The mechanism of anti-tumor immunity induced by varlilumab, a CD27 agonist mAb, is model dependent

Li-Zhen He, Anna Wasiuk, James Testa, Jeff Weidlick, Crystal Sisson, Andrea Crocker, Jenifer Widger, Eric Forsberg, Catherine Pilsmaker, James Boyer, Lawrence Thomas, Henry Marsh, and Tibor Keler

Celldex Therapeutics, Inc., Hampton, NJ and Needham, MA

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Varlilumab (CDX-1127): A Human Monoclonal Antibody to CD27

- CD27 is a potent co-stimulatory molecule that drives T cell activation and survival through interaction with it's ligand, CD70
- Varlilumab is an agonist anti-CD27 human IgG1 mAb that induces activation and proliferation of human T cells when combined with T-cell receptor stimulation
- Varlilumab is effective in syngeneic murine tumor models alone, and in combination with chemotherapy or check-point inhibitors
- In a Phase 1 clinical trial of advanced cancer patients varlilumab demonstrated:
  - Excellent safety profile at doses from 0.1 to 10 mg/kg
  - Biomarker data consistent with immune activation and Treg depletion
  - One durable CR (Hodgkin's), One durable PR (RCC), and 13 pts with SD.
- Multiple combination studies with varlilumab are on-going

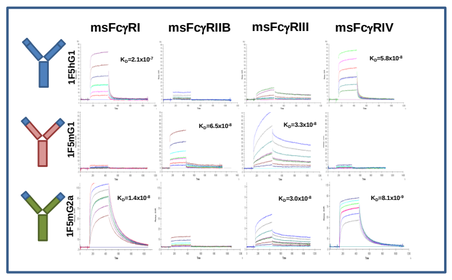
The following studies were performed to better understand the contribution of immune stimulating properties vs the Treg depletion of varlilumab in different tumor models.

Varlilumab Isotype Variants

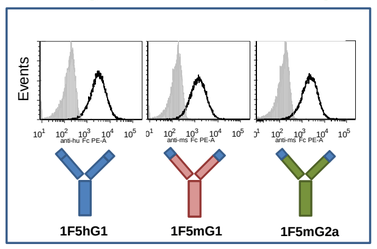
Mouse isotype variants of varlilumab were constructed to differentiate between pure agonist function and Treg suppression activity.

Differential Fcγ receptor binding

Varlilumab (1F5)



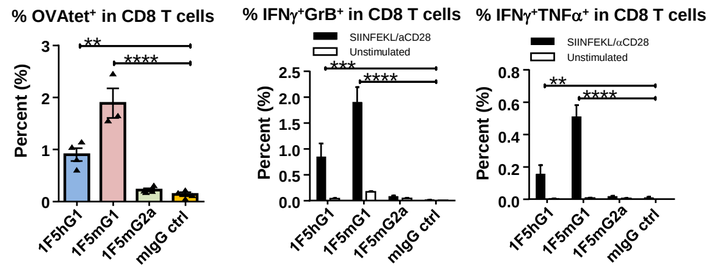
CD27 binding is maintained by the variants



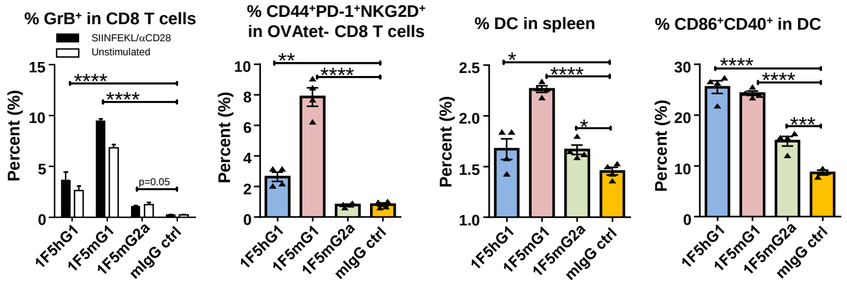
Varlilumab mIgG2a: Decreases Treg Number and Activity

Varlilumab mIgG1: Dominant Agonist Activity

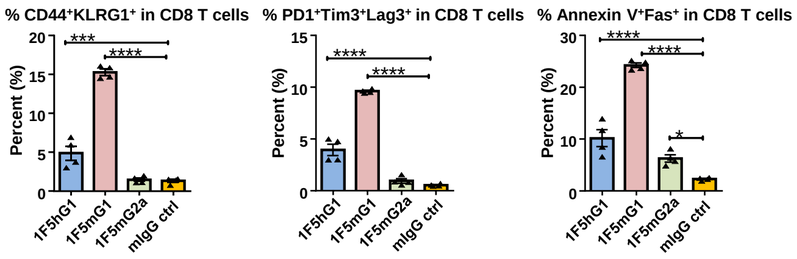
Increased antigen specific T cell response



Increased "non-specific" immune profile



Drives T cells towards terminal differentiation



hCD27-Tg mice were i.p. injected with 1F5 variants 50 μg plus ovalbumin 5 mg on day 0. Spleens were harvested for assessment on day 7. Surface markers and SIINFEKL-tetramer staining and ICCS were performed with or without ex vivo stimulation as indicated. Compared to mIgG ctrl by one-way ANOVA test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Varlilumab mIgG2a: Potent Anti-tumor Activity in Subcutaneous Models

A, 0.5x10⁶ E.G7 cells were s.c. inoculated on day 0. 1F5 variants 0.2 mg were i.p. injected on day 3, 10 and 17.
B, 1.5x10⁴ CT26 cells were s.c. inoculated on day 0. 1F5 variants 0.2 mg were i.p. injected on day 3, 5, 7, 9 and 11.
C, 0.5x10⁶ Colon26 cells were s.c. inoculated on day 0. 1F5 variants 0.1 mg were i.p. injected on day 7, 14, 21 and 28. Tumor growth were monitored twice a week and survival was compared by Mantel-Cox test.

A, hCD27-Tg mice were injected with 1F5 variants 0.1 mg on day 0. Spleens were harvested for assessment on day 7. Compared to mIgG ctrl by one-way ANOVA test: *p<0.05, **p<0.01, ***p<0.001.
B, hCD27-Tg mice were injected with 1F5 variants 0.2 mg on day -7. E.G7 tumor cells were inoculated on day 0. Tumor and draining lymph nodes were harvested for assessment on day 20. Compared to mIgG ctrl by one-way ANOVA test: *p<0.05, ****p<0.0001.
C, hCD27-Tg mice were injected with 1F5 variants 0.1 mg on day 0. Treg cells were isolated from the spleens of Ab-treated mice and co-cultured with CFSE-labeled naïve CD8 T cells at a 1:1 ratio in the presence of immobilized αCD3 and soluble αCD28 Abs for 72 hrs. ICCS was performed after Golgi blocking for 4 hrs. Compared to mIgG ctrl by one-way ANOVA test: **p<0.01, ***p<0.001, ****p<0.0001.

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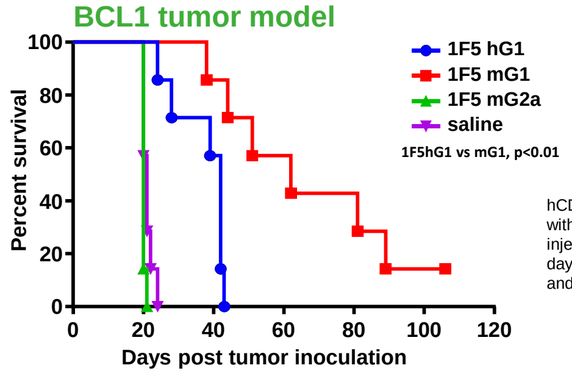
Varlilumab mIgG1: Potent Anti-tumor Activity in Lymphoma Model

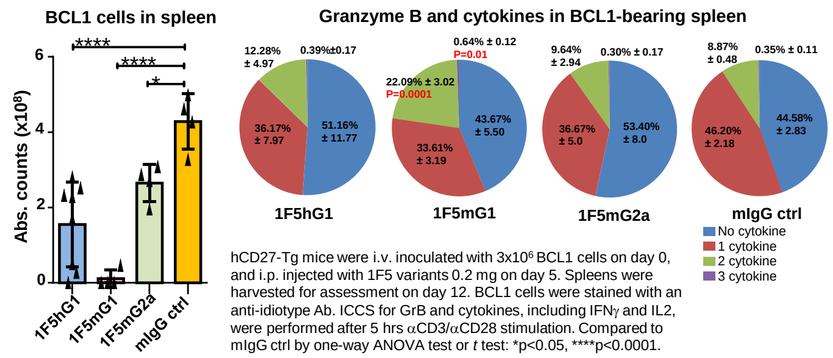
BCL1 tumor model



hCD27-Tg mice were i.v. inoculated with 5x10⁶ BCL1 cells on day 0 and i.p. injected with 1F5 variants 0.2 mg on day 5. Survival was monitored daily and compared by Mantel-Cox test.

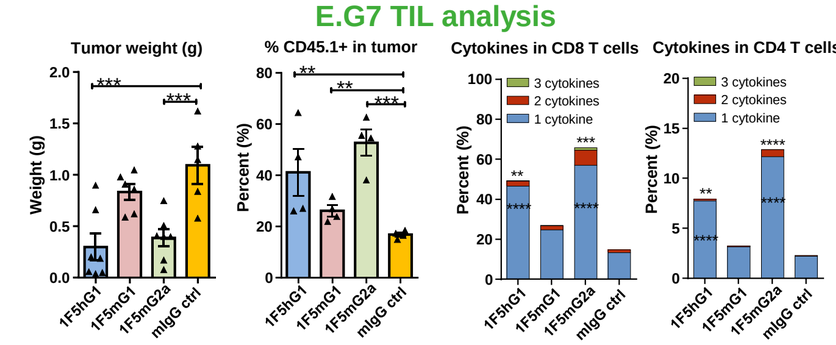
Correlates of Anti-tumor Activity

BCL1-bearing spleen analysis



hCD27-Tg mice were i.v. inoculated with 3x10⁶ BCL1 cells on day 0, and i.p. injected with 1F5 variants 0.2 mg on day 5. Spleens were harvested for assessment on day 12. BCL1 cells were stained with an anti-idiotype Ab. ICCS for GrB and cytokines, including IFNγ and IL2, were performed after 5 hrs αCD3/αCD28 stimulation. Compared to mIgG ctrl by one-way ANOVA test or *t* test: *p<0.05, ****p<0.0001.

E.G7 TIL analysis



E.G7 TdLN analysis

hCD27-Tg mice were i.p. injected with 1F5 variants 0.2 mg on day -7 and s.c. inoculated with 0.5x10⁶ E.G7 cells on day 0. Tumor and draining lymph nodes were harvested on day 20 for assessment. CD45.1 is a congenic marker for tumor infiltrated leucocytes. ICCS for GrB, IFNγ, TNFα and IL2 were performed after 5 hrs Golgi blocking in the absence of stimulants for TIL or in the presence of SIINFEKL peptide or αCD3/αCD28 for TdLN. Compared to mIgG ctrl by one-way ANOVA test or *t* test: **p<0.01, ***p<0.001, ****p<0.0001.

Conclusions and Implications

- Varlilumab (1F5) expressed on a mouse IgG1 or mouse IgG2a backbone retains the same binding to CD27, but its activity is fundamentally changed due to interaction with different Fc receptors.
  - Varli as a ms IgG1 acts as a pure agonist with no observable impact on Treg
  - Varli as a ms IgG2a has low agonist activity and decreases Treg numbers and function
- These two mouse IgG variants of varlilumab are effective in different tumor models suggesting that some tumors are more responsive to Treg suppression, while others are more sensitive to strong CD27 agonist activity.
- Varlilumab as a human IgG1, has both good agonist activity and decreases Treg numbers and function. As such it has good anti-tumor activity in each tumor model.
- The Phase 1 biomarker data are consistent with varlilumab having both of these mechanisms in patients.
- Different Human cancers are also likely to vary in their sensitivity to immune activation vs Treg suppression suggesting that multiple mechanisms of action may be an advantage for a CD27 immune modulating antibody.

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