Phase 1 First-in-Human Trial Of LV305 In Patients With Advanced Or Metastatic Cancer Expressing NY-ESO-1

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Abstract #3021

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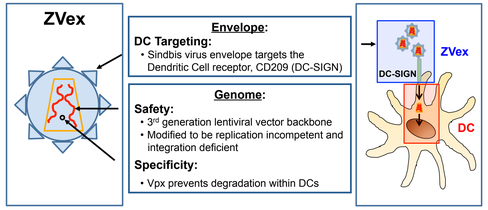
I. ABSTRACT

Background: Generation of tumor-specific cytotoxic T cells (CTLs) in vivo is a major goal of cancer immunotherapy. LV305 is a replication-incompetent, integration-deficient, hybrid viral vector based on the ZVex platform designed to target dendritic cells (DC) in vivo via CD209 (DC-SIGN) and induce the full-length expression of the cancer testis antigen, NY-ESO-1, in order to generate and expand anti-cancer CTLs. In preclinical models, LV305 is a potent inducer of CTLs and anti-tumor immunity. In this first-in-human study, the safety, immunogenicity and efficacy of LV305 are being examined in patients (pts) with cancer. **Methods:** Adults with previously treated, advanced or metastatic melanoma, sarcoma, breast, lung or ovarian cancers expressing the NY-ESO-1 protein by IHC were eligible. Following a 3+3 dose escalation design, 3 pts were enrolled into 4 cohorts to receive 3 or 4 intradermal injections every 3 weeks of 10^8, 10^9 or 10^{10} vector genomes (vg) per dose. Expansion of up to 53 pts at the 10^{10} dose is underway. **Results:** During dose escalation, 12 pts with NY-ESO-1 positive sarcomas were treated. No DLT or SAEs were observed and all related AEs were CTCAE grade 1 or 2. Common AEs were fatigue (58%), injection site reaction (33%), and myalgia (33%). After review by an independent DSMB, the highest dose, 10^{10} vg, was determined to be safe. Immunogenicity data from the initial 6 pts at the low dose (10^8 vg) demonstrated increased NY-ESO-1-specific CD8 and/or CD4 T cells in 3 pts by ELISPOT, tetramer assay of unstimulated blood, and/or TCR sequencing of blood and TILs. Data indicate the induction of T cells to new epitopes and boosting of TIL TCR sequences in blood. Of these initial 6 pts, 4 had a best response of SD (range 12-34+ weeks) with 1 pt achieving regression of 13.8%, and 2 had PD. The 6 pts dosed at 10^9 and 10^{10} vg are completing therapy. **Conclusions:** LV305 demonstrated acceptable safety at all doses up to 10^{10} vg. At the lowest dose, LV305 generated strong T cell responses and preliminary evidence of anti-tumor effect. Data from the mid and high dose are pending. These encouraging results will be followed by studies of LV305 (10^{10} vg) alone, in prime/boost with G305 (NY-ESO-1 protein-TLR4 agonist) and with anti-PD-1 therapy.
(ClinicalTrials.gov # NCT02122861)

II. RATIONALE / BACKGROUND

- LV305 is a novel hybrid viral vector gene delivery system (ZVex™) that expresses NY-ESO-1 RNA and is designed to target DCs ***in vivo*** and stimulate CD8 T cell responses against this cancer testis antigen.

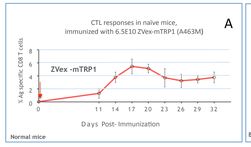


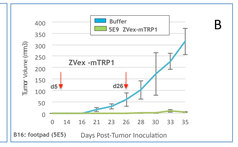
Clinical Tumor Target Antigen: NY-ESO-1

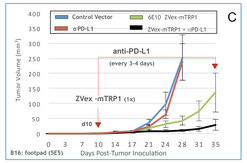
- NY-ESO-1 is a tumor-associated antigen whose function is unknown. It is expressed only in cancer and in normal testis in adults.
- Expression (by IHC) observed in many different malignancies with high expression of 70-100% reported in the sarcoma subtypes: synovial sarcoma and myxoid / round cell liposarcoma and moderate expression in melanoma (24-45%), ovarian (14-43%), and NSCLC (11-25%).
- Excellent immunologic target: preclinical and clinical data demonstrate CTLs directed against NY-ESO-1 can specifically target and kill antigen bearing cancer cells with no off-target effects.

(Expression data derived from CT Database (http://www.cta.lncc.br/index.php) or from Jungbluth, Int J Ca 2001; Nicholaou, Imm Cell Bio 2006; Hudolin, J Trans Med 2013; Gjerstorff, BMC Can 2013; Demirovic, Path Res Prac 2010)

ZVex Breaks Tolerance To Self-antigen & Induces CTLs





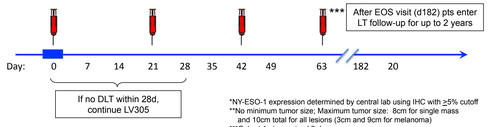


ZVex encoding mouse TRP1 (tyrosinase related protein) in B16 melanoma footpad model

- Panel A: Single dose of ZVex-TRP1 induces long-lasting TRP1 specific CTLs.
- Panel B: 2 therapeutic doses of Zvex-TRP1 prevent growth of B16 melanoma.
- Panel C: additive or synergistic effect when ZVex-TRP1 is combined with anti-PD-1 antibody in a therapeutic setting

III. LV305 TRIAL DESIGN AND RESULTS

- **Indication:** Locally advanced, recurrent or metastatic melanoma, sarcoma, ovarian, or lung cancers (breast cancer allowed in Part 1 dose escalation) expressing NY-ESO-1* s/p at least one prior cancer therapy (2 for lung) with low tumor burden**
- **Treatment/Study Measurements:**
  - Part 1, Dose Escalation: 4 cohorts, 3 dose levels
    Cohorts 1 (10^8 vg x 3 doses), 1A (10^8 vg x 4), 2 (10^9 vg x 4), and 3 (10^{10} vg x 4)
  - LV305 administered q21d intradermally; 28d DLT observation period
  - Blood samples collected for safety and immunologic testing at multiple time points including leukapheresis pre- and post- LV305.
  - Disease status measured by irRC criteria modified to use RECIST.
  - Follow-up: 2 yrs monitoring safety, disease status and LV305 persistence in blood



*NY-ESO-1 expression determined by central lab using IHC with ≥5% cutoff
**No minimum tumor size; Maximum tumor size: 8cm for single mass and 10cm total for all lesions (3cm and 9cm for melanoma)
***Cohort 1 pts received 3 doses

Characteristics of Dose Escalation (Sarcoma) Patients

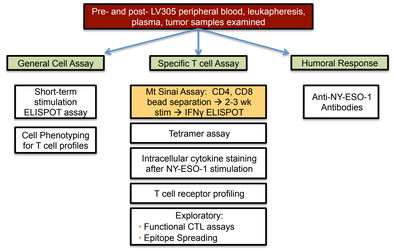
	Cohort 1 $1x10^8$ vg (N=3)	Cohort 1A $1x10^8$ vg (N=3)	Cohort 2 $1x10^9$ vg (N=3)	Cohort 3 $1x10^{10}$ vg (N=3)	Total (N=12)
Age (years)					
Mean (Median)	46.8 (46.7)	44.3 (36.6)	52.2 (60.4)	53.4 (53.9)	49.2 (49.2)
Min, Max	42, 52	29, 67	30, 67	34, 72	29, 72
Doses Administered, n (%)					
2	0	1	0	0	1 (8%)
3	3	0	0	0	3 (25%)
4	0	2	3	3	8 (67%)
Tumor Type, n (%)					
Ewing's Sarcoma	0	1	0	0	1 (8%)
Leiomyosarcoma	0	0	1	1	2 (17%)
Myxoid Liposarcoma	1	0	1	0	2 (17%)
Rhabdomyosarcoma	0	1	0	0	1 (8%)
Spindle Cell	0	0	1	0	1 (8%)
Synovial Sarcoma	2	1	0	2	5 (42%)
Stage, n (%)					
Stage IV	2	2	3	3	10 (83%)
Recurrent	1	1	0	0	2 (17%)
Completed Study, n (%)					
Yes	3	2	3	3	11 (92%)
NY-ESO-1 (%)					
Mean (Median)	90 (99)	39 (30)	31 (8)	48 (30)	52 (49)
Min, Max	70, 100	20, 68	6, 80	20, 95	6, 100

Treatment Emergent AEs Occurring in ≥2 Patients

	Cohort 1 $1x10^8$ vg (N=3)	Cohort 1A $1x10^8$ vg (N=3)	Cohort 2 $1x10^9$ vg (N=3)	Cohort 3 $1x10^{10}$ vg (N=3)	Total (N=12)
Fatigue					
Grade 1 or 2	3	2	1	1	7 (58%)
Injection site pain/discomfort					
Grade 1	2	0	1	1	4 (33%)
Injection site pruritus					
Grade 1	2	0	0	2	4 (33%)
Myalgia					
Grade 1 or 2	2	1	1	0	4 (33%)
Irritability					
Grade 1 or 2	1	0	1	0	2 (17%)
Nausea					
Grade 1 or 2	1	1	0	0	2 (17%)

- Table includes all AEs considered at least possibly related to LV305.
- There were no DLTs or treatment related SAEs reported.
- All AEs were CTCAE grade 1 or 2.
- An Independent DSMB determined that the highest dose level 10^{10} vg was the maximum safe dose examined in the study.

Immune Response Measurements



LV305 Induced Specific Anti-NY-ESO-1 T Cell Immune Responses

Overall Anti-NY-ESO-1 Specific Immune Response			
Humoral (Ab)	**CD4 T Cell**	**CD8 T Cell**	**CD4 and/or CD8**
0/12	**5/11**	**6/11**	**8/11**

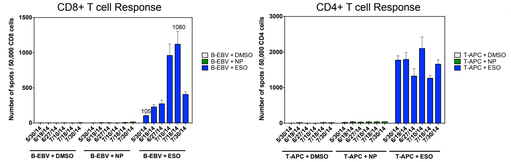
- 6/11 pts developed new T cell responses (ie. No pre-existing T cells).
  - Of these 6, 4 pts received the mid or high dose of LV305; possibly indicating a dose response (red box).

		Antibody: 0/12		CD4: 5/11		CD8: 6/11	
	Patient	Pre	Response	Pre	Response	Pre	Response
Cohort 1 10^8 vg x 3	1	+	=	+	++	+	++
	2	+	=	-	++	-	++
	3	-	-	-	-	-	-
Cohort 1A 10^8 vg x 4	4	+	=	-	++	-	-
	5	+	=	+	++	-	-
	6	-	-	-	-	-	-
Cohort 2 10^9 vg x 4	7	-	-	-	-	-	++
	8	+	=	-	-	-	++
	9	+	=	N/E	N/E	N/E	N/E
Cohort 3 10^{10} vg x 4	10	-	-	-	++	-	++
	11	+	=	-	-	-	-
	12	-	-	-	-	-	++

"+" in the "Pre" column indicates a pre-existing measureable immune response.
"++" in the "Response" indicates – **Ab:** 4-fold rise or newly positive response;
T cell: ELISPOT >50 spots & >2-fold rise and/or ICS >2-fold over baseline.
"=" indicates Pre-existing response that did not boost, **N/E:** not evaluable
"+" Represent new T **cell** responses (ie. no pre-existing measureable response).

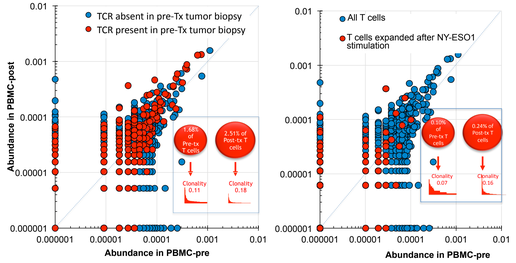
NY-ESO-1 Specific T Cell ELISPOT Assay

LV305 Induced 10 fold Increase of NY-ESO-1 Specific CD8 T cells in Pt 1-1



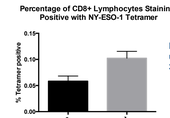
- Cryogenically preserved PBMC were separated into CD8 and CD4 populations by bead fractionation and stimulated with EBV transformed B cells or T cells pulsed with overlapping NY-ESO-1 (ESO) peptides or control peptides (NP) for 2-3 weeks.
- T cells were then stimulated with NY-ESO-1 or NP, DMSO or PMA/ionomycin and examined for IFN-gamma by ELISPOT.

LV305 Induced New T Cells And Increased The Frequency Of TILs And NY-ESO-1 Reactive Cells (Pt 1-1)



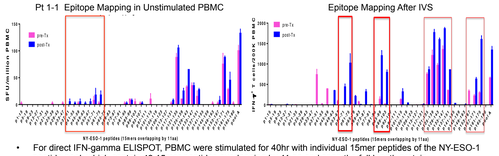
- T cell receptor (TCR) sequencing is performed on PBMC obtained pre- and post-LV305 treatment and the frequency of individual TCR sequences are plotted.
- Dots represent unique TCR sequences and those above the dashed line indicate an increase following LV305 therapy.
- Red dots represent: Left panel – TCR sequences of pre-treatment tumor TILs; Right Panel – NY-ESO-1 reactive T cells as determined by ELISPOT following in vitro expansion by peptide stimulation

LV305 Induced 2 Fold Increase of Tetramer Positive CD8 T-cells in Unstimulated PBMC (Pt 1-1)



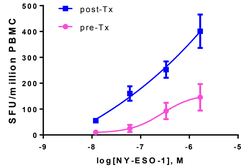
HLA-A201 tetramer on unstimulated PBMC.
3 separate expts, p<0.01

LV305 Induced T Cells Against Previously Unrecognized Epitopes of NY-ESO-1 (Pt 1-1)



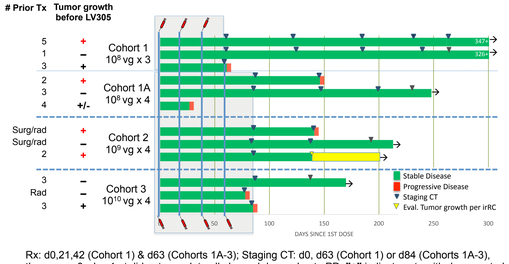
- For direct IFN-gamma ELISPOT, PBMC were stimulated for 40hr with individual 15mer peptides of the NY-ESO-1 peptide pool, which contain 43 15mer peptides overlapping by 11 aa and span the full length protein.
- For IVS (in vitro stimulation) ELISPOT, PBMC were stimulated in vitro for one week in Optmizer T cell expansion medium in the presence of NY-ESO-1 peptide pool and cytokines (IL-2 and IL-7, 10 ng/mL). Then the expanded cells were stimulated with individual peptide overnight for the IFN-gamma ELISPOT.
- Boxes represent NY-ESO-1 epitopes that had increased T cell response following LV305.

LV305 Induced T Cells With Increased Polyclonal Avidity (Pt 1-1)



Clinical Outcome: LV305 Stabilized Tumor Growth

- **Dose Escalation: n= 12 sarcoma pts**
  - **Stable disease: 8/12 (67%) patients achieved a best response of SD (defined as stable for at least 84 days). Median duration of SD was 208 days (range: 139-347+).**
  - **4 of 6 pts with evidence of growing disease at study entry stabilized their tumor growth following LV305.**
  - **Pt 1-1 remains with SD for 347+ days and had tumor regression up to 14%.**



Rx: d0,21,42 (Cohort 1) & d63 (Cohorts 1A-3); Staging CT: d0, d63 (Cohort 1) or d84 (Cohorts 1A-3), then every 8 wks; 1 pt did not complete all planned doses due to PD; **"+"** indicates pts with documented pre-LV305 tumor growth: **"+"** indicates those pts who stabilized following LV305.

IV. SUMMARY

- LV305 is a novel hybrid viral gene delivery vector that targets and transduces DCs *in vivo* with full length NY-ESO-1 RNA for MHC Class I presentation.
- In this first-in-human study, LV305 has proven to be safe, immunologically active in generating anti-NY-ESO-1 CD4 and CD8 T cells, with preliminary evidence of durable stable disease in a subset of patients.
  - 4 of 6 patients with evidence of growing disease prior to LV305 stabilized and stopped progression with the longest >347+ days; tumor regression up to 14% observed in 1 patient.
  - 8/12 (67%) patients achieved a best response of SD with a median duration of 208 days (range: 139-347+) and the progression-free rate (PFR) of all 12 patients at 6 months was at least 42%.
  - Although this is a small study and the patient population was restricted by tumor burden and ECOG PS, the observed 6 month PFR of >=42% compares favorably to the historical PFR for active agents in these sarcomas as reported by Van Glabbeke, et al., where active agents for first- and second-line treatment exhibited PFR of >= 30-56% (histology dependent) and > 14% at 6 months, respectively.
  (Note; the LV305 study consisted of patients who had all received at least one prior treatment, total tumor burden was restricted to <10cm and ECOG status was <2)
- This is an ongoing study and additional patients with sarcoma, lung, ovarian and melanoma are being examined at the highest dose level in Part 2 Patient Expansion.

(‡ Van Glabbeke,et al. EuJCa, "Progression-free rate as the principal end-point for phase II trials in soft-tissue sarcomas", 2002)

V. FUTURE PLANS

CMB305:

- An effective approach to maximally activate T cells may be to prime and boost with two different active immunotherapy methods that complement each other in generating CTLs.
- LV305 and G305 (NY-ESO-1 protein plus GLA-SE, a TLR4 agonist) are biologically unique active immunotherapy approaches that have shown up to 5 fold additive/synergistic effects in preclinical models when combined in a sequential prime-boost immunization approach called **CMB305.**
- G305 can increase CD4 help and induce humoral immunity and can synergize with the CD8 inducing effects of LV305.
- The potentially more potent CMB305 prime-boost is now being examined in a Phase 1b trial: #NCT02387125.
- The LV305 first-in-human study is described here and the G305 Phase 1 trial is being presented as ASCO #3073.

Additional Studies with Checkpoint Inhibitors:

- These agents may be synergistic with checkpoint inhibitors like anti-PD-1/L1 agents.
- LV305 with or without anti-PD-1 antibody therapy in melanoma patients who have had an inadequate response to anti-PD-1 therapy – open and enrolling
- Phase 2 studies of these approaches in sarcoma and other cancers – planned