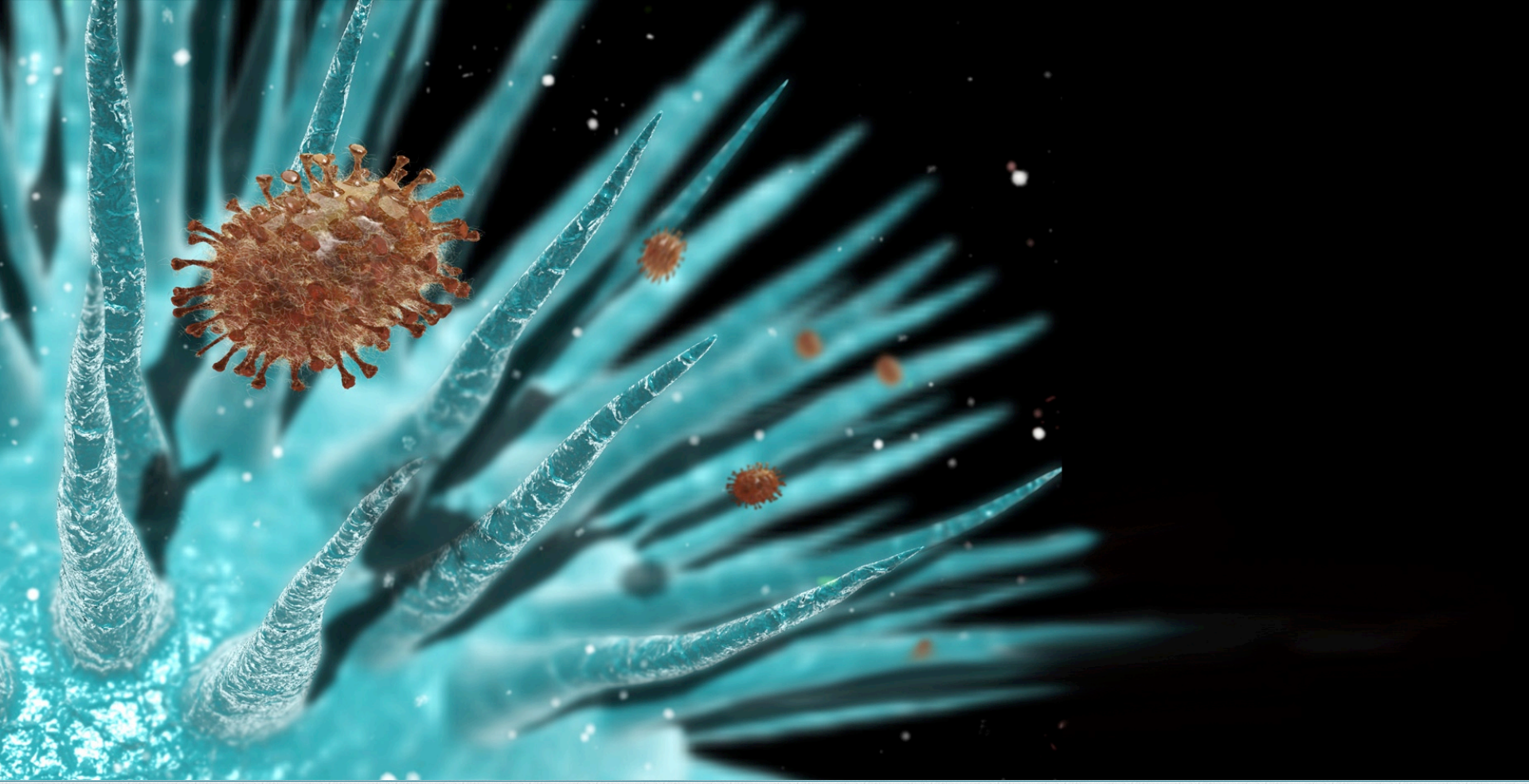

ID-LV305: A LENTIVECTOR THERAPEUTIC CANCER VACCINE

SAFETY BY DESIGN

DECEMBER 05, 2013

IMMUNE DESIGN

Breakthrough Therapeutic Vaccines

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ID-LV305 PRESENTATION TO THE NIH RAC

Discussion of Human Gene Transfer Protocol #1310-1266, 5 December 2013:

A Phase 1, Open-Label Clinical Trial Evaluating the Safety, Tolerability and Immunogenicity of Intradermally Administered ID-LV305 in Patients with Locally Advanced or Metastatic Cancer Expressing NY-ESO-1

Principal Investigator

- Neeta Somaiah, MD
  Principal Investigator, Assistant Professor, Sarcoma Medical Oncology
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Sponsor

- Jan H. ter Meulen, MD, Dr. habil., DTM&H
  Chief Scientific Officer, Immune Design Corp. (Seattle, WA)

- Richard T. Kenney, MD, FACP
  Chief Medical Officer, Immune Design Corp. (San Francisco, CA)

OVERVIEW OF THE PRESENTATION

1. Rationale for ID-LV305 Approach

2. Features of the Lentivirus Vector ID-VP02
   - DC-SIGN targeting and selectivity
   - Dendritic cell transduction
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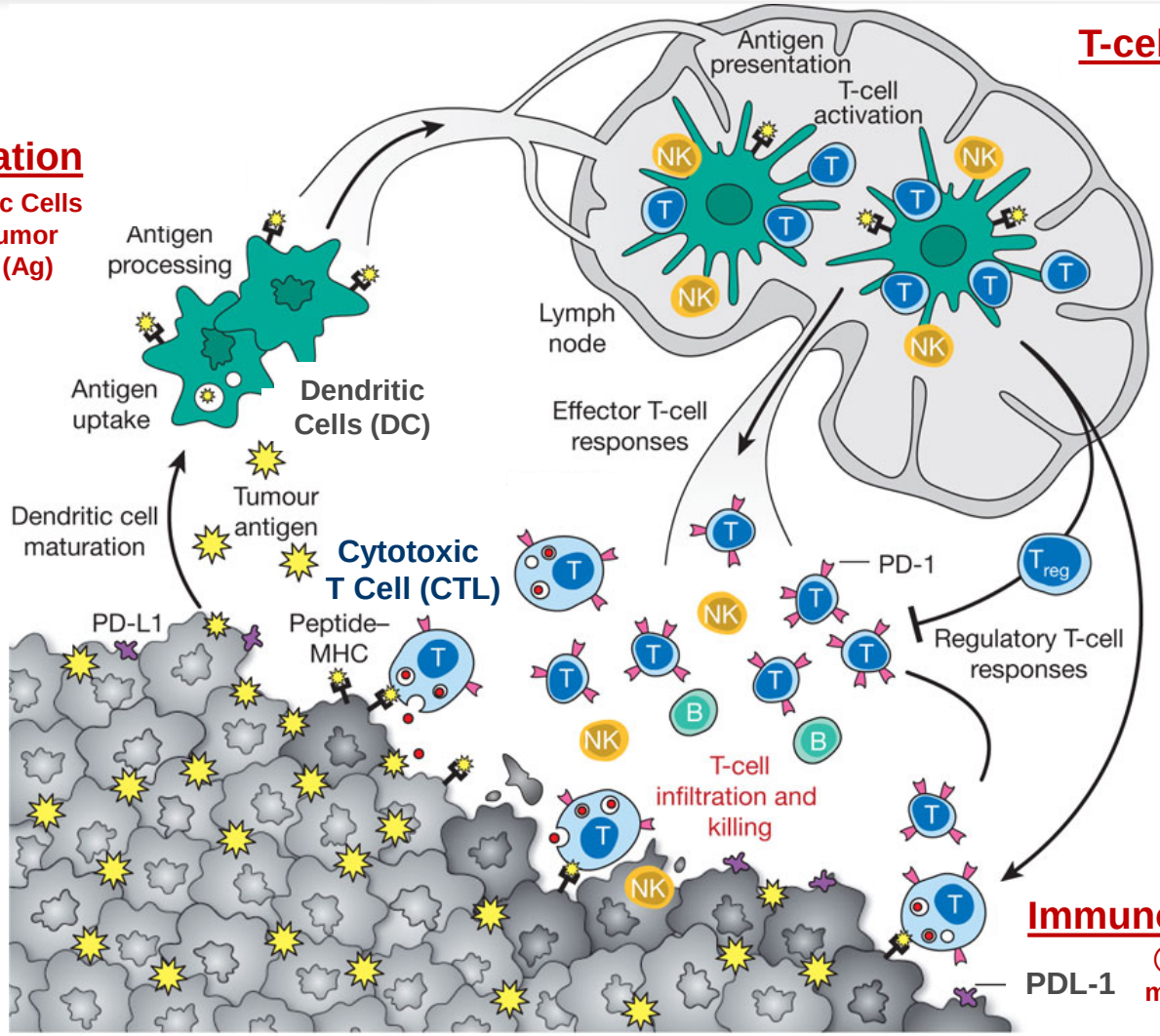
THE CANCER IMMUNOTHERAPY PREMISE

Immunization

① Dendritic Cells capture tumor antigens (Ag)

T-cell response

② Activated DC present tumor Ag to CD8 T cells, which then become anti-tumor CTLs



Immunosuppression

③ Tumor expresses molecules (e.g. PDL1) that fend off CTLs

PDL-1

The Dendritic Cell is at the core of making a tumor antigen/s being recognized as "foreign"
The Cytotoxic T Cell (CTL) is a major effector of "cancer immunotherapy"

*Mellman, Ira et al, Nature 2011

ID-LV305: RATIONALE FOR APPROACH

Cancer immunotherapy suffers from limited efficacy

- Inefficient generation and function of CTLs
- Clinical PoC with *ex vivo generated* dendritic cell (DC) vaccines
- *Ex vivo* DC have low migration capacity from blood, procedure is difficult to standardize and can be costly

ID-LV305 is a first-in-class approach to CTL generation

- Selective targeting of tumor antigens to DCs with novel LV-vector through *in vivo* immunization
- Preclinical tumor control (B16, CT26 models) mediated by very robust, polyfunctional CD8 T-cell responses

Validated tumor-associated antigen used as a target

- NY-ESO-1 selectively expressed in tumors; highly immunogenic in patients
- Major target for CD8+ T-cells (TIL, TAL)
- Immunological / clinical PoC of vaccination

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DESIGN OF THE LENTIVECTOR ID-VP02 PLATFORM

Attenuation and Dendritic Cell *Tropism*

- Modified Sindbis virus (SINV) envelope: SINVar1
- DC-SIGN* (CD209) dependency (DCs, macrophages, sinusoidal endothelial cells
- Terminal high-mannose dependency

Dendritic Cell *Transduction* Adds Specificity

- Anti-restriction factor (Vpx) dependency

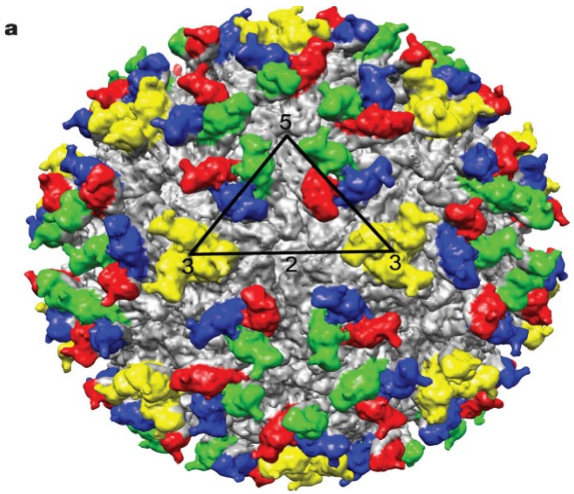
Molecular Safety Features

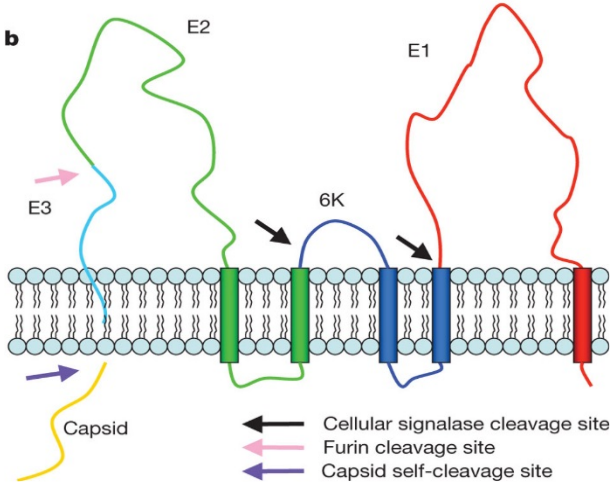
- State-of-the art replication-incompetent 3rd generation lentivirus vector backbone derived from HIV-1 lab strain NL4-3, extensively modified
- *Enhanced* safety features further reduce the risk of recombination
- *Novel* safety feature*: Integration deficient*

DC-Specific Intercellular adhesion molecule-3 Grabbing Nonintegrin; mannose-binding receptor

SINVar1: ENGINEERED ENVELOPE GLYCOPROTEIN

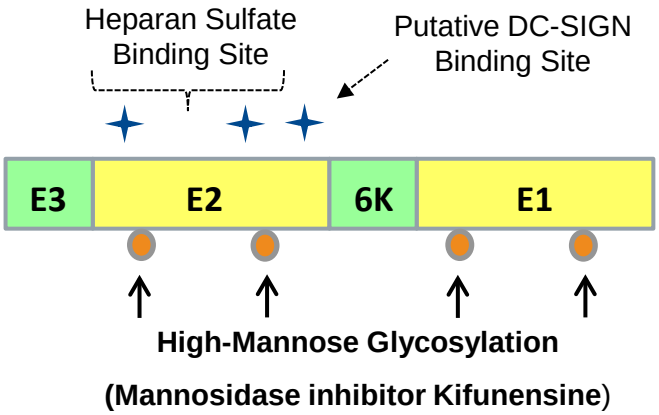
Cryo-EM of Sindbis virus





Long Li et al., Nature, 2010

SINVar1 envelope



Envelope modifications	Phenotyopic changes	Reference
K70E	Reduced Heparan Sulfate binding	Klimstra et al. 1999
K159E	Reduced Heparin-Sepharose binding Reduced virulence in mice Reduced replication in CNS in mice	Bear et al. 2006
E160G	Increased DC targeting	Gardner et al. 2000 Morizono et al. 2010
Terminal high mannosylation E1 and E2	Increased DC targeting Potential targeting of other C-type lectins on APC	Morizono et al. 2010 Tai et. al 2011

IMMUNE DESIGN

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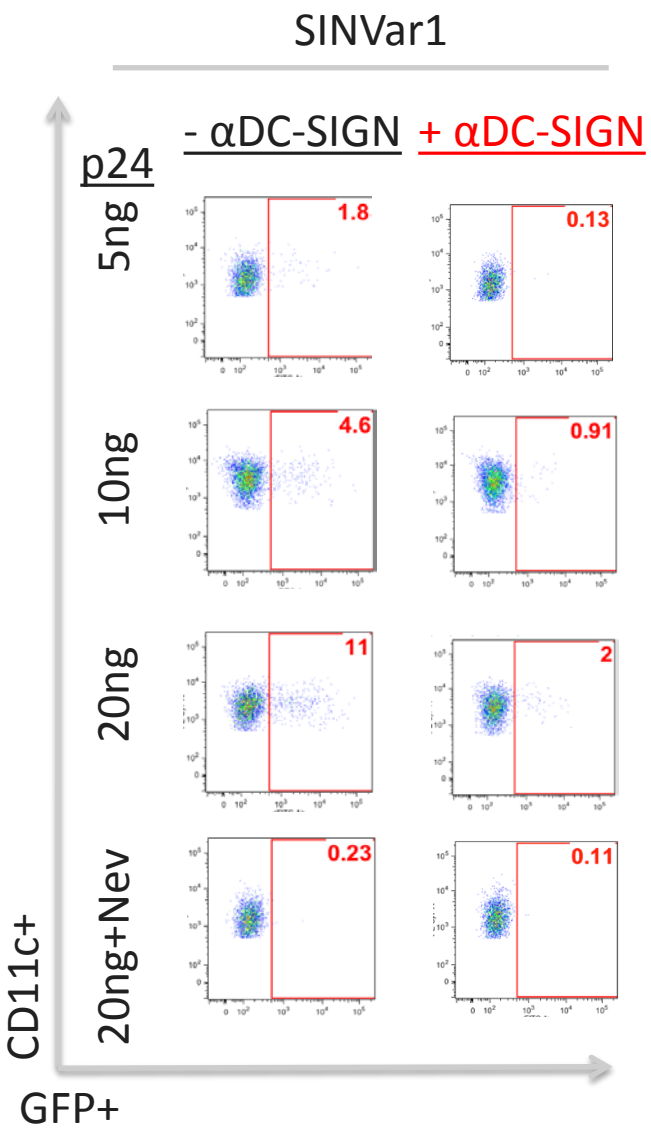
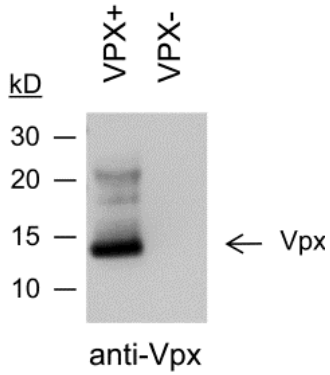
ID-VP02: DC TRANSDUCTION IS DC-SIGN DEPENDENT

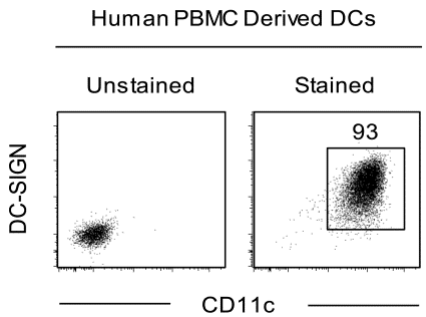
- Primary human monocyte-derived DC (moDC) (IL4/GM-CSF)
- 1 hr pretreatment with DC-SIGN blocking mAb
- ID-VP02 with Vpx and high-mannose
- αDC-SIGN antibody blocks transduction of primary human moDC



ID-VP02: DC TRANSDUCTION IS VPX DEPENDENT

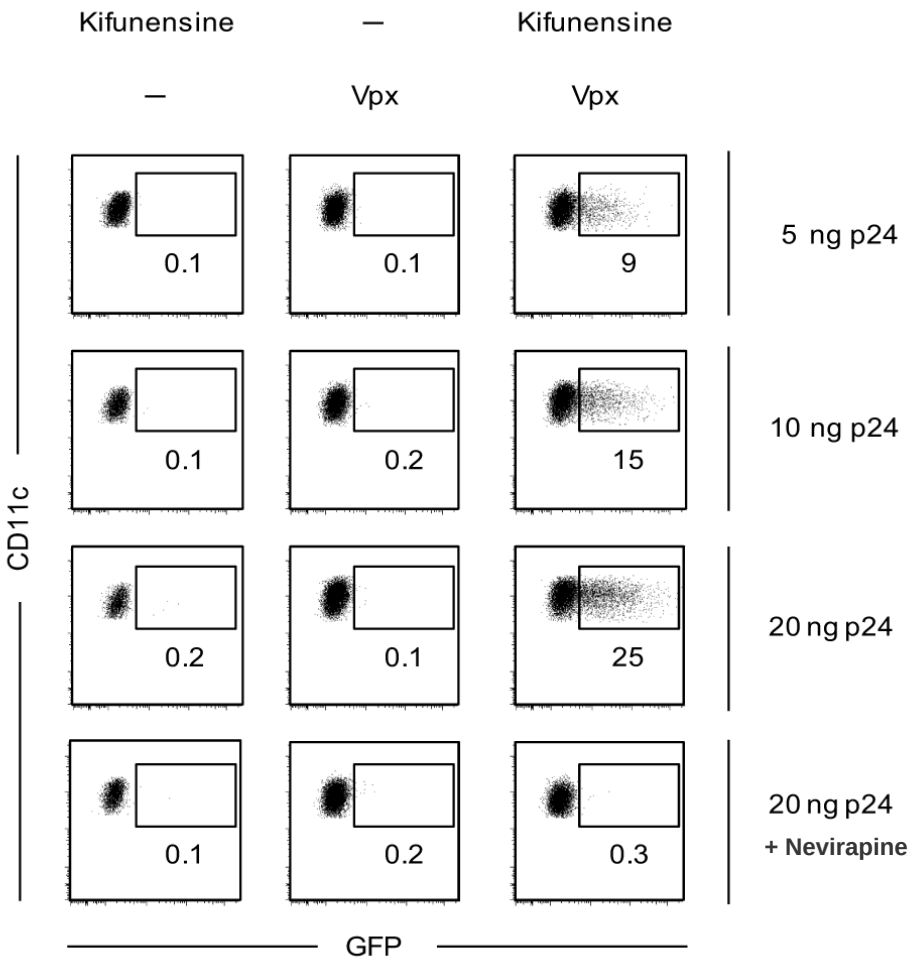
Vpx packaged into vector particles





IL4/GM-CSF

Transduction of primary human moDC requires Vpx (SAMHD1 restriction)



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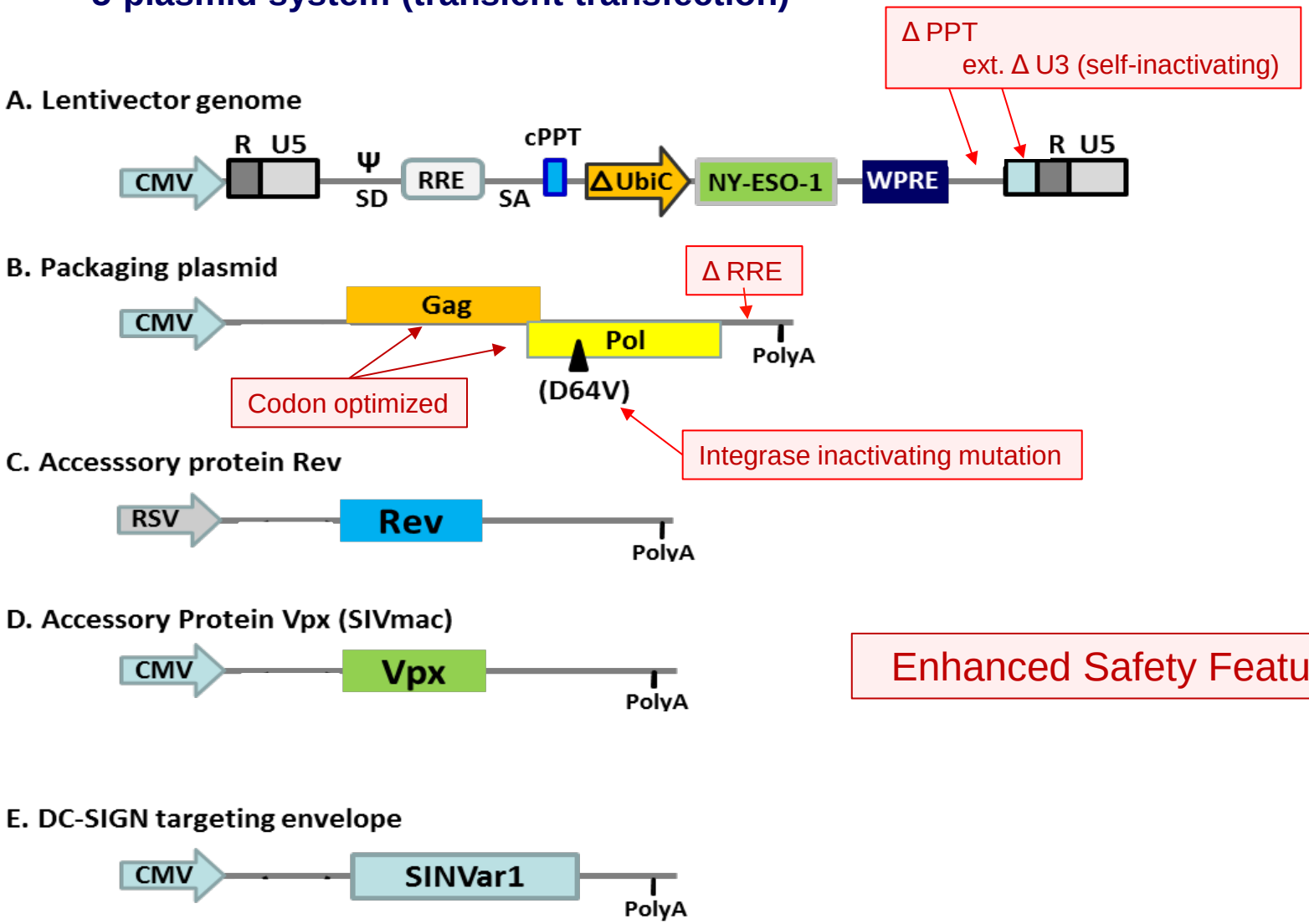
ID-VP02: SAFETY-ENHANCED, 3RD GENERATION LENTIVECTOR

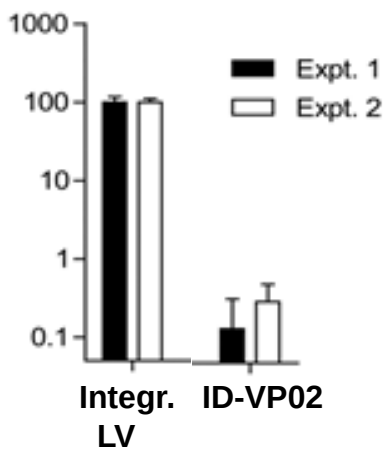
5-plasmid system (transient transfection)



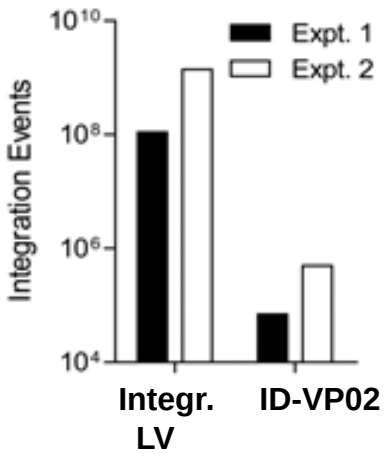
Enhanced Safety Features

ID-VP02: EVIDENCE FOR INTEGRATION DEFICIENCY (*in vitro*)

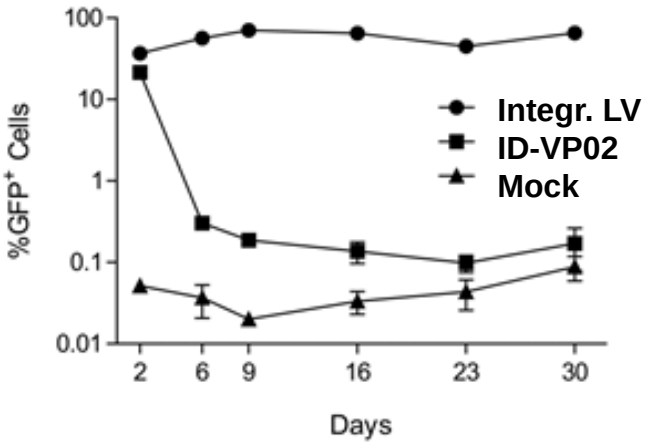
Alu-PCR
(integrated vector)



Neo^R loss
(expressed protein)



GFP loss over time
(expressed protein)



	Fold reduction
Expt 1	731
Expt 2	324

	Fold reduction
Expt 1	1571
Expt 2	2800

Fold reduction					
D2	D6	D9	D16	D23	D30
1.7	188	440	462	450	386

- Design results in 2-3 log reduction in integration

ID-LV305: SUMMARY OF MAIN SAFETY FEATURES

	Split genome	3' LTR deletions	DC specific tropism	Integration deficient
3rd gen LV	Yes 4 plasmid	Yes	No	No
ID-LV305	Yes 5 plasmid	Yes Extended	Yes	Yes

ID-LV305 targets non-dividing cells of limited lifespan, and is integration deficient:

→ Very low genotoxic risk

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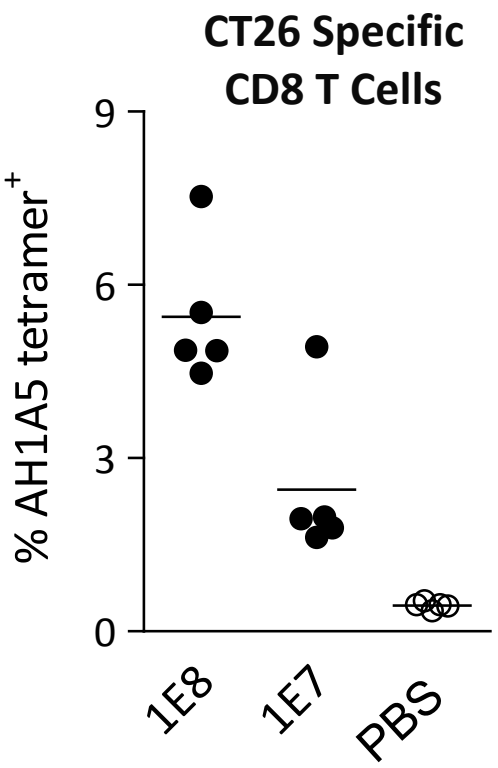
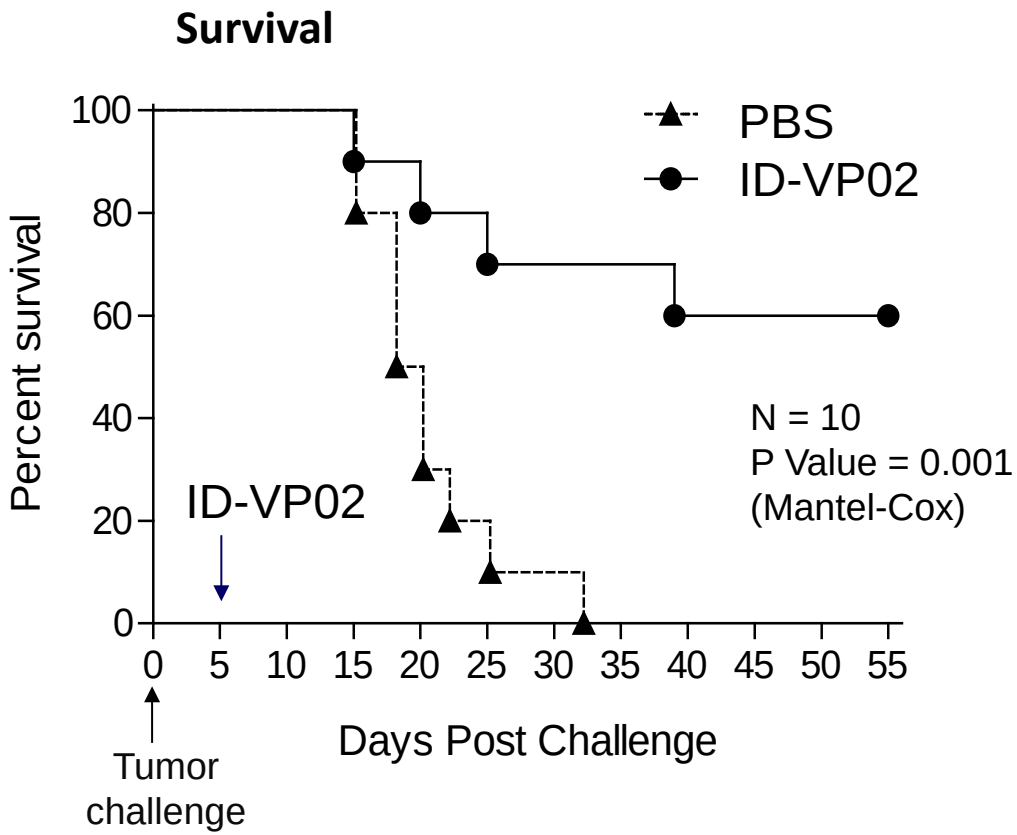
ID-VP02: CD8 T CELL LEVEL CORRELATES WITH SURVIVAL

- Challenge: 1.5E5 CT26 cancer cells implanted s.c. in flank on d0
- Immunization: Single dose ID-VP02-AH1A5 day 5 post challenge (1E8, 1E7 genomes)
- Dose-dependent CD8 T-cell response, 60% protection from death





ID-LV305: DOSE-SPECIFIC DECREASE IN TUMOR BURDEN

- Challenge: 1.5E5 CT26/NY-ESO-1 cancer cells i.v. to induce lung metastases
- Immunization: Single dose ID-LV305 injection day 3 post challenge in three doses
- Dose-dependent, polyfunctional CD8 T-cell response

No tumor challenge

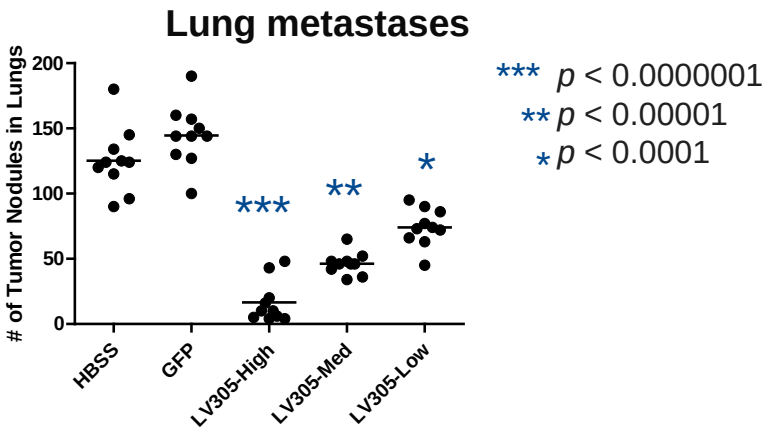
ID-VP02-GFP

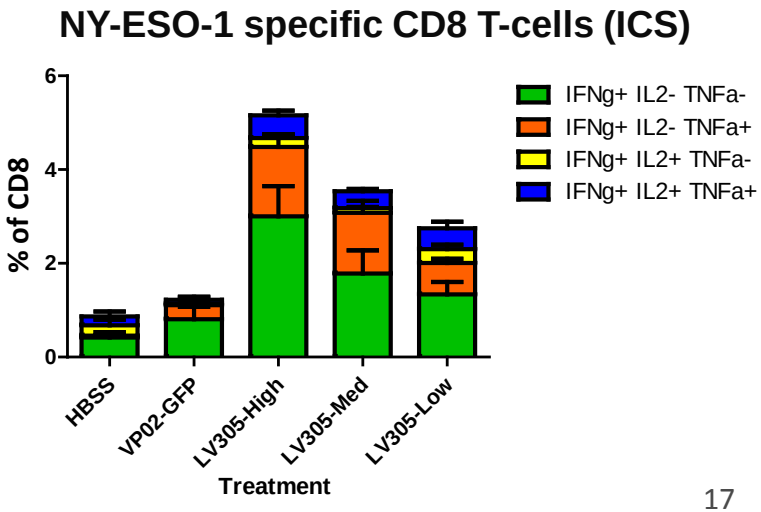
LV305 5E9 gen

LV305 1.5E9 gen

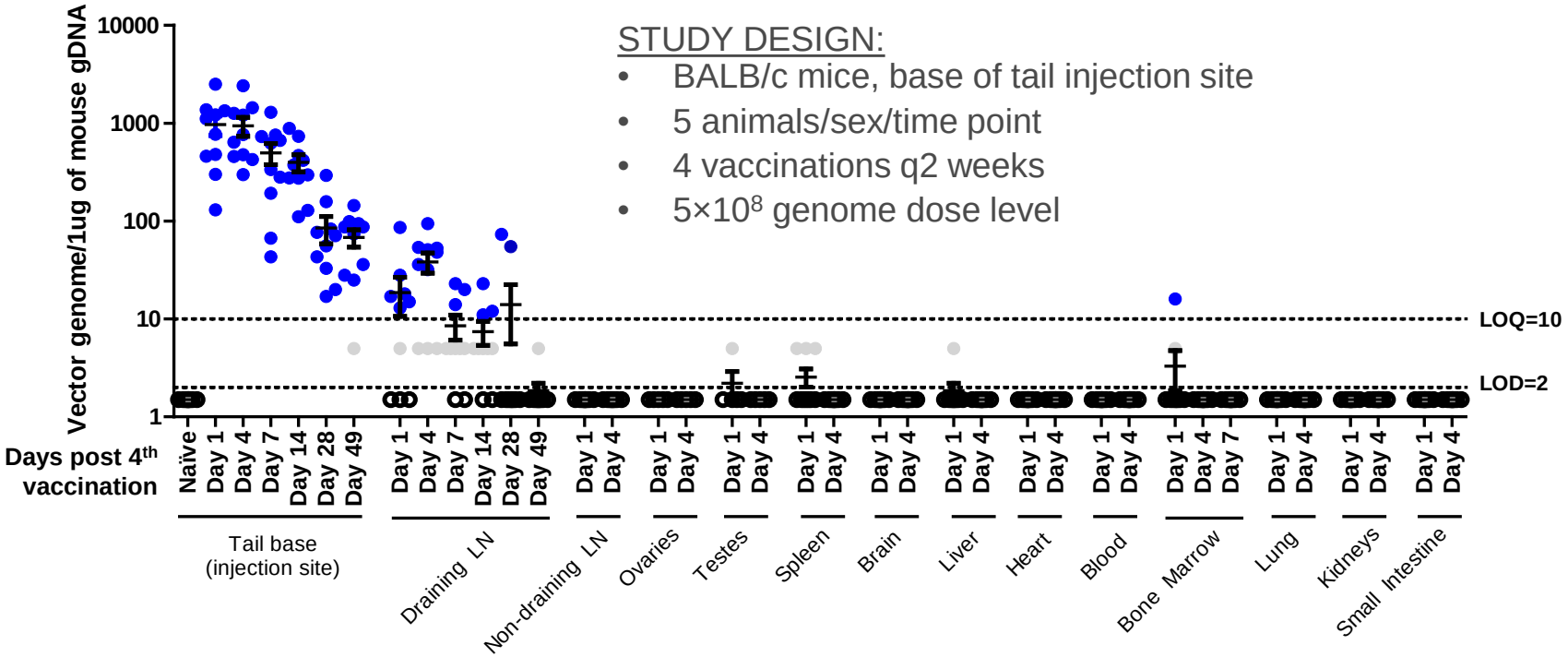
LV305 5E8 gen

Mouse lungs (India Ink)





ID-LV305: BIODISTRIBUTION LIMITED TO INJECTED AREA



STUDY DESIGN:

- BALB/c mice, base of tail injection site
- 5 animals/sex/time point
- 4 vaccinations q2 weeks
- 5×10^8 genome dose level

- Non-draining lymph node, ovaries, brain, heart, blood, lung, kidney, and small intestines were negative for ID-LV305
- Testes, spleen, liver, and bone marrow had very low levels of ID-LV305 in some animals at Day 1 post-4th vaccination
  - No detectable ID-LV305 on Day 4 or later
- ID-LV305 measured at injection site and draining lymph node with on-going clearance 7-weeks post-4th vaccination

ID-LV305 PRIMARY HUMAN CELL SCREENING SHOWS A LACK OF SIGNIFICANT OFF-TARGET TRANSDUCTION

A Total Transduction Events (Real-Time qPCR)

Cell Line	293T-MLV-DC-SIGN	100.0
	293T	**0.7**
Primary Cell	Mesangial	0.2
	Hepatocyte	-0.4
	Astrocyte	0.6
	Pulmonary Microvascular Endothelial	-0.2
	Dermal Microvascular Endothelial	0.1
	Ovarian Surface Epithelial	0.0
	Dermal Fibroblasts	0.1
	Renal Glomerular	0.1
	Aortic Smooth Muscle	-0.1
	Dendritic	15.4
	Liver Sinusoidal Endothelial	-0.3
	Lymph Node Endothelial	0.3

Transduction events <1% are shaded gray.

B Productive Transduction Events (GFP expression in FACS)

Cell type	Origin	
	Human	Cynomolgus Macaque
Dendritic cell	36.7	0.4

(A) Transduction events expressed as % of a control cell line (293T-MLV-DC-SIGN)

(B) Productive transduction events expressed as percentage of total cells expressing GFP

ID-LV305: GLP TOX STUDY SUPPORTS PHASE 1 STUDY

Study Design:

- BALB/c mice, 4 vaccinations, q2 weeks
- 5×10^8 and 5×10^7 genome dose levels
- Safety evaluated 1 day post-each vaccination and after a 7 week recovery period

Findings:

- The NOAEL was defined as the maximum dose administered = 5×10^8 genomes
- **By weight, this is 28-fold greater than the highest proposed clinical dose (5×10^{10})**
- No test article-related changes/effects in hematology, coagulation, clinical chemistries, organ weight, macroscopic findings, body weight, or food consumption
- Very slight edema and/or erythema at site of injection, not considered adverse
- Microscopic findings limited to draining lymph node follicular hyperplasia and injection site inflammation consistent with proposed MOA, resolving at recovery necropsy and not considered adverse

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ID-LV305: TRADITIONAL GMP ADHERENT CELL SYSTEM

Drug Substance

- Transient transfection of HEK 293 cells with a 5-plasmid system
- Multiple sequential harvests followed by depth filtration and benzonase treatment
- Purification by two chromatography steps and tangential flow filtration
- Resulting bulk drug substance is stored at -60 °C (or colder)

Drug Product

- Product concentration
- Sterile filtration followed by dilution to desired drug product strengths
- Fill into pre-sterilized containers
- Final drug product stored at -60 °C (or colder)

Release

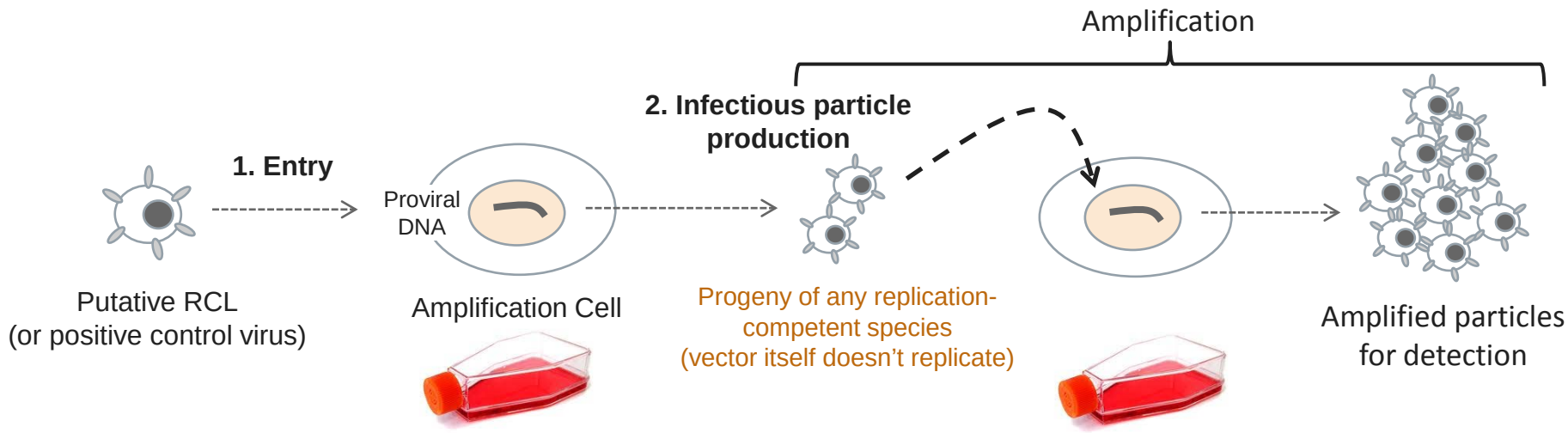
- Extensive QC testing and stability program

ID-LV305: FULL SET OF QC RELEASE ASSAYS

	GENERAL	IDENTITY	PURITY	POTENCY
DRUG SUBSTANCE	Appearance	SINVar1 envelope	Endotoxin	Vector titer (genomes)
	Osmolality	Vector identity	Bioburden	p24 content
	pH		Replication competent lentivirus (RCL)	
			Residual host cell DNA	
			Residual plasmid DNA	
			Residual T-Ag DNA	
			Residual E1a DNA	
			Residual host cell protein	
			Residual BSA	
			Residual benzonase	
			Residual kifunensine	
DRUG PRODUCT	Appearance	Vpx *	Endotoxin	Vector titer (genomes)
	Osmolality	Vector identity	Sterility	Vector titer (infectivity)
	pH		Residual host cell DNA	p24 content

* Performed on bulk drug product

REPLICATION COMPETENT LENTIVECTOR (RCL) ASSAY



- Standard C8166-based RCL assay is inappropriate for ID-LV305 (no receptor for SINVar1)
- Assay format consists of DC-SIGN expressing HEK 293F cells and MLV-4070A positive control virus
  - The assay qualification is designed to demonstrate a sensitivity of detecting (PERT) with 95% probability 1 RCL in the equivalent of 100 mL of harvest per FDA guidance
- Immune Design has consulted with FDA regarding assay development and final format

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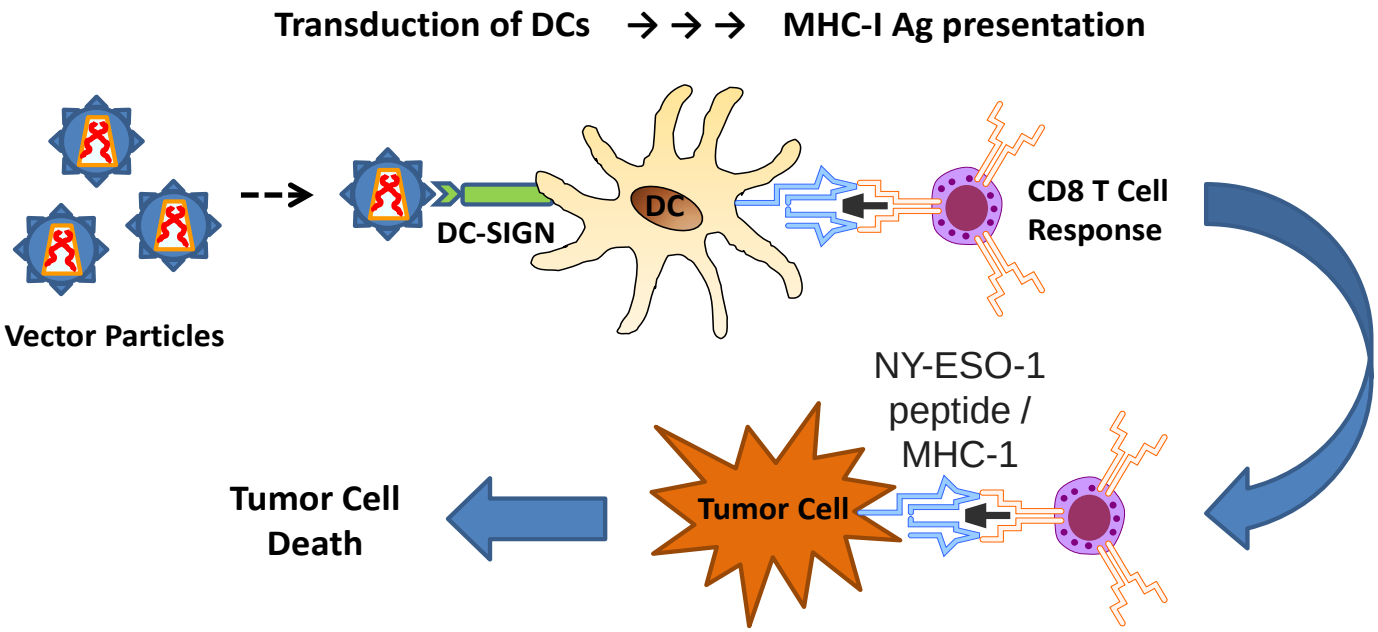
CLINICAL STRATEGY FOR INITIAL DEVELOPMENT



Phase 1 Safety Study

- Establish the safety of escalating doses of the ID-LV305 lentivector
  - Replication-incompetent, integration-deficient vector injected intradermally
- Study the mechanism of action to show proof of concept
  - Show induction of peripheral blood CTLs
  - Assess intratumoral CD8s, reduction of Tregs
- Evaluate preliminary efficacy in several tumor types
  - Melanoma, synovial sarcoma, NSCLC, ovarian, renal, and breast cancer

TARGET ANTIGEN: SELECTION OF NY-ESO-1

Antigen selection

- NY-ESO-1 first identified in a patient with squamous cell carcinoma of the esophagus
- Function unknown, expressed only in cancer and testis in adults
- Highly immunogenic outside of testis in a subset of cancer patients

Cancer Indication	IHC	PCR
Bladder (TCC)	14-22%	32-43%
Breast	3-14%	10-44%
Cholangiocarcinoma	NR	10%
Colorectal	0% (0/10)	0-20%
Endometrial Cancer	6%	NR
Gastric	NR	0-12%
HNSCC	0-2%	7-20%
Hepatocellular CA	19%	Lg range 0-67%
Melanoma	24-45%	24-43%
Multiple Myeloma	NR	60%
Neuroblastoma	82%	28-55%
NHL (DLCL)	38%	NR
NSCLC	11-25%	20-39%
Esophageal SCCA	21-32%	24-33%
Ovarian	14-43%	25-39%
Prostate CA	localized 15% HRPC 86%	25-38%
Renal (oncocytoma)	88% (15/17)	NR
Sarcoma (synovial, MRCL)	22-80+%	0-36%
Thyroid (medullary)	NR	40-65%
Uterine	32% (6/19)	0% (0/8)

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

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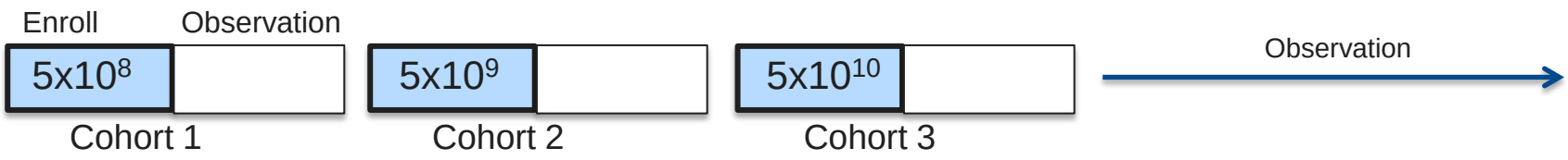
ID-LV305 PHASE 1 STUDY DESIGN

Safety will be established in stages

- Dose escalation 3+3 design (Ivy, 2010) to show safety in small cohorts
- Expansion in several tumor types with LV305 at each dose

Dose Escalation

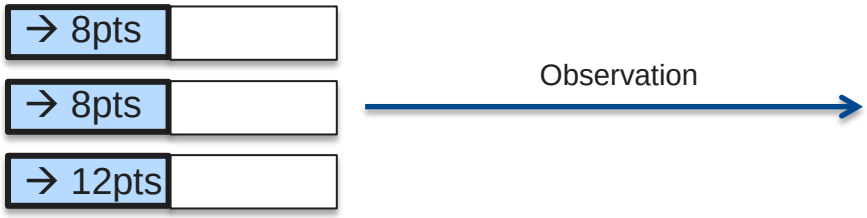


Safety and Immune Monitoring Expansion (Tumor indications may change)

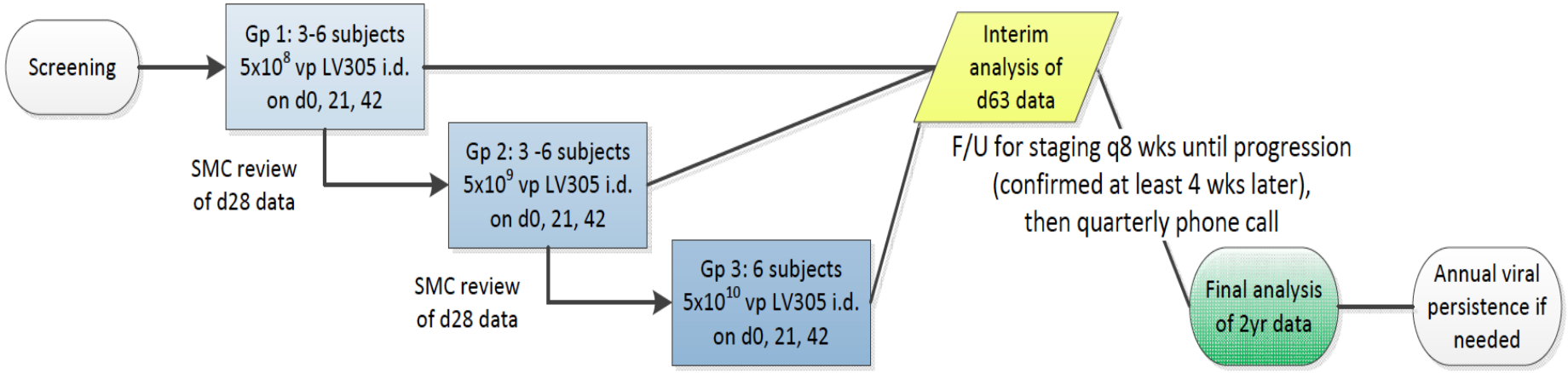
Cohort 1: $5x10^8$ genomes/mL

Cohort 2: $5x10^9$ genomes/mL

Cohort 3: $5x10^{10}$ genomes/mL



PROTOCOL ID-LV305-2013-001 OUTLINE



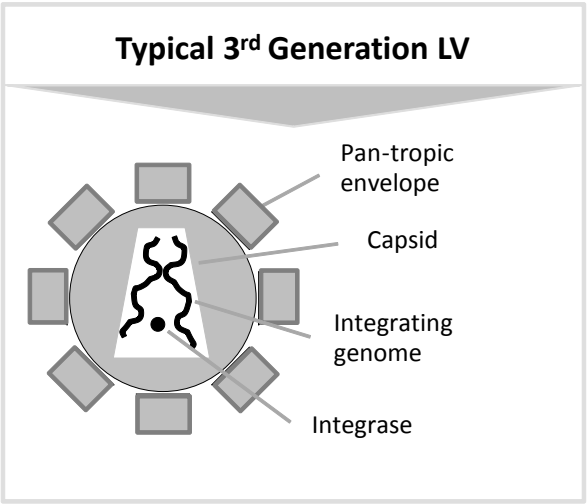
- Staggered escalating dose administration, pending input from review bodies
- Objectives include
  - 1° Safety and tolerability of three doses of i.d. ID-LV305 in locally advanced or metastatic cancer expressing NY-ESO-1 in dose escalation groups
  - 2° Evaluation of cellular and humoral immunogenicity
  - Exp. Assessment of clinical responses (by RECIST and IrRC) and TTP
  - Evaluation of available pre- and post-regimen tumor tissue
- F/U over 2 years for clinical response, evidence of integration
- Cohort expansion to gain experience in safety and immunogenicity

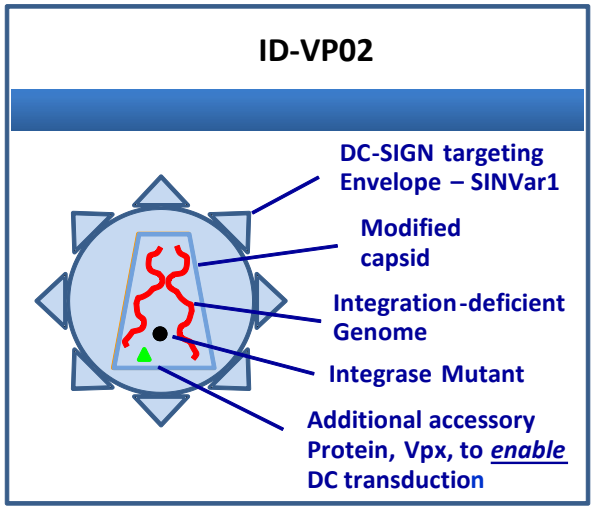
PROTOCOL ID-LV305-2013-001 KEY POINTS

- Inclusion
  - Locally advanced and/or metastatic cancer following prior treatment(s)
  - Tumor histology consistent with breast cancer, melanoma, non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), ovarian cancer or sarcoma
  - Tumor specimen positive for NY-ESO-1 gene expression by RT-PCR or IHC
  - Performance status 0 or 1
- Exclusion
  - Investigational therapy within 3 weeks prior to ID-LV305 dosing
  - Prior administration of other NY-ESO-1 targeting immunotherapeutics
  - Significant immunosuppression
- Dose Limiting Toxicity
  - AE severity by NCI CTCAE v4.03
  - Unacceptable toxicity defined by ≥ 2 of 6 with organ-specific, Grade 3+ related AE
- Readouts
  - Safety: tabulation and descriptive statistics
  - Immunogenicity: NY-ESO-1 specific responses in blood and biopsy (if available)
  - Tumor response: RECIST 1.1, IrRC, TTP, and disease control rate reported

ID-LV305 IN CONTEXT

Safety by Design





- The 3rd generation lentivector in this trial has a modified surface protein to make it DC-specific for direct i.d. injection
- Multiple enhancements have been incorporated to improve safety *in vivo*
  - ID-LV305 is replication-incompetent and integration-deficient
- Lentivector technology is being used safely and has shown clinical efficacy in multiple diseases as integrating vectors
- The proposed ID-LV305 clinical trial design exposes few patients, yet establishes preliminary safety and immunogenicity for future development