Gynecologic Oncology Reports 19 (2017) 42–45

Contents lists available at ScienceDirect

Gynecologic Oncology Reports

journal homepage: www.elsevier.com/locate/gynor

Short communication

Predicted neoantigen load in non-hypermutated endometrial cancers: Correlation with outcome and tumor-specific genomic alterations

Sachet A. Shukla [a,b], Brooke E. Howitt [c], Catherine J. Wu [a,b], Panagiotis A. Konstantinopoulos [d,*]

[a] The Broad Institute of Harvard and MIT, Cambridge, MA 02142, USA
[b] Department of Medical Oncology, Dana Farber Cancer Institute, Harvard Medical School, USA
[c] Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, USA
[d] Medical Gynecologic Oncology Program, Dana Farber Cancer Institute, Harvard Medical School, USA

ARTICLE INFO

Article history:
Received 17 November 2016
Received in revised form 12 December 2016
Accepted 27 December 2016
Available online 29 December 2016

ABSTRACT

Elevated neoantigen load has been previously correlated with improved outcome and response to immune checkpoint blockade in various tumor types. In endometrial cancer, previous studies of neoantigen load prediction have shown that the hypermutated MSI and *POLE*-mutated tumors harbor significantly higher predicted neoantigen load compared to the hypomutated CN-low/endometrioid and CN-high/serous-like tumors. Here, we report that predicted neoantigen load may be a prognostic factor in hypomutated endometrial cancers, both in CN-low/endometrioid and CN-high/serous-like tumors. Specifically, in the TCGA dataset, CN-low/endometrioid tumors with neoantigen load in the highest tertile were associated with significantly improved progression free survival (PFS) ($p = 0.031$), while CN-high/serous-like tumors with neoantigen load in the lowest tertile were associated with worse PFS ($p = 0.041$). Importantly, certain tumor-specific genomic alterations were enriched in tumors with lower neoantigen load, including *CTNNB1* mutations in CN-low/endometrioid tumors and *MYC* amplification and *PIK3CA* mutations in CN-high/serous-like tumors. These findings suggest that predicted neoantigen load and specific genomic alterations (such as *CTNNB1* mutations, *MYC* amplification and *PIK3CA* mutations) may be biomarkers of response to immunotherapy in hypomutated endometrial cancers, and argues that these exploratory biomarkers should be incorporated in clinical trials of immune checkpoint blockade in this disease.

1. Introduction

The Cancer Genome Atlas (TCGA) project (Cancer Genome Atlas Research Network et al., 2013) has identified two groups of endometrial cancers with high mutation frequency (i.e., *POLE*-mutated and MSI endometrial cancers) and two groups with lower mutation frequency (hypomutated tumors): a group with low degree of somatic copy number alterations (SCNAs) which consisted of the majority of the microsatellite stable (MSS) endometrioid cancers (Copy-number-low (CN-low)/endometrioid group), and a group with extensive SCNAs that consisted primarily of serous-like cancers (CN-high/serous-like group) (Cancer Genome Atlas Research Network et al., 2013). These hypomutated CN-low/endometrioid and CN-high/serous-like endometrial cancers represented approximately 39% and 26% of all tumors in the endometrial TCGA dataset respectively, and exhibited a significantly lower mutation frequency of 2.9 and 2.3 mutations per megabase (Mb) compared to the 18 and 232 mutations per Mb observed in the hypermutated MSI and *POLE*-mutated tumors respectively.

Cancer specific neoantigens result from genetic alterations accumulated by tumor cells that create altered open reading frames (ORFs), i.e. neoORFs, which encode novel stretches of amino acids that are not present in the normal genome and therefore have not been previously recognized by the immune system. Total neoantigen load may be inferred/predicted by applying bioinformatics algorithms of in silico peptide translation on whole-exome sequencing data from patients' tumors (Shukla et al., 2015; Nielsen et al., 2007). Previous studies of neoantigen load prediction in endometrial cancer have shown that the hypermutated MSI and *POLE*-mutated endometrial cancers harbor significantly higher predicted neoantigen load compared to the hypomutated CN-low/endometrioid and CN-high/serous-like tumors (Howitt et al., 2015; van Gool et al., 2015). Furthermore, *POLE*-mutated endometrial cancers, which harbor the highest predicted neoantigen load, are associated with significantly improved outcome (Cancer Genome Atlas Research Network et al., 2013; Church et al., 2014); a similar association between elevated predicted neoantigen load and improved survival has also been noted in other tumors (Cancer Genome Atlas Research Network et al., 2013; Brown et al., 2014). Importantly,

* Corresponding author at: Dana Farber Cancer Institute, YC-1424, 450 Brookline Ave, Boston, MA 02215, USA.
E-mail address: panagiotis_konstantinopoulos@dfci.harvard.edu (P.A. Konstantinopoulos).

http://dx.doi.org/10.1016/j.gore.2016.12.009

higher predicted neoantigen load has been associated with clinical response to immune checkpoint blockade in various tumor types, including colon cancer, melanoma and non-small cell lung cancer (Desrichard et al., 2015).

In this study, we focused on the non-hypermutated endometrial cancers (i.e., the CN-low/endometrioid and the CN-high/serous-like groups), and assessed whether predicted neoantigen load may correlate with outcome and specific genotypes of endometrial cancer with the ultimate goal of identifying subsets of hypomutated endometrial cancers that may be good candidates for immunotherapy.

2. Methods

2.1. Tumors in the TCGA dataset

We accessed whole-exome sequencing data from 90 CN-low/endometrioid and 60 CN-high/serous-like endometrial tumors as defined in the endometrial TCGA dataset (Cancer Genome Atlas Research Network et al., 2013). Progression free survival (PFS) data were not available for 3 CN-low/endometrioid and 8 CN-high/serous-like endometrial cancers. These 11 tumors were included in the analysis of the neoantigen load and the definition of the cut-offs, but were not included in the survival analysis.

2.2. Prediction of HLA type and neoantigen load

Inference of HLA type was performed by applying the POLYSOLVER (POLYmorphic loci reSOLVER) tool (Rajasagi et al., 2014) to whole-exome sequencing (WES) data generated from TCGA consortium as previously described (Cancer Genome Atlas Research Network et al., 2013; Shukla et al., 2015). For prediction of neoantigen load, we used previously curated lists of somatic mutations (somatic single nucleotide variants and somatic insertions and deletions) for each of these samples (Sage Bionetworks' Synapse resource (http://www.synapse.org/#!synapse:syn1729383 and Lawrence et al. (2014))) from which individual-specific HLA-binding peptides were identified by a neoantigen prediction pipeline (Rajasagi et al., 2014) that uses detected somatic mutations in the individual. Binding affinities of all possible 9 and 10-mer mutant peptides to the corresponding POLYSOLVER-inferred HLA alleles were predicted using NetMHCpan (v2.4) (Nielsen et al., 2007). All predicted binders with affinity < 500 nM were used to evaluate the neoantigen load.

2.3. Statistical analyses

The *t*-test was used to analyze the difference in predicted neoantigen load between CN-low/endometrioid and CN-high/serous-like tumors while the Fisher's exact test was used to assess whether certain genetic alterations were enriched among tumors with high versus low predicted neoantigen load. The correlation between predicted neoantigen load and mutational load was assessed using the Pearson's correlation coefficient. Progression free survival (PFS) curves were generated using the Kaplan-Meier method, and statistical significance was assessed using the log-rank test. Significance was defined as a $p < 0.05$; all reported p values are two sided.

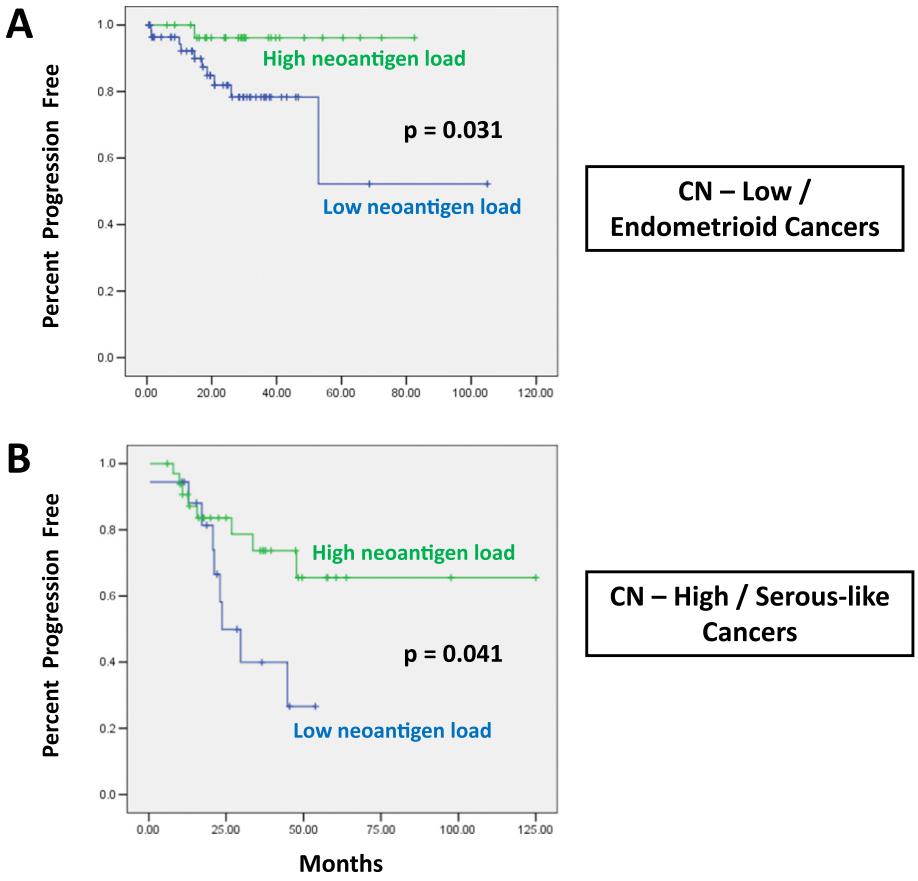

Fig. 1. Association between neoantigen load and PFS in hypomutated endometrial cancers. (A) CN-low/endometrioid tumors with neoantigen load in the highest tertile are associated with significantly improved PFS ($p = 0.031$). (B) CN-high/serous-like tumors with neoantigen load in the lowest tertile are associated with worse PFS ($p = 0.041$).