# Conditioning Treatment with A CD27 Antibody Enhances *in vivo* Expansion and Antitumor Activity of Adoptively Transferred T Cells

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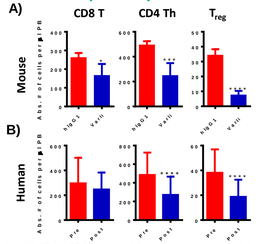
## Study Rationale

- Conditioning treatment to induce lymphodepletion is critical in adoptive cell therapy (ACT).
- Lymphodepletion enhances expansion of the transferred T cells through
  - ➢ Removing a potential cytokine sink
  - ➢ Eliminating regulatory T cells ($T_{reg}$)
  - ➢ Activating the innate immune system
- Cyclophosphamide (Cy) and fludarabine (Flu) are current conditioning agents, which cause
  - ➢ Neutropenia
  - ➢ B cell loss
  - ➢ General cytotoxicity
- A safer and more selective T cell depletion conditioning regimen is an unmet need in ACT.

## CD27 mAb – Varlilumab Background

- CD27 is a member of TNFRSF, constitutively expressed on most T cells with the highest level on $T_{reg}$ > $CD4_{Th}$ > CD8.
- CD70-CD27 signaling provides costimulation important in T cell activation
- CD27 mAb varlilumab (human IgG1)
  - ✓ Agonistic activity (T cell costimulation)
  - ✓ Ligand blocking activity
  - ✓ Clinical responses in monotherapy and combination with PD-1 blockade
  - ✓ A favorable safety profile in its class of agonistic mAbs in clinical development
  - ✓ $T_{reg}$ preferential T cell depletion activity in hCD27 transgenic mice and cancer patients

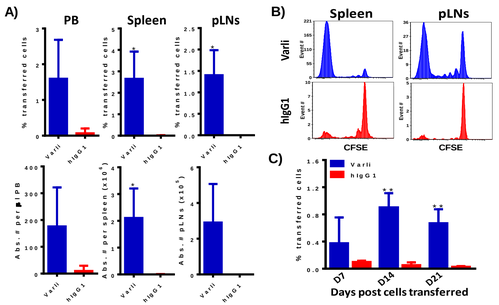



A) hCD27$^{+/+}$mCD27$^{-/-}$ mice, 14 days post varli or hIgG1 injection (N=5).
B) Cancer patients enrolled into phase II clinical trial of varlilumab in combination with nivolumab, before 1$^{st}$ dose and 14 days later (N=73).

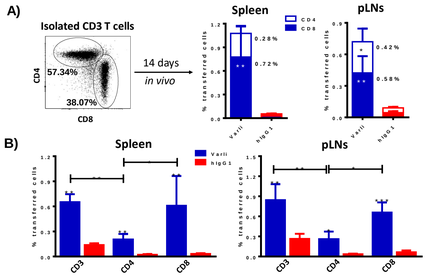
## Hypothesis

Does more selective Treg depletion plus agonistic activity make varlilumab a better conditioning regimen?

## Remarkable Expansion of Transferred T Cells Following Varlilumab Pretreatment

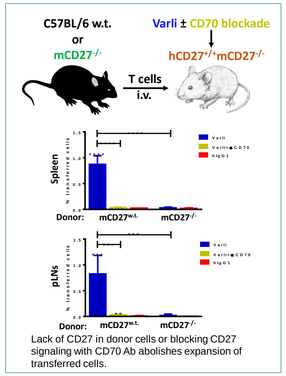



## Varlilumab Pretreatment Favors CD8 T Cell Expansion



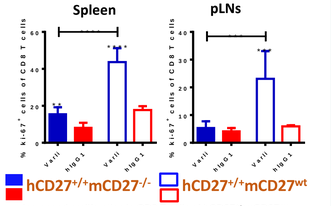

A) CD3 T cells were infused on day 0. Shown are the relative expansion of CD4 and CD8 T cells in same recipients.
B) Same number of CD3, CD4 or CD8 T cells were infused on day 0. Shown are their expansion in different recipients.

## CD27 Signal in Donor or Recipient Cells Plays Distinct Role on Transferred T Cell Expansion upon Varlilumab Pretreatment

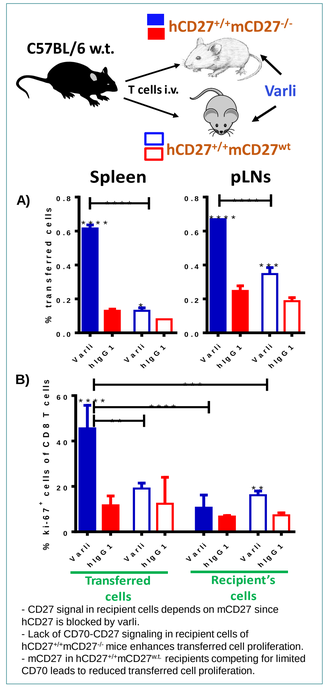



Lack of CD27 in donor cells or blocking CD27 signaling with CD70 Ab abolishes expansion of transferred cells.

### Ki-67$^+$ CD8 T Cells in the Presence or Absence of CD27 Signal after Varli Injection w/o Cell Transfer



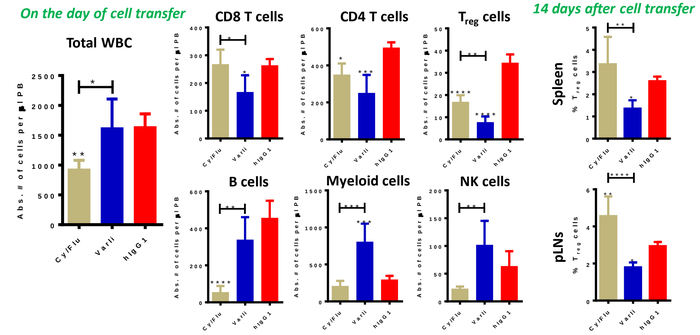

Increased proliferation in CD8 T cells of hCD27$^{+/+}$mCD27$^{wt.}$ mice (CD70-CD27 signal competent), compared to that in hCD27$^{+/+}$mCD27$^{-/-}$ mice (lack of CD70-CD27 signal) following varli treatment.



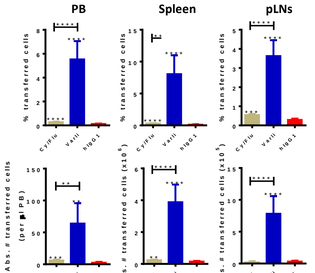

- CD27 signal in recipient cells depends on mCD27 since hCD27 is blocked by varli.
- Lack of CD70-CD27 signaling in recipient cells of hCD27$^{+/+}$mCD27$^{-/-}$ mice enhances transferred cell proliferation.
- mCD27 in hCD27$^{+/+}$mCD27$^{wt.}$ recipients competing for limited CD70 leads to reduced transferred cell proliferation.

## Varlilumab Versus Current Regimen for Conditioning Treatment

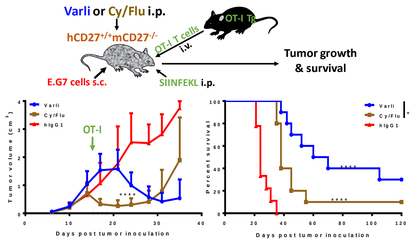
### A) More Selective and Longer-lasting T cell Depletion




### B) Greater Transferred Cells Expansion



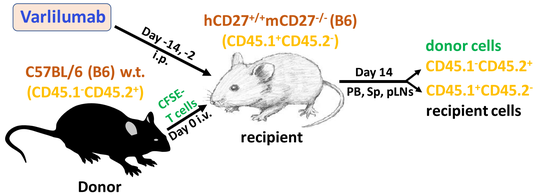

### C) Stronger Antitumor Activity of Transferred Cells



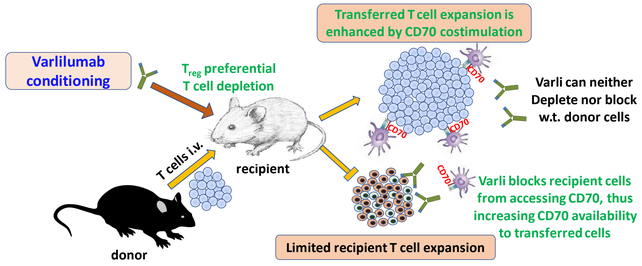

**A) & B)** hCD27$^{+/+}$mCD27$^{-/-}$ mice were injected i.p. with varli or hIgG1 on day -14 and -2; Cy/Flu on day -5, -4, -3; CD8 T cells were injected i.v. on day 0.

**C)** E.G7 tumor cells were inoculated s.c. into hCD27$^{+/+}$mCD27$^{-/-}$ mice on day 0. Varli or hIgG1 was injected on day 7 and 14; Cy/Flu on day 13 and 14; OT-I cells were injected on day 16, and SIINFEKL peptide on day 17.

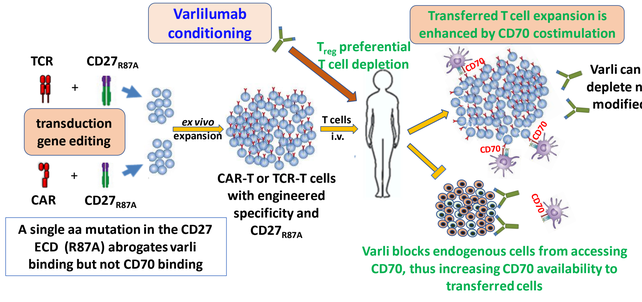
## Adoptive T Cell Transfer Schema




## Working Model of Varlilumab Conditioning Treatment




## Translation of Varlilumab Conditioning Treatment into ACT Clinical Practice

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