Peripheral Blood T cell responses to immunotherapy related adverse events in NSCLC patients treated with Immunotherapy
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**Background**

Non small cell lung cancer is the leading cause of cancer(NSCLC) mortality in both men and Women1. Immunotherapy has emerged as standard of care not only in the metastatic setting, but also in the earlier stages of NSCLC2. There is prospective and retrospective evidence for the correlation of immunotherapy (IO) related adverse events (irAE) and efficacy of anti PD1 and PD L1 monoclonal antibodies3. The incidence of irAE in these studies ranged anywhere from 30-44%. There have been attempts to cluster irAEs into distinct subtypes by T cell profiling 4. Our observation, which is part of another project of investigating a biomarker for irAEs, is along the same lines of predictive capability of T cell shifts in the peripheral blood for irAEs, which we consider as valid reporting in this context.

**Aim**

We observed the trend of CD4/CD8 changes during immunotherapy with PD1 and PDL1 monoclonal antibodies with each cycle and the related adverse events in patients with NSCLC. This observation suggested that there may be a predictive trend of T cells with irAE. Our null hypothesis based on this was “there will be no drop in the CD4/CD8 T cell ratio right before irAE compared to the cycle before”5. Our alternate hypothesis was “there will a drop in the the CD4/CD8 ratio before the incidence of irAE compared to the cycle before” thus predicting the occurrence of irAE.

**MATERIALS & METHODS**

We have collected blood samples from 20 patients of NSCLC before each cycle of immunotherapy with informed consent as part of a related project after our institutional review board approval. We have isolated the cellular components such as CD4 and CD8 positive cells using magnetic bead technique, from these samples in our research lab while the regular patient testing is done via flow cytometry (usually only total lymphocyte number without CD4 or CD8 T cell numbers) and therefore cross correlation is required before large scale studies are conducted.

**RESULTS**

In the cohort of 20 patients that were treated with immunotherapy for NSCLC, 9 experienced irAE, out of which 5 had grade 2, including thyroiditis, pneumonitis, cytokine release syndrome (CRS), 1 had grade 3 pneumonitis, 1 had grade 3 dermatitis, 1 had grade 1 encephalopathy and 1 had grade 1 CRS. When we looked at the CD4/CD8 ratio, the one prior to the incidence of the grade 2-3 irAE had atleast 30% drop in the ratio consistently from the ratio of the preceding cycle. The two patients that had grade 1 irAE (R and S) did not quite reach the 30% threshold but were close. When compared to the percentage change of the ratio in the preceding cycle, this was statistically significant. This is preliminary data and it is unclear if this would be statistically significant with a larger sample and if the grade correlates with the degree of the change. Analysis of the trend of the CD4/CD8 ratio during other cycles of those that developed irAE and the trend in the patients that did not have irAE may also help clarify this question. However, a more larger sample is needed to establish the statistical significance.

**DISCUSSION**

Although most irAEs are treated and reversed with steroids and other immunosuppressive agents, prolonged immunosuppression can lead to reduced efficacy if IO and development of undue opportunistic infections5. Experience with IO has shown that there is association between the irAE and efficacy of PD1 and PDL1 antibodies 6 and earlier initiation of immunosuppression shortens the required treatment. However, given the challenge in the subtlety of the earlier presentation, therapies are frequently delayed. Hence, biomarker to identify the early manifestations is of critical importance for early intervention. Studies suggest there is clonal expansion of CD8 T cells preceding grade 2-3 irAEs7. Studies also indicate that increased T cells in the tumor is indicative of response to immunotherapy8. Our observation suggests that increased CD8 in proportion to CD4 in the peripheral blood precedes the onset of irAE. It is unclear as to how this leads to increased toxicity when the immunotherapy treatment works by affecting T cell function. One possible explanation is that the T cell response in the tumor tissue is beneficial, however, T cell response in the peripheral blood may indicate response against self antigens leading to toxicities in the form of irAE

**Limitations**

This observation by no means is a conclusive evidence of biomarker status given the small sample size, observational findings and the lack of statistical analysis. However, this could be considered as a potential area of investigation to identify predictable markers of irAE that are easy to obtain using readily available tests and are cost effective to the patient as well as informative enough that the treating physician can act upon immunotherapy in this to mitigate the adverse events without compromising the efficacy of patient population. It is to be noted that the measurement of T cells from the peripheral blood in this study is obtained by magnetic bead isolation technique in a research lab while the regular patient testing is done via flow cytometry (usually only total lymphocyte number without CD4 or CD8 T cell numbers) and therefore cross correlation is required before large scale studies are conducted.

**References**