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Commentary OMICS International

The Kynurenine/Tryptophan Ratio and Glioblastoma Patients Treated with Hsppc-96 Vaccine

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ABSTRACT

The discovery that immunotherapy is a clinically-relevant approach for the treatment of malignant tumors is revolutionizing patient care. In adults diagnosed with glioblastoma (GBM), an aggressive and incurable primary brain tumor, autologous HSPPC-96 vaccination provides a significant increase in overall survival. However, all GBM patients eventually succumb to their disease, providing rationale for discovering new methods that proactively identify individuals that will respond, optimally. Of the immunosuppressive mediators that contribute to the inhibition of productive tumor immunity, indoleamine 2,3 dioxygenase 1 (IDO1), a rate-limiting enzyme that catabolizes tryptophan (Trp) into kynurenine (Kyn), has been demonstrated to be expressed at elevated levels in patients with malignant glioma. Recently, our group determined that a correlation exists between peripheral blood Trp and Kyn levels in GBM patients and the association with overall survival after HSPPC-96 treatment. Our findings indicate that the Kyn/Trp ratio may be a useful benchmark for identifying GBM patients with a higher likelihood to survive longer after vaccination. The relevance to future clinical trials, the limitations of brain tumor models to address these findings and the role of IDO1 versus tryptophan dioxygenase (TDO) in the maintenance of peripheral Trp and Kyn levels, is discussed.

Keywords: IDO; Glioma; Immunotherapy; T cell; Treg; Immunosuppression

Abbreviations: IDO1: Indoleamine 2,3 dioxygenase 1; GBM: Glioblastoma; Trp: Tryptophan; Kyn: Kynurenine; CNS: Central Nervous System; OS: Overall Survival; HSPPC-96: Heat Shock Protein Peptide Complex-96; 48h: 48hours; 10w+: 10 week; IDO2: Indoleamine 2,3 dioxygenase 2; TDO: Tryptophan Dioxygenase; HGG: High Grade Glioma

Background

Glioblastoma (GBM) is the most common and aggressive form of brain tumor in adults. Despite maximal surgical resection, irradiation and chemotherapy, median overall survival (OS) remains at 14.6 months for newly diagnosed patients [1] and only 30 weeks for recurrent patients [2], emphasizing the need for novel treatments. With multiple observations indicating the immunospecialization, rather than immunoprivilege of the central nervous system (CNS) [3], the discovery that immunotherapy is a clinically-relevant treatment for patients with end-stage cancers refractory to other treatment strategies [4], have hastened the pace of clinical trial deployment utilizing immunotherapy in GBM patients [5,6]. A new immunotherapy approach is vaccine therapy, specifically heat shock protein peptide complex (HSPPC) vaccines. With an overexpression in brain tumor cells, heat shock protein peptides are target antigens and have been implicated in the activation of both innate and adaptive immune systems. Vaccines derived from autologous tumor have generated both prophylactic and therapeutic immunity against individual cancer and promising studies in adult patients with GBM are ongoing [7]. However, less is known with respect to the analytic tools required for predicting overall responsiveness and prognosis to these novel immunotherapeutic agents.

Research in GBM cells has demonstrated that IDO1 is highly responsive to the proinflammatory T cell effector cytokine, interferon-gamma, suggesting that T cell-mediated inflammatory-drivers act to facilitate IDO1 expression/activity [8]. Notably, if this hypothesis is true, then it stands to reason that immunosensitizing agents including radiotherapy and/or chemotherapy, as well as immune-boosting strategies such as PD-(L)1 blockade, would effectively induce and/or amplify IDO1 expression and/or activity in patients treated with the relevant clinical regimen(s).

IDO1 is canonically-characterized as a rate-limiting cytoplasmic enzyme that catabolically converts tryptophan (Trp) into kynurenine (Kyn) [9]. In vitro, the depletion of Trp and/or accumulation of Kyn have been shown to suppress tumor immunity by inducing CD^{8+} cytolytic T cell deactivation and/or apoptosis, in addition to the conversion of naïve $CD^{4+}FoxP3^{-}$ conventional T cells into inducible $CD^{4+}FoxP3^{+}$ regulatory T cells (iTreg) [10-12]. Additionally, both Trp and Kyn are actively transported across the blood brain barrier via the

large amino acid transporter system [13]. Given these collective findings, our study hypothesized that serological Trp and/or Kyn levels would provide prognostic value for physicians planning to enroll GBM patients in immunotherapy.

Commentary

In our recently published study, we described the comparison of Trp and Kyn levels in individuals without tumors to recurrent GBM patients who were scheduled to receive the gp-96 heat shock protein peptide complex (HSPPC-96) vaccine [14]. Quantification of Trp and Kyn levels was conducted using reverse phase high performance liquid chromatography and obtained at time intervals including pre-surgical resection, as well as at 48 hours (48 h) and 10 week+ (10w+) post-GBM resection (Figure 1). We hypothesized that, the increased GBM expression of IDO1 would lead to the depletion of Trp and increase of Kyn levels in the peripheral blood, translating into a higher Kyn/Trp ratio. In GBM patients, Trp levels were universally decreased when compared to non-GBM patients. Unexpectedly, Kyn levels were also decreased in the pre-surgical and 48 h post-resection GBM patients, while 10w+ patients had higher levels similar to healthy individuals. Overall, Kyn/Trp ratios were highest in 10w+ patients (mean 7.32 ± 1.1) and were found to be bi-modally distributed. Interestingly, those patients with a high Kyn/Trp ratio (≥ 9.5) had a substantially decreased OS (mean 23.6 ± 6.8 months) compared to GBM patients with a lower Kyn/Trp ratio (mean 38.7 ± 4.9 months). These novel data suggest that, a time point well after surgical resection, is clinically-relevant for utilizing the Kyn/Trp index to predict overall response and prognosis in GBM patients treated with immunotherapy.

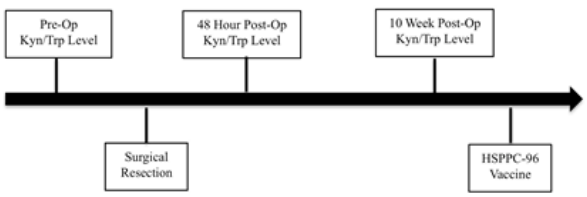

Figure 1: Time Course of Kyn/Trp blood work, surgical resection of tumor and HSPPC-96 vaccine treatment. All blood work completed prior to vaccine immunotherapy.

Conclusion & Future Directions

With recent advancements in the understanding of brain tumor immunity, rational immunotherapeutic strategies to fight incurable tumors such as GBM, are finally showing promise. However, it is equally important to continue investigating novel tools for identifying, stratifying and improving the OS of these patients. Notably, and to our knowledge, our research is the first to show that, the Kyn/Trp ratio possesses a correlation with OS in human GBM patients treated with immunotherapy. Although this finding requires further vetting in a larger GBM patient study utilizing HSPPC-96 vaccination, it provides initial evidence that Kyn-pathway profiling is a rational tool for identifying longer-lived patient responders to immunotherapy.

Our work confirms that GBM patients possess altered serological Trp and Kyn levels at the time of surgery when compared to control individuals. Previous studies suggested that, IDO1 mediates immunosuppression by depleting Trp and Kyn accumulation [10,11], though the exact levels of Trp and Kyn required to physiologically promote immunosuppression have yet to be established. While some of this work can be further investigated in brain tumor models, caveats abound. Given the hypothesis that tumor-infiltrating effector T cells are required to optimally-express IDO1 in GBM cells, *in vivo*, the analysis of this mechanism requires the immunocompetent mouse setting. Importantly, our group has noted substantial differences in the IDO1-mediated enzymatic potential of the popular mouse GL261 glioma cell line (syngeneic to C57BL/6 mice) versus human (U87 and DBTRG) GBM cells. Specifically, although GL261, U87 and DBTRG universally express IDO1 mRNA after IFNγ treatment, mouse GL261 is defunct for IDO1 enzymatic activity, while both human U87 and DBTRG readily catabolize Trp into Kyn (unpublished findings). Adding to the challenge, studies indicate that immunocompetent mice engrafted GL261 succumb to disease within 4-6 weeks post-tumor cell implantation. However, the prognostically-important Kyn/Trp ratio is not observed in GBM patients until 10 weeks post-surgical resection.

Pursuing future clinical trials is important for establishing a better understanding of Trp and Kyn regulation in GBM patients treated with immunotherapy. Our sample size (n=10) was too small for statistical significance (P=0.1), although the data demonstrated a strong trend. Additionally, the majority of our analysis focused on recurrent GBM patients, highlighting the future goal of comparing to newly-diagnosed individuals. It is also necessary to compare our current findings to the pediatric setting in newly diagnosed high-grade glioma (HGG), recurrent HGG, or recurrent ependymoma patients planning to enroll in HSPPC-96 treatment (NCT02722512). Notably, with many trials initiating immunotherapeutic agents no later than 6 to 8 weeks post-tumor resection or post-irradiation, the analysis of the 10w+ time point will likely have to be adjusted in future studies.

It was surprising to find an elevated Kyn/Trp ratio in GBM patients well after surgery, but prior to HSPPC-96 vaccine treatment. Understanding that median progression free survival in recurrent GBM patients is ~10 weeks [2], this could represent disease progression leading to immune activation coincident with IDO1 induction. It could also be indicative of multiple tryptophan catabolic enzymes contributing to Trp degradation, since tryptophan dioxygenase (TDO) is constitutively expressed in the liver and thought to serve as a primary mediator of systemic Kyn levels [15]. Previous studies have demonstrated that the Trp→Kyn pathway, and specifically TDO, is induced in all states of chronic immune activation and is responsive to non-specific inflammation including infection and nutritional state [16]. Additionally, the use of glucocorticoids and other drugs are known to systemically induce TDO and interfere with Trp conversion [17]. Therefore, the patient's overall condition, including standard of care treatment with irradiation, dexamethasone and chemotherapy, greatly affect the complexity of determining the sole determinant(s) of systemic Kyn/Trp levels.

As more clinical trials seek safety, efficacy and impact in GBM patients treated with novel immunotherapeutic approaches, it is critical to re-evaluate the connection between brain tumor, IDO1 and TDO, versus peripheral expression for these enzymes and their impact on one another. Moreover, developing mouse models that better reflect the immune status of human GBM patients, as well as intratumoral heterogeneity, is essential for increasing the prediction power against immune-sensitive immunomodulators; such as IDO1. Overall, the future is bright for patients with malignant brain tumors with our rapidly increasing understanding of compensatory

immunosuppressive pathways and their response to different therapeutic interventions.

Availability of Data and Material

All data generated or analyzed during this study are included in the published article. The kynurenine to tryptophan ratio as a prognostic tool for glioblastoma patients enrolling in immunotherapy, Journal of Clinical Neuroscience.

Authors' Contributions

AL and DW drafted the manuscript and edited the final version. LZ, KL, GG, EL, MG, CDJ and OB made substantial contributions with acquisition of data and interpretation.

Ethics Approval and Consent to Participate

The trial was approved by Institutional Review Board at all participating sites (Northwestern University Medicine, Chicago, and University of California San Francisco).

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Not applicable.

Competing Interests

The authors declare that they have no competing interests.

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