Molecular profiling of breast and lung cancer in women with HIV reveals high tumor mutational burden

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Objective: This study compared the mutation profile and tumor mutational burden (TMB) in women with HIV (WWH) diagnosed with lung adenocarcinoma ($n = 8$) or breast ductal neoplasm ($n = 13$) who were enrolled into the Women's Interagency HIV Study (WIHS).

Design: Previous studies tended to focus on single institutions based on sample availability. This study is based on a representative, multicenter cohort that represents the racial and ethnic composition of women with HIV in the United States

Methods: The study sequenced the complete human exome of $n = 26$ cancer samples from HIV-positive women, using Ion torrent next-generation sequencing. The study cohort was compared with a HIV-negative cohort obtained from the Genomic Data Commons Data Portal of the NCI.

Results: There were no differences in known cancer mutations between breast cancer and lung cancer that developed in WWH and those that developed in HIV-negative (HIV-) women; however, WWH presented a significantly higher TMB in comparison to HIV- patients. Seventy-five percent of lung cancers and 61% of breast cancers were defined as TMB-high (more than 10 mutation/mb of DNA).

Conclusion: This study affirms the recommendation that WWH be included in clinical trials of novel treatments for these cancers. Although these data are preliminary, the high TMB in WLHV suggests, paradoxically, that this immune challenged population may benefit greatly from immune checkpoint inhibitor therapies.

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Introduction

Infection with HIV increases the risk of cancer in people with HIV (PWH). The oncogenic properties of HIV go beyond systemic immunodeficiency due to $CD4^+$ depletion during the end stage of HIV disease. They may be attributable to viral proteins directly, or to events over the long preclinical course of infection. Little is known how cancers that develop in PWH differ from cancers that develop in persons who were not exposed to HIV-related immune suppression and persistent low-level inflammation. Consequently, HIV was classified an oncogenic virus [1]. Using whole exome sequencing, this study ascertained the mutational status of lung and breast cancers that developed in a well characterized cohort of women with HIV (WWH) and compared it with the general population. We hypothesized that cancers in HIV-infected persons would exhibit genomic patterns that could inform cancer therapy in this population.

The introduction, early, and lifelong application of effective combination antiviral therapy (cART) reduced the risk of severe immune deficiency and progression to AIDS, which was defined as a combination of opportunistic infections and viral associated cancers, such as Kaposi sarcoma and lymphoma. In low and middle-income countries (LMIC) with less than optimal access to cART and a less than ideal public health structure, most cancers that develop in PWH are still those associated with viral infections and loss of immune function [2]. In countries with ready access to cART noninfection-associated cancers, such as breast cancer and lung cancer are on the rise and are predicted to become the most prevalent cancer types in PWH [3]. This rise in breast and lung cancer is primarily attributable to the increased median age of PWH [4,5]. Lung cancer is emerging as one the leading causes of deaths among PWH, as it already is in the general population. The increased incidence of lung cancer has been attributed to the more frequent smoking in PWH [6–10]. However, some studies found that lung cancer incidence remained significantly elevated in PWH, even after adjustment for smoking habits [11]. Adenocarcinoma, the most common type of lung cancer in PWH, is not strongly linked to smoking. These epidemiological observations raise the possibility that HIV-infection may be an additional risk factor for lung cancer [12,13].

Another common cancer in PWH is breast cancer. Here, the epidemiological data are less clear. Breast cancer is the most common cancer in women world-wide. WWH do not have a higher risk of developing breast cancer than HIV-seronegative (HIV-) women [14]; however, this may be explained by competing comorbidities in HIV-positive women prior to the introduction of cART. The proportion of HIV-positive women with breast cancer is increasing over time. Importantly, HIV-positive women with breast cancer have a two-fold lower survival relative to cases in the general population, despite – presumably – equal access to cancer treatment and cART. This survival difference motivated this study of common genomic alterations that define breast cancer in HIV-positive women as compared to HIV- women.

The assertion of a direct role for HIV in oncogenesis, beyond modulating the immune system, is controversial. No HIV-specific mechanisms driving increased lung and breast cancer risk in PWH has been identified. Few genomic studies of lung and breast cancer in WWH have been reported [15,16]. They tended to be single institution-based and relied on convenience samples or case reports. By comparison, this study is based on a multicenter cohort of women with HIV in the USA. The Women's Interagency HIV Study (WIHS) is a large, prospective cohort study designed to investigate the consequences of HIV disease in women.

Within the sample size limitations of this study, no evidence for specific genomic differences between breast cancer and lung cancer were discovered; however, tumor mutational burden (TMB) was far greater in PWH than matched controls. It remains unclear why both cancer risk and cancer survival is worse among PWH than for the general population. This study supports the recent recommendation that PWH should be included in all clinical trials of novel treatment approaches for these two cancers and receive the same consistent clinical treatment according to the same standards of care as HIV-negative persons.

Materials and methods

Samples

The WIHS is a cohort of women with and at risk for HIV infection [17]. Seven WIHS sites (New York, Chicago, Washington, DC, San Francisco, Chapel Hill, Atlanta, and Miami) contributed 26 tissue blocks as well as matching PBMC from breast and lung cancer cases between 2000 and 2017 after obtaining participant consent. Specimens were de-identified replacing the patient information with a WIHS ID number. The study was IRB approved at each participating WIHS site. Lung

($n = 219$) and breast ($n = 719$) cancer sequencing data from HIV-negative individuals were obtained from Genomic Data Commons Data Portal.

DNA extraction

Tumor DNA was extracted using the QIAamp DNA FFPE Tissue Kit (Qiagen Inc., Venlo, The Netherlands) per manufacturer protocol with 3 h tissue digestion. PBMC DNA was extracted using a MagNA Pure Compact Instrument and Nucleic Acid Isolation Kit I - Large Volume (Roche Inc., Mannheim, Germany).

Exome analysis

Barcoded libraries were prepared from 100 ng DNA with an Ion AmpliSeq Exome RDY library preparation kit (Thermo Fisher, Waltham, Massachusetts, USA) quantitated by Qubit dsDNA HS assay, sized with an Agilent Bioanalyzer 2100 high-sensitivity DNA assay (Agilent Technologies, Santa Clara, California, USA), and pooled to 80 pmol/l final concentration before sequencing on the Ion Chef and S5 sequencer (Thermo Fisher). Base calling, quality filtering, and demultiplexing were performed on the instrument with default parameters. Reads greater than 50 bp were mapped to the human genome (NCBI build hg38_2016) using CLC Genomics Workbench 9 (Qiagen Inc.) and low-frequency variant detection tool with the parameters: minimum frequency = 10%, and minimum coverage = 100x. Only nonsynonymous variants with a minimum average quality score of at least 19 and a forward/ reverse balance of 0.5 were included. Tumor variants were filtered against germline variants obtained from PBMC for each patient.

Statistical analysis

Results are reported as mean ± standard deviation (SD). The unpaired two-tailed *t*-test with Welch correction was used to compare groups.

Results

The study sequenced the human exomes of initially $n = 26$ tumor biopsies from HIV-positive women provided as formalin-fixed paraffin-embedded (FFPE) blocks or slides, as well as matched PBMC samples. Two samples were excluded due to the slides being stained, two samples were excluded due to low DNA yield, and one additional sample was excluded due to low library quality. A total of 21 samples, 8 lung and 13 breast cancers, and matched normal PBMC, were included in the final analysis. All samples were primary tumors and confirmed by disease. The clinical information for the study cohort is provided in Table 1. The participants were between 41 and 73 years old, with an average of 54.5 and 56.8 years for the lung and breast cohort, respectively. Most patients were either white or African–American. Only one person identified as Hispanic. For the lung cohort, 87.5% were adenocarcinomas and 12.5% were squamous cell carcinomas. For the breast cancer cohort, 100% were ductal carcinomas, 38.5% were classified as *in situ*, and 61.5% as invasive.

All samples were subjected to targeted amplification and next-generation sequencing. The reads were aligned to the human genome (NCBI build hg38_2016) and single nucleotide variants (SNVs) identified. A median of $42\,106\,610 \pm 13\,447\,761$ reads were obtained for each tumor sample, translating to $88.07\% \pm 0.03\%$ median coverage at 10x. A median of $33\,157\,434 \pm 11\,861\,795$ reads was obtained for each PBMC sample, translating to $93.08\% \pm 0.04\%$ median coverage at 10x. Any tumor SNVs that were also present in the matched PBMC samples were removed. To focus the data set further, all nonsynonymous SNVs were removed as well.

The study cohort was compared to a HIV- cohort obtained from the Genomic Data Commons Data Portal of the NCI. This cohort contained $n = 719$ breast and $n = 219$ lung cancer samples. The HIV seronegative

Table 1. Patient characteristics.

	Lung		Breast	
Characteristics	HIV- ($N = 219$)	HIV+ ($N = 8$)	HIV- ($N = 719$)	HIV+ ($N = 13$)
Age mean (range)	61.6 (41–73)	54.5 (41–73)	57.0 (41–73)	56.8 (41–73)
Ethnicity				
White	160 (73.1%)	5 (38.5%)	512 (71.2%)	2 (25.0%)
African–American	24 (11.0%)	5 (38.5%)	115 (16.0%)	6 (85.7%)
Other	35 (16.0%)	3 (23.1%)	92 (12.8%)	0 (0.0%)
Race				
Hispanic	3 (1.4%)	1 (7.69%)	48 (6.7%)	1 (12.5%)
Non-Hispanic	716 (99.6%)	12 (92.3%)	671 (93.3%)	7 (87.5%)
Subtype				
Adenocarcinoma	219 (100%)	7 (87.5%)	NA	NA
Ductal Carcinoma	NA	NA	719 (100%)	13 (100%)
Other	NA	1 (12.5%)	NA	NA

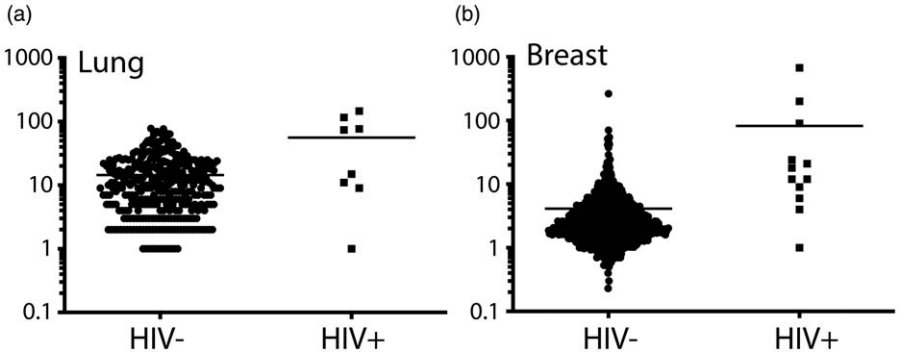

Fig. 1. Tumor mutational burden of HIV-positive vs. HIV- patients. Dot plot showing the total number of mutations per Mb observed in whole-exome sequencing of HIV-positive patients included in this study and HIV- patient obtained from the TCGA database for (a) breast and (b) lung malignancies. Each dot represents a tumor.

cohort was filtered by the following parameters sex: female, age: 41–74 years, sample type: primary tumor, subtype: breast ductal and lobular neoplasms, and lung adenocarcinomas, respectively. The HIV- group in lung cancer was further divided into smokers (>1 cigarette/day) and nonsmokers (<1 cigarette/day).

The most mutated gene in lung and breast samples was for TP53. There were no significant differences between the HIV-positive and HIV- cohorts, regarding the frequencies of key oncogenes and tumor suppressor genes.

Increased tumor mutational burden in breast and lung cancer in people with HIV

The term TMB describes the total number of mutations in DNA. It has emerged as a useful clinical predictor in certain cancer types and therapy types, such a checkpoint inhibitor therapy [18,19]. For lung cancer, the TMB for the HIV+ cohort (mean = 53.13/MB; range: 146.13–1.07/MB) was significantly higher than HIV- both nonsmoker (mean = 14.09/MB; range: 77.97–0.03/MB) and smoker (mean = 15.23/MB; range: 54.40-0.43/MB) (Fig. 1). However, TMB did not differ significantly between of nonsmokers and smokers in the HIV- cohort. For breast cancer, the same phenomenon is observed, where the HIV-positive cohort (mean = 82.46/MB; range: 673.7-0.40/MB) has a significantly higher TMB in comparison to the HIV- cohort (mean = 4.38/MB; range: 264.93-0.23/MB).

Discussion

Cancer has become the leading cause of mortality and morbidity in PWH (reviewed in [20]). Recent improvements to cART, such as long-lasting injectables, promise further advancements in the lifelong suppression of HIV viral loads; however, HIV infection rates are no longer declining in many countries, and a HIV cure or an HIV vaccine remains elusive. Hence, one can project an increase in the number of patients living with HIV and cancer [21].

This study tried to address a fundamental question in the field. Do cancers that develop in PWH differ from those that develop in the general population, and should we make cancer treatment recommendations specific to PWH?

In our cohort of HIV-positive women with lung adenocarcinoma or breast ductal cancer, we observed no significant differences in the frequency of the most common mutated oncogenes; however, this study observed a significantly higher TMB in the HIV-positive vs. the matched HIV- cohort. Seventy-five percent of lung cancer cases and 61.5% of breast cancer cases were TMB-high, while the matched HIV- cohort were 52.4 and 31% for lung and breast cancer, respectively. The main limitation of this study is the small sample size, as both HIV infection and cancer are rare events in the USA.

The incidences of breast and lung cancer have been rising in PWH and are projected to become the leading cause of mortality for this population. As TMB-high cancers are more susceptible to immune checkpoint inhibitor therapies, the results from this study reemphasize the notion that HIV-positive cancer patients on successful cART should not *a priori* be excluded from immune therapies. This conjecture is supported by recent successful clinical trials with pembrolizumab and nivolumab in HIV-positive KS, HIV-positive lung cancer, and in Merkel cell carcinoma [22,23].

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Conflicts of interest

There are no conflicts of interest.

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