Regulatory T cells in endogenous mouse lymphoma are antigen-specific nTregs and provide a target for immune checkpoint-inhibiting therapies

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Purpose/Objectives: Foxp3+ regulatory T cells (Tregs) play an important role in maintaining immune homeostasis. In malignant disease, however, Tregs contribute to the generation of an immuno-suppressive microenvironment. To establish therapeutic approaches it is necessary to uncover activating and suppressive mechanisms. Immune checkpoint-blocking (ICB) monoclonal antibodies (mAbs) have been shown to elicit cancer regression and long-lasting tumor control. Particular interest was focused to the mutual influence of Treg cells and therapy by ICB.

Materials/Methods: We used a c-MYC transgenic mouse model of spontaneously arising B-cell lymphoma, which mirrors key features of human Burkitt lymphoma. To assess treatment effects mice were intraperitoneally injected with mAbs against PD-1 and CTLA-4. Mice splenocytes and lymphocytes were analyzed by flow cytometry.

Results: An augmented fraction of CD4+Foxp3+Tregs was detected in spleens and lymph nodes of tumor-bearing c-MYC mice. Tregs were involved in suppressing antitumor response because specific ablation of Tregs significantly delayed tumor development. Compared to wildtype mice, c-MYC Treg cells showed an activated phenotype as evidenced by upregulated CD69 and CD137. The high expression of the costimulatory molecule CD137 indicated TCR-specific activation. As intratumoral Tregs were predominantly Nrp-1+Helios+nTregs, we suggested that Tregs recognized tumor-associated self-antigens. We identified MHC class II-restricted self-epitopes, which were prevalent in lymphoma as compared to normal B cells and could be recognized by Treg cells resulting in enhanced proliferation (Ki-67) in vitro. Interestingly, effector T cells (Teffs) were able to recognize the

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same epitopes. Antigen contact apparently led to upregulation of IL-10. In vitro suppression assays showed that Tregs from c-MYC and wt mice potently suppress proliferation of Teffs in a dose-dependent manner. When preventing cell contacts, Teff proliferation was increased and almost restored upon additional IL-10 neutralization. Tregs from c-MYC mice treated with anti-CTLA-4/anti-PD-1 mAbs revealed a lower suppressive capacity.

**Conclusion:** In malignant lymphoma, nTregs directed against self epitopes are involved in cancer immune escape. Taken together, the Treg population is a promising target in future immunotherapies and needs to be further investigated.