

POSTER PRESENTATION

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# Elucidating the role of Neuropilin-1 in intra-tumoral regulatory T cell stability

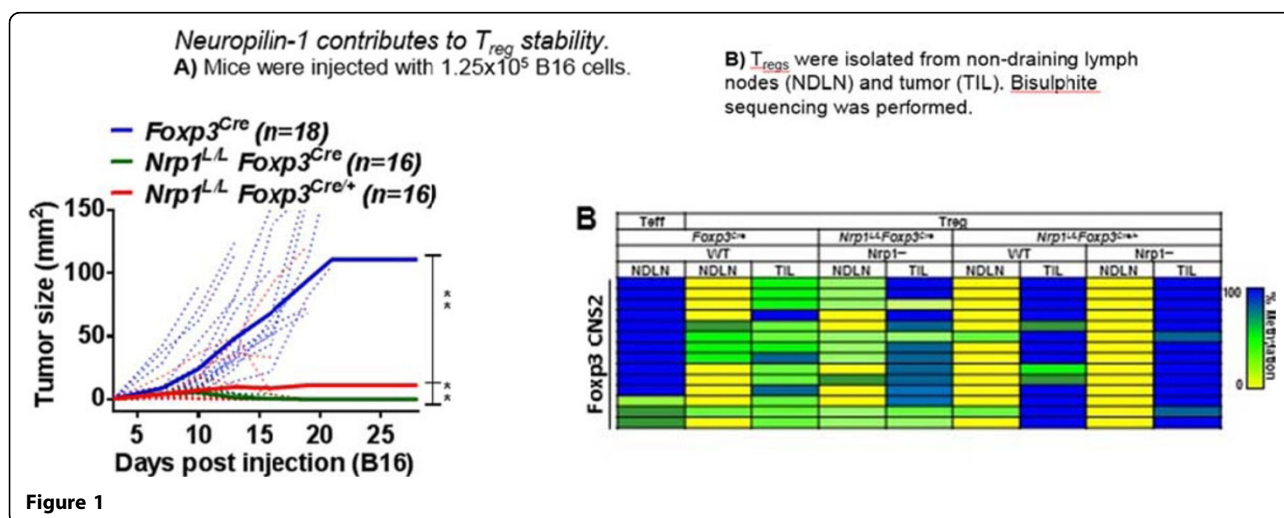
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Regulatory T cells ( $T_{regs}$ ) play an integral role in the adaptive immune system through suppression of self-reactive immune responses in order to prevent autoimmunity and maintain homeostasis. However, they are deleterious in cancer through suppression of the anti-tumor immune response. In fact, we show that deletion of 50% of  $T_{regs}$  results in normal tumor growth. Therefore, it is advantageous to understand the role of  $T_{regs}$  in the tumor microenvironment in order to create targeted cancer therapies. Our lab has shown that the Neuropilin-1 (Nrp1) pathway is required for  $T_{reg}$  stability in the tumor microenvironment, but is dispensable for maintaining immune homeostasis in the periphery, identifying it as a prime therapeutic target.

In order to further understand the role of Nrp1-deficient  $T_{regs}$  intratumorally, we constructed a competitive

environment by utilizing *Foxp3*, which is located on the X chromosome, and as a result of X-inactivation, female *Foxp3<sup>Cre-YFP</sup>* heterozygous mice are cellular heterozygotes. We generated *Nrp1<sup>L/L</sup>Foxp3<sup>Cre-YFP/+</sup>* heterozygous mice comprised of 50% WT  $T_{regs}$  and 50% Nrp1-deficient  $T_{regs}$ . Surprisingly, when given B16 melanoma, heterozygous mice **phenocopy** *Nrp1<sup>L/L</sup>Foxp3<sup>Cre-YFP</sup>* homozygous mice (Figure 1A). This suggests that **Nrp1-deficient  $T_{regs}$  are playing an active role in shifting the anti-tumor immune response by destabilizing surrounding WT  $T_{regs}$  as determined by DNA methylation status** (Figure 1B). Neither WT nor Nrp1-deficient  $T_{regs}$  in the tumor from *Nrp1<sup>L/L</sup>Foxp3<sup>Cre-YFP/+</sup>* mice can suppress in a standard microsuppression assay *ex vivo*, unlike WT  $T_{regs}$  from *Foxp3<sup>Cre-YFP</sup>* mice. Through various co-culture experiments, we revealed that destabilization of WT  $T_{regs}$



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is possibly due to a soluble factor derived from Nrp1-deficient  $T_{regs}$ . Our data revealed that Nrp1-deficient  $T_{regs}$  produce large amounts of  $IFN\gamma$  in the tumor microenvironment. Indeed, **when treated with  $IFN\gamma$ , WT  $T_{regs}$  lose suppressive capacity.** In order to uncover potential novel pathways leading to this phenotype, we are performing global transcript studies using RNASeq. Overall, we have shown that Nrp1 is required for intratumoral  $T_{reg}$  stability, and in its absence, there is an alteration in the tumor microenvironment, leading to an **enhanced anti-tumor immune response.** These studies uncover a novel potential target for future cancer immunotherapies that preserves peripheral immune health.

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