL- axis in lymph node metastasis in tongue cancer. ***Commun Biol***. 2023 Jan 17;6(1):57. ***IF: 6.1***

About: Lymph node metastasis is a major factor in predicting the severity of tongue cancer. We identified a biomarker for early-stage tongue cancer that predicts lymph node metastasis. The protein *MMP10* is over-expressed in 86% of patients with metastasis, making it a potential marker to spare 70-80% of patients from unnecessary surgery. We also found *miR-944*, which reduces *MMP10* and inhibits cancer cell spread. Mouse experiments supported these findings, showing reduced metastasis by lowering *MMP10*. *MMP10* also influences genes related to metastasis. *MMP10* and *AXL* overexpression correlate with poor survival in tongue cancer patients. This axis could improve prognosis through *miR-944/MMP10/AXL* expression screening, reducing misdiagnoses and enhancing treatment.

3. Desai S, Ahmad S, Bawaskar B, Rashmi S, Mishra R, Lakhwani D, **Dutt A***. Singleton mutations in large-scale cancer genome studies: uncovering the tail of cancer genome. ***NAR Cancer*.** 2024 Mar 12;6(1):zcae010. doi: 10.1093/narcan/zcae010. ***IF: 5.1***

About: The study introduces a tool called the Domain Driver Mutation Estimator (DOME), designed to identify rare driver mutations, which are difficult to detect due to their low frequency. DOME analyzes mutations by considering known statistical hotspots, resistant mutations, and their functional and biochemical context within protein structures. It integrates the CADD scoring system to enhance prediction accuracy. When compared to seven other tools, DOME demonstrated superior or comparable accuracy in identifying functional cancer driver mutations, with only one tool outperforming it. DOME identified 32,917 high-confidence driver mutations across 1,331 genes, including many genes not typically associated with cancer, highlighting its distinctive role in cancer genome analysis. In a broader analysis of tumor samples, DOME detected 847 potential driver mutations, particularly in tyrosine kinase genes, which are significant in cancer. Overall, DOME effectively complements existing methods for detecting novel, low-frequency driver and resistant mutations, contributing to personalized cancer therapy.

Best selected papers of the applicant highlighting important discoveries

4. Desai S, Dharavath B, Manavalan S, Rane A, Redhu AK, Sunder R, Butle A, Mishra R, Joshi A, Togar T, Apte S, Bala P, Chandrani P, Chopra S, Bashyam MD, Banerjee A, Prabhash K, Nair S, **Dutt A***. Fusobacterium nucleatum is associated with inflammation and poor survival in early-stage HPV-negative tongue cancer. ***NAR Cancer***. 2022 Mar 4;4(1):zcac006. doi: 10.1093/narcan/zcac006. ***IF: 5.1***

About: We present an in-depth infectious pathogen analysis of 349 whole exomes and 1,058 transcriptomes of samples derived from breast, lung, gallbladder, cervical, colorectal (CRC) and head and neck cancer (HNSC) patients of Indian origin and TCGA sample set. In the first, in addition to known cancer-associated pathogens, like HPV in cervical and HNSC or *Bacteroides, Fusobacterium* and pathogenic *Escherichia* in CRC, we identify a significant prevalence of *Fusobacterium* in head and neck cancer (HNSC) samples, comparable to colorectal tumours. In the TCGA-HNSC tumors, *Fusobacterium nucleatum* occurs mutually exclusive to infection with HPV, which is known to have distinguished genomic and immune characteristics.

5. Upadhyay P, Gardi N, Desai S, Chandrani P, Joshi A, Dharavath B, Arora P, Bal M, Nair S, **Dutt A***. Genomic characterization of tobacco/nut chewing HPV-negative early stage tongue tumors identify MMP10 asa candidate to predict metastases. ***Oral Oncol***. 2017 Oct;73:56-64. doi: 10.1016/j.oraloncology.2017.08.003. ***IF: 5.97; CI: 36***

About: We present the first and most comprehensive glance to genomic alterations and mutational signature across 57 early stage (pT1 and pT2) derived from HPV-negative early stage tongue cancer patients habitual of chewing betel nuts, areca nuts, lime or tobacco using by whole exome and whole transcriptome sequencing followed by validation using orthologous methods. We present several lines of distinct features underlie this study attributing to unique aetiology, subsite, and specific population, which have been previously described for HNSCC The mutational profile of large fraction of patients display high frequency (53%) of C:G > A:T transversion in exome sequencing data—a hallmark of tobacco usage—reflecting tobacco as the most predominant etiological agent. We present the first report to describe *EGFR* amplification in TSCC mutually exclusive to 11q13.3 (*CCND1, FGF19, ORAOV1, FADD*) amplification among HPV-negative early TSCC tumors. Most significantly, we identify gene-sets involved in EMT processes with significant overexpression of MMP10 in 48% early stage TSCC tumors (n=50) as a potential candidate prognostic biomarker in early stage tongue cancer patients to predict nodal metastases.

6. Yadav N, Sunder R, Desai S, Dharavath B, Chandrani P, Godbole M, **Dutt A***. Progesterone modulates the DSCAM-AS1/miR-130a/ESR1 axis to suppress cell invasion and migration in breast cancer. ***Breast Cancer Res***. 2022 Dec 28;24(1):97. ***IF: 8.3***

About: To understand the molecular basis of the beneficial effects of progesterone, we performed a differential analysis of whole transcriptome dataset for non-coding RNAs in 30 breast cancer patients and tumor cell lines treated with hydroxy progesterone. We identify down-regulation of a long non-coding RNA, *Down Syndrome Cell Adhesion Molecule* (*DSCAM-AS1),* upon progesterone treatment in hormone receptor-positive breast cancer cells. The *DSCAM-AS1* sponges the activity of a miRNA, *miR-130a* that targets the 3'-UTR region of the estrogen receptor gene (*ESR1*) impeding breast cancer cell invasion and migration similar to progesterone treatment. Additionally, we show that breast cancer patients with high expression of *miR-130a* or low expression of *DSCAM-AS1* correlate with better survival

outcomes similar to progesterone treatment. Taken together, this study is the first lead to describe the progesterone-responsive long non-coding RNAs and their mechanical functional insight involving the *DSCAM-AS1/miR-130a/ESR1* genomic axis downstream to progesterone to impede breast cancer cell invasion and migration.

7. Desai S, Rashmi S, Rane A, Dharavath B, Sawant A, **Dutt A***. An integrated approach to determine the abundance, mutation rate and phylogeny of the genome. ***Brief Bioinform***. 2021 Mar 22;22(2):1065-1075. doi: 10.1093/bib/bbaa437. ***IF: 13.9; CI: 17***

About: The plurality of advanced sequencing datasets-such as short and long reads-presents a formidable computational challenge to uniformly perform quantitative analysis. To address this, we developed Infectious Pathogen Detector (IPD), a graphical user interface (GUI) based automated quantification for 1060 pathogens from heterogeneous NGS data. The IPD predicts the occurrence and dynamics of variability among infectious pathogens to help automate the NGS based pathogen analysis and in responding to public health threats, efficaciously.

8. Godbole M, Togar T, Patel K, Dharavath B, Yadav N, Janjuha S, Gardi N, Tiwary K, Terwadkar P, Desai S, Prasad R, Dhamne H, Karve K, Salunkhe S, Kawle D, Chandrani P, Dutt S, Gupta S, Badwe RA, **Dutt A***. Up-regulation of the kinase gene SGK1 by progesterone activates the AP-1-NDRG1 axis in both PR-positive and -negative breast cancer cells. ***J Biol Chem***. 2018 Dec 14;293(50):19263-19276. doi:10.1074/jbc.RA118.002894. ***IF: 5.5; CI: 24***

About: We performed an integrated analysis of micro array based mRNA expression profile and deep sequencing of non-coding small RNA of breast cancer cells. We present an intricate convergence model indicating a dual-phase regulation downstream to progesterone treatment to regulate the expression of a *Serum- and Glucocorticoid-regulated Kinase* gene 1, *SGK1*: predominantly driven as a direct transcriptional target, in PR-positive breast cancer cells; and, down-regulation of *miR-29a* and *miR-101-1* targeting *SGK1* with relatively distinct effect in PR-negative breast cells in response to progesterone. We demonstrate with a series of over expression and shRNA knockdown assays, along with biochemical validations, that the stringent up-regulation of *SGK1* in response to progesterone lead to the activation of a tumor metastasis suppressor gene, *NDRG1,* via a set of AP-1 network genes that inactivates AKT1, ERK1/2 and EGFR kinases, impeding the invasion and migration of breast cancer cells. Thus, we propose a model for the mode of action of progesterone in breast cancer deciphering the molecular basis of a randomized clinical trial studying the effect of progesterone in breast cancer.

9. Iyer P, Shrikhande SV, Ranjan M, Joshi A, Gardi N, Prasad R, Dharavath B, Thorat R, Salunkhe S, Sahoo B, Chandrani P, Kore H, Mohanty B, Chaudhari V, Choughule A, Kawle D, Chaudhari P, Ingle A, Banavali S, Gera P, Ramadwar MR, Prabhash K, **Dutt A***. ERBB2 and KRAS alterations mediate response to EGFR inhibitors in early stage gallbladder cancer. ***Int J Cancer***. 2019 Apr 15;144(8):2008-2019. doi:10.1002/ijc.31916. ***IF: 7.4; CI: 38***

About: The uncommonness of gallbladder cancer in the developed world has contributed to the generally poor understanding of the disease and thus lends itself to the need for further research. We describe the landscape of somatic alterations among a clinically distinct early staged pT1/pT2 Indian gallbladder cancer patients. We report a novel recurrent activating *ERBB2* (V777L) somatic mutation in 6 of 44 gallbladder primary tumors with an overall

mutation frequency of 13%; along with *KRAS* activating mutations in 3 gallbladder cancer samples, which show constitutive phosphorylation of ERBB2 and EGFR. We demonstrate that treatment with *ERBB2*-specific, *EGFR*-specific shRNA or with a covalent EGFR family inhibitor BIBW-2992 inhibits transformation, survival, migration and invasion characteristics of gallbladder cancer cells harboring wild type or *KRAS* (G13D) but not *KRAS* (G12V) mutation. Our studies implicate *ERBB2* as an important therapeutic target in early stage gallbladder cancer. Most significantly, we present the first evidence that the presence of *KRAS* (G12V), but not *KRAS* (G13D) mutation, may preclude gallbladder cancer patients to respond to anti-EGFR treatment, similar to the clinical algorithm commonly practiced to opt for anti-EGFR treatment in colorectal cancer.

10. Rekhi B, Upadhyay P, Ramteke MP, **Dutt A***. MYOD1 (L122R) mutations are associated with spindle cell and sclerosing rhabdomyosarcomas with aggressive clinical outcomes. ***Mod Pathol.*** 2016 Dec;29(12):1532-1540. doi: 10.1038/modpathol.2016.144. ***IF: 7.8; CI: 92***

About: We analyzed a cohort of 49 rare primary RMS tumor samples of various subtypes, collected over a period of 9 years, for presence of *MYOD1* (L122R), *PIK3CA* (H1047) and *PIK3CA* (E542/E545) mutations, along with immunohistochemical analysis of desmin, myogenin and MYOD1, and clinical outcome. We report 20.4% *MYOD1* (L122R) mutation in RMS, found exclusively in spindle cell/sclerosing RMS subtype tumor. Moreover, a striking correlation was found between *MYOD1* mutation and clinical outcomes. Based on these findings, *MYOD1* (L122R) mutation may preclude the requirement to perform IHC analysis to identify an aggressive subset of spindle cell/sclerosing RMS patients early on to help inform adoption of appropriate therapeutic regimen. In over all, we present the first report of *MYOD1* (L122R) mutation in a large cohort of 49 RMS reported so far, as a single study, that are associated with a relatively aggressive clinical course.

11. Chandrani P, Prabhash K, Prasad R, Sethunath V, Ranjan M, Iyer P, Aich J, Dhamne H, Iyer DN, Upadhyay P, Mohanty B, Chandna P, Kumar R, Joshi A, Noronha V, Patil V, Ramaswamy A, Karpe A, Thorat R, Chaudhari P, Ingle A, Choughule A, Prabhash, K **Dutt A***. Drug-sensitive FGFR3 mutations in lung adenocarcinoma. ***Ann Oncol***. 2017 Mar 1;28(3):597-603. doi: 10.1093/annonc/mdw636. ***IF: 51.8; CI: 38***

About: We present the first mutational landscape of actionable alterations and the first systematic evidence that mutations of *FGFR3* are present in 20 of 363 (5.5%) lung adenocarcinoma patients of Indian origin. Our study establishes that *FGFR3* extracellular and kinase domain mutations present in lung adenocarcinoma patients are oncogenic and sensitive to small molecule inhibitors *in vitro* and *in vivo* systems, using mouse xenograft assays. Interestingly, the *FGFR3* mutations appear to be significantly higher in proportion of patients less patients less than 45 years old ($P = 0.048$), and show a trend towards better overall survival of 17 months (n= 8; 95% CI: 6.4-27.5; HR: 0.6; P= 0.5). These findings implicates *FGFR3* as a novel therapeutic target in lung adenocarcinoma.