A First-in-Human Phase 1 Dose-escalating Trial of G305 in Patients with Solid Tumors Expressing NY-ESO-1

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I. ABSTRACT # 152974

Background: A major way to expand *in vivo* tumor antigen-specific CD8 cytotoxic T cells (CTLs) is to induce and enhance the presence of CD4 helper T cells that are specific for the same antigen. G305 is recombinant full-length NY-ESO-1 protein mixed with a proprietary formulation of the synthetic TLR4 agonist, glucopyranosyl lipid A (GLA). GLA, when administered with other recombinant antigens in healthy volunteers, induces antigen-specific CD4 T and B cells and activates innate immunity.

Methods: Adults with advanced or metastatic melanoma, sarcoma, ovarian, breast, bladder, or NSCLC expressing NY-ESO-1 by IHC were enrolled using a 3+3 dose-escalation design. Patients were dosed i.m. every three weeks for three doses. NY-ESO-1 was fixed at 250 µg and GLA was increased from 2 to 5 to 10 µg. Safety, immunogenicity, and clinical responses were assessed prior to, during, and at the end of therapy.

Results: 12 patients were treated; mild local reactogenicity was most common (92%) and all related adverse events were CTCAE Grade 1 or 2, with no DLTs or related SAEs. Serology results show that G305 was immunogenic at all doses; 8 of 12 patients had preexisting antibody titers at baseline, 5 of which increased with treatment, and 3 of 4 with no baseline titer seroconverted. 4 of 6 patients tested to date had high baseline CD4 T cells that increased in 2 with G305 treatment; 1 of 8 tested developed antigen-specific CD8 T-cell responses. 6 of 12 patients (50%) have had SD for 2.5 to 8+ months and 6 patients progressed after a mean of 99 days with no relationship between dose, reciprocal antibody titers, or efficacy. One patient had decreasing CA125 titers and SD for 8 months, and subsequently developed brain metastases.

Conclusions: G305 was shown to be safe and well-tolerated at doses up to 10 µg GLA and was associated with a satisfactory clinical response. Anti-NY-ESO-1 titers suggest that a 5 µg dose of G305 is suitable for subsequent trials. The immunogenicity results support the mechanism of action for G305, which is planned to complement LV305, a hybrid novel DC-tropic viral vector expressing NY-ESO-1, in a sequential prime-boost regimen to generate and expand NY-ESO-1-specific CTLs that target solid tumors.
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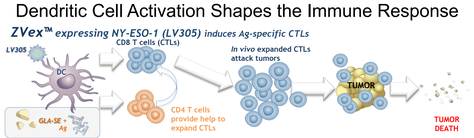
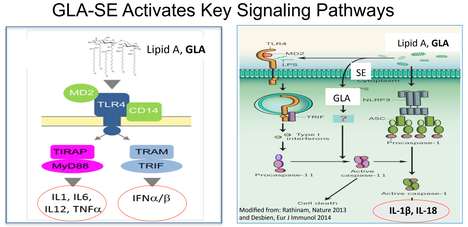
II. RATIONALE / BACKGROUND

Dendritic Cell Activation Shapes the Immune Response



- Development of effective CTLs requires the coordinated efforts of DCs, CD4 T cell help, and CD8 T cells.
- **G305** is a novel immunotherapy agent, composed of GLA-SE – a TLR4 agonist – and recombinant full length NY-ESO-1 to target DCs to stimulate a synergistic, NY-ESO-1-specific, CD4 T-cell and humoral response.
- **LV305** is a novel Sindbis/lentiviral vector from the ZVex platform expressing NY-ESO-1 that selectively targets DC *in vivo* to generate NY-ESO-1 specific CTLs. (ASCO #153150).
- Priming with LV305 and boosting with G305 is expected to be synergistic in generating NY-ESO-1 specific CTLs. This combination is being tested in the clinic as **CMB305**.

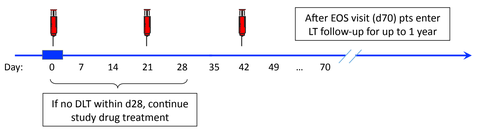
GLA-SE Activates Key Signaling Pathways



- TLR4 recognizes GLA to activate MyD88 pathway through TIRAP to induce inflammatory cytokines and activates TRAM and TRIF to induce type I interferon by activating IRF3. (Coler et al., 2011)
- In addition, GLA-SE can signal through an unidentified intracellular receptor, activating the non-canonical NLRP3 inflammasome, which results in secretion of IL1β and IL18. (Desbien et al., 2015)
- GLA is a synthetic analog of Lipid A, and is a more potent immunostimulant than MPL on a weight basis.
- Formulation with recombinant NY-ESO-1 stimulates the activation of dermal dendritic cells and induction of NY-ESO-1-specific CD4 T cells that can enhance the formation of NY-ESO-1-specific CTLs by LV305.

III. G305 TRIAL DESIGN AND POPULATION

- **Indication:** Adults with unresectable, relapsed, or metastatic melanoma, sarcoma, ovarian, bladder or lung cancers expressing NY-ESO-1 with low disease burden s/p at least one prior cancer therapy (2 for lung)
- **Treatment/Study Measurements:**
  - 3+3 design using 3 GLA-SE doses (2, 5, and 10 µg), all with rNY-ESO-1 250 µg
  - Bloods drawn for safety and immunologic testing at multiple timepoints
  - Disease status measured by RECIST 1.1 on d0 and d70
  - Long-term follow-up (1 yr) to monitor safety and disease status



Dose-escalation Cohorts had Similar Tumor Types and Disease Status

	Cohort 1 2 µg GLA-SE (N=3)	Cohort 2 5 µg GLA-SE (N=3)	Cohort 3 10 µg GLA-SE (N=6)	Total (N=12)
Age (years)				
Mean	61.8	50.1	65.8	60.9
Tumor Type, n (%)				
Melanoma	0	0	1 (16.7%)	1 (8.3%)
Ovarian	2 (66.7%)	2 (66.7%)	3 (50.0%)	7 (58.3%)
Synovial Sarcoma	1 (33.3%)	1 (33.3%)	1 (16.7%)	3 (25.0%)
Urothelial Carcinoma	0	0	1 (16.7%)	1 (8.3%)
Stage, n (%)				
Stage III	2 (66.7%)	1 (33.3%)	1 (16.7%)	4 (33.3%)
Stage IV	1 (33.3%)	2 (66.7%)	5 (83.3%)	8 (66.7%)
NY-ESO-1 (%)				
Mean	71.0	73.0	48.7	60.3
Completed Day 70, n (%)				
Yes	3 (100.0%)	3 (100.0%)	6 (100.0%)	12 (100.0%)

Most Common Treatment-emergent AE was Mild Local Pain

	Cohort 1 2 µg GLA-SE (N=3)	Cohort 2 5 µg GLA-SE (N=3)	Cohort 3 10 µg GLA-SE (N=6)	Total* (N=12)
Injection site pain				
Grade 1	3 (100.0%)	3 (100.0%)	6 (100.0%)	12 (100.0%)
Any Grade	3 (100.0%)	3 (100.0%)	6 (100.0%)	12 (100.0%)
Fatigue				
Grade 1	1 (33.3%)	1 (33.3%)	0	2 (16.7%)
Any Grade	1 (33.3%)	1 (33.3%)	0	2 (16.7%)
Nausea				
Grade 1	1 (33.3%)	0	1 (16.7%)	2 (16.7%)
Any Grade	1 (33.3%)	0	1 (16.7%)	2 (16.7%)
Pain				
Grade 1	1 (33.3%)	1 (33.3%)	0	2 (16.7%)
Any Grade	1 (33.3%)	1 (33.3%)	0	2 (16.7%)

*Only related AE's occurring in 2 or more patients are shown

IV. NY-ESO-1-SPECIFIC IMMUNE RESPONSES

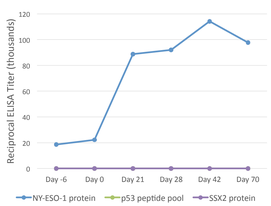
Overall Immune Response to G305			
Humoral (Ab)	CD4 T cell	CD8 T cell	Ab and/or CD4
9/12	5/11	2/10	9/12

G305 Induced Significant Antibody and CD4 T-cell Responses

		Antibody		CD4		CD8		Clinical
	Patient	Pre	Response	Pre	Response	Pre	Response	Follow Up
Cohort 1 2 µg x 3	1	+	=	+	=	-	-	162
	2	+	++	+	=	-	-	305
	3	+	++	N/E	N/E	N/E	N/E	365+
Cohort 2 5 µg x 3	4	+	++	+	++	-	++	161
	5	-	++	-	-	-	-	335+
	6	-	++	-	++	-	-	70
Cohort 3 10 µg x 3	7	-	-	-	-	-	-	62
	8	-	++	-	++	-	-	70
	9	+	++	+	=	-	N/E	70
	10	+	++	-	++	-	++	191
	11	+	++	-	++	-	-	247+
	12	+	=	+	=	+	=	252+

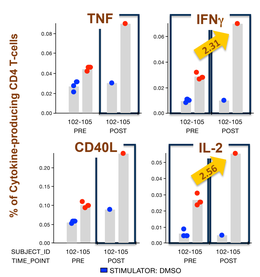
"+" in the "Pre" column indicates a pre-existing measureable immune response.
"++" in the "Response" indicates for **Ab:** 4-fold rise or newly positive response;
T cell: ELISPOT >50 spots & >2-fold rise and/or ICS >2-fold over baseline
"=" indicates a pre-existing response that did not boost, **N/E:** not evaluable
Follow-up: A '+' indicates stable disease as of 13 May 2015

Serum Antibodies and Cytokine Production in PBMCs (Pt #4)



- Patient was seropositive for NY-ESO-1 at baseline (> 100)
- ELISA against full-length recombinant NY-ESO-1
- SSX2 and p53 controls remained negative (titer < 100) throughout the study.
- Serum antibody titers significantly increased (>4x) against NY-ESO-1 protein from d21 onward compared to baseline (d-6 and d0), going from 1/18,000 to 1/110,000.

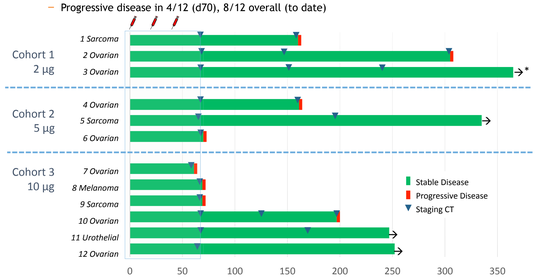
- PBMCs obtained before treatment and at various times during the trial
- Overnight incubation without/with NY-ESO-1 peptides
- Boxes represent NY-ESO-1-specific responses with SI > 2.
- Production of cytokines was assessed by ICS and FACS analysis
- Arrows show enhancement of the response after vaccination (>2x)



V. CLINICAL RESPONSE TO G305 IMMUNOTHERAPY

Patients showed extended stable disease, one CA125 response

- **Patients:** n=12 enrolled: 7 ovarian, 3 synovial sarcoma, 1 melanoma, 1 urothelial carcinoma
- **Efficacy:**
  - Stable disease: 8/12 patients (67%) achieved a best response of SD (defined as stable for at least 70 days). Median duration of SD was 245+ days (range: 161 to 365+ days). One patient (#3) had a CA125 response on d70 (based on GCIG Criteria).
  - Progressive disease in 4/12 (d70), 8/12 overall (to date)



Rx: d0, 21, 42
Staging CT:
d0, d70, then per standard care

* CA125 response 600 on D0 → 281 on D70 → 207 on D242

VI. CONCLUSIONS

Summary

- G305 is a potent immunotherapeutic agent that is taken up by DCs *in vivo* for MHC II presentation to stimulate NY-ESO-1-specific antibody and CD4 T-cell responses.
- In this first-in-human study, G305 was shown to be safe, immunologically active, and showed evidence of clinical benefit in the form of durable stable disease in a subset of patients.
  - **Safety**: There were no treatment emergent DLTs or related SAEs; all AEs were grade 1 or 2.
  - **Immunogenicity**: G305 was immunogenic at all doses. Patients developed or boosted antibodies in 9/12 pts (75%) and CD4 T-cell responses in 5/11 pts (45%).
  - **Clinical Benefit**: 8/12 pts (67%) had SD at Day 70. Median duration of SD was 245+ days (range: 161-365+ days). Pt #3 remains with SD for > 1 year with a CA125 response by GCIG criteria.

Future Plans

- 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 (a hybrid Sindbis/lentiviral vector with an NY-ESO-1 insert delivered *in vivo*) and G305 are biologically separate active immunotherapy approaches that have shown up to 5-fold additive/synergistic effects in preclinical models when dosed sequentially in a prime-boost immunization approach called CMB305.
- The potentially more potent CMB305 prime-boost is now being examined clinically in a Phase 1b trial (#NCT02387125).
- These agents may be synergistic with checkpoint inhibitors like anti-PD-1/L1.

We gratefully acknowledge the support of the staff at each of the clinical sites and the patients for their participation.