Advances in Radiation Oncology (2025) **10**, 101677

advances in radiation oncology

www.advancesradonc.org

Research Letter

Effect of External Beam Radiation Therapy and Brachytherapy on Circulating Myeloid-Derived Suppressor Cell Populations in Patients Treated Definitively for Cervical Cancer

Kelsey M. Wanhainen, MD, PhD,[a] Matthew Berkseth, PhD,[b] Nicole Sando, BS,[b] Lydia Golden, BS,[b] Amy Techam, RN, PHN,[c] Jennifer Wieworka, DNP,[c] Kyra M. Boorsma Bergerud, BS,[b] Peter Argenta, MD,[a,d,e] Andrea O'Shea, MD,[a,d] Britt K. Erickson, MD,[a,d,e] Sally Mullany, MD,[a,d] Colleen Rivard, MD,[a,d,e] Rahel Ghebre, MD, MPH,[a,d,e] Deanna Teoh, MD, MS,[a,d,e] Margaret Reynolds, MD,[a,d,e] Stephanie Terezakis, MD,[a,b,e] Jianling Yuan, MD, PhD,[a,b,e,1] and Lindsey Sloan, MD, PhD[a,b,e,1,*]

[a]University of Minnesota Medical School, Minneapolis, Minnesota; [b]Department of Radiation Oncology, University of Minnesota, Minneapolis, Minnesota; [c]M Health Fairview, Minneapolis, Minnesota; [d]Department of Obstetrics, Gynecology and Women's Health, University of Minnesota, Minneapolis, Minnesota; and [e]Masonic Cancer Center, University of Minnesota, Minneapolis, Minnesota

Received 30 May 2024; accepted 28 October 2024

Purpose: The immunosuppressive function of myeloid-derived suppressor cells (MDSCs) has been implicated in the regulation of immune responses against cancer and is associated with poor prognosis. Radiation treatment is known to alter immune cell populations within the tumor; however, whether this results in the recruitment of immunosuppressive MDSC populations is not well understood. Here we evaluate the response of circulating MDSC populations in patients treated per standard-of-care cisplatin chemoradiation therapy (CRT) for locally invasive cervical cancer.

Methods and Materials: Newly diagnosed, treatment-naïve patients with locally advanced cervical cancer were enrolled. Blood samples were collected from patients prior to starting CRT (T_0), after external beam radiation therapy (T_1), and after high-dose-rate brachytherapy (T_2). Samples from each time point were processed, and the prevalence of MDSC subsets was determined using flow cytometry. MDSC populations were identified using Live/Dead-CD11b+CD33+HLA-DR- staining. MDSC subsets were further subdivided into granulocytic (g-, CD15+CD14-), monocytic (m-, CD15-CD14+), or early-MDSCs (e-, CD15-CD14-).

Results: Most patients in our study were Caucasian nonsmokers with human papillomavirus-associated squamous cell carcinoma of the cervix. We saw a trend for increased MDSC frequency in patients with more advanced-stage disease at the time of initiating treatment. MDSCs increase in response to CRT and peak after brachytherapy (T_2). In particular, the g-MDSC subset increases by 6.44 times relative to the baseline. There was no correlation between MDSC expansion and response to therapy.

Sources of support: Data acquisition and analysis funded by the Sloan Laboratory at the University of Minnesota Medical School, Minneapolis, Minnesota.

Research data are stored in an institutional repository and will be shared upon request to the corresponding author.

[1]J.Y. and L.S are Co-senior authors.

*Corresponding author: Lindsey Sloan, MD, PhD; Email: sloan153@umn.edu

https://doi.org/10.1016/j.adro.2024.101677

Conclusion: Our study confirms other reports that circulating MDSCs in patients with cervical cancer increase in response to CRT and are associated with more advanced stages. Additionally, we show that MDSC expansion is driven by the g-MDSC subset. We did not see any correlation between MDSC expansion and treatment response, though this may be because of the limited sample size for this study.

Introduction

Myeloid-derived suppressor cells (MDSCs) are immature myeloid cells that develop in the context of chronic inflammation, such as cancer or infection. They are a heterogeneous population and are often subdivided into 2 subsets, monocytic- and granulocytic-MDSCs, based on phenotypic characteristics.[1] MDSCs are present in numerous types of cancer, both within the tumor stroma and associated blood/lymphoid tissue. They contribute to immune suppression and tumor progression by stimulating tumor cell proliferation and angiogenesis.[2,3] The presence of MDSCs within tumors is associated with an increased likelihood of progression during chemotherapy or radiation therapy and a high probability of future recurrence.

Cervical cancer is known as an immunogenic tumor due in part to the role of human papillomavirus (HPV) infection in its carcinogenesis and progression. The HPV-induced chronic inflammation also likely promotes the development of MDSCs in the tumor and peripheral blood of patients with cervical cancer. Consistent with this, several studies have shown that MDSCs are elevated in patients with cervical cancer and are associated with poor prognosis.[4-6]

There has been growing evidence that radiation therapy (RT) can augment anti-tumor immune responses through the immunostimulatory effect of damage-associated molecular patterns release and radiation-induced DNA damage, which lead to increased production of inflammatory cytokines, increased antigen presentation, and recruitment of cytotoxic T cells.[7,8] RT also results in the recruitment of immunosuppressive populations such as MDSCs. The outcomes of clinical studies investigating the interactions between MDSCs and RT have identified context-dependent heterogeneity based on both intrinsic (eg, tumor type) and extrinsic (eg, dose-fractionation) factors.[7,9] In cervical cancer, van Meir et al[10] showed that circulating MDSCs in patients with cervical cancer increase in response to CRT. However, this study did not report patient outcomes, and it remains unknown whether the increased frequency of MDSCs results in poor responses to RT. Here we investigated how circulating MDSC populations change in response to chemoradiation therapy (CRT) and whether changes in MDSC frequency or subsets are predictive of cervical cancer treatment responses.

Methods and Materials

Study design

After obtaining institutional review board approval from the University of Minnesota, we conducted a prospective, single-arm, single-institution, observational study of treatment-naïve patients undergoing primary CRT for locally advanced carcinoma of the cervix. Patients were recruited from either the gynecologic oncology or radiation oncology clinics. All provided written informed consent prior to any sample collection.

Patients were all treated per the standard of care at our institution for locally advanced disease. Briefly, external beam radiation therapy (EBRT) of 45 Gy in 25 fractions was delivered to the pelvis, with a simultaneous integrated boost of 52 to 55 Gy to involved regional lymph nodes and parametria, concurrent with weekly cisplatin (40 mg/m^2). Gross tumor was boosted via interstitial or tandem ring high-dose-rate brachytherapy (22.5-27.5 Gy) after completion of EBRT.

Blood samples were collected prior to initiating therapy (T_0), post-EBRT, prebrachytherapy (T_1), and 1 month postbrachytherapy (T_2). Treatment response was determined based on pretreatment positron emission tomography-computed tomography scans compared with a 3-month follow-up positron emission tomography-computed tomography scan after completing treatment. Tumor measurements in pretreatment pelvic magnetic resonance imaging were used to calculate tumor volume. Complete response was defined as the resolution of hypermetabolism in the cervix and any previously involved lymph nodes. A response less than a complete response was scored as a partial response.

Sample processing and analysis

Peripheral blood mononuclear cells were isolated from whole blood using density gradient centrifugation and stained for analysis using flow cytometry. MDSC populations were identified using Live/Dead$^-$CD11b$^+$CD33$^+$HLA-DR$^-$ staining. MDSC subsets were further subdivided into granulocytic (g-, CD15$^+$CD14$^-$), monocytic (m-, CD15$^-$CD14$^+$), or early-MDSCs (e-, CD15$^-$CD14$^-$). Fluoresce minus one and isotype controls were used for proper gating of MDSCs and MDSC subpopulations. Flow cytometric analysis was performed on a Fortessa, and data were analyzed using FlowJo software v10.8.

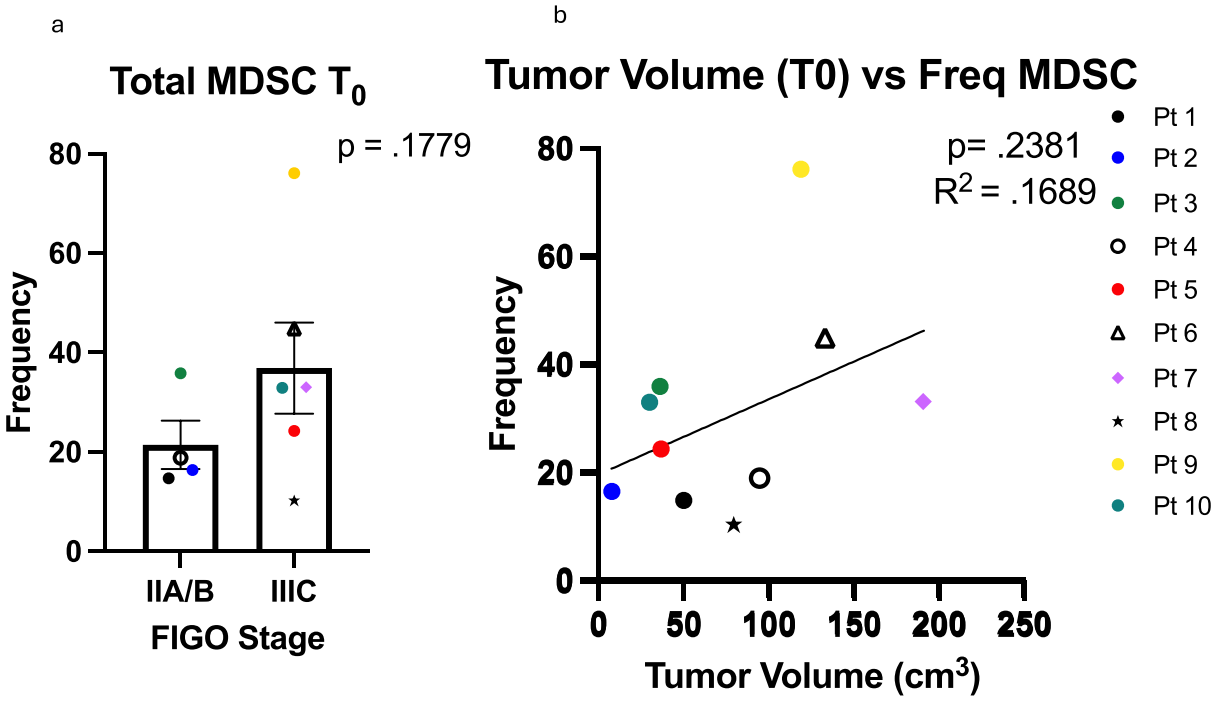

Figure 1 Increased circulating myeloid-derived suppressor cells (MDSCs) correlate with a more advanced stage of cervical cancer. Frequency (freq) total MDSC (Live/Dead$^-$CD11b$^+$CD33$^+$HLA-DR$^-$) was measured in the peripheral blood of the 10 enrolled patients (pts) at T_0 (prior to initiating CRT). (a) relative to tumor FIGO stage and (b) relative to estimated tumor burden on pre-treatment MRI scan. Statistical analysis was calculated using an unpaired *t* test (a) and simple linear regression (b).
Abbreviations: CRT = chemoradiation therapy; FIGO = International Federation of Gynecology and Obstetrics; MRI = magnetic resonance imaging.

Statistics

This effort was a pilot study with the primary goal of assessing circulating MDSC frequencies in patients undergoing CRT and providing an estimate of the effect size. Time constraints for this study limited the enrollment of additional patients. Data were subject to the Kolmogorov-Smirnov test to assess the normality of samples. Statistical differences were calculated using an unpaired 2-tailed T test. Correlation analyses were conducted using simple linear regression. All statistical analyses were performed using Prism 9. Graphical data are shown with error bars indicating the standard error of the mean.

Results

Patients

We enrolled 12 patients between July 2022 and July 2023. Two patients withdrew from the study prior to initial sample collection, leaving a final sample size of 10. One patient withdrew after initial sample collection and is only included in Fig. 1. Patient characteristics are reported in Table 1, but most patients had HPV-associated squamous cell carcinoma

Table 1 Patient characteristics

Characteristic	All subjects (n = 10)
Age (y), Mean (SD) Range	47.8 (10.4) 35-73
Presence of high risk HPV	7 (70%)*
Former/Current smoker	2 (20%)
Ethnicity	Caucasian: 7 (70%) Hispanic/Latino: 2(20%) Other: 1 (10%)
Tumor histology	Squamous cell carcinoma: 8 (80%) Adenocarcinoma: 2 (20%)
FIGO stage (2018)	IIA/B: 4 (40%) IIIC: 6 (60%)
Type of brachytherapy	Tandem and Ring: 6 (60%)† Interstitial: 3 (30%)
Response	Complete: 6 (60%) Partial: 4 (40%) Progression: 0 (0%)

Abbreviations: FIGO = International Federation of Gynecology and Obstetrics; HPV = Human Papillomavirus.
*HPV status unavailable for one patient
†One patient underwent 1 tandem and ring treatment initially and then 4 interstitial treatments

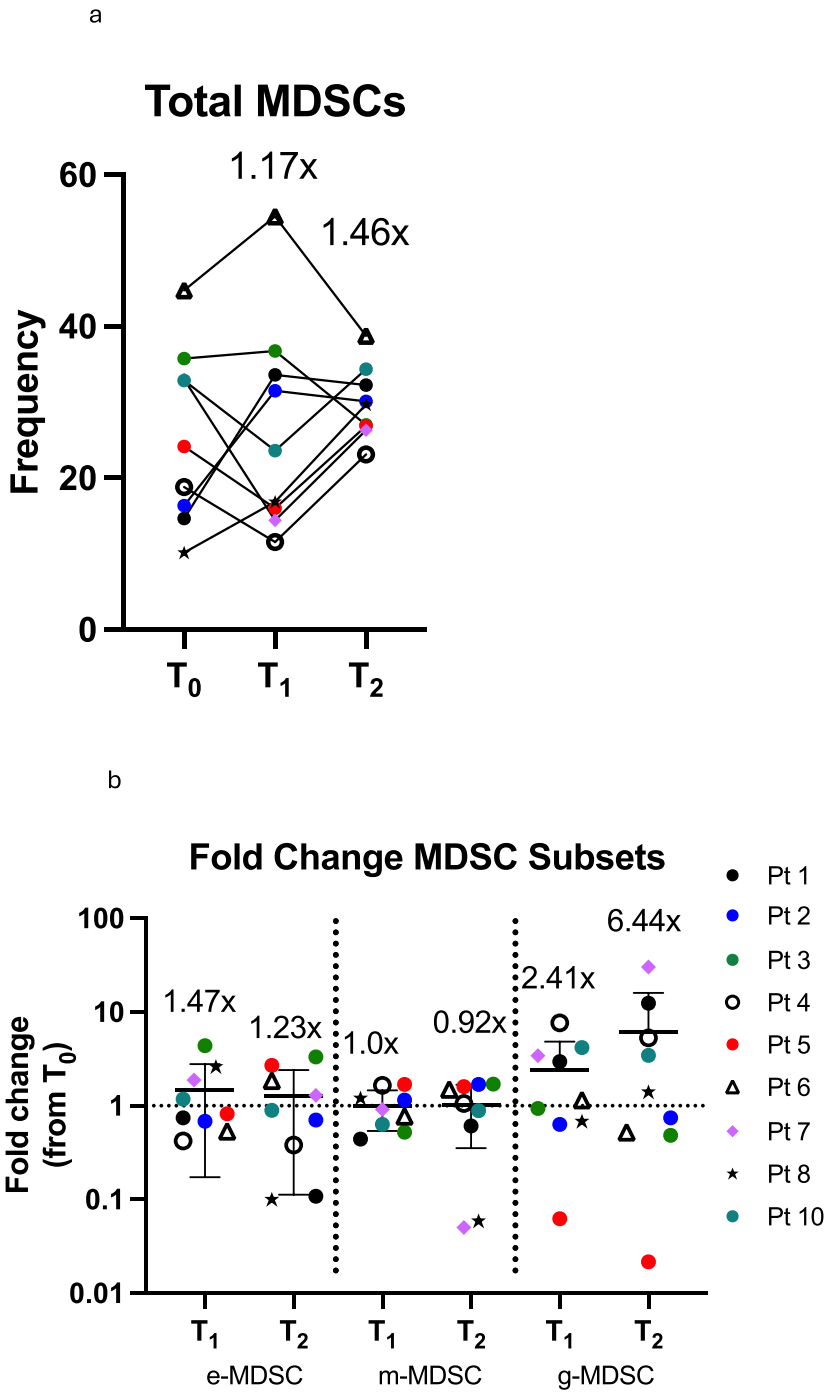

Figure 2 Granulocytic myeloid-derived suppressor cells (g-MDSCs) expand in response to external beam radiation therapy (EBRT) and brachytherapy. The frequency of total MDSC (Live/Dead$^-$CD11b$^+$CD33$^+$HLA-DR$^-$) was measured in the peripheral blood of patients (pts) with cervical cancer and then was further divided into MDSC subsets e-MDSC (CD14$^-$CD15$^-$), m-MDSC (CD14$^+$CD15$^-$), and g-MDSC (CD14$^-$CD15$^+$). Fold change of (a) total MDSC or (b) each MDSC subset from T_1 (post-EBRT) and T_2 (post brachytherapy) relative to T_0 (prior to initiating CRT) was calculated, and the average fold change indicated on the graph. Pt 9 was excluded from the analysis because they withdrew from the study after T_0.

Abbreviations: CRT = chemoradiation therapy; e-MDSC= early myeloid-derived suppressor cell; m-MDSC = monocytic myeloid-derived suppressor cell.

of the cervix and were Caucasian nonsmokers. International Federation of Gynecology and Obstetrics (FIGO) 2018 stage at diagnosis ranged from IIB to IIIC.

Circulating MDSCs are elevated in patients with advanced tumor stage

Fresh peripheral blood samples were analyzed from the 10 participants prior to initiating CRT to establish baseline MDSC frequencies (T_0). There was a trend for increased total MDSC frequency in patients with more advanced FIGO 2018 stage at the time of diagnosis (Fig. 1a). However, there was no obvious relationship between tumor volume at T_0 and circulating MDSC frequencies (Fig. 1b).

Frequency of g-MDSCs increases in response to RT

Peripheral blood samples were analyzed after completing EBRT (T_1) and 1 month after completing brachytherapy (T_2). Total circulating MDSCs in this cohort increased 1.46 times from baseline in response to EBRT and brachytherapy (Fig. 2a). MDSC expansion occurred primarily in the g-MDSC subset. The g-MDSCs increased 2.41 times relative to baseline in response to EBRT ($T_0 \rightarrow T_1$), and 6.44 times 1 month after completing brachytherapy ($T_0 \rightarrow T_2$) (Fig. 2b). There was no correlation between total MDSC or g-MDSC expansion and response to CRT (Fig. E1a, b). However, one patient with adenocarcinoma of the cervix (Patient 8) had an increased frequency of g-MDSCs at all time points relative to other patients with squamous cell carcinoma of the cervix (Fig. E2).

Discussion

We investigated the response of circulating MDSC populations to CRT in patients with cervical cancer. A previous study has demonstrated that circulating MDSC levels are increased in patients with more advanced-stage disease.[11] Though not statistically significant, in our cohort of patients, we also observed a trend for increased MDSC levels with more advanced FIGO 2018 stage. van Meir et al[10] demonstrated that MDSC levels peak at the end of EBRT and brachytherapy in patients with cervical cancer. Our data confirms that MDSCs expand in response to CRT but also shows that MDSC expansion is largely driven by g-MDSCs. Interestingly, the presence of g-MDSCs has been associated with increased tumor burden and poor survival in patients with cervical cancer.[6] We did not see any relationship between g-MDSC levels and the FIGO 2018 stage or response to CRT in our study.

Our study enrollment was time-limited, yielding a relatively small sample size and limiting our power to detect potentially relevant treatment effects. Additionally, our study analyzed circulating MDSCs in the peripheral blood, which may not be representative of MDSC populations within the tumor. In future studies, we would like to analyze the immune composition of pretreatment biopsy specimens and compare them to peripheral blood samples. However, our study is novel in that we show the dynamics of MDSC subsets at multiple time points throughout a patient's treatment course.

The patients in our study were treated with RT and concurrent cisplatin chemo-sensitization. A recent clinical trial demonstrated that checkpoint inhibitor pembrolizumab used in combination with CRT was efficacious for improving progression-free survival in patients with locally advanced cervical cancer,[12] prompting US Food and Drug Administration approval. MDSCs have been shown to directly interfere with T-cell- and B-cell-mediated responses against the tumors in cervical cancer.[13,14] Future studies should address the impact of adding immunotherapy to CRT regimens in modulating MDSC populations in patients with cervical cancer.

Disclosures

Lindsey Sloan receives research support from Research Support—GT Medical Technologies. Britt K. Erickson reported as an advisory board member of AstraZeneca, Merck, Gilead, and GSK. Deanna Teoh reported as an advisory board member of Asieris and received clinical trial support from Tesaro/GSK, Jounce Therapeutics, and Moderna. All other authors declare they have no competing interests.

Acknowledgments

We acknowledge funding from the Department of Radiation Oncology, Masonic Cancer Center, and Medical School at the University of Minnesota as well as the National Institutes of Health (grant nos. P01 CA254849, U54 CA268069).

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.adro.2024.101677.

References

1. Hegde S, Leader AM, Merad M. MDSC: Markers, development, states, and unaddressed complexity. *Immunity*. 2021;54:875-884.

2. De Cicco P, Ercolano G, Ianaro A. The new era of cancer immunotherapy: targeting myeloid-derived suppressor cells to overcome immune evasion. *Front Immunol*. 2020;11:1680.
3. Mabuchi S, Sasano T. Myeloid-derived suppressor cells as therapeutic targets in uterine cervical and endometrial cancers. *Cells*. 2021;10:1073.
4. Wu L, Liu H, Guo H, et al. Circulating and tumor-infiltrating myeloid-derived suppressor cells in cervical carcinoma patients. *Oncol Lett*. 2018;15:9507-9515.
5. Mabuchi S, Matsumoto Y, Kawano M, et al. Uterine cervical cancer displaying tumor-related leukocytosis: a distinct clinical entity with radioresistant feature. *J Natl Cancer Inst*. 2014;106:dju147.
6. Liang Y, Lü B, Zhao P, Lü W. Increased circulating GrMyeloid-derived suppressor cells correlated with tumor burden and survival in locally advanced cervical cancer patient. *J Cancer*. 2019;10:1341-1348.
7. Kang C, Jeong SY, Song SY, Choi EK. The emerging role of myeloid-derived suppressor cells in radiotherapy. *Radiat Oncol J*. 2020;38:1-10.
8. Liu C, Li X, Huang Q, et al. Single-cell RNA-sequencing reveals radiochemotherapy-induced innate immune activation and MHC-II upregulation in cervical cancer. *Signal Transduct Target Ther*. 2023;8:44.
9. Jiménez-Cortegana C, Galassi C, Klapp V, Gabrilovich DI, Galluzzi L. Myeloid-derived suppressor cells and radiotherapy. *Cancer Immunol Res*. 2022;10:545-557.
10. van Meir H, Nout RA, Welters MJ, et al. Impact of (chemo)radiotherapy on immune cell composition and function in cervical cancer patients. *Oncoimmunology*. 2016;6:e1267095.
11. Du T, Shen Y, Qu Q-X, Huang Q. The increase in circulating myeloid-derived suppressor cells correlated with clinical stage of cervical carcinoma. *Eur J Inflamm*. 2017;15:262-266.
12. Lorusso D, Xiang Y, Hasegawa K, et al. Pembrolizumab or placebo with chemoradiotherapy followed by pembrolizumab or placebo for newly diagnosed, high-risk, locally advanced cervical cancer (ENGOT-cx11/GOG-3047/KEYNOTE-A18): a randomised, double-blind, phase 3 clinical trial. *Lancet*. 2024;403:1341-1350. A.
13. Jianyi D, Haili G, Bo Y, et al. Myeloid-derived suppressor cells crosstalk with B10 cells by BAFF/BAFF-R pathway to promote immunosuppression in cervical cancer. *Cancer Immunol Immunother*. 2023;72:73-85.
14. Liang Y, Wang W, Zhu X, Yu M, Zhou C. Inhibition of myeloid-derived suppressive cell function with all-trans retinoic acid enhanced anti-PD-L1 efficacy in cervical cancer. *Sci Rep*. 2022;12:9619.