• Although Treg are essential for the prevention of autoimmune diseases, their immunoregulatory function may hinder the induction of immune responses against cancer and infectious agents.
• FOXP3 transcription factor is essential for the specification and maintenance of Treg cells, and thus, it is considered as the “master regulator” of Treg cells.
• Development of inhibitors of FOXP3 might give new therapeutic opportunities for these diseases.
• We identified a peptide (named P60) able to enter into the cells, bind to FOXP3, and impair Treg activity in vitro and in vivo (Patent ES2328776, WO2009065982).
• P60 binds to the intermediate region of FOXP3 and inhibits its homodimerization and interaction with the transcription factor AML1/Runx1.
• Characterization has been performed to identify those residues which contribute to the stability of P60-FOXP3 interaction.
• Key concepts and Target Identification
  - FOXP3 dimerization is required for its function as a transcriptional regulator and it has been described that the leucine zipper region is necessary and sufficient to mediate homo-dimerization.
  - AML1 is required for IL-2 and IFN-γ gene expression in conventional CD4+ T cells and its interaction with Foxp3 is needed for the immunosuppressive activity of Tregs.
  - P60 derived peptides inhibit FOXP3 homodimerization and FOXP3/AML1 interaction impairing the immunosuppressive activity of Treg cells and enhancing T cell proliferation and cytokine production upon TCR stimulation.
  - P60/P60 peptides derived are identified as regulators of Treg and synergizes with anti-PD1 to cure colon cancer tumors in a murine model. P60/P60 peptides derived can be considered as new agents for tumor immunotherapy.

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**Target Validation**
- Treg inhibitory peptide P60 interacts with the intermediate region of FOXP3 inhibiting homodimerization and its association with AML1. Key residues implicated in the interaction of P60 with FOXP3 have been identified and allowed us to identify P60 derived peptides with higher Treg inhibitory capacity and anti-tumor activity.

*Figure 1. Region of interaction of the FOXP3 inhibitory peptide P60 (A) Effect of different concentrations of P60 or a control peptide on FOXP3 homodimerization (B) and FOXP3/AML1 interaction (C).*

*Figure 2. Heat map summarizing the effect of point mutations in the indicated in vitro assays.*