Immunological Activity of an Activating Anti-CD27 Antibody (Varlilumab [CDX-1127]) In Patients With Solid Tumors

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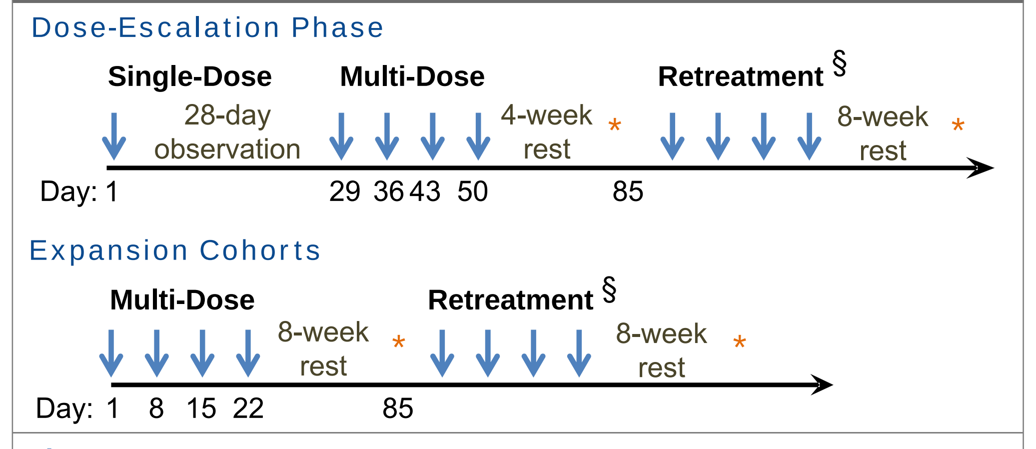
Varlilumab (CDX-1127): A Fully Human Monoclonal Antibody to CD27

- CD27 is a potent co-stimulatory molecule that drives T cell activation and survival through interaction with CD70.
- Varlilumab is an agonist anti-CD27 IgG1 mAb that induces activation and proliferation of human T cells when combined with T cell receptor stimulation.
- Varlilumab has been shown effective in murine tumor models alone and in combination with chemotherapy or check-point inhibitors.

Phase 1 Clinical Study Design

- Two study arms: Solid Tumors and Hematologic Malignancies (Abstract #3024/Poster Board: #16)
- Solid tumor patient eligibility:
  - Progressive disease subsequent to previous therapies; no remaining approved therapy options
  - Washout from prior therapies including:
    - ≥4 weeks for chemotherapy (or 5 half-lives, if longer), monoclonal based therapies and systemic radiation
    - ≥2 weeks for all other immunotherapy
- Standard 3+3 dose-escalation (0.1, 0.3, 1, 3 or 10 mg/kg)
  - Weekly dosing to establish safety with maximum exposure
- Expansion cohorts enrolled to estimate single agent activity and better define safety in potential combination study populations
  - Patients with metastatic melanoma refractory to (or who refused) check-point blockade (n=16) and metastatic renal cell carcinoma (n=15)
  - 3 mg/kg dose selected based upon immunological activity in dose escalation and preclinical modeling

Treatment Schema



Dose-Escalation Phase

Single-Dose 28-day observation; **Multi-Dose** 4-week rest *; **Retreatment** § 8-week rest *

Day: 1, 29, 36, 43, 50, 85

Expansion Cohorts

Multi-Dose 8-week rest *; **Retreatment** § 8-week rest *

Day: 1, 8, 15, 22, 85

↓ *Varlilumab: 90-minute IV infusion*

* *Diagnostic Imaging/Staging performed every 85 days*

§ *Up to 4 retreatment cycles (5 cycles total) permitted for patients with Stable Disease*

Patient Characteristics/ Disease History

		Dose-escalation (n=25)	Melanoma Expansion (n=16)	RCC Expansion (n=15)
Age, years [median (range)]		66 (42-83)	69 (29-83)	61 (45-68)
≥ 65 [n(%)]		16 (64)	11 (69)	5 (33)
Male [n(%)]		16 (64)	10 (63)	13 (87)
ECOG Performance Status [n (%)]	0	11 (44)	7 (44)	8 (53)
	1	14 (56)	9 (56)	6 (40)
	2	-	-	1 (7)
Tumor Types [n (%)]	CRC	10 (40)	-	-
	Melanoma	7 (28)	16 (100)	-
	Ovarian	3 (12)	-	-
	Prostate	2 (8)	-	-
	RCC	2 (8)	-	15 (100)
	NSCLC	1 (4)	-	-
Stage at Study Entry [n (%)]	III	1 (4)	-	-
	IV	24 (96)	16 (100)	15 (100)
Duration of Disease, years [median (range)]		4.6 (1-24)	4.0 (0.6-26.3)	4.5 (2.4-17.9)
Lines of treatment [median (range)]	Anticancer therapy	5 (0-8)	1 (0-5)	3 (1-5)
	Cytotoxic chemotherapy	3 (0-8)	0 (0-1)	-
Prior treatments received [n (%)]	Check-point blockade (CTLA-4 or PD-1)	5 (20)	13 (81)	1 (7)
	Kinase inhibitor	5 (20)	2 (13)	15 (100)
	Cytotoxic chemotherapy	22 (88)	5 (31)	-
	Cytokine (IL-2 or IFN)	4 (16)	4 (25)	4 (27)
	Other mAbs	13 (52)	-	3 (20)
	Other investigational	11 (44)	2 (13)	2 (13)
Prior radiation [n (%)]		14 (56)	10 (63)	9 (60)

Dosing and Toxicity

- No identification of a Maximum Tolerated Dose (MTD)
  - One DLT: Grade 3 transient asymptomatic hyponatremia 14 days after the single dose (1.0 mg/kg)
- Infrequent treatment-related AEs
  - Nearly all mild to moderate in severity
  - No indication of immune-mediated adverse events (colitis, endocrinopathies, etc.) typically associated with check-point blockade
- Two treatment-related SAEs (both RCC expansion patients treated with varlilumab at 3 mg/kg):
  - Recurrence of asthma/bronchospasm; patient with history of asthma, lung metastases and previous grade 4 anti-PD-1 monoclonal antibody-associated infusion reaction including bronchospasm
  - Reversible Grade 2 infusion reaction; patient who went on to receive additional varlilumab infusions with pre-medication without further infusion reactions

Treatment-Related Adverse Events

	Dose-escalation (n=25)		Melanoma Exp. (n=16)		RCC Exp. (n=15)		All Patients (n=56)	
	All Grades	Grade ≥ 3	All Grades	Grade ≥ 3	All Grades	Grade ≥ 3	All Grades	Grade ≥ 3
Any Treatment-Related Event	12(48)	2(8)	11(69)	0(0)	10(67)	1(7)	33(59)	3(5)
Fatigue	3(12)		9(56)		4(27)		16(29)	
Rash	3(12)		4(25)		2(13)		9(16)	
Nausea	1(4)		2(13)		4(27)		7(13)	
Headache			4(25)		1(7)		5(9)	
Diarrhea	2(8)				2(13)		4(7)	
Vomiting	1(4)				2(13)		3(5)	
Chills	2(8)		1(6)				3(5)	
Peripheral edema	2(8)				1(7)		3(5)	
Pyrexia	1(4)		2(13)				3(5)	
Decreased appetite	2(8)		1(6)				3(5)	
Pruritus			1(6)		2(13)		3(5)	
Asthenia	1(4)				1(7)		2(4)	
Influenza-like illness	1(4)				1(7)		2(4)	
Arthralgia			1(6)		1(7)		2(4)	
Erythema	1(4)				1(7)		2(4)	
Hyperhidrosis	2(8)						2(4)	
Lymphopenia	1(4)	1(4)					1(2)	1(2)
Hyponatremia	1(4)	1(4)					1(2)	1(2)
Asthma					1(7)	1(7)*	1(2)	1(2)

** One grade 4 treatment-related event was reported.*

Table does not include events that occurred in one patient only at grade 1-2 severity.

Clinical Activity

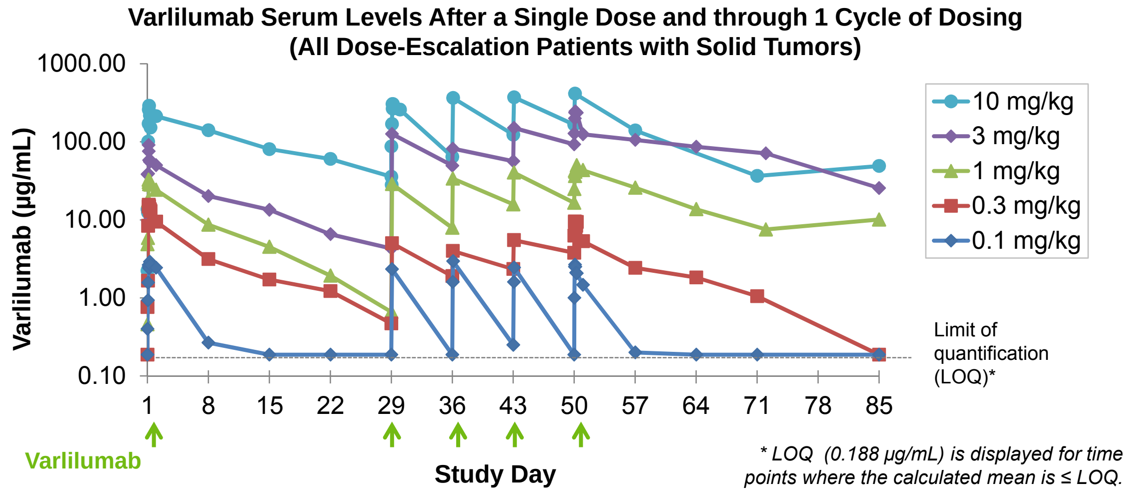
- Heavily pre-treated population of patients with progressive, metastatic disease
- Dose-Escalation (n=25):
  - All patients have completed treatment
  - Four patients with stable disease across dose levels

Tumor Type	Dose Level	Duration of Stable Disease
Renal cell carcinoma	3.0 mg/kg	22.4+ months
Colorectal cancer	1 mg/kg	5.7 months
Melanoma	0.1 mg/kg	3.8 months
Colorectal cancer	1.0 (single dose), then 0.3 (multi-dose)	3.0 months

- Melanoma expansion (n=16):
  - All patients have completed treatment
  - A patient with uveal melanoma (Stage M1c) who previously failed ipilimumab and temozolomide chemotherapy had 12% shrinkage in measurable disease, and has experienced stable disease for 11.5+ months
  - 2 additional patients with SD (duration 7.3 and 2.7+ months)
- Renal cell carcinoma expansion (n=15)
  - 5 patients continue treatment
  - 1 patient had a PR at 2.7 months; confirmatory scans are pending
  - 3 patients with SD (duration of 8.4+, 5.6 and 2.8+ months)
  - 1 patient not yet seen for 1st response assessment

Varlilumab Pharmacokinetics

Varlilumab Serum Levels After a Single Dose and through 1 Cycle of Dosing (All Dose-Escalation Patients with Solid Tumors)

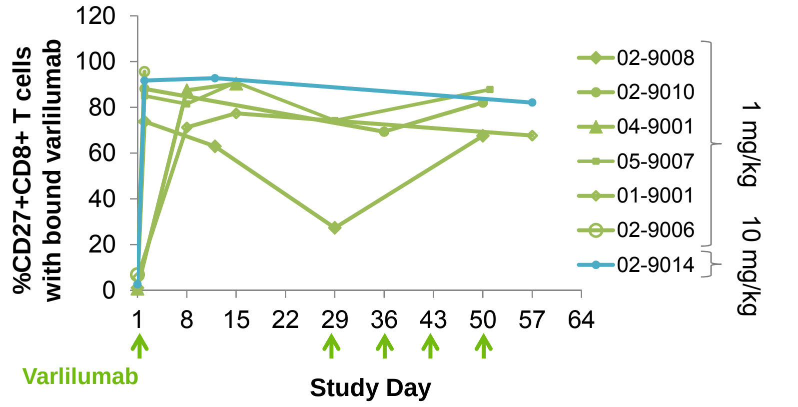


** LOQ (0.188 μg/mL) is displayed for time points where the calculated mean is ≤ LOQ.*

Pharmacokinetics similar for patients with solid tumors and hematologic malignancies. For all pooled patients:

- $T_{1/2}$ ranged from 6 days at 1.0 mg/kg to 10.6 days at 10 mg/kg in day 1 dosing
- Vd range 21-51 mL/kg (1680-4080 mL) ≅ serum volume of 3000 mL
- Exposure was linear across dose groups from 0.3-10 mg/kg

Varlilumab Maintains Receptor Occupancy at Doses ≥ 1mg/kg



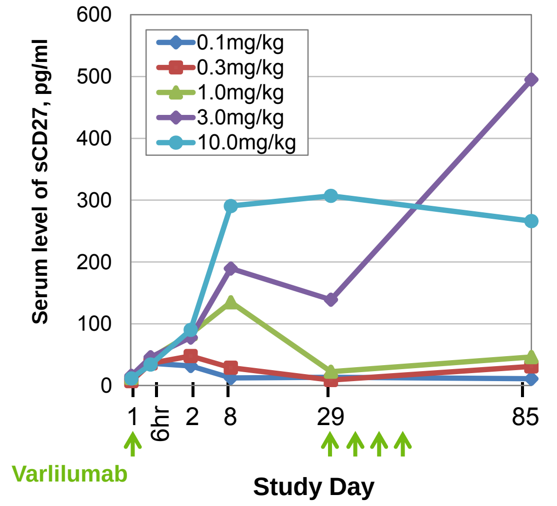
- 1 mg/kg varlilumab sufficient to achieve saturation for one month as seen with highest dose level
- Dose levels below 1 mg/kg did not maintain saturation through day 29

Proportion of CD27+CD8+ T cells binding varlilumab as measured by flow cytometry. Total CD27 expression was determined using a CD27-specific antibody that does not compete with varlilumab. Varlilumab binding was determined using anti-huIgG.

No Significant Anti-Varlilumab Antibody Responses Observed

- 2/77 (3%) patients tested had positive responses higher than baseline anti-varlilumab; these were just over baseline (OD values 0.112 and 0.116 relative to plate specific cut-point of 0.110).
- Positive titers were not associated with any discernable impact on PK parameters.
- Patients with extended retreatment show no changes in PK profiles.

Varlilumab Increases the Serum Level of Soluble CD27



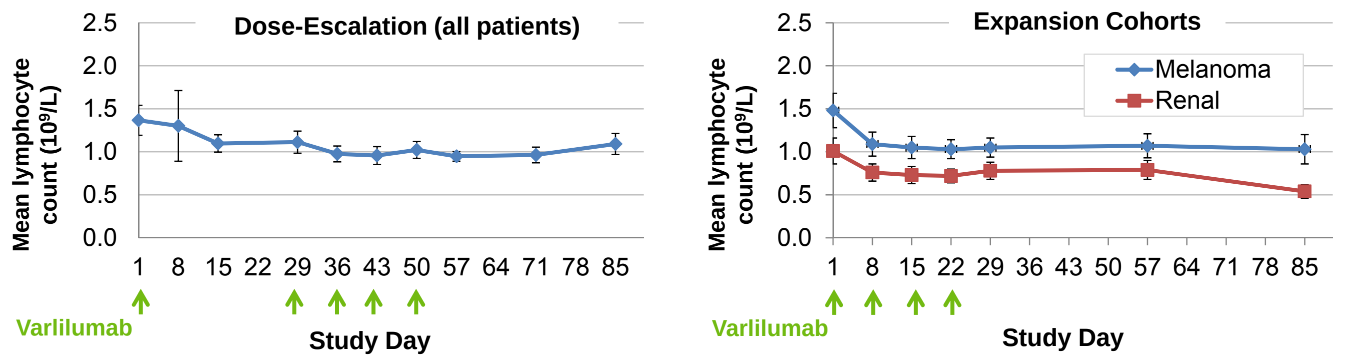
- sCD27 was measured with an ELISA using antibodies that bind to CD27 at non-competing sites with varlilumab.
- Increases in sCD27 are dose dependent and increase with time.
- Nonclinical models indicate that the increase in sCD27 is largely mediated through stabilization of sCD27 by complexing with the antibody. The anti-VEGF antibody (bevacizumab) similarly stabilizes serum VEGF levels (ref. 1,2).
- Increased sCD27 is consistent with an inflammatory effect and has been recently associated with improved response to immunotherapy (ref. 3).

CD27 is Expressed by Tumor Infiltrating Lymphocytes

	Rare/few (<5%) CD27+ cells	Intermediate (5-25%) CD27+ cells	Many (>25%) CD27+ cells
Melanoma (n=13)	3	4	6
Renal (n=7)	3	3	1
Colon (n= 5)	1	3	1

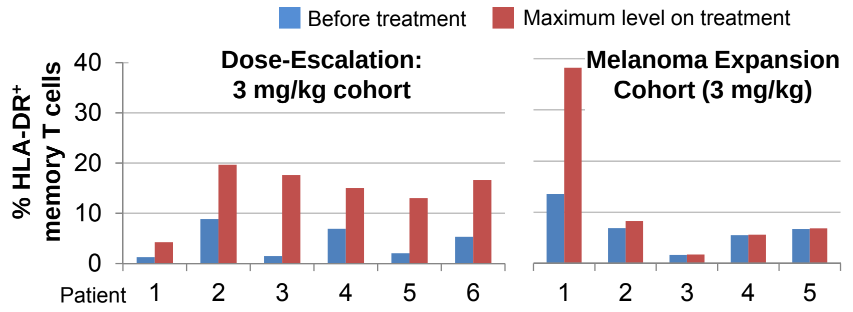
Immunohistochemistry for CD27 expression was performed on archived tumor specimens from patients. Table shows number of patients at each staining intensity.

No Significant Depletion of Lymphocytes



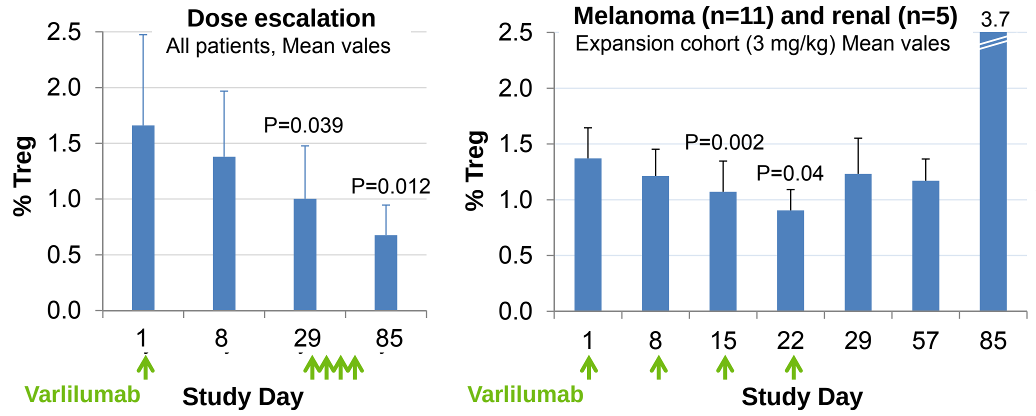
- Flow cytometry showed no change in CD8+ T cells or B cells, and a small decrease in CD4+ T cells

Increased Expression of T-cell Activation Marker



- Expression of the activation marker, HLA-DR, was assessed by flow cytometry
- Most significant up regulation was observed on memory T cells (CD45 RO+)
- More consistent increases in T cell activation observed in dose-escalation than in the expansion cohorts

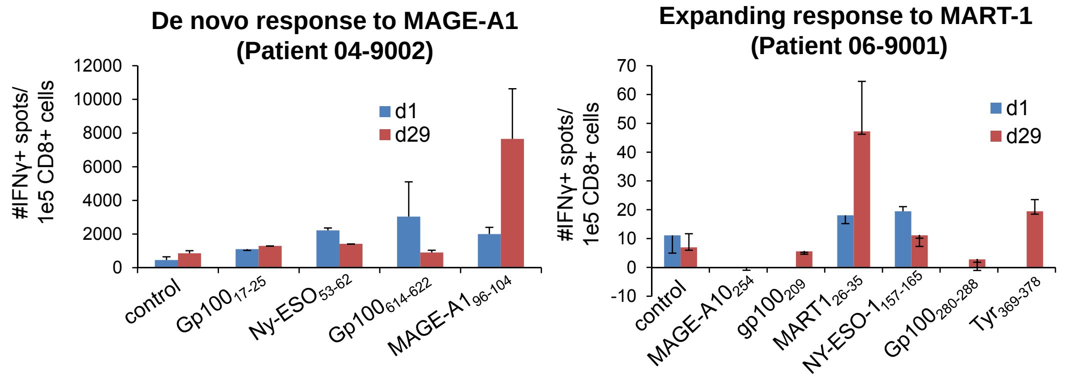
Decrease in T regulatory Cells



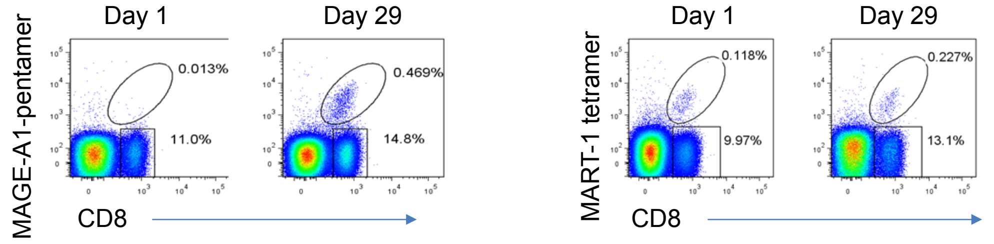
Flow cytometry analysis of regulatory T cells defined as CD3+CD4+CD25+CD127-FoxP3+

Evidence of Increased Response to Melanoma Antigens in Melanoma Patients

- 4 of 5 patients tested in the dose-escalation phase showed enhanced antigen activity



ELISPOT: PBMC from Day 1 and 29 treatment time points were assayed for IFNγ production in response to APC pulsed with the indicated peptide after 2 weeks in vitro stimulation with a peptide cocktail (pt 04-9002) or directly ex vivo (pt 06-9001)



Flow Cytometry: To confirm ELISPOT data, PBMC were directly stained for MHC-multimer binding.

Acute Induction of Pro-inflammatory Cytokines

Cytokine/chemokine	Pre	2hrs	24hrs	Day 29
IP-10 (pg/ml serum)	214	608 (P=0.003)	337 (P=0.005)	174
IL-6 (pg/ml serum)	10.4	34.8 (P=0.015)	8.5	9.1
MCP-1 (pg/ml serum)	399	752 (P<0.0001)	384	419

Serum samples were tested for cytokine and chemokine levels by Luminex. P values indicate statistical significance by paired T-Test.

- Increase in serum IP-10 levels implicates INF-γ release by T or NK cells activated via CD27
- Also increases in IL-6 and MCP-1 levels with similar kinetics, other cytokines showed variable patterns among patients

Conclusions

As a first in man study of an agonist anti-CD27 antibody, varlilumab has achieved proof of concept;

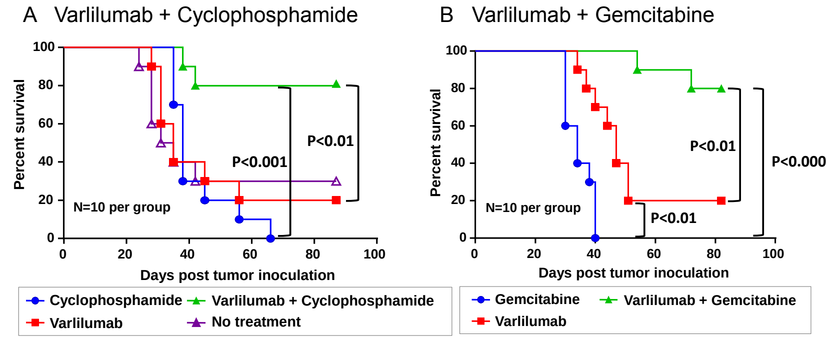
- Varlilumab administration (up to 21 infusions over 14 months) associated with minimal toxicity, even in the elderly
- PK profile is dose proportional, consistent with human antibodies and similar in patients with solid tumors and hematologic malignancies
  - Good drug exposure even at low dose levels with accumulation during multi-dose phase
  - Clearance is seen at low doses given less frequently than weekly, while higher dose levels (> 1 mg/kg) maintain receptor occupancy
- No significant anti-varlilumab antibody responses detected to date
- Stimulation of immune cell activation
  - Upregulation of HLA-DR expression by T cells
  - Decrease in T regulatory cells
  - Induction of pro-inflammatory cytokines
  - Enhanced or new responses to melanoma-associated antigens
- Evidence of single agent clinical activity, including an early PR in a RCC patient

Future Directions

- Optimal dosing regimen to be defined
  - Future studies to explore continuous receptor saturation vs. ‘on-off’ signaling
  - Immune monitoring data suggest a dose-dense regimen may not be optimal
- Combination studies targeting multiple non-redundant pathways regulating tumor burden and immune responses may be synergistic and enhance anti-tumor immune responses (preclinical data shown below)
- Planned phase 1/2 studies include:
  - Combination with nivolumab in patients with melanoma, NSCLC, colorectal, head and neck and ovarian tumors
  - Combination with ipilimumab and CDX-1401 (human anti-DEC-205 monoclonal antibody conjugated to NY-ESO-1) in melanoma
  - Combination with dabrafenib and trametinib followed by a checkpoint inhibitor in BRAF mutated melanoma
  - Additional studies in renal and lung cancer with approved agents
  - Additional combinations with experimental agents also in discussion

Support for Combination Studies of Varlilumab

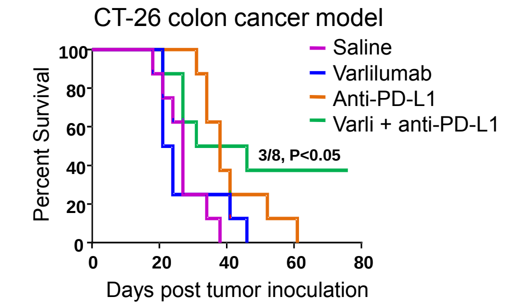
Synergistic Activity of Varlilumab and Chemotherapy



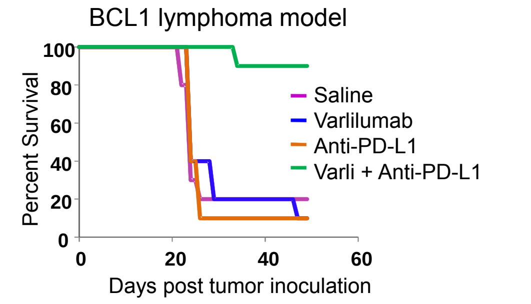
EG7 Thymoma tumor model

E.G7 cells 10^6 were s.c. inoculated to hCD27-Tg mice on day 0. P values determined by Mantel cox test.
A. Varlilumab (0.1 mg) on day 14, 28, 35, 42; cyclophosphamide (1 mg) on day 13, 20, 27, 34, 41 and the combination.
B. Varlilumab (0.1 mg) and/or gemcitabine (2.5 mg) on day 4, 7, 11, 14 and 18.

Synergistic Activity of Varlilumab and Checkpoint Blockade



CT-26 model: K-M survival curves hCD27-Tg BALB/c mice (n=8) administered 1.5×10^4 CT26 cells s.c. on day 0. Varlilumab (600 mg) or saline was administered i.p. on days 9, 11, 13, 15 and 17 and anti-PD-L1 (100 ug) was administered i.p. on days15, 17 and 19.



BCL1 model: K-M survival hCD27-Tg BALB/c mice (n=10) administered 1×10^7 BCL1 cells i.v. on day 0. Varlilumab (200 ug) or saline was administered i.p. on days 4, 6, 8, 10 and 12 and anti-PD-L1 (100 ug) was administered i.p. on days 4, 6 and 8. Mice were followed for survival.

References:
1. Gordon, et al. *J Clin Oncol.* 2001; 19:843-50.
2. Yang, et al. *N Engl J Med.* 2003; 349:427-34.
3. Huang, et al. *J Immunol* 2013 Jun 15;190(12):6250-8.

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