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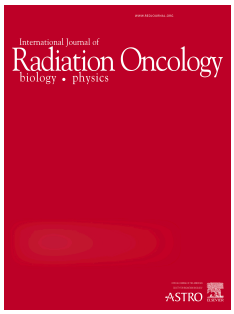

Absolute Lymphocyte Count Predicts Abscopal Responses and Outcomes in Patients Receiving Combined Immunotherapy and Radiotherapy: A prospective-retrospective analysis of 3 phase I/II Trials

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Absolute Lymphocyte Count Predicts Abscopal Responses and Outcomes in Patients Receiving Combined Immunotherapy and Radiotherapy: A prospective-retrospective analysis of 3 phase I/II Trials.

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Running Title: ALC Predicts Abscopal Responses

Conflicts of Interest: JWW reports research support from GlaxoSmithKline, Bristol Meyers Squibb, Merck , Nanobiotix, Mavu Pharma and Checkmate Pharmaceuticals. JWW serves on the scientific advisory board for RefleXion Medical, MolecularMatch, OncoResponse, CheckMate, Mavu Pharmaceuticals, Alpine Immune Sciences. He is co-founder of Helios Oncology, MolecularMatch, and OncoResponse and serves as an advisor to Astra Zeneca, Merck, MolecularMatch, Incyte, Aileron and Nanobiotix. JWW has the following patents; MP470 (amuvatinib), MRX34 regulation of PDL1, XRT technique to overcome immune resistance. MD Anderson Cancer Center has a trademark for RadScopal™. Remainder of authors declare no conflicts of interest.

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Absolute Lymphocyte Count Predicts Abscopal Responses and Outcomes in Patients Receiving Combined Immunotherapy and Radiotherapy: A Retrospective Analysis of 3 Phase I/II Trials

Abstract

Background: Research to elucidate predictive factors of the abscopal effect is an essential first step toward potentially modifying these factors to increase the incidence of systemic anti-tumor effects. This study, utilizing data from three institutional phase I/II trials, examined the predictive capacity of recorded parameters in patients undergoing combined radiotherapy (RT) and immunotherapy and explored outcomes based on those predictive factors.

Methods: All patients underwent combined immunotherapy and RT and had at least one nonirradiated noncontiguous lesion to evaluate out-of-field (abscopal) responses, defined as the best RECIST response.

Results: Altogether, 153 patients met the study criteria, and the median follow-up was 21.1 months. The most common cancer types were NSCLC (n=62), SCLC (n=25), head/neck cancers (n=16), and renal cell carcinoma (n=13). Immunotherapies included ipilimumab (n=98) or pembrolizumab (n=55). Multivariable linear regression indicated that post-RT ALC, when analyzed as a continuous variable, correlated with abscopal responses ($p<0.001$). For post-RT absolute lymphocyte count (ALC), the abscopal response rate was 34.2% in the cohort with ALC higher than the median value, compared to 3.9% in patients with ALC lower than the median ($P<0.0001$). Corresponding figures for pre-RT ALC were 30.3% vs. 7.8%, respectively ($P=0.0004$). Cox multivariate analysis confirmed that lower post-RT ALC also associated with poorer PFS ($p=0.009$) and OS ($p=0.026$).

Conclusion: Lymphopenia, measured as the continuous variable of post-RT ALC, may impact the occurrence of abscopal responses and thus influence prognosis in patients treated with RT and immunotherapy. Although this hypothesis-generating finding requires corroboration by additional data, it suggests the importance of ALC monitoring and the potential of therapeutic manipulation of this parameter to induce abscopal effects.

Keywords: Absolute lymphocyte count (ALC), abscopal effect, radiotherapy, immunotherapy

Introduction

First coined in 1953, the abscopal effect refers to local therapy (e.g., radiation therapy (RT)) that induces a systemic anti-tumor response. (1) It is thought to be the result of several mechanisms functioning in concert. (2) First, antigens released due to local tumoricidal activity are recognized and processed by antigen-presenting cells (e.g., dendritic cells or certain tumor-associated macrophages). (3) Presentation of these tumor antigens to T lymphocytes then results in the generation and organization of an anti-tumor immune response. Finally, lymphocytes (particularly cytotoxic T cells) enter the circulatory system, infiltrate distal tumor microenvironments, and destroy neoplastic tissue with high specificity.

The rarely occurring abscopal effect is of great clinical interest due to its potential for targeted immune destruction of the tumor environment. Consequently, work during the past decade has produced several novel strategies to make this phenomenon more common and thus to improve outcomes for cancer patients. One of the most important developments in this realm has been the rapid rise of immunotherapy, including the optimization of compounds that disrupt inhibitory signals used by tumor cells to suppress immune cells (i.e., immune checkpoints) and thus galvanize the immune system. (4) The addition of RT to immunotherapy may enhance the release of tumor antigens along with cytokines that promote immune cell trafficking. (5) In a recent randomized trial, the out-of-field response rate for immunotherapy combined with RT was double the rate for immunotherapy alone. (6)

Despite the excitement surrounding these advances, abscopal responses remain uncommon, and it is thus a critical research priority to evaluate whether modifiable factors affecting the occurrence of this phenomenon exist. This study analyzed data from three institutional phase I/II trials to determine the potential of recorded parameters to predict abscopal responses in patients undergoing combined RT and immunotherapy and to explore outcomes based on those predictive factors. In particular, we hypothesized that absolute lymphocyte count (ALC) may predict abscopal responses, given that these effector cells are essential for the proposed mechanisms of the abscopal effect (5), and that numerous studies have associated RT-induced lymphopenia with negative outcomes in several malignancies. (7)

Methods

After obtaining Institutional Review Board approval, subjects were selected from three prospective phase I/II studies (Clinicaltrials.gov identifiers NCT xxxxxx, NCT xxxxxx, and NCT xxxxxx). Protocol details for each trial are described in Supplemental Files 1-3. All trials utilized combined immunotherapy/RT and required at least one nonirradiated noncontiguous lesion to evaluate out-of-field (abscopal) responses. The first trial, which focused on metastatic solid tumors, consisted of two cycles of ipilimumab, then stereotactic body radiation therapy (SBRT) (50 Gy in 4 fractions or 60 Gy in 10 fractions), and finally two further cycles of ipilimumab. In the second trial, stage IV non-small cell lung cancer (NSCLC) patients with thoracic/liver metastasis underwent concurrent pembrolizumab and RT treatment (phase I) or were randomized into groups receiving pembrolizumab with or without concurrent RT (phase II); all patients received maintenance pembrolizumab thereafter. Only the patients that received both immunotherapy and RT were included in our analysis. RT was delivered as SBRT (50 Gy in 4 fractions) or as hypofractionated RT (45 Gy in 15 fractions). The third trial assessed limited- or extensive-stage small cell lung cancer (SCLC), although only the latter was included in our study since there is by definition no out-of-field disease in the former. Patients received induction chemotherapy followed by concurrent chemotherapy/pembrolizumab/RT (45 Gy in 15 fractions) and then maintenance pembrolizumab.

All studies involved the collection of salient clinical parameters as part of the enrollment workup. These parameters included pathologic confirmation of disease and baseline hematological parameters. Obtaining a complete blood count (including pre-RT and post-RT ALC, neutrophils, monocytes, white blood cells and platelets) was standard practice for all trials and occurred during each cycle of immunotherapy along with the most recent first day of RT (range, 0-2 days from start of RT) and the most recent last day of RT (range, -3 to 2 days). In all trials, patients generally were followed up every three months after therapy with imaging (most commonly, computed tomography of the chest, abdomen, and pelvis). Assessment of abscopal responses in nonirradiated lesions was based on the best treatment response according to RECIST.

Statistical analyses aimed to address several objectives. First, univariable and multivariable linear regression analyses were performed to identify variables associated with an

abscopal response. Spearman's correlation coefficients were used to quantify these associations. Significance was evaluated with Fisher's exact test to compare abscopal responses in different groups and with Student's t-test to compare changes in the sum of longest diameter of abscopal tumors. Second, for candidate variable(s) with significant association after regression analysis, a linear correlation model was graphed for visual description, and Kaplan-Meier analyses for progression-free survival (PFS) and overall survival (OS) were graphed with stratifying for the candidate variable(s). Univariate and multivariate Cox regression was also done to examine the effect of the variable(s) on outcomes.

Results

In all, 153 patients treated from 2013 to 2018 as part of the three trials were included in this analysis (Figure 1). The median follow-up of all patients was 21.1 months. Table 1 shows characteristics of the total study population. The most common cancer types were NSCLC (n=62), SCLC (n=25), head/neck cancers (n=16), and renal cell carcinoma (n=13). A total of 98 patients received ipilimumab, and 55 received pembrolizumab. RT was most commonly directed to the lung (n=119), and 90% of patients received RT at a single site. The most common fractionation was 50 Gy in 4 fractions (n=99).

Table 2 shows the results of univariable and multivariable linear regression analyses to determine potential predictors of abscopal responses. Following multivariable analysis, two parameters remained significantly associated with an abscopal response: no prior chemotherapy ($p=0.022$) and higher post-RT ALC (measured as a continuous variable; $p<0.001$). Of note, pre-RT ALC and the percent change in ALC from pre-RT to post-RT were significant by univariable assessment but did not retain significance following multivariable analysis. In order to better visualize the correlation between out-of-field lesion responses and ALC prior to (Figure 2A) and following (Figure 2B) RT, the linear correlation was graphed, yielding $r=-0.23$ for pre-RT ALC and $r=-0.41$ for post-RT ALC.

Because ALC as a continuous variable demonstrated significance on multivariable regression analysis above, this parameter was then dichotomized around the median (pre-RT: 1.3 $\times 10^3$ cells/μL, range 0.28-4.84 $\times 10^3$ cells/μL; post-RT: 0.56 $\times 10^3$ cells/μL, range 0.12- 4.7 $\times 10^3$ cells/μL) in order to further quantify abscopal response rates and changes in tumor size. For

post-RT absolute lymphocyte count (ALC), the abscopal response rate was 34.2% in the cohort with ALC higher than the median value, compared to 3.9% in patients with ALC lower than the median (Fisher's exact test P<0.0001; Figure 2C). Corresponding figures for pre-RT ALC were 30.3% vs. 7.8%, respectively (Fisher's exact test P=0.0004; Figure 2D). A parallel analysis was performed to explore the percent change in the sum of the longest diameter for out-of-field lesions at different ALCs. For post-RT ALC, the median change for the higher ALC cohort was +13.41% vs. +67.23% in the lower ALC cohort (P<0.0001, Figure 2E). For pre-RT ALC, the median change for the higher ALC cohort was +26.01% vs. +54.79% in the lower ALC cohort (P=0.02, Figure 2F).

Kaplan-Meier curves for PFS and OS, based on pre-RT and post-RT ALC (above or below the median), are displayed in Figure 3. Pre-RT ALC did not seem to associate with either PFS (7.8 vs. 6.6 months, p=0.21) or OS (19.2 vs. 22.8 months, p=0.82). However, lower post-RT ALC was associated with poorer PFS (12.3 vs. 6.0 months, p=0.0004) and OS (27.4 vs. 15.7 months, p=0.005). Cox regression analysis performed to identify potential predictors of PFS and OS is shown in Table 3. Following multivariable adjustment, age (p=0.001) and post-RT ALC (p=0.003) were significantly associated with PFS. Zubrod performance status (p=0.009) and post-RT ALC were also associated with OS (p=0.026).

Discussion

Despite the clinical interest in exploiting the abscopal effect for cancer therapy, this phenomenon remains uncommon; therefore, research to elucidate predictors of the effect is an essential first step to discovering factors that can be modified to increase the incidence of systemic anti-tumor responses. This analysis of three phase I/II trials showed that radiation-induced lymphopenia, measured by post-RT ALC (a continuous variable), may impact the occurrence of abscopal responses and thus influence prognosis in patients treated with RT and immunotherapy. While this hypothesis-generating finding requires further corroboration, it has implications for the monitoring of ALC and the therapeutic manipulation of this parameter.

Lymphocytes play a key role in abscopal phenomena (5), and the degree of RT-induced lymphopenia correlates with prognosis in several tumors (7), potentially due to ineffective

systemic anti-neoplastic responses unable to address micrometastatic and pre-existing gross disease. There are multiple potential strategies to modify ALC in order to promote more frequent abscopal responses. First, lymphopenia is associated with larger RT volumes exposed to a "low-dose bath" (8), which should be minimized to the greatest extent possible. To this end, it is interesting that SBRT (versus hypofractionated RT) was not associated with an increased incidence of abscopal responses especially because 1) SBRT implies smaller treatment volumes and potentially a smaller "low-dose bath" and 2) preclinical data have indicated ablative dosing is more conducive to abscopal responses. (9) One caveat of our analysis is that dosimetric data were not included; it is certainly plausible that ALC is a direct surrogate for (and/or result of) dosimetric factors and RT volumes. (8) Additionally, it is difficult to extrapolate animal research data to humans, and it is possible that the SBRT in the trials we analyzed delivered fractional doses too high to make an impact. Second, these data suggest that boosting ALC will induce stronger abscopal responses. Golden and colleagues performed a prospective study on the delivery granulocyte-macrophage colony-stimulating factor for this purpose, which resulted in an abscopal response rate of 27%. (10) It should be noted that the study utilized neither immunotherapy nor ablative RT (35 Gy was delivered in 10 fractions), which might further increase the response rate.

There are also other factors that did not associate with the incidence of abscopal responses in our analyses, despite hypothesis-generating data showing the contrary. For instance, prior radiotherapy has been thought to increase the activity of immunotherapy and to associate with increased PFS or OS. (11) Furthermore, (12) irradiation of the lung was posited to be less immunogenic than that of the liver. (13) Although neither factor was significant in this analysis, which was considerably larger than either aforementioned study, it should be contextualized that metastatic cancer patients are extremely heterogeneous, not only in terms of histology but also with respect to molecular factors that were not assessed in these studies, such as tumor mutational burden and PD-L1 levels. Although these heterogeneities could explain the discrepancy between our and other results, given the compelling data associating multi-site RT with abscopal responses (14), it should also be noted that the vast majority of these studies, including the present investigation, irradiated only one site.

Despite the statistical significance in Figure 2, it is acknowledged that the correlation coefficient is relatively weak, indicating that additional unknown factors are involved that cannot be explained by the ALC correlation. These include some factors mentioned above, but also underscore that unforeseen factors are more difficult to address, such as manipulating the tumor microenvironment, which is hostile to T-cell infiltration even if the immune system is galvanized. (15) Additionally, RT upregulates regulatory T cells, myeloid-derived suppressor cells, and M2 tumor-associated macrophages; these cells promote an immunosuppressive milieu despite the presence of adequate ALC. (15) Identifying and addressing these unforeseen variables in addition to ALC may enable more robust abscopal responses than would addressing only ALC. (16)

This study has several limitations in addition to the relatively small subgroup sample sizes. First, despite the prospectively collected study population, this study was a retrospective analysis thereof and hence cannot exclude selection biases. Second, as mentioned above, metastatic cancer patients are highly heterogeneous in many different ways (e.g., molecular factors), which limits the applicability of our results. Third, the PFS/OS analyses based on the ALC are exploratory, given that the stratification was simply performed *a priori* on the median values. Although the association between ALC post RT and abscopal response remained significant, no such assessment can accurately encompass all possible confounding factors. Fourth, this study is not meant to reliably evaluate specific mechanisms of abscopal responses (since ipilimumab and pembrolizumab act differently) or synergy thereof and also cannot comment on the timing of immunotherapy and RT, given that the vast majority of subjects received concurrent therapy, with at most 1-2 weeks of RT without immunotherapy. Lastly, hematologic parameters are often influenced by numerous other factors not controllable in clinical trials, and thus potential confounding can never be excluded. Nevertheless, these shortcomings diminish neither the potential of our findings nor the importance of dedicated prospective investigations to corroborate these findings.

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Figure Legends

Figure 1. Patient selection diagram.

Figure 2. Higher ALC post RT was associated with abscopal response. Linear correlation between post- (A) and pre-radiotherapy (B) ALC and the abscopal response. Abscopal rate in the higher and lower ALC cohorts post- (C) and pre-radiotherapy (D). Change in sum of longest diameter for abscopal tumors in the higher and lower ALC cohorts post- (E) and pre-radiotherapy (F).

Figure 3. Higher ALC post RT was associated with better outcomes. Kaplan-Meier analysis for progression-free survival (PFS) (A, B) and overall survival (OS) (C, D) based on pre- (A, C) and post- (B, D) radiotherapy absolute lymphocyte count.

Table 1. Patient characteristics

Characteristic	All Patients (n=153)
Demographics	
Gender	
Male	56
Female	97
Age	
median (IQR), years	63(29-91)
Race	
White	131
Black	11
Asian	11
ECOG PS score	
0	55
1	88
2	10
Primary Tumor	
NSCLC	62
SCLC	25
HN	16
RCC	13
HCC	5
GYN	7
CRC	6
Pancreatic cancer	4
Prostate	4
Esophageal	3
Bone	3
Other	5
Immunotherapy drug	
Ipilimumab	98
Pembrolizumab	55
RT Scheme	
12.5Gy*4f	99
6Gy*10f	20
3Gy*15f	34
RT Site	
Lung	119
Liver	31
Other	3
Prior RT	
Yes	43
No	110
Prior Chemotherapy	
Yes	142
No	11
Prior Immunotherapy	
Yes	8
No	145
Pretreatment laboratory findings	
ALC, median (range), $\times 10^3/\mu L$	1.3 (0.28-4.84)
WBC, median (range), $\times 10^3/\mu L$	7.8 (2.4-20.2)
Monocyte (range), $\times 10^3/\mu L$	0.9 (0.15-2.77)
Neutrophils, median (range), $\times 10^3/\mu L$	4.8 (0.28-10.19)
Platelets, median (range), $\times 10^3/\mu L$	278 (53.7-588)
ALC post radiotherapy (range), $\times 10^3/\mu L$	0.56 (0.12-4.7)

Abbreviations: ALC, absolute lymphocyte count; ECOG PS, Eastern Cooperative Oncology Group performance status; SABR, stereotactic ablative radiation therapy; WBC, white blood cells.

Table 2. Univariate and multivariate linear regression associating baseline variables and blood parameters with abscopal response

	Univariate analysis			Multivariate analysis		
	Regression coefficient	95% CI	P Value	Regression coefficient	95% CI	P Value
Characteristics						
Age (≥65)	-0.10700	-0.024 to 0.121	0.188	NI		
Gender (Male)	-0.10600	-0.454 to 0.092	0.193	NI		
Race (White)	0.13100	-0.042 to 0.43	0.106	NI		
Prior Radiotherapy	0.08000	-0.147 to 0.44	0.325	NI		
Prior Chemotherapy	0.20500	0.151 to 1.153	0.011	0.165	0.076 to 0.973	0.022
PS Score	0.54000	-0.182 to 0.368	0.507	NI		
SBRT	-0.35000	-0.134 to 0.632	0.4812	NI		
RT Site	0.058	-0.179 to 0.381	0.477	NI		
Immunotherapy Agent	0.07100	-0.154 to 0.396	0.385	NI		
ALC prior to RT*	-0.24400	-0.443 to -0.097	0.002	0.153	-0.077 to 0.412	0.178
ALC post RT*	-0.41200	-0.538 to -0.256	<0.0001	-0.432	-0.64 to -0.193	<0.0001
% ALC Change*	-0.25800	-0.5 to -0.124	0.001	-0.141	-0.352 to 0.01	0.084
Neutrophils*	-0.07200	-0.08 to 0.031	0.383	NI		
Monocyte*	-0.025	-0.352 to 0.257	0.759	NI		
WBC*	0.13700	-0.006 to 0.078	0.092	NI		
Platelet*	0.064	-0.001 to 0.002	0.435	NI		

Abbreviations: HR, hazard ratio; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; ALC, absolute lymphocyte count.

* Indicates a continuous variable with units indicated in parenthesis.

Table 3. Univariate and multivariate COX regression associating baseline variables and blood parameters with PFS and OS

	PFS						OS					
	Univariate analysis			Multivariate analysis			Univariate analysis			Multivariate analysis		
	HR	95% CI	P Value	HR	95% CI	P Value	HR	95% CI	P Value	HR	95% CI	P Value
Characteristics												
Age (≥65)	0.50400	0.339 to 0.749	0.001	0.501	0.335 to 0.751	0.001	0.80400	0.517 to 1.252	0.334	NI		
Gender (Male)	1.10500	0.745 to 1.639	0.619	NI			0.94600	0.603 to 1.487	0.811	NI		
Race (White)	0.92000	0.636 to 1.331	0.66000	NI			0.89900	0.562 to 1.438	0.657	NI		
Prior Radiotherapy (Yes)	1.16600	0.76 to 1.789	0.481	NI			1.14200	0.699 to 1.867	0.596	NI		
Prior Chemotherapy (Yes vs no)	1.80400	0.733 to 4.444	0.199	NI			0.73400	0.317 to 1.698	0.47	NI		
PS Score (0)	1.05800	0.71 to 1.575	0.783	NI			1.63900	1.024 to 2.622	0.039	1.862	1.17 to 2.96	0.009
SBRT (Yes)	1.23000	0.822 to 1.84	0.313	NI			0.77900	0.464 to 1.309	0.346	NI		
RT Site (Lung)	1.24100	0.583 to 1.742	0.543	NI			1.06100	0.73 to 1.642	0.462			
Immnotherapy Agent (Ipilimumab)	0.78100	0,523 to 1.166	0.227	NI			0.98800	0.622 to 1.568	0.958	NI		
ALC prior to RT (Higher)*	0.77500	0.597 to 1.005	0.054	0.719	0.478 to 1.08	0.112	0.98000	0.625 to 1.537	0.931	NI		
ALC post RT (Higher)*	0.74100	0.578 to 0.95	0.018	0.514	0.363 to 0.815	0.003	0.51600	0.327 to 0.813	0.004	0.677	0.48 to 0.955	0.026
% ALC change (Higher)*	0.82800	0.562 to 1.22	0.34	NI			0.76900	0.493 to 1.201	0.249	NI		
Monocyte (Higher)*	0.689	0.465 to 1.022	0.064	0.678	0.453 to 1.015	0.059	0.478	0.302 to 0.754	0.002	0.814	0.659 to 1.106	0.12
WBC (Higher)*	1.13600	0.771 to 1.672	0.51900	NI			1.24700	0.803 to 1.937	0.325	NI		
Platelet (Higher)*	1.115	0.757 to 1.642	0.582	NI			1.799	1.151 to 2.811	0.01	1.121	0.798 to 1.43	0.25

Abbreviations: HR, hazard ratio; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; ALC, absolute lymphocyte count; * Indicates a continuous variable.

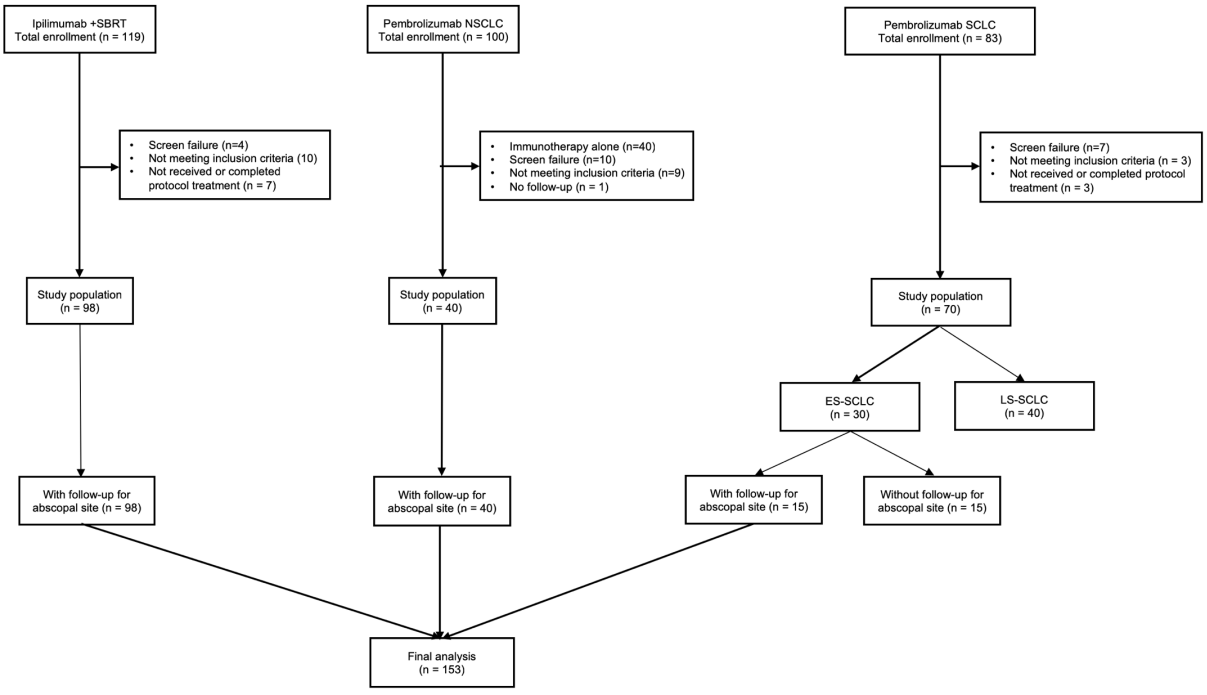


Figure. 2

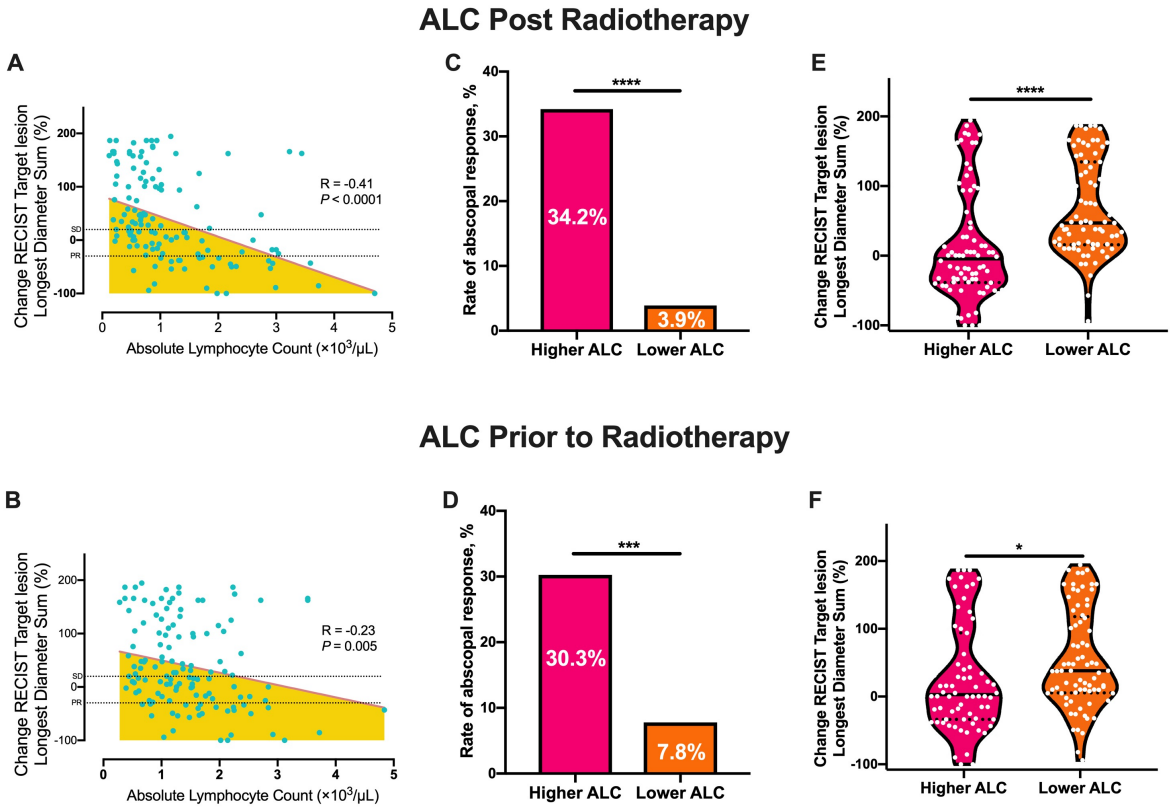
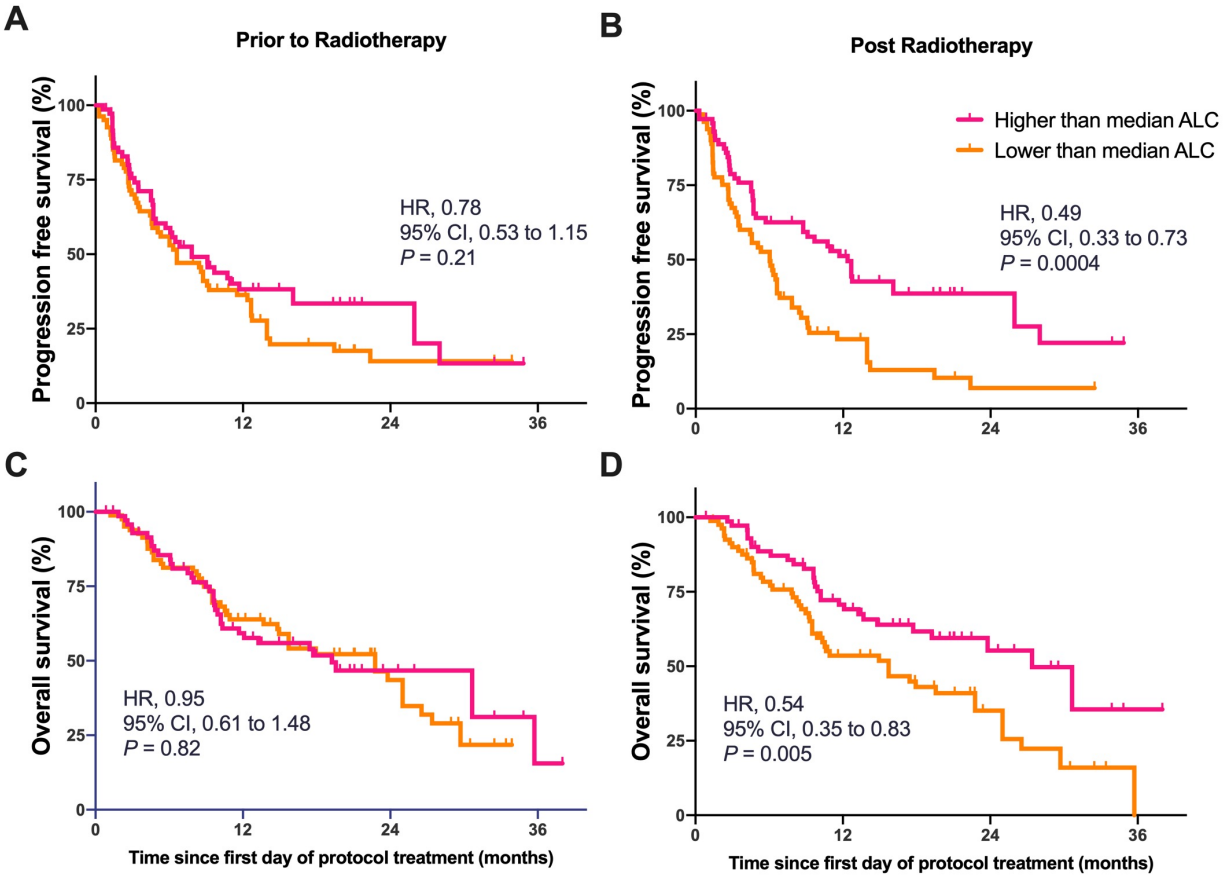


Figure. 3


A
Prior to Radiotherapy
Progression free survival (%)
HR, 0.78
95% CI, 0.53 to 1.15
P = 0.21
B
Post Radiotherapy
Higher than median ALC
Lower than median ALC
HR, 0.49
95% CI, 0.33 to 0.73
P = 0.0004
C
Overall survival (%)
HR, 0.95
95% CI, 0.61 to 1.48
P = 0.82
Time since first day of protocol treatment (months)
D
Overall survival (%)
HR, 0.54
95% CI, 0.35 to 0.83
P = 0.005
Time since first day of protocol treatment (months)