

SITC 2019

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Society for Immunotherapy of Cancer

#039



Soluble TNF α induced mucin 4 is a mediator of trastuzumab resistance and of an immunosuppressive tumor microenvironment in HER2+ breast cancer

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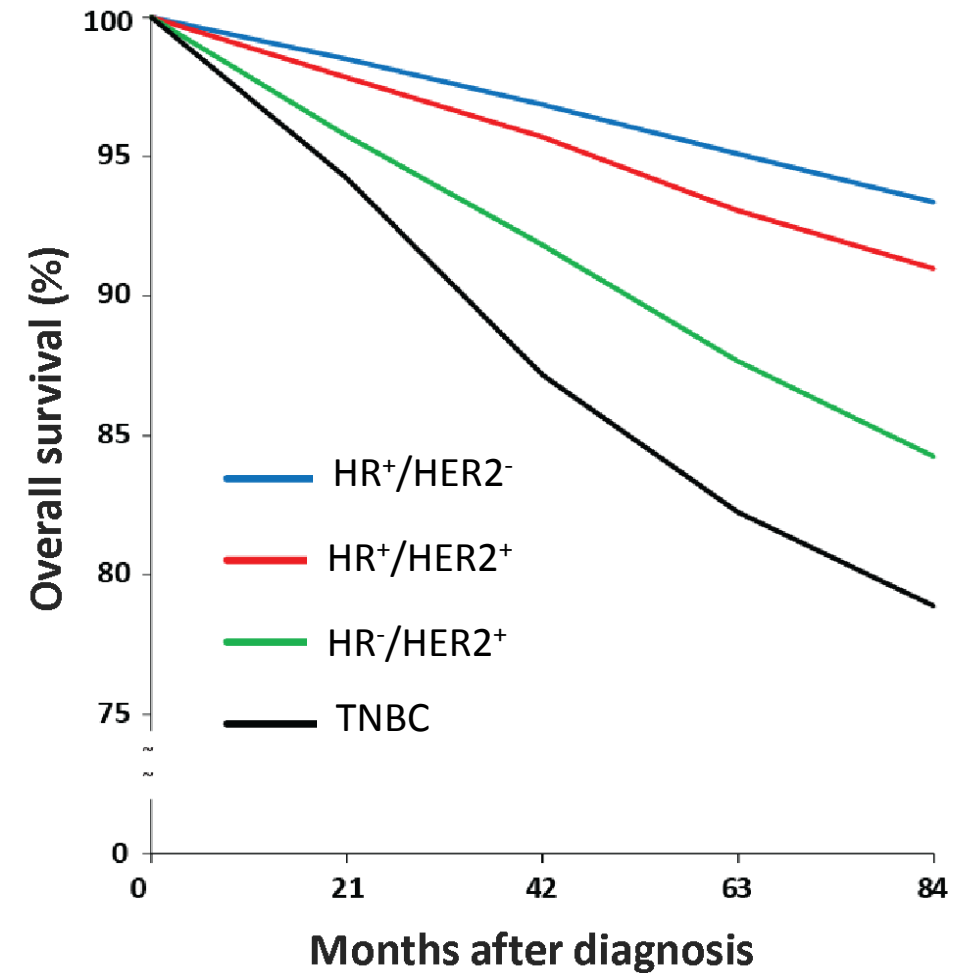
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INTRODUCTION

HER2 positive (HER2+) is a breast cancer (BC) subtype that affects 13-20% of the patients

This BC subtype has an aggressive behaviour and poor prognosis.



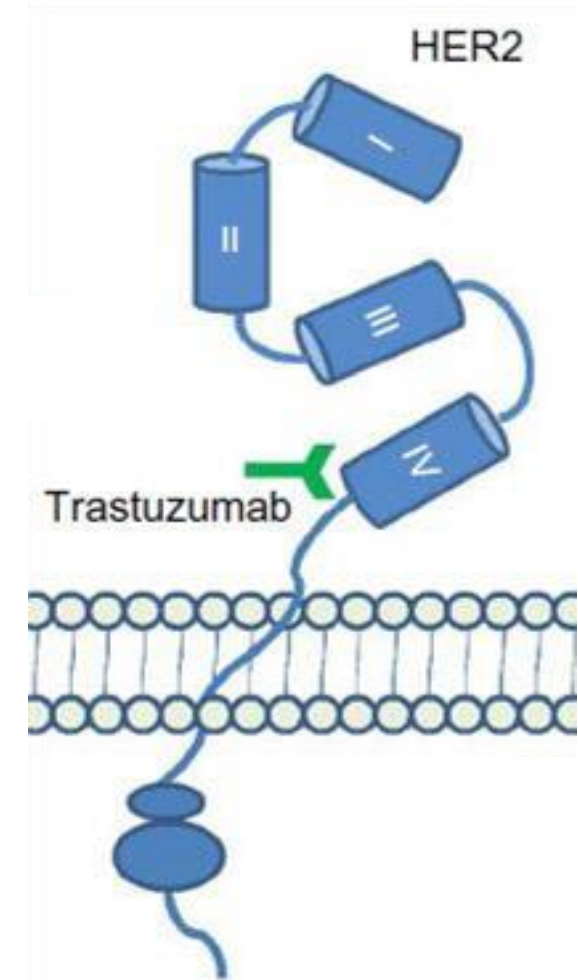
Adapted from Howlader, et al. *Cancer Epidemiol Biomarkers Prev.* 2018

INTRODUCTION

Trastuzumab (T) is an anti-HER2 monoclonal antibody that binds to the IVth domain of HER2 molecule.

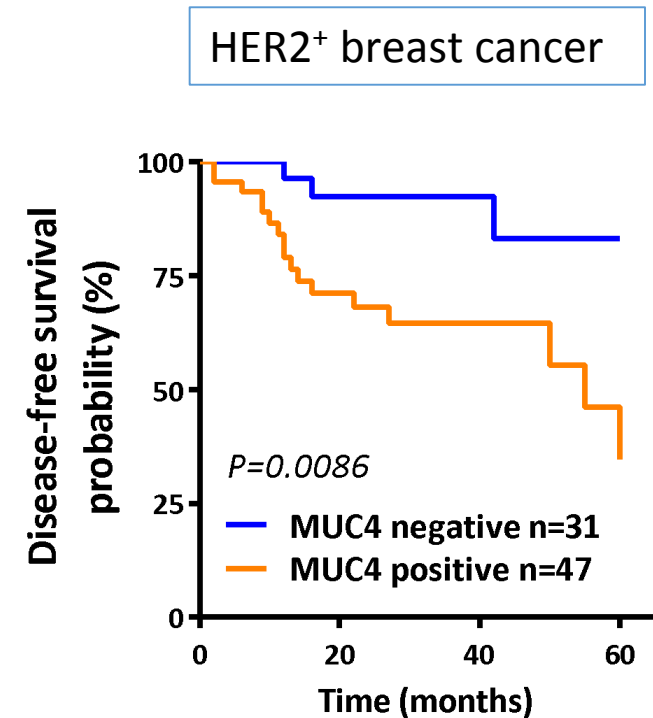
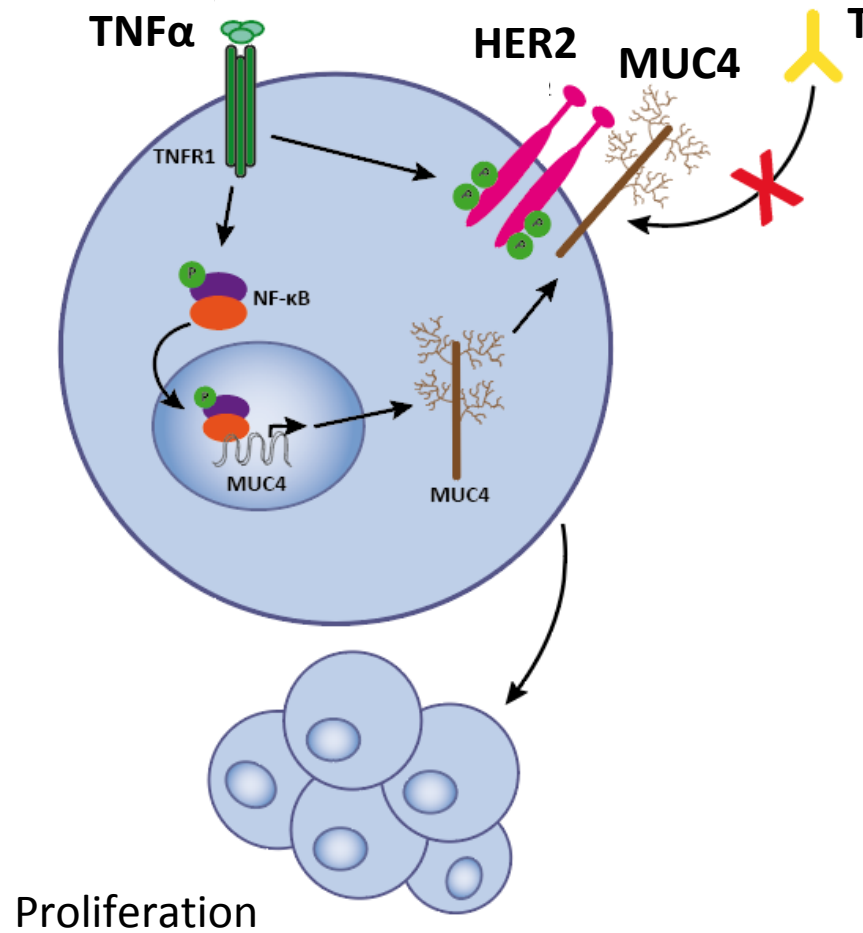
It is used as a first line treatment, but resistance events hamper its clinical benefit in 27-42% of the cases.

Gianni L. et al. Lancet Oncol 2014



BACKGROUND

TNF α expression induces the upregulation of the expression of the glycoprotein mucin 4 (MUC4), impairing T binding to HER2 and ADCC.



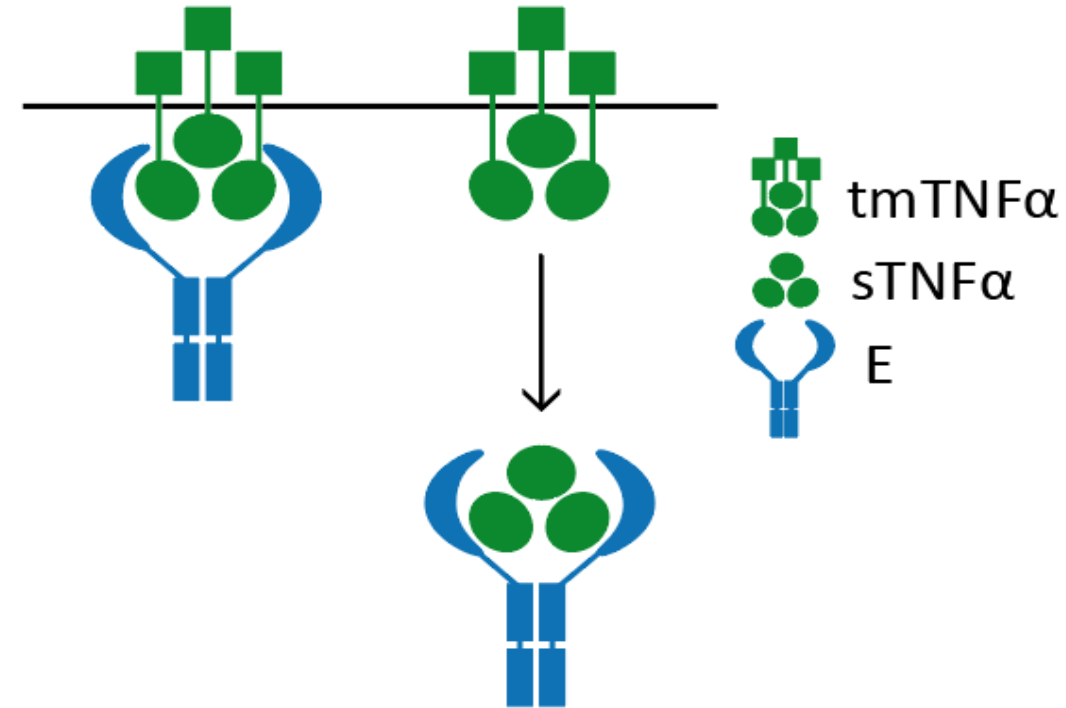
Mercogliano et al. Clin Cancer Res 2017

BACKGROUND

Etanercept (E) inhibits transmembrane (tmTNF α) and soluble TNF α (sTNF α)

E downregulates MUC4 expression and sensitizes T-resistant BC cells and xenografts to T

Mercogliano et al. Clin Cancer Res 2017



TNF α role in innate immune response and tumorigenesis

- TNF α receptors bind both TNF α forms, but sTNF α and tmTNF α preferentially trigger TNFR1 and TNFR2, respectively (*Brenner, et al Nat Rev Immunol 2015*)
- tmTNF - TNFR2 interaction is necessary for proper dendritic cell-natural killer cell cross-talk (*Xu, et al Blood 2007*)
- sTNF α induces expansion of myeloid-derived supresor cells (MDSCs), development of its immunosuppressive activity and promotion of carcinogenesis (*Sobo-Vujanovic, et al. Cancer Immunol Res , 2016*)

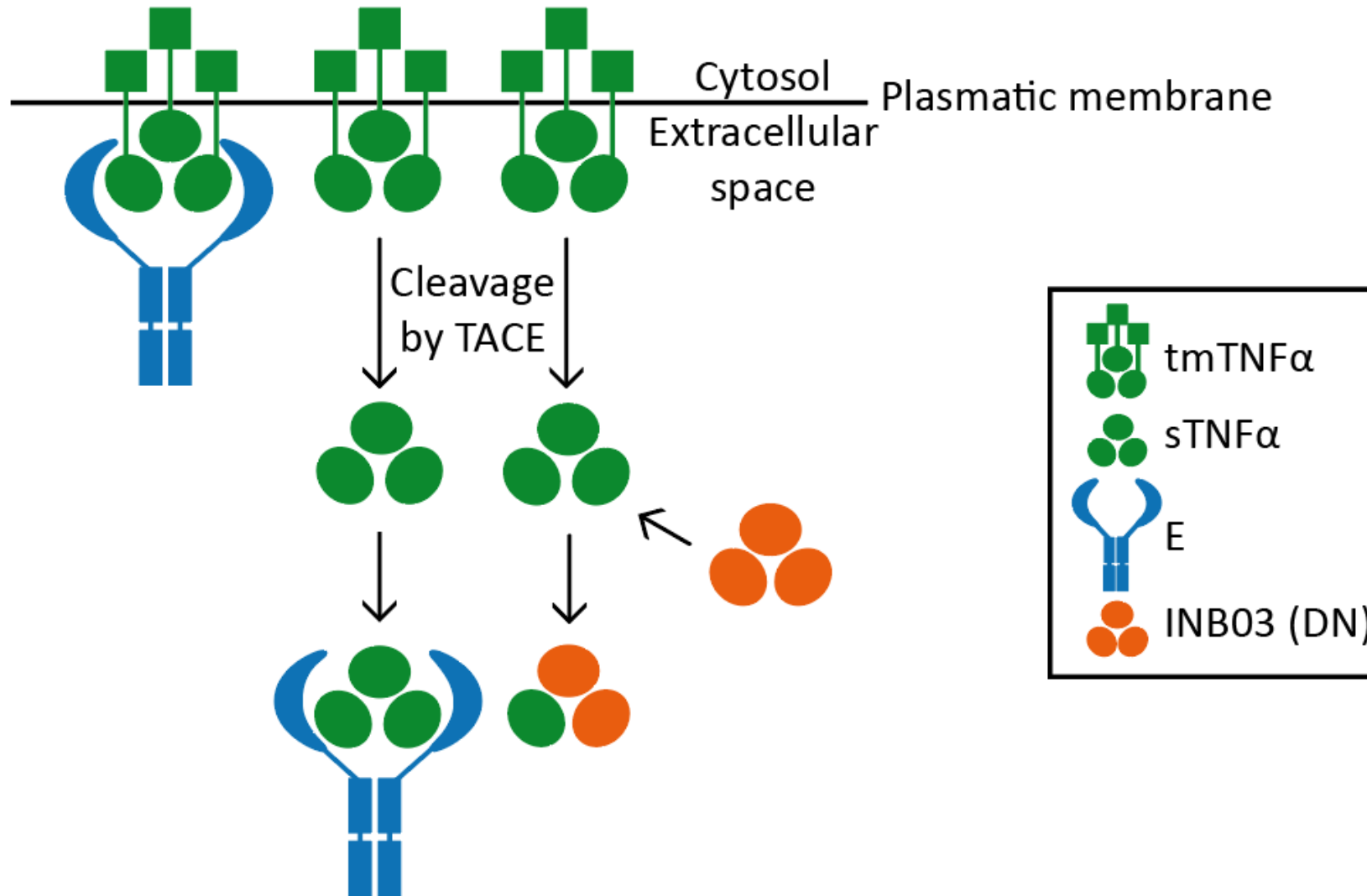
OBJECTIVES

To evaluate the role of sTNF α on MUC4 expression

To study the participation of MUC4 on

- ✓ T resistance *in vivo*
- ✓ T-mediated antitumor innate immune response

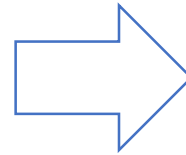
TNF α blocking agents



STRATEGY - MODEL

JIMT-1 cells

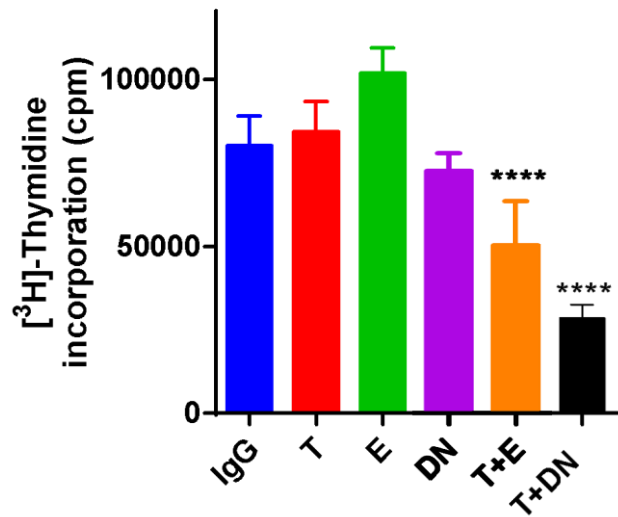
- HER2+ human BC cell line
- T resistant
- MUC4+
- Produces TNF α



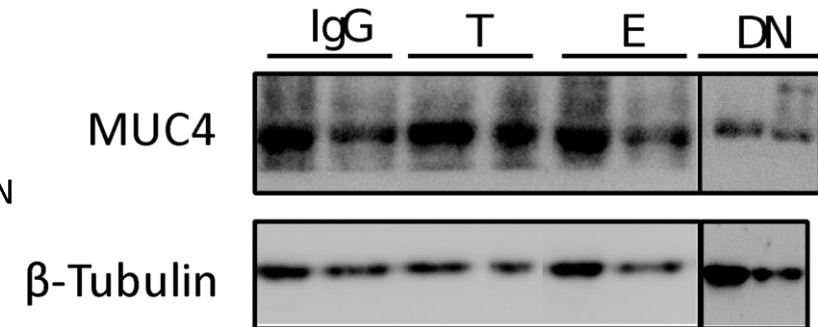
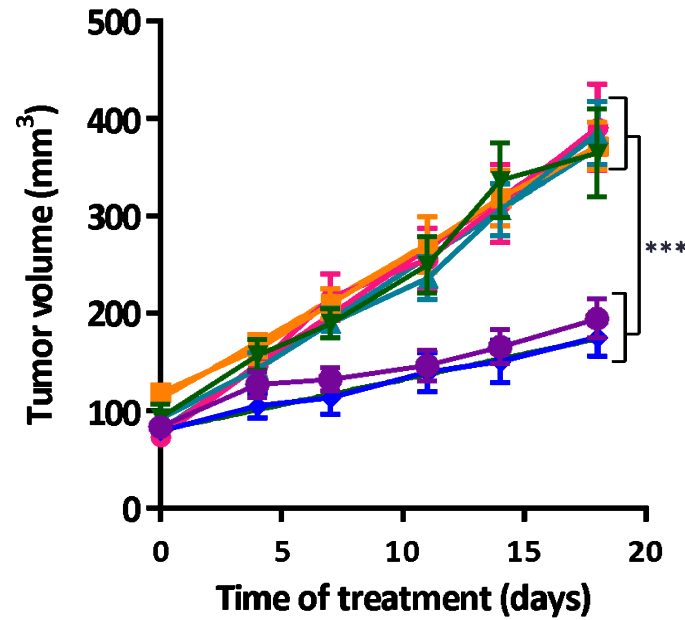
**DN molecule tested *in vitro*
and *in vivo***

sTNF α blockade sensitizes cells and tumors to trastuzumab

WT JIMT-1 cells



WT JIMT-1 tumor



*** $p < 0.0001$, **** $p < 0.00001$ vs. IgG

STRATEGY - MODEL

JIMT-1 cells

- HER2+ human BC cell line
- T resistant
- MUC4+
- Produces TNF α

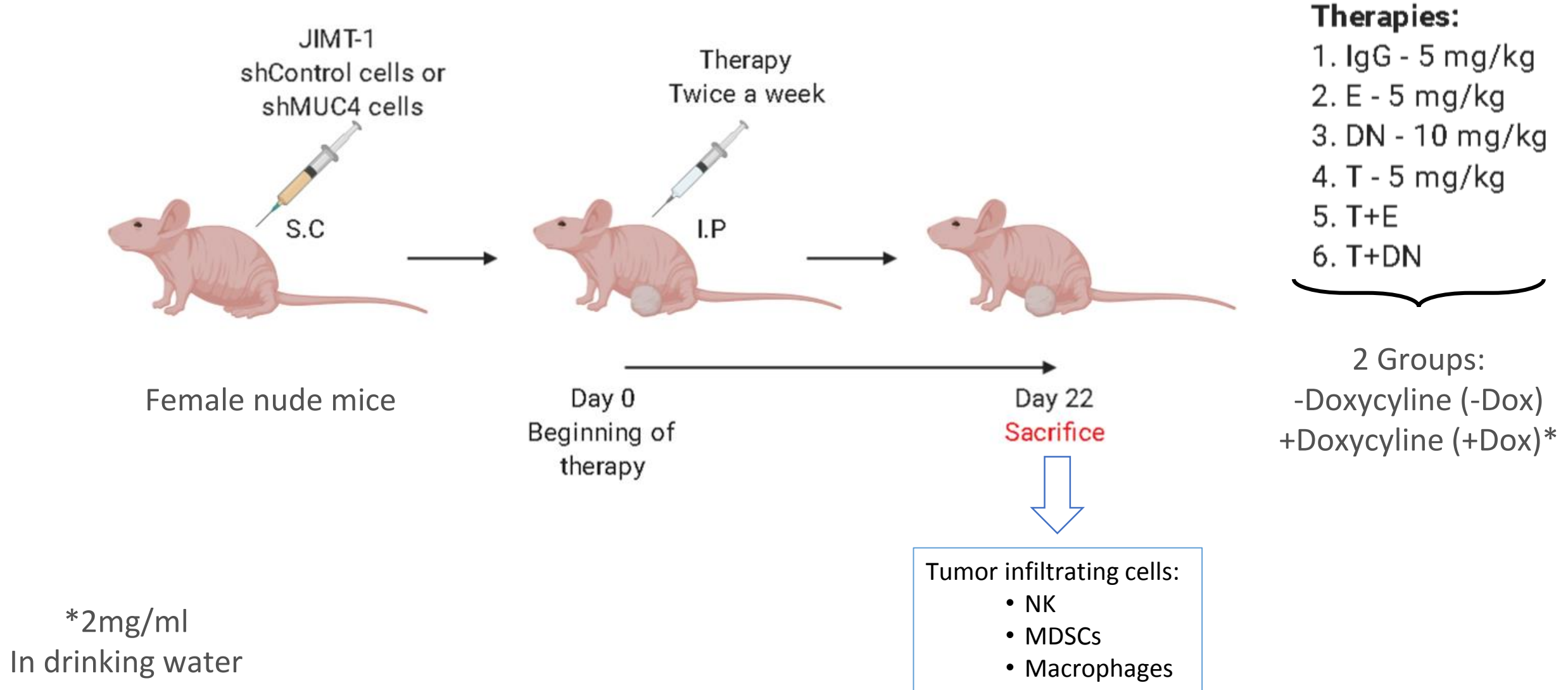


Doxycycline-inducible MUC4 or Control shRNA



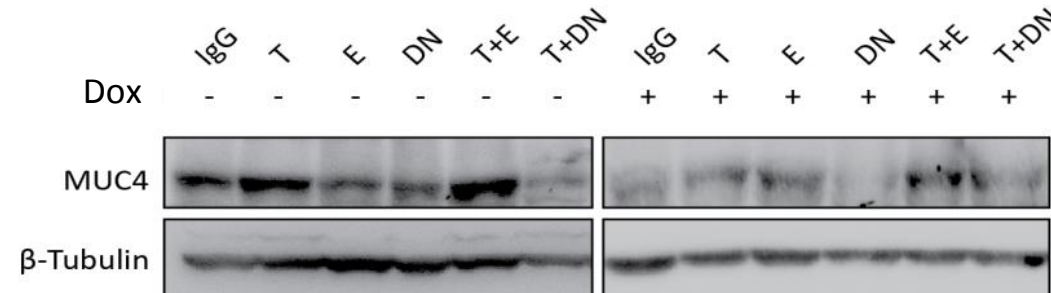
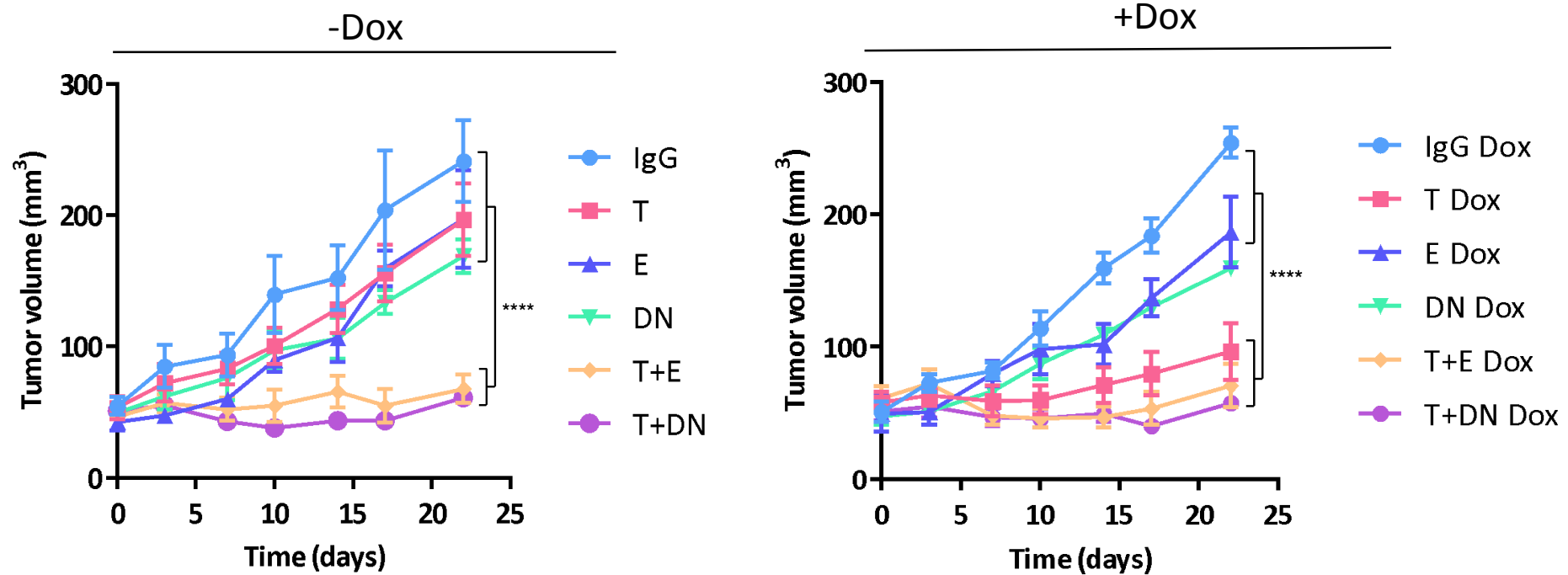
JIMT-1 shMUC4
JIMT-1 shControl

STRATEGY - MODEL



MUC4 silencing sensitizes tumors to trastuzumab

JIMT-1-shMUC4

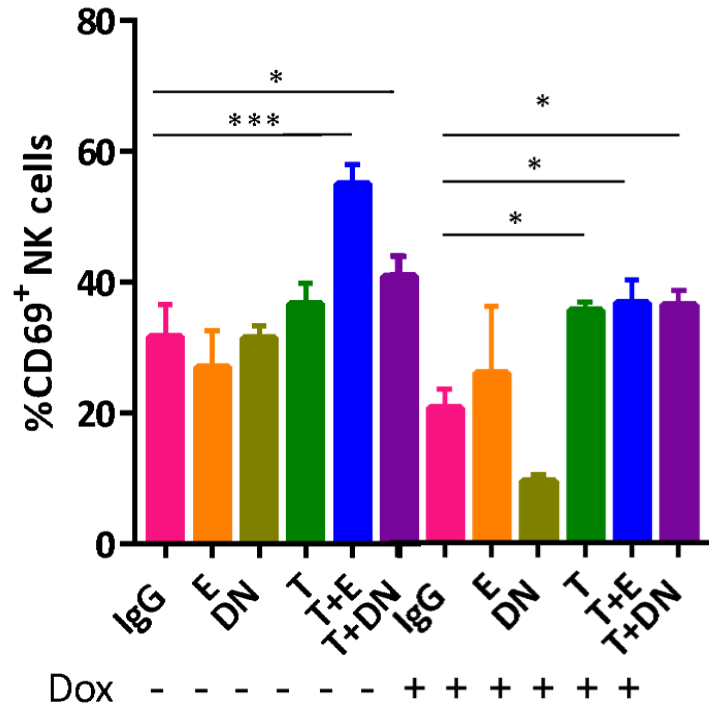


****p<0.00001 vs. IgG

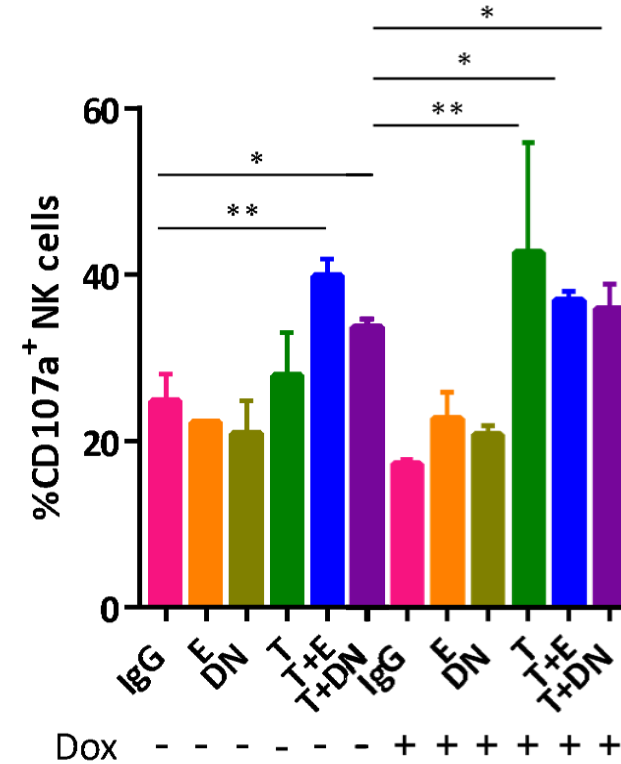
MUC4 downregulation increases NK cell activation and degranulation in combination with trastuzumab

JIMT-1-shMUC4

NK cell activation



NK cell degranulation

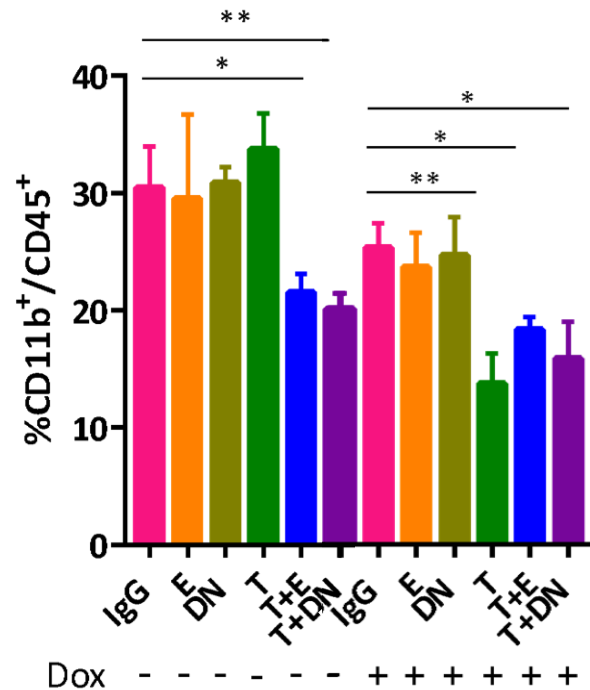


*p<0.05, **p<0.01, ***p<0.001 vs. IgG

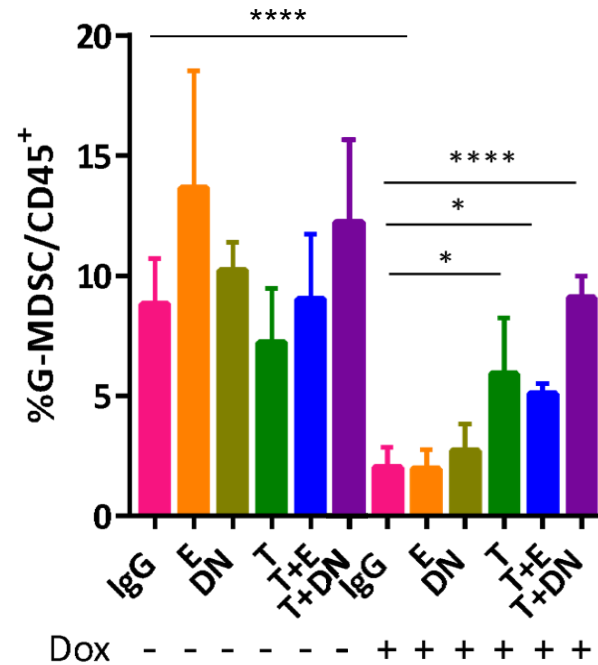
MUC4 downregulation decreases MDSCs in the TME

JIMT-1-shMUC4

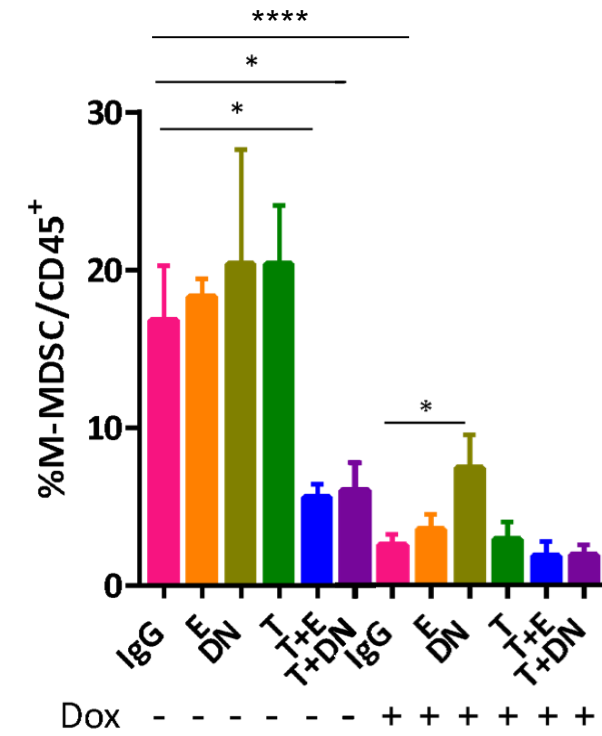
Myeloid cells



G-MDSC



M-MDSC

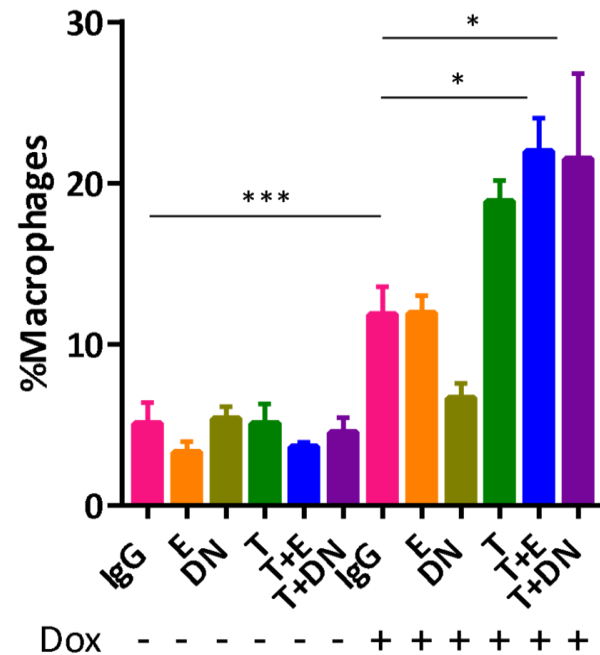


*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs. IgG

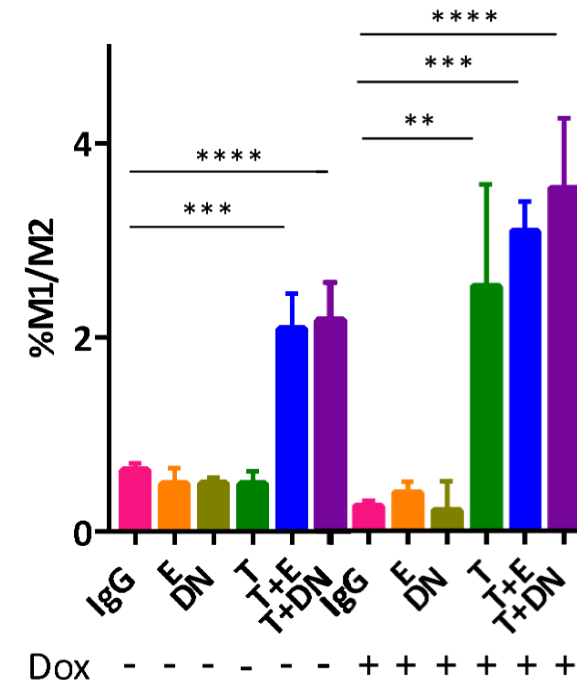
MUC4 downregulation increases macrophage M1/M2 ratio in the TME in combination with trastuzumab

JIMT-1-shMUC4

Macrophages



M1/M2 ratio

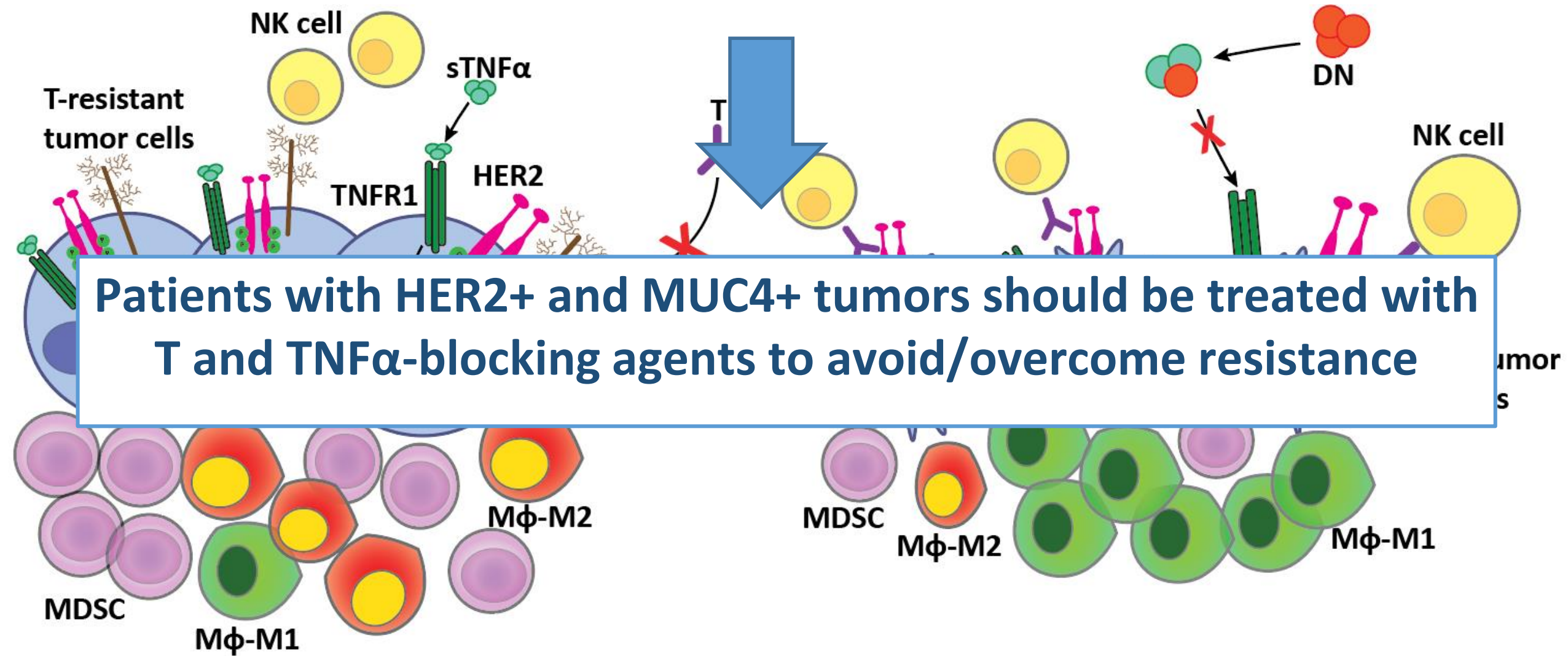


*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs. IgG

CONCLUSIONS

- sTNF α blockade accounts for MUC4 downregulation
- sTNF α blockade has similar effect in sensitizing T-resistant tumors to T than blockade of both sTNF α and tmTNF α
- MUC4 is the major player in TNF α -induced T resistance *in vivo*

MUC4 downregulation favors a less immunosuppressive TME, increasing the macrophage recruitment, M1 differentiation and decreasing the MDSCs



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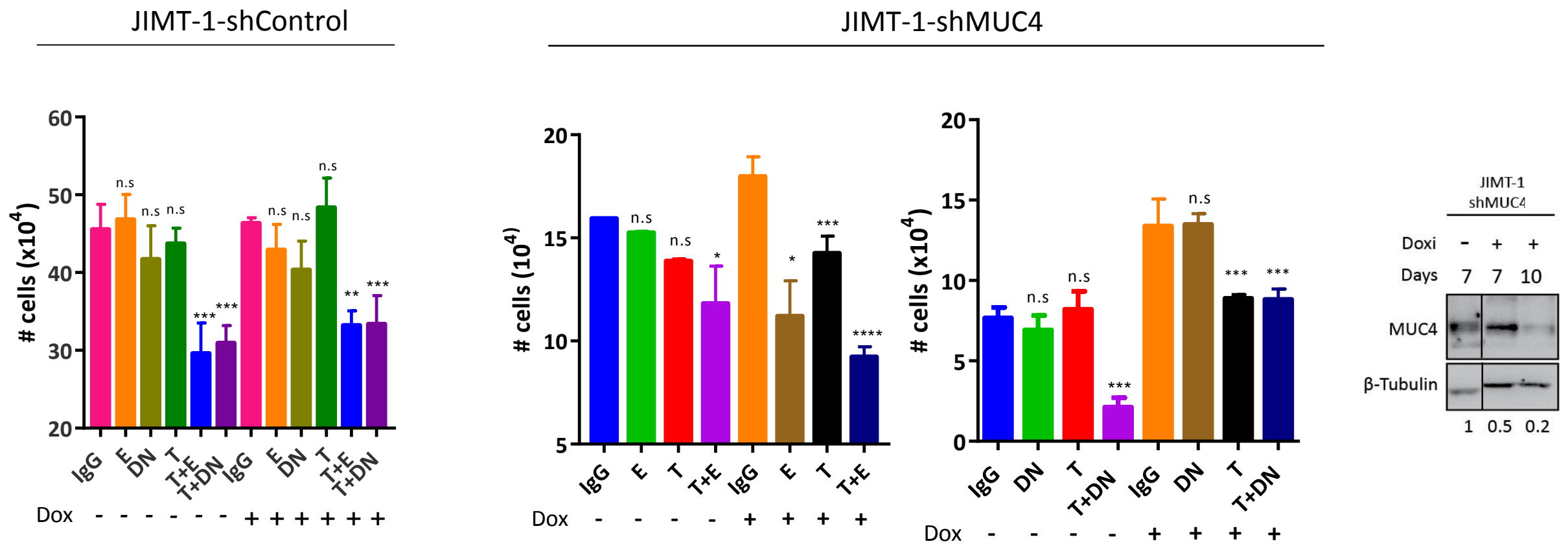
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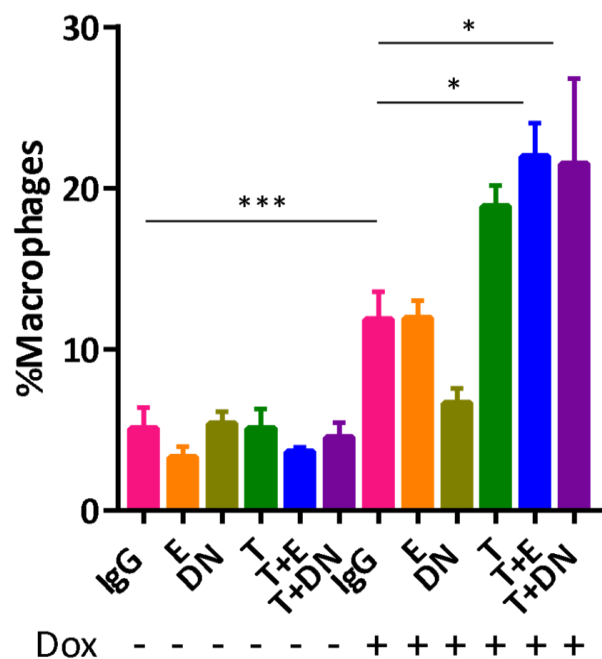
MUC4 silencing sensitizes cells to trastuzumab *in vitro*



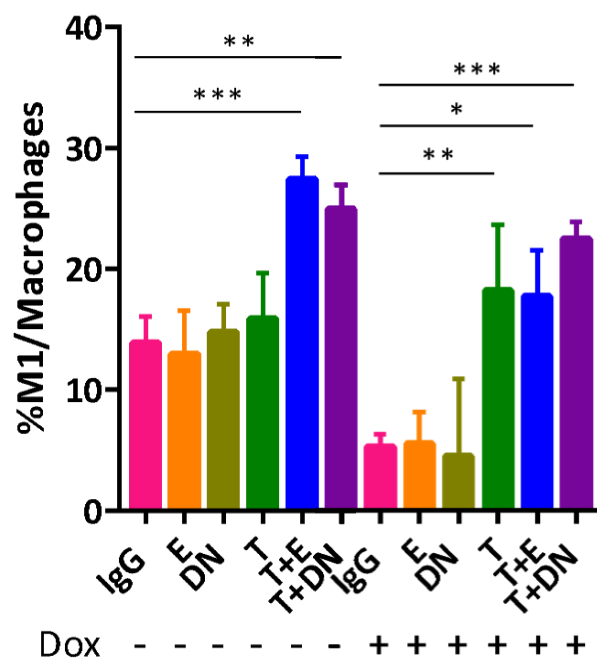
*p<0.05, **p<0.01, ***p<0.0001, ****p<0.00001 vs. IgG

RESULTS – Tumor infiltrating immune cells

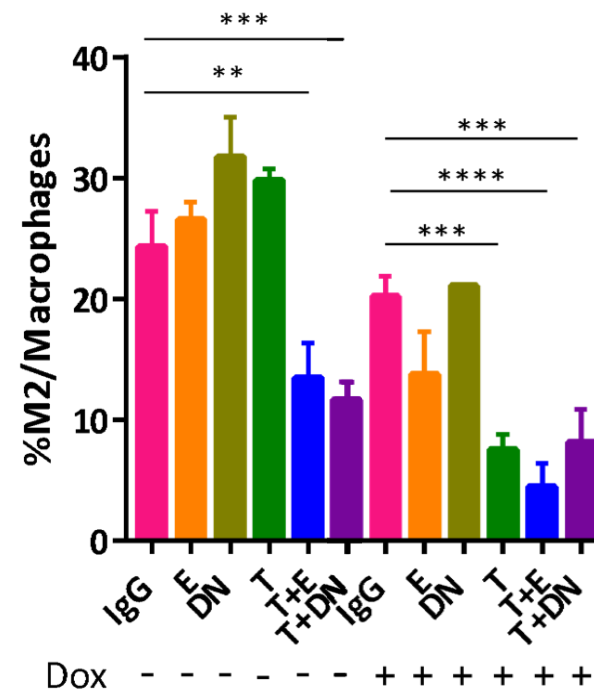
Macrophages



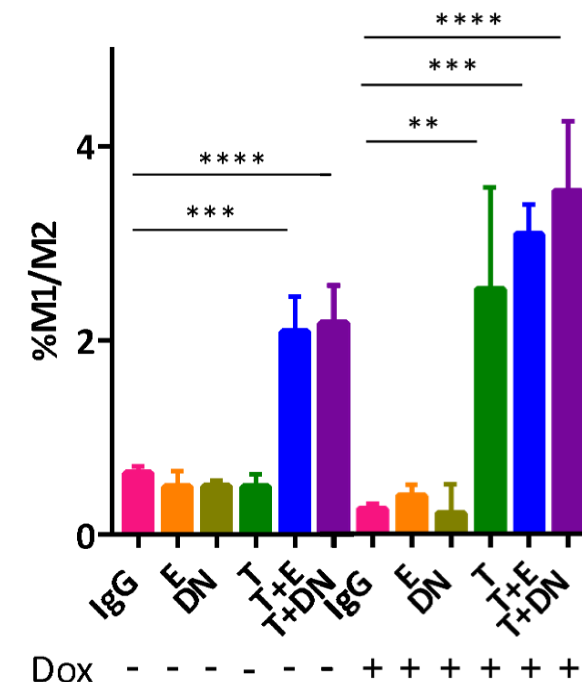
%M1



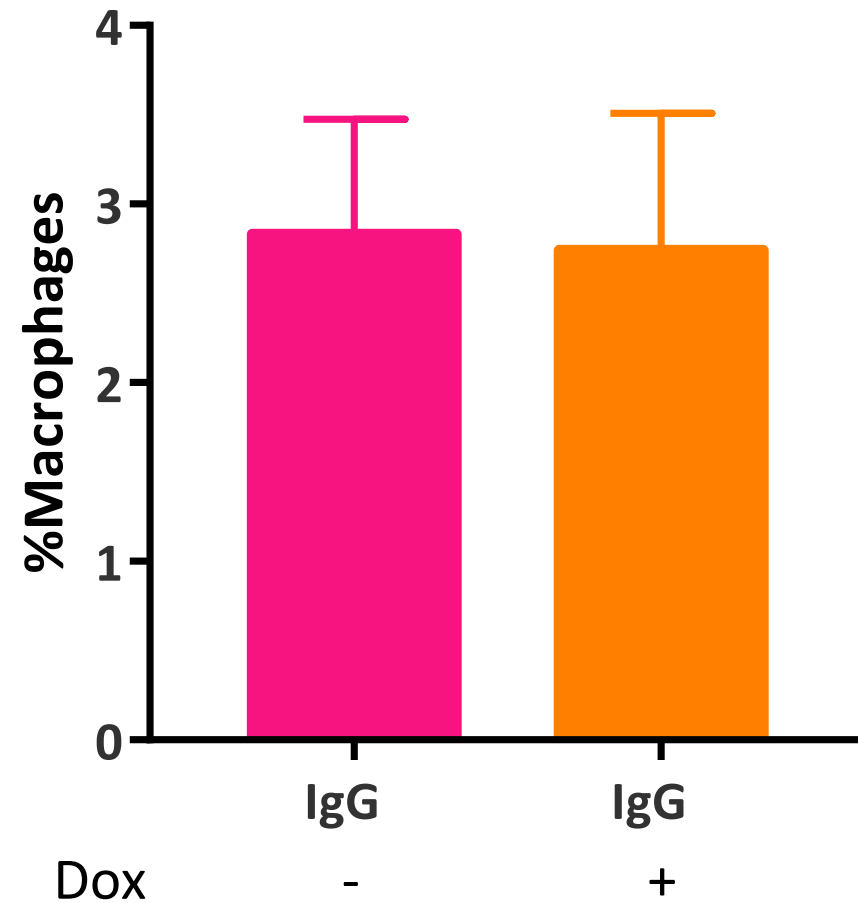
%M2






M1/M2 ratio



*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs. IgG



RESULTS – Tumor infiltrating immune cells

 No change
 Increase
 Decrease
 vs. IgG MUC4+ tumors

| Population S | MUC4+ tumor | | | MUC4- tumor | | | |
|--------------------|-------------|-----|------|-------------|---|-----|------|
| | T | T+E | T+DN | IgG | T | T+E | T+DN |
| NK act+deg | = | ↑ | ↑ | = | ↑ | ↑ | ↑ |
| MDSCs | = | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ |
| G/M-MDSCs ratio | = | ↑ | ↑ | = | ↑ | ↑ | ↑ |
| Macrophages | = | = | = | ↑ | ↑ | ↑ | ↑ |
| M1/M2 ratio | = | ↑ | ↑ | = | ↑ | ↑ | ↑ |

Change in tumor microenvironment (TME)
 LESS IMMUNOSUPPRESSIVE