Association of NY-ESO-1 Expression with Baseline Immunity and Clinical Outcomes in Soft Tissue Sarcoma Patients Treated with LV305 or CMB305

Abstract #2804760

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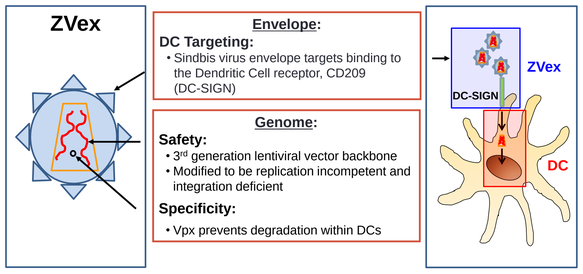
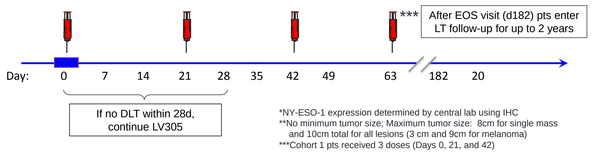
RATIONALE / BACKGROUND

- NY-ESO-1 is a tumor-associated antigen whose function is unknown. It is expressed only in cancer and in normal testis in adults.
- NY-ESO-1 expression (by IHC) is observed in many different malignancies with high expression of 70-100% reported in the sarcoma subtypes: synovial sarcoma and myxoid / round cell liposarcoma (MRCL) and moderate expression in melanoma (24-45%), ovarian (14-43%), and NSCLC (11-25%).
- NY-ESO-1 is an excellent immunologic target: preclinical and clinical data demonstrate cytotoxic T lymphocytes (CTLs) directed against NY-ESO-1 can specifically target and kill NY-ESO-1 antigen bearing cancer cells with no off-target effects.
- LV305 is a novel hybrid viral vector from the ZVex® platform that expresses NY-ESO-1 RNA and is designed to target DCs ***in vivo*** and stimulate CD8 T cells against this cancer testis antigen.
- CMB305 is a prime-boost immunotherapy targeting NY-ESO-1 which utilizes LV305 priming with sequential dosing with G305 boosting. G305 is a potent TLR-4 agonist co-formulated with NY-ESO-1 full length protein to enhances the activity of LV305 to induce T cell immunogenicity and anti-NY-ESO-1 antibodies
- We have evaluated the baseline tumor expression of NY-ESO-1 in STS patients enrolled in either the LV305 or CMB305 Phase 1 with clinical outcomes.



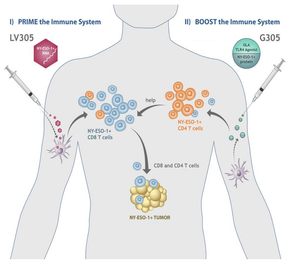
LV305 TRIAL DESIGN

- **Rationale:** In this first-in-human study, we examined the safety, immunologic activity, and preliminary efficacy of LV305 in patients whose cancers expressed NY-ESO-1.
- **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 1 prior cancer therapy (2 for lung) with low tumor burden**.
- **Treatment / Study Measurements**:
  - Part 1, Dose Escalation: 4 cohorts, 3 dose levels
  - Cohort 1 (10^8 vg x 3 doses), Cohort 1A (10^8 vg x 4), Cohort 2 (10^9 vg x 4), and Cohort 3 (10^{10} vg x 4)
  - Part 2, Patient expansion: the dose (10^{10} vg x 4) determined in Part 1 to be safe was used to treat additional cancer patients
  - 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 years monitoring safety, disease status, and LV305 persistence in blood



*NY-ESO-1 expression determined by central lab using IHC
**No minimum tumor size; Maximum tumor size: 8cm for single mass and 10cm total for all lesions (3 cm and 9cm for melanoma)
***Cohort 1 pts received 3 doses (Days 0, 21, and 42)

CMB305: Prime-Boost Immunotherapy Targeting NY-ESO-1



- LV305 Priming:
  - Dendritic cell (DC) targeting NY-ESO-1 lentiviral vector encoding full length NY-ESO-1 RNA
  - Integration deficient, replication incompetent
  - Induces and expands NY-ESO-1 specific CD8 and CD4 T Cells
- G305 boosting:
  - Potent TLR-4 agonist co-formulated with NY-ESO-1 full length protein
  - Enhances LV305 T cell immunogenicity and triggers anti-NY-ESO-1 antibodies
- No ex vivo manipulation or HLA matching required

C131 (CMB305 Monotherapy) TRIAL DESIGN

Indication:
- Recurrent locally advanced, relapsed and/or metastatic solid tumors, limited tumor burden (<10 cm)
- ECOG PS 0-1
- NY-ESO-1+ by IHC
- Received prior therapy

Part 1 Dose Escalation

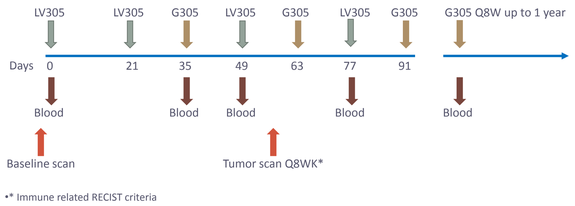
Cohort	LV305 Dose (vg per dose)	G305 Dose (GLA/Ag)
1	10^9 ID	5 µg/250 µg IM
2	10^{10} ID	5 µg/250 µg IM

- **Primary Objective**: Safety and tolerability

Part 2 Expansion
- **Arm A**: Up to 9 each NSCLC*, ovarian cancer*, synovial sarcoma or MRCL
- **Arm B:** SQ route, 9 synovial or MRCL
- **Arm C**: CMB305 + oral metronomic cyclophosphamide
- **Arm D:** CMB305 + Intratumoral G100
- **Objectives**:
  - Safety
  - Efficacy
  - Immune response and biomarkers

ID=Intradermal; IM=Intramuscular; SC=Subcutaneous

CMB305 Treatment and Biomarker Schedule



*** Immune related RECIST criteria

METHODS

- Patients with advanced, recurrent STS tumors were tested for NY-ESO-1 expression performed using a mouse monoclonal antibody E978 (Sigma).
- Patient characteristics for subjects with soft tissue sarcoma enrolled in C131 and LV305 are listed in Table 1.
- The summary for NY-ESO-1 levels of expression are summarized in Table 2 and depicted in Figure 2.
- The potential association between tumor NY-ESO-1 level of expression and clinical outcomes were evaluated. The survival outcomes listed in Figure 3. and Table 3. and for those subjects who had pre-existing immunity are reported by level of NY-ESO-1 expression in Table 4.

RESULTS

Table 1. Pretreatment Characteristics

- Patients enrolled with STS enrolled in the C131 Study and LV305 Study are listed in Table 1. excluding Arm C and Arm D patients

	C131 Soft Tissue Sarcoma	LV305 Soft Tissue Sarcoma
n	34	23
Median age (range)	42 years (22, 76)	48 years (25,72)
Female	8 (32%)	12 (52.2%)
ECOG PS1	14 (56%)	13 (54%)
>2 prior lines of chemotherapy	17 (52%)	8 (34.7%)
Disease progression at study start	19 (56%)	14 (60.8%)

Table 2. Percent of Soft Tissue Positive for NY-ESO-1

Results:
- A total of 148/327 STS pts (n=72 liposarcoma, n=119 synovial sarcoma, n=136 other) were screened positive for NY-ESO-1 IHC shown in Table 2. (includes patients enrolled in Arm C and D n=6)
- After meeting eligibility criteria, 63 STS pts (n=17 myxoid/round Cell liposarcoma, n=39 synovial sarcoma, n=7 Other) treated with at least 1 line of prior therapy were enrolled in the two trials and included in the analysis of survival outcomes by NY-ESO-1 level of expression.

	C131 Study		LV305 Study		Overall	Enrolled
NY-ESO-1 Result	Positive	Negative	Positive	Negative	Positive	Positive
Synovial Sarcoma	47	26	31	15	78/119 (65.5%)	39/119 (32.8%)
Myxoid/Round Cell Liposarcoma	34	14	14	10	48/72 (66.6%)	17/72 (23.6%)
Other STS*	10	28	12	86	22/136 (15.4%)	6/136 (4.4%)
Total	91	68	57	111	148/327 (45.2%)	62/327 (18.9%)

*Other STS = spindle cell (3); rhabdomyosarcoma (1); Leiomyosarcoma (2)

Figure 2. High Levels of NY-ESO-1 Expression in Synovial Sarcoma

- Levels of NY-ESO-1 Expression did not differ by number of prior lines of chemotherapy

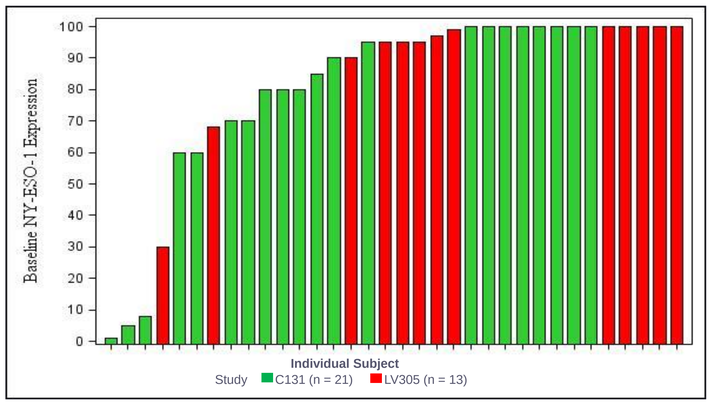


Table 3. STS Outcomes by Levels of NY-ESO-1 Expression

Median Overall Survival by Level of NY-ESO-1 Expression						
NY-ESO-1 Expression Level (%)	n	C131 Study (months, 95% CI)	n	LV305 Study (months, 95% CI)	n	Overall (months, 95% CI)
1-25	5	NR (11.4, NR)	3	31.1 (27.8, NR)	8	31.1 (11.4, NR)
>25-50	1	22.1 (NR, NR)	2	10.3 (10.3, NR)	3	22.1 (10.3, NR)
>50-75	5	23.7 (12.2, NR)	2	NR (NR, NR)	7	NR (12.5, NR)
>75-100	23	22.4 (10.7, NR)	16	26.3 (16.9, NR)	39	23.7 (17.6, NR)
All patients	34	22.4 (15.8, NR)	23	31.1 (18.8, NR)	57	27.8 (22.1, NR)
Median Progression Free Survival by Level of NY-ESO-1 Expression						
NY-ESO-1 Expression Level (%)	n	C131 Study (months, 95% CI)	n	LV305 Study (months, 95% CI)	n	Overall (months, 95% CI)
1-25	5	7.4 (2.1, 9.2)	3	4.6 (2.8, 4.7)	8	4.6 (2.1, 7.8)
>25-50	1	5.4 (NR, NR)	2	NR (0.9, NR)	3	5.4 (0.9, NR)
>50-75	5	2.0 (1.3, 3.7)	2	16.6 (14.3, 18.9)	7	2.1 (1.3, 14.3)
>75-100	23	3.7 (2.1, 14.0)	16	2.8 (2.1, 8.6)	39	3.6 (2.2, 7.2)
All patients	34	3.7 (2.1, 7.2)	23	4.5 (2.5, 8.6)	57	3.7 (2.5, 5.6)

NR = not reached. Excludes patients enrolled in Arm C and Arm D n=6

Clinical Outcomes

Immune Response Assessments

Pre- and Post Peripheral PBMC and Plasma

T-cell assays
- NY-ESO-1 IFNγ ELISPOT (ex vivo ELISPOT without in vitro stimulation (IVS) and ELISPOT after IVS)
- Intracellular cytokine staining (ICS)

Antibody assays
- NY-ESO-1 ELISA (recombinant protein & peptides)
- Antigen epitope spreading: 24 recombinant Tumor Associated Antigens ELISA (p53, MAGE, SSX, PRAME, etc.)

- Anti-NY-ESO-1 Abs detected by ELISA against either the whole NY-ESO-1 protein or to peptides. A 4-fold rise or newly positive response was scored as positive (induced response)
- Anti-NY-ESO-1 CD4 and CD8 responses were determined to be positive by the following assays:
  - IFNγ production detected by ELISPOT in separated T cell fractions
  - Intracellular cytokine staining for IFNγ or TNFα after stimulation with NY-ESO-1 peptides
  - ELISPOT >50 spots & >2-fold rise and/or ICS >2-fold over baseline were considered positive (induced response)
- Antigen spreading by ELISA with 24 recombinant tumor associated antigens & selected ELISPOT assays.

Table 4. STS Outcomes by Pre-existing Anti–NY-ESO-1 Immunity

- The association of survival by pre-existing immunity was evaluated by level of NY-ESO-1 expression
- The IHC expression of NY-ESO-1 was significantly associated with baseline anti-NY-ESO-1 antibodies (p=0.0367).

Median Overall Survival by Level of NY-ESO-1 Expression for Subjects Positive and Negative Pre-existing Anti-NY-ESO-1 Immunity			
NY-ESO-1 Expression Level (%)	Total (n)	Positive for Pre-existing Immunity (months, 95% CI)	Negative for Pre-existing Immunity (months, 95% CI)
1-25	8	31.1 (27.8, NR) n=5	NR (11.4, NR) n=3
>25-50	3	NA n=0	22.1 (10.3, NR) n=3
>50-75	7	NR (13.0, NR) n=5	NR (12.2, NR) n=2
>75-100	39	NR (15.8, NR) n=16	22.4 (7.5, 26.3) n=2
Median Progression Free Survival by Level of NY-ESO-1 Expression for Subjects Positive and Negative Pre-existing Anti-NY-ESO-1 Immunity			
NY-ESO-1 Expression Level (%)	Total (n)	Positive for Pre-existing Immunity (months, 95% CI)	Negative for Pre-existing Immunity (months, 95% CI)
1-25	8	4.7 (2.8, 7.8) n=5	4.1 (2.1, 9.2) n=3
>25-50	3	NA n=0	5.4 (0.9, NR) n=3
>50-75	7	3.7 (1.3, 18.9) n=5	2.0 (2.0, 2.1) n=2
>75-100	39	12.0 (2.6, 20.3) n=16	2.6 (2.1, 4.5) n=22

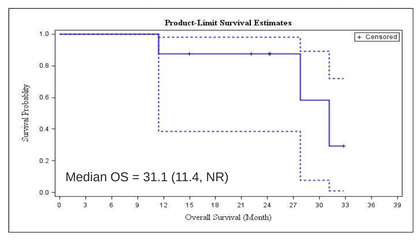
NA = not applicable; NR = not reached. Analysis excludes patients enrolled in Arm C and Arm D n=6

Figure 3. Clinical Outcomes by Levels of NY-ESO-1 Expression

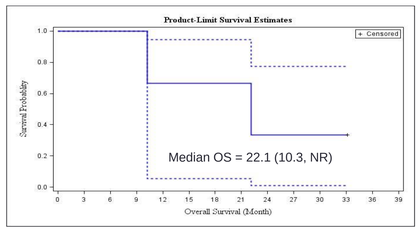
- The Kaplan-Meier curves for Overall Survival and Progression Free Survival (PFS) by level of NY-ESO-1 expression in soft tissue sarcoma patients. (Excluding patients enrolled in Arm C and Arm D n=6)

Overall Survival

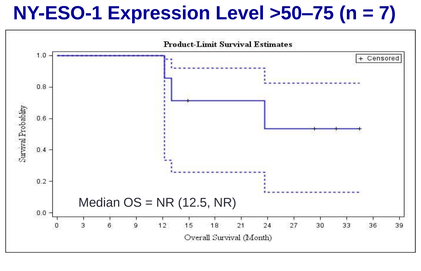
NY-ESO-1 Expression Level 1–25 (n = 8)



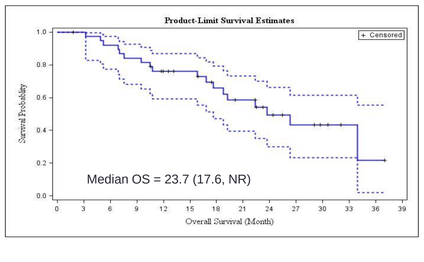
NY-ESO-1 Expression Level >25–50 (n = 3)



NY-ESO-1 Expression Level >50–75 (n = 7)

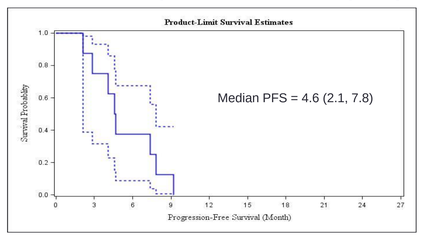


NY-ESO-1 Expression Level >75–100 (n = 39)

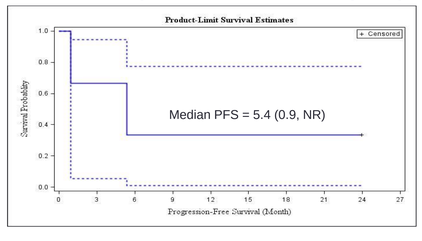


Progression-Free Survival

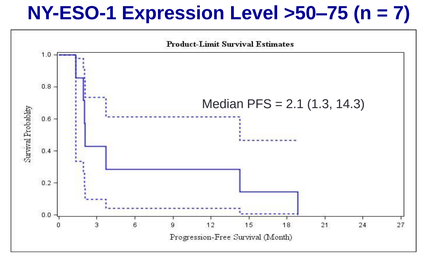
NY-ESO-1 Expression Level 1–25 (n = 8)



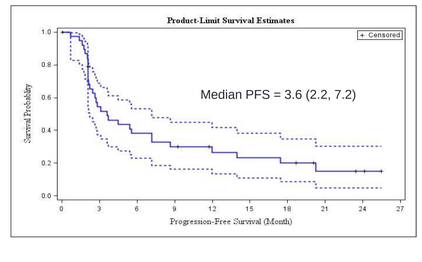
NY-ESO-1 Expression Level >25–50 (n = 3)



NY-ESO-1 Expression Level >50–75 (n = 7)



NY-ESO-1 Expression Level >75–100 (n = 39)



SUMMARY

- Synovial sarcomas are highly enriched for NY-ESO-1 positive patients and consistently demonstrate high levels of expression.
- NY-ESO-1 expression by IHC is significantly associated with a baseline anti-NY-ESO-1 antibody response.
- Despite a poorer prognosis, the patients on C131 and LV305 appear to have clinical outcomes similar across all levels of NY-ESO-1 expression
- When the NY-ESO-1 level of expression is positive and expressed at a higher level (>75%) the subjects who have pre-existing anti-NY-ESO-1 Immunity may have improved PFS.