Original Article

A brief report on the mutational landscape in non-small cell lung cancer of South Asian patients: Comparison at a US and an Indian Institution

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ABSTRACT

Background: Various molecular underpinnings of lung cancer have been noted in Asian populations, especially with targetable oncogenic drivers such as EGFR mutations and ALK rearrangements, although they have been lesser described in South Asian/Indian patients. **Methods:** Tumour molecular testing results from non-small cell lung cancer (NSCLC) patients with a name of South Asian origin and diagnosed from 2005 to 2019 at the Stanford Cancer Center in the United States were retrospectively reviewed and compared to the results of molecular testing from PGIMER in Chandigarh, India, from the patients diagnosed from 2011 to 2019. **Results:** We identified 72 patients of South Asian (largely Indian) origin, of whom 64 patients (51% female) had mutational testing at Stanford. Of the tested patients, 33% of cases harboured either an EGFR exon 19 deletion or exon 21 L858R mutation, and 12.5% had ALK rearrangements. At PGIMER, a larger sample of 1,264 patients was identified (33% female), with 22.5% of patients having two main EGFR activating mutations, and 9.5% harbouring an ALK rearrangement. **Conclusions:** South Asian, largely Indian, patients with NSCLC appear to have a higher chance of harbouring EGFR mutations and ALK translocation as compared to Caucasians. The percentage of South Asian patients with these molecular abnormalities was largely similar in two different geographical locations. These findings corroborate prior single-institution findings and emphasise the importance of molecular testing.

KEY WORDS: ALK, EGFR, mutational analysis, NSCLC

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INTRODUCTION

The range of estimated prevalence of *EGFR* mutations and *ALK* translocations across South Asian populations with non-small cell lung cancer (NSCLC) varies. Estimates range from 20 to 35% for EGFR and 4-8% for ALK.[1,2] These results come largely from tertiary referral centres in India, however, there is limited data on patients of

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Indian (or more largely, South Asian) origin and mutational characteristics from areas outside of the country. This is of specific interest given the interest in exploring the impact of ethnicity on tumour mutational frequency, noting the significant difference with *EGFR*-activating mutations as an example, where up to 50% of lung adenocarcinomas in East Asians may harbour this mutation, while only 15% North Americans and Europeans are diagnosed with lung adenocarcinoma harbouring these mutations.[3] These discrepancies in mutational rates in different populations led to this brief report, evaluating the differences in mutational changes in South Asians (largely those of Indian origin) at a United States (US) academic institution compared to an Indian academic institution.

METHODS

We performed a retrospective review of NSCLC cases at the Stanford Cancer Centre (Stanford, CA, US) and the Postgraduate Institute of Medical Education and Research (PGIMER) (Chandigarh, India). At Stanford, we identified 1,915 patients with a self-identified race of "Asian" in the electronic medical record, who also had a diagnosis of "adenocarcinoma", and who had had an encounter with a thoracic medical oncologist at the Stanford Cancer Centre from January 2005 to December 2019. The "adenocarcinoma" criteria included all types of cancers, and resulted in a large number of patients, as a thoracic medical oncologist may have had an encounter with non-thoracic oncology patients also. From this cohort of 1,915 patients, we identified 72 patients with NSCLC, who had a South Asian name and who we confirmed with manual chart review as having identified their ethnicity as originating from India, Pakistan, Bangladesh, or Sri Lanka. Patients were excluded if they did not have a confirmation of their ethnicity in the electronic medical record. Staging was done using the 7th edition of the TNM classification at both centres. Patients diagnosed at PGIMER (all of whom identified from Indian ethnicity) were included if diagnosed from January 2011-December 2019. At both sites, molecular testing results were recorded and compared. We collected data on sex, smoking status, pathologic diagnosis, tumour histology, disease stage and the type of molecular testing conducted. Molecular testing was performed as part of routine clinical care at Stanford but the methodologies have varied over the study period: DNA sequencing (2007-2011), SNaPshot (2011-2013) and Stanford Solid Tumour Actionable Mutation Panel (STAMP) (2013-Present). For EGFR mutation testing at PGIMER, real-time polymerase chain reaction (RT-PCR) and Sanger sequencing were mainly used with the rising use of next-generation sequencing (NGS). The latter, however still comprised a minority in the testing methods used. ALK relocations were ascertained using immunochemistry (D5F3 clone). The ROS1 rearrangement was tested using immunochemistry (D4D6 clone) and positive samples were confirmed by fluorescence *in situ* hybridisation (FISH). Frequencies of oncologic mutations and alterations were compared among different subgroups with descriptive statistics.

RESULTS

Demographics and testing characteristics for both cohorts are shown in Table 1. The Stanford cohort included 72

Table 1: Baseline demographics and disease characteristics

	***n*=72 (Stanford)**	***n*=1264 (PGIMER)**
Female	37 (51%)	415 (33%)
Median age of diagnosis, years (range)	64 (IQR 50-74)	60 (IQR 50-65)
Non-Smoker	62 (86%)	513^ (46%)
Country of Origin/Ethnicity	India- 68 (94%) Pakistan- 3 (4%) Bangladesh-1 (1%)	India (100%)
Pathology	Adenocarcinoma- 63 (88%) Adenocarcinoma with Mixed Features- 4 (6%) Squamous 3 (4%) Non-Small Cell NOS/Other: 2	**Adenocarcinoma- 1075 (94.6%) Squamous- 33 (2.9%) Non-Small Cell NOS- 17 (1.5%) Large Cell- 11 (1.0%)
Stage of Disease	I or II- 19 (26%) III- 8 (11%) IV-45 (63%)	*n*=1101 where staging data available I or II- 50 (4.5%) III: 259 (23.5%) IV: 792 (71.9%)
Mutation Testing Resulted/Number Tested	64/64 patients*	1163/1233 for EGFR 928/973 for ALK 62/62 for ROS1
	Type of Testing for Positive Mutations	
For *EGFR*:	RT-PCR: 16/28 (57.1%) NGS: 12/28 (42.9%)	Sanger Sequencing- 234/1163 (20.1%) RT-PCR: 984/1163 (84.6%)
For *ALK*:	FISH: 2/8 NGS: 4/8 (50%)	NGS: 12/1163 (1%) FISH: 53/928 (5.7%)
For *ROS1*:	NGS: 2/2 (100%) Other Mutations aside from above all detected through NGS	IHC: 919/928 (99%) IHC for ROS1: 62/62, with FISH confirmation if positive

IQR=interquartile range, NOS=not otherwise specified. *All patients had *EGFR/ALK* testing, others with broader panels as noted in Table 1. **Data available for 1136 patients. ^Data available for 1108 patients

South Asian patients (diagnosed 2005-2019), of whom, 64 patients had mutational testing (from 2009 to 2019). Twenty-one of the sixty-four patients (33%) had the two main activating EGFR mutations (14 with Exon 19 deletions and 7 with exon 21 L858R). Other molecular alterations included eight ALK rearrangements (12.5%), seven KRAS mutations (amplifications, G12V and G12C, 20.5% of NGS testing performed), two ROS1 fusions (3.1%), and two BRAF V600E mutations (5.9%, of NGS testing performed). In assessing the co-mutations, three ARID1A and three STK11 mutations were noted. At the progression of the disease (n = 61), the most common resistance mutations, if tested and resulted, included TP53, EGFR (T790M,), ERBB2 and KRAS.

The PGIMER cohort included 1,264 patient cases with mutational testing (2011-2019). Twenty-six percent (298/1163) had EGFR mutations, with 22.5% (262/1163) being the two main EGFR-activating mutations (exon 19 deletion and exon 21 L858R mutation). Nine and a half percent (88/928) of the patients tested had ALK rearrangements, and one patient (out of 62 tested) had a confirmed ROS1 fusion [Figure 1]. At the progression of the disease (n = 90), the most common mutations were EGFR (T790M in 35% of cases) in 81 patients with progression on EGFR-targeted treatments. EGFR was also detected in three patients who were initially EGFR wild-type.

Both cohorts largely included adenocarcinomas (94% in the Stanford cohort and 95% in the PGIMER cohort), and presented with advanced-stage disease (63% in the Stanford cohort and 72% in the PGIMER cohort). Figure 2 shows the distribution of all EGFR mutations (including non-exon 19 and exon 21 mutations) and ALK mutations by sex. In assessing the EGFR mutation prevalence on only men in the PGIMER cohort, the current or former smokers had a prevalence of 15.9% (79/497) while non-smokers had a prevalence of 32.6% (60/184) (x^2 23.09, $P < 0.0001$). There was limited broader NGS panel testing in the PGIMER cohort (n = 25), limiting frequency analysis of rarer and secondary mutations. For patients with EGFR mutations at PGIMER and experiencing disease progression on first/second generation EGFR TKIs, liquid biopsy detected the presence of exon 20 T90M resistance mutation in 35% (data presented in a separate scientific communication).

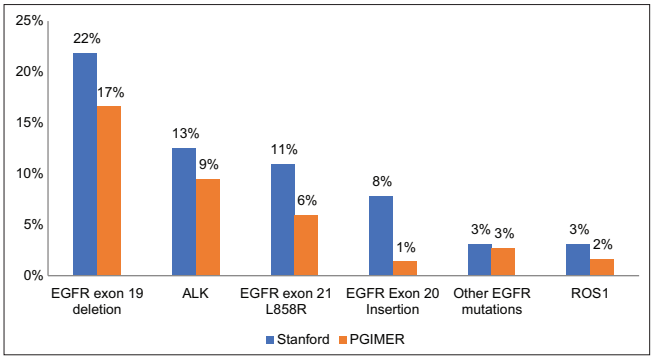

Figure 1: Mutation frequencies for common mutations at the Stanford Cancer Institute and PGIMER Chandigarh

CONCLUSIONS AND DISCUSSION

South Asian (largely Indian) patients with NSCLC appear to have a high chance of harbouring oncogenic drivers, especially *EGFR* mutations and *ALK* rearrangements, at two distinct geographical locations. These findings corroborate before single-institution findings, including those at a large tertiary cancer centre in western India.[4,5] Given the paucity of data on comparing frequencies of these oncogenic drivers from different institutions in different geographical settings, this collaboration adds to the data related to the impact of ethnicity on these oncogenic drivers. To our knowledge, this is one of the first reports of EGFR mutation and ALK rearrangement prevalence in the South Asian/Indian population based out of the US.

While our data are limited given their retrospective analysis, and susceptible to selection bias, we do report on the full available data at both institutions. The Stanford cohort is limited by its small sample size (about 3.7% of the screened Asian cohort), however, with the strength of manual verification of Indian/South Asian ethnicity through electronic medical record review. We may have missed patients who did not self-identify as "Asian" in the medical record and those with non-traditional names or those who changed their names through marriage or otherwise. The PGIMER cohort is limited by the scope of mutation testing for patients, hence, we report on those tested for each mutation type.

There are also some notable differences between these two populations: the PGIMER cohort had notably more men (67%) and also more smokers (54%), which reflects both cultural and social differences between the two populations. There is a high prevalence of smoking in North India, and by extension, in India in general, which has been documented in prior work.[6,7] For example, in a large cohort of over 73,000 subjects aged 15 years or older, the prevalence of ever smoking was 28.5% in men versus 2.1% in women.[8] Therefore, most lung cancer patients seen in North India are men (estimated ratio of 4.4 to 1 for male to female), with also a high prevalence of smoking *bidi* (a hand-rolled form of tobacco). In a prior work from PGIMER, we have noted that up to 56% of current or ex-smokers were exclusively *bidi* smokers.[7] Bidi smoking, however, still appears to have a similar risk

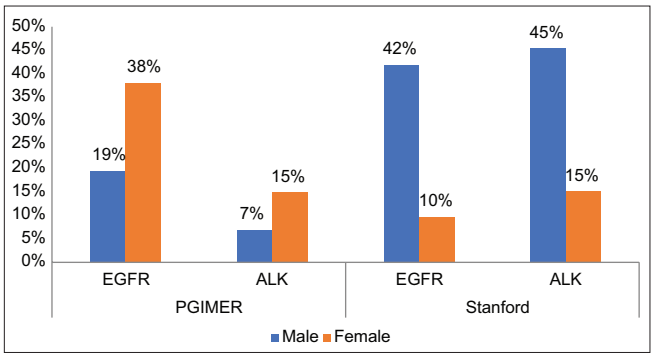

Figure 2: Prevalence of EGFR and ALK mutations by sex

association for developing lung cancer, compared with cigarette smoking.[9] Comparatively, Stanford's location in the greater Bay Area in California has more female patients. In the United States (US) as a whole, there has been a higher lung cancer incidence noted in young women, with the historical trend of higher rates in men reversing among non-Hispanic Whites and Hispanics.[10] The estimated prevalence of smoking in the greater Bay Area is estimated to be 10% in Asians/Pacific Islanders, however, there are no specific data on the specific prevalence in the Indian/South Asian community, or among women in that community.[11] The PGIMER cohort confirms the known association of EGFR mutations in non-smoking patients, and thus, the overall lower prevalence of these mutations compared to the Stanford cohort, where there were no smokers among women and only 28.6% of male smokers.

We also note that the PGIMER cohort has one of the lowest rates of EGFR mutation in India. Patients from the southern parts of India have been reported to have much higher rates of EGFR mutation (40-50%).[12,13] The observed higher mutation rate in the Stanford cohort could be because of a greater diversity in the cohort as well. As for ALK rearrangements/fusions, prior reports note an estimated prevalence of about 4-8% in the Indian populations.[14,15] Interestingly, in an exclusively South Indian population, the ALK rates were also slightly higher at 11% of patients, although with the limitation of this being a single report.[16] We report a rate of 9% at PGIMER and 13% at Stanford, which again shows a similar distribution, although with the same considerations of the Stanford cohort being a mixed population.

Lastly, there is also a substantial difference in the testing methodology employed at the two centress, with largely PCR methodology used at PGIMER, but rising use of NGS at Stanford over the years of this cohort analysis. However, even in considering these limitations, we believe that these similar rates of mutations seen here in two distinct geographical populations underline the importance of ethnicity despite geographical differences. For example, earlier work has shown that polymorphism frequencies within the *EGFR* gene are influenced by ethnicity.[17] These frequencies also mirror what is known about NSCLC in East Asia, where approximately one-third of patients are non-smokers, and many are diagnosed with adenocarcinoma with oncogenic driver mutations (as high as 90% in some analyses). Interestingly though, *EGFR* mutations are seen at higher rates (around 50%) for the East Asian populations (including Vietnamese, Thai, Chinese, and Filipino), but are much lower in the Indian and South Asian patients.[18] These findings prompt the question of why the mutation rate (at least for *EGFR)* appears to be lower in the Indian and South Asian populations, than in those of other Asian countries. Further research is needed to better elucidate these differences in the mutation rates and underlying mechanisms as treatment of these subsets of NSCLC continues to advance.

Ethical approval

IRB approval for molecular analysis September 2020 at Stanford, revised February 2022. (PGIMER : Ethics Committee Approval date June 2020).

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Conflicts of interest

There are no conflicts of interest.

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