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## Reading the Tea Leaves of Tumor-Mediated Immunosuppression

**Rimas J. Orentas**

Pediatric Oncology Branch National Cancer Institute, CCR, NIH Bethesda, MD 20892 USA

### Summary

Polyphenol E, available as Polyphenon E, is a green tea extract whose activity can be benchmarked to the presence of specific catechins such as EGCG (epigallocatechin 3-gallate). Herein, Polyphenon E is shown to reverse myeloid-derived suppressor cell (MDSC) activity, linking the activity of a natural product extract to cell-mediated immunity.

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In this issue of *Clinical Cancer Research*, Santilli and colleagues tackle the problem of tumor-mediated immunosuppression with the novel application of compounds found in tea. Using mouse models of neuroblastoma, they show that the immunosuppression mediated by MDSCs can be reversed by Polyphenon E (1).

Neuroblastoma is a leading cause of cancer death in children, and progress is desperately needed. One of the most recent therapeutic advances has been the use of antibody specific for the cell-surface glycolipid GD2. In the context of bone marrow transplantation for advanced disease, including cytokine support with interleukin-2, GM-CSF, and isotretinoin, two-year event-free survival increased from 46% to 66%, and overall survival increased from 75% to 86% in those receiving antibody (2). Other studies have shown that vaccination, depletion of T-regulatory cells (Treg), and immunotherapy with T cells modified to express anti-GD2 chimeric antigen receptor (CARs) impact disease (3,4). This has further encouraged immunotherapeutic approaches to treating neuroblastoma.

The tumor lesion itself is a source of local and systemic immune misdirection. The non-cancerous stromal cells in the tumor, Tregs, tumor-associated fibroblasts, tumor-associated macrophages and MDSC, create an environment in which mounting an effective cell-based immune response is blocked. Tumor-associated stromal cells expressing fibroblast activation protein-alpha (FAP) are part of normal tissue inflammation and repair; but when present in a tumor blunt cell-mediated immunity (5). Macrophages expressing the M2 phenotype similarly blunt tumor immunity (6). The cell type that is perhaps the most perplexing is the MDSC. The origin and fate of MDSC has been much debated (7). MDSCs are present in many cancers, and produce soluble mediators that inhibit cellular immunity such as nitric oxide, arginase, TGF- $\beta$ , and prostaglandin E2. MDSCs are immature cells that are thwarted from fully differentiating into either macrophages or neutrophils. As MDSC accumulate, they help form a cellular network that blocks anti-tumor immunity. Thus, current research is focused on preventing MDSC induction and recruitment, and on promoting MDSC differentiation. The current report by Santilli et al., shows the potential impact of inactivating MDSC in neuroblastoma (1).

Polyphenon E<sup>TM</sup> is a pharmaceutical grade natural product extract of green tea that contains a mixture of five different catechins, including EGCG, produced by Mitsu Norin Co., Ltd. of

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Contact: Rimas J. Orentas, Pediatric Oncology Branch, CCR, NCI, National Institutes of Health, 1W3840 10 Center Dr., Bethesda, MD 20892, Telephone: 301-451-5897, Telefax: 301-451-7010, Rimas.Orentas@nih.gov.

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Japan. In 2006, Polyphenon ointment was FDA approved for HPV-associated skin lesions. In 2009, Shanafelt, et al., normalized dosage to EGCG levels present in the mixture and reported a decline in absolute lymphocyte count and/or lymphadenopathy in chronic lymphocytic leukemia patients, indicating potential therapeutic activity (8). Recently, using xenografted PC3 prostate cancer cells in immunodeficient mice, EGCG was used to nucleate therapeutic gold particles composed of the radioactive isotope  $^{198}\text{Au}$ . These particles bound to the prostate cancer antigen Laminin 67R and inhibited tumor growth (9). This supports earlier prospective studies in prostate cancer patients with early stage lesions (10).

Santilli et al., began by supplementing the drinking water of MYCN-transgenic mice (which spontaneously develop neuroblastoma) with Polyphenon E. The effective plasma concentration of the agent was far lower than that seen for in vitro activity against tumor lines. Moreover, Polyphenon E did not inhibit the growth of a human neuroblastoma cell line xenografted into immunodeficient mice, indicating a possible immune mechanism. When developing tumors in MYCN-Tg mice were immunostained, there was a decrease in the number of cells bearing the MDSC markers CD11b and Gr-1 in treated mice. Using the strain A-derived Neuro2A neuroblastoma cell line, implanted subcutaneously, flow cytometry showed a decrease in CD11b+/Gr-1+ cells while T cells were more abundant in the tumor and lymphoid tissues of treated mice. In a very insightful experiment, bone marrow cells from strain A mice were cultured in the presence of neuroblastoma supernatants to induce MDSC, and then co-injected with Neuro2a. The in vitro-induced MDSC promoted tumor growth, unless they were cultured with Polyphenon E. This effect was reversed when anti-CD8 antibody was administered, indicating that CD8 T cells play a major role in how polyphenon E-treated MDSC lose the ability to promote tumor growth. Importantly, the investigators also showed that changes in cell surface markers on MDSC induced by Polyphenon E were blocked by antibody to Laminin 67R. While the investigators argued that induced changes from a more granulocytic to a more monocytic MDSC lineage are beneficial, this may not be the case as others have demonstrated both subtypes can inhibit tumor immunity (11). IL-16, G-CSF, and IL-6 were all sharply upregulated in the monocytic MDSCs of treated animals, and other immunosuppressive characteristics of MDSC were inhibited such as arginase production and the recruitment of Treg.

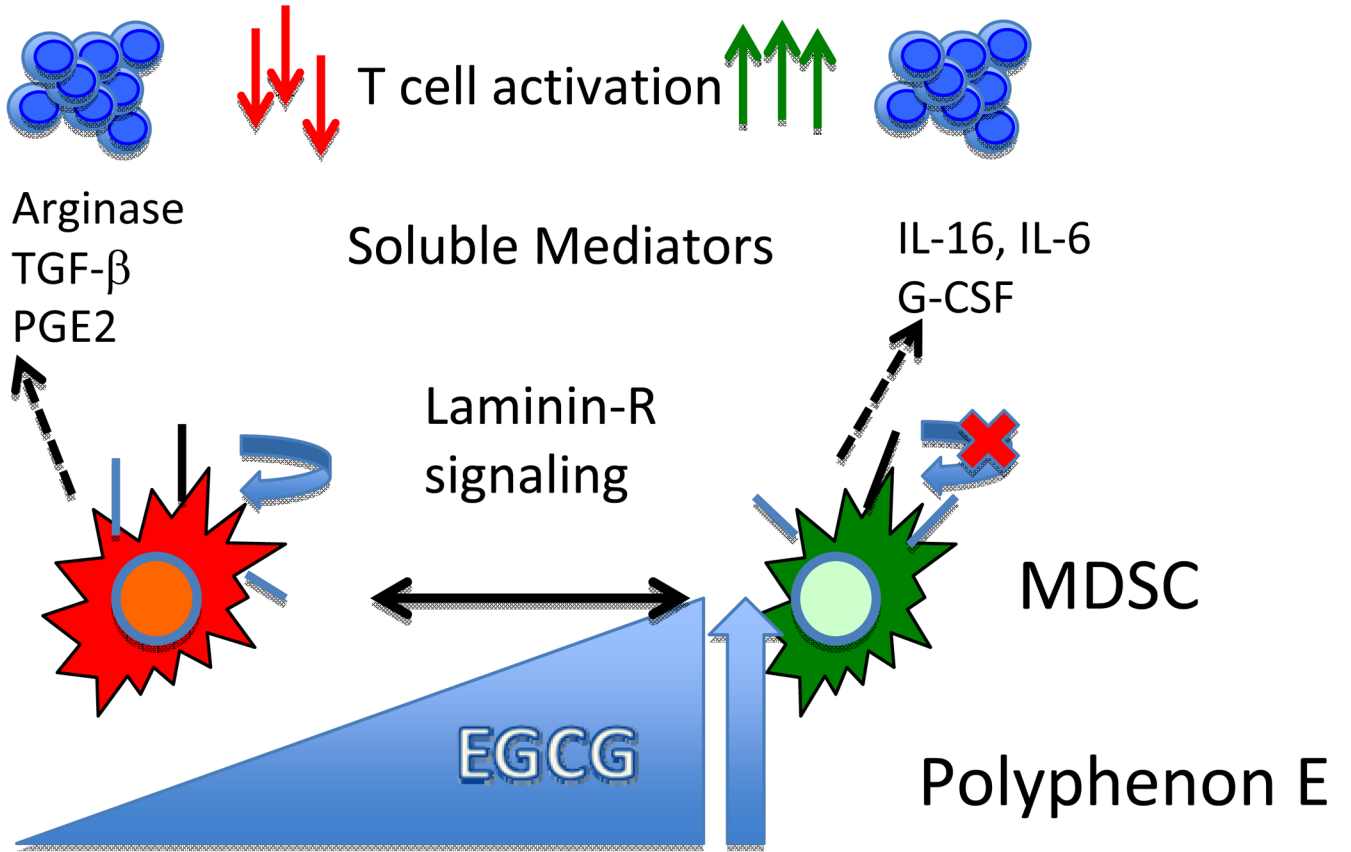
Important first steps in translating these findings to clinical research were taken. When Lechner et al., analyzed the induction of MDSC by co-culture of PBMC with tumor lines, the expression of CD66b (along with CD11b) on immature CD33+ myeloid cells was part of the canonical phenotype they described (12). Santilli et al., found increased expression of CD66b in the blood of neuroblastoma patients. In a single patient they demonstrated that depletion of CD66b+ cells restored proliferation of T lymphocytes stimulated with CD3/CD28 beads. In another patient, a metastatic lesion was disaggregated, and CD3/CD28 beads added to activate tumor-infiltrating lymphocytes. Here, Polyphenon E reversed T cell suppression, but only if the CD66b+ cells were left in the assay. This again implies that Polyphenon E rescue of immune responsiveness further benefits from differentiation of inhibitory MDSC to stimulatory APC. These individual cases successfully develop the hypothesis that Polyphenon E is able to promote anti-tumor immunity by its direct effects on MDSC. This hypothesis allows a number of factors to be tested, including: the impact of EECG on MDSC adhesion receptors, the ability to differentiate MDSC, the ability to alter the production of cytokines in the tumor lesion, and the ability to co-stimulate tumor infiltrating lymphocytes (figure 1). With this new approach to differentiating MDSC, one hopes that the “tea leaves” of Polyphenon E activity have been read correctly and new mechanistic insights as to how MDSC-mediated immunosuppression can be reversed will arise.

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**Figure 1. PolyphenonE reverses MDSC-mediated immunosuppression in neuroblastoma**  
 Active agents present in green tea extract include EGCG, which interacts with the 67 KDa laminin receptor, and modulates MDSC function such that an immunosuppressive phenotype (red MDSC) is reversed and a phenotype supportive of immuneactivation (green) is induced; including alteration of the soluble mediators and cell-cell interactions that give rise to T cell mediated anti-tumor immunity.