

## Synergistic inhibition of cancer invasion and metastasis by combined anti-PD1/TRC105-mediated Endoglin targeting on cancer-associated fibroblasts and endothelial cells

### Introduction

Endoglin is a co-receptor for Transforming Growth Factor (TGF)- $\beta$  and Bone Morphogenetic Protein (BMP)-9 and has a crucial role in developmental and tumor angiogenesis.

Endoglin is highly expressed by angiogenic endothelial cells, but also by cancer-associated fibroblasts (CAFs) at the invasive margins of colorectal tumors (Paauwe *et al* Clin Ca Res 2018) and several other cells in the tumor microenvironment. Expression of endoglin has been correlated with metastases and poor prognosis in cancer patients.

TRC105 (carotuximab, Tracon pharmaceuticals) is a humanized IgG1 antibody directed against endoglin and was previously shown to inhibit tumor angiogenesis *in vitro* and *in vivo*. The effects of TRC105 seem to be dependent on an immune response against the target cells. TRC105 is currently in clinical development in multiple phase 2 and -3 clinical trials as well as a phase 1 trial with nivolumab.

In this project we have evaluated if immune responses against endoglin can be enhanced by simultaneously activating anti-tumor responses using anti-PD1 therapy. We used three different mouse colorectal cancer (CRC) models to evaluate therapeutic responses.

### Aim:

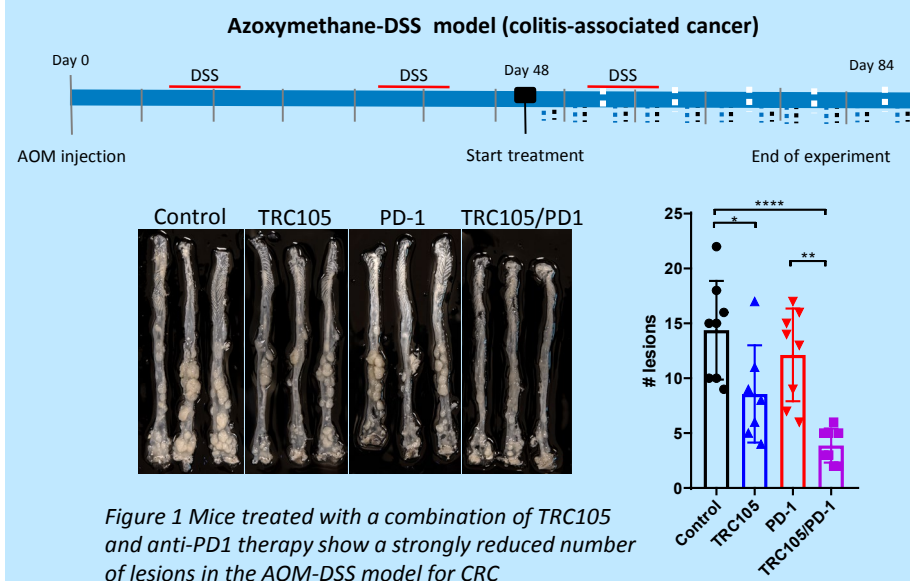
1. To evaluate if combining TRC105 with anti-PD1 enhances anti-tumor responses in three mouse models for different stages of colorectal cancer.
2. To evaluate the underlying mechanism of action of synergistic effects.

### Materials and methods

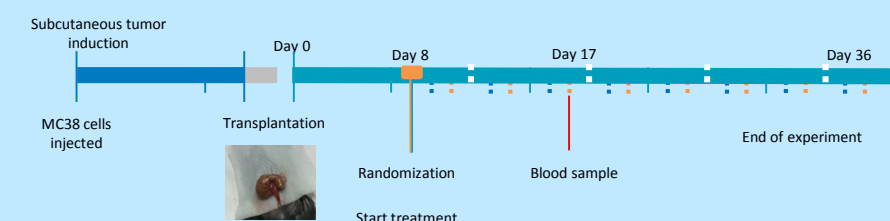
- Chemically induced CRC model, using azoxymethane (AOM) followed by dextran sodium sulphate (DSS) induced inflammation
- Syngeneic orthotopic or subcutaneous tumor models using MC38 or CT26 mouse CRC cells
- Upon tumor formation (palpable tumors) treatment with TRC105 and/or anti-PD1 therapy (or isotype control antibodies)
- Endpoint: tumor > 1,5 cm<sup>3</sup> (survival) or short term (9 days)
- Flow cytometry analysis, immunohistochemical analysis

### Results

Tumor formation was induced by injection with AOM, followed by 3 cycles of DSS. After 84 days mice were sacrificed and number of lesions was evaluated.



### Orthotopic MC38 CRC model (late stage/metastatic cancer)



After tumor transplantation mice were divided in groups based on equal bioluminescence signal and treated with antibodies. After 36 days mice were sacrificed and tumor volume (A), vascularization (B) and the number of tumor-free animals was determined.

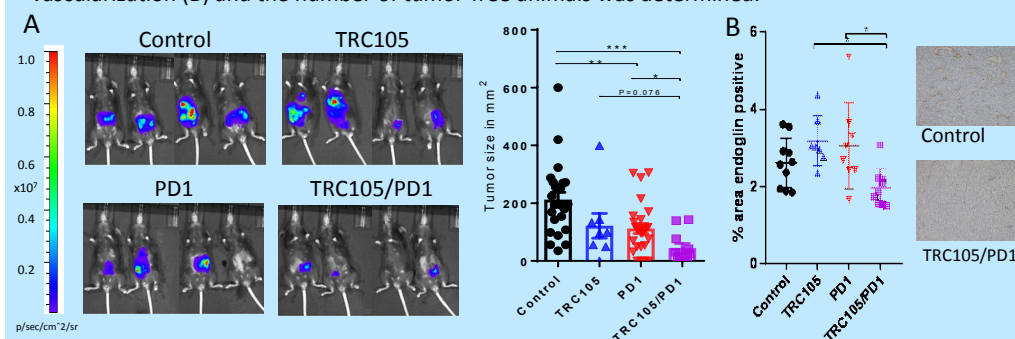
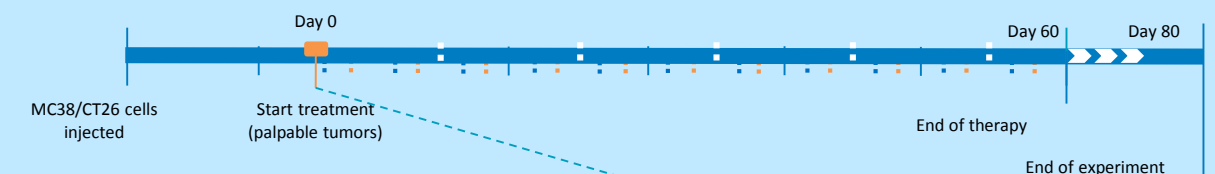


Figure 2 Mice treated with a combination of TRC105 and anti-PD1 therapy display significantly reduced tumor volume. An increased number of tumor-free mice is detected.

### MC38/CT26 subcutaneous tumor model



After MC38 or CT26 tumor inoculation, when palpable tumors were present mice were treated and sacrificed when tumors reached 1.5 cm<sup>2</sup>. In a second experiment (Fig 3E) the immune infiltrate was analyzed 9 days after start of treatment, when tumors had equal volume.

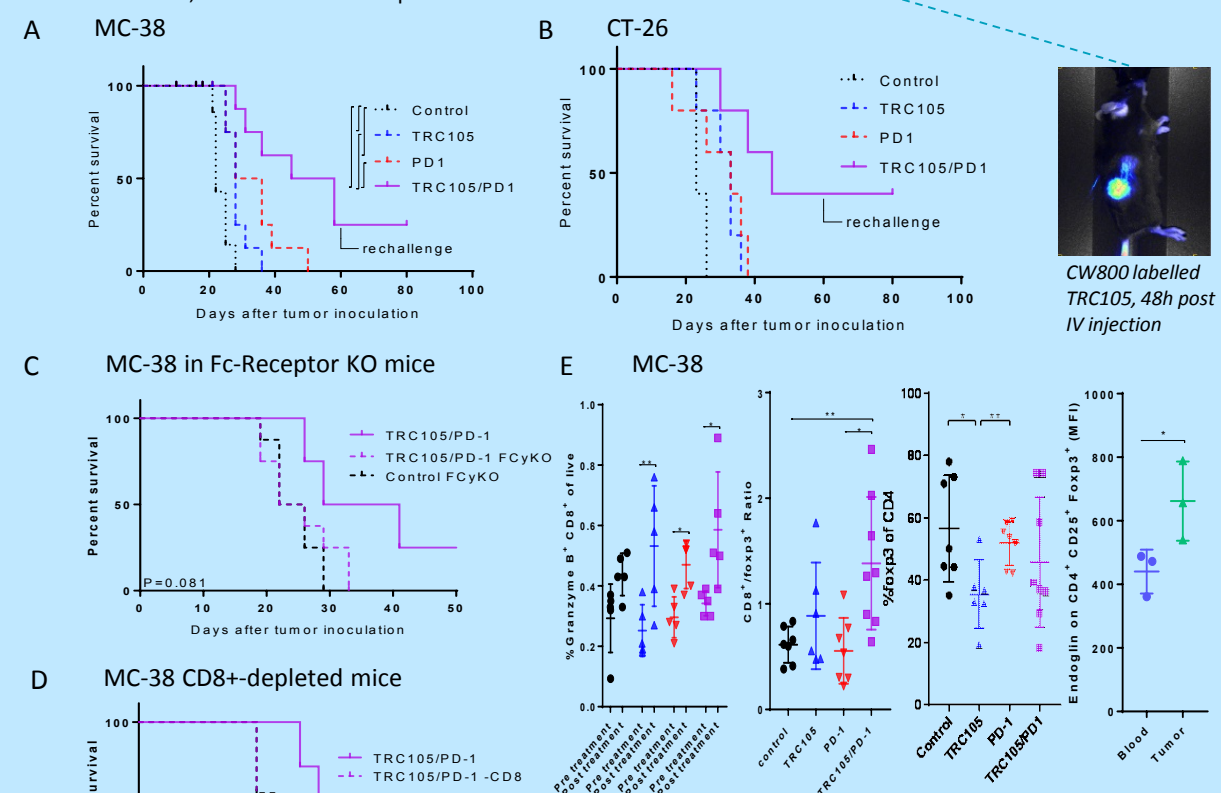


Figure 3 Mice with subcutaneous MC38 (A) or CT26 (B) tumor treated with a combination of TRC105 and anti-PD1 therapy showed significantly increased survival and resistance to a rechallenge with tumor cells. These effects were dependent on the presence of Fc receptors (C), CD8+ T-cells (D) and involves an increase in activated CD8 cells together with a decrease in endoglin-expressing FoxP3+ Treg cells (E).

### Conclusions:

- TRC105/PD1 significantly reduced number of lesions in a model of early stage CRC
- TRC105/PD1 combination therapy reduced tumor growth and increased survival
- Effects of TRC105/PD1 combination therapy was dependent on ADCC responses and the presence of CD8+ T cells
- TRC105 significantly reduced tumor resident endoglin- and FoxP3-expressing regulatory T-cells (Treg)

