Detalimogene Voraplasmid in Non-Muscle Invasive Bladder Cancer: Bench-to-Bedside Translation of Molecular Mechanisms

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Introduction

- enGene has developed Dually Derivatized Oligochitosan® (DDX), a non-viral, non-integrating gene delivery platform designed for local administration to the bladder mucosa via intravesical instillation (IVI).
- Detalimogene voraplasmid (EG-70) consists of a plasmid DNA (pDNA) complexed with DDX® polymer to form nanoparticles, which then undergo reversible PEGylation to enhance mucus penetration and stability while preserving their ability to enter cells (**Figure 1**).
- The pDNA in detalimogene voraplasmid is designed to stimulate innate and adaptive immunity by simultaneously expressing a secretable interleukin (IL)-12 protein and non-coding RNAs that activate the retinoic acid-inducible gene I (RIG-I) pathway in urothelial cells. (**Figure 2**).
- Non-muscle invasive bladder cancer (NMIBC) remains challenging to treat, with high recurrence rates and limited therapeutic options following BCG failure. Detalimogene voraplasmid, an investigational, non-viral gene therapy, is being evaluated in the ongoing LEGEND Phase 1/2 trial (NCT04752722) in high-risk, BCG-unresponsive, non-muscle invasive bladder cancer (NMIBC).

Detalimogene: a novel, non-viral gene therapy

Figure 1. Reversible coating of DDX® nanoparticles with PEG-b-PLE di-block copolymers improves mucus penetration without affecting potency

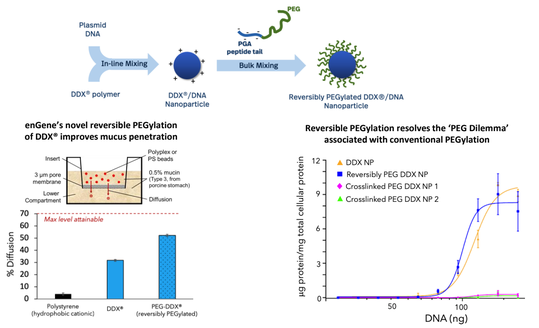
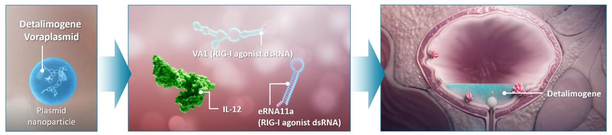


Figure 2. Detalimogene voraplasmid is designed for bladder-localized immune activation[1–5]



- Detalimogene **delivers plasmid DNA** encoding 3 genes that activate the RIG-I pathway and drive expression of IL-12.
- RIG-I activation **triggers innate immune signaling** and recruitment of antitumor NK and T cells.
- Localized IL-12 expression drives Th1 polarization of tumor-infiltrating cells, antitumor cytokine production, and **immune memory formation**.

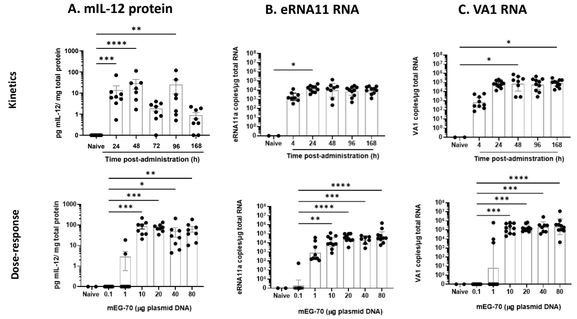
Methods

- A murine surrogate to detalimogene (mEG-70) encoding for murine IL-12 (mIL-12) was used to evaluate transgene expression, bioactivity, and antitumor efficacy in an orthotopic MB49-Luc model.
- Transgene expression and downstream immune signaling were quantified using immunoassays and RT-qPCR assays in preclinical models. Immune cell infiltration in murine bladders was characterized via flow cytometry.
- Human IL-12 (hIL-12) transgene expression and activation of RIG-I and IL-12 pathways were also evaluated in non-human primates following IVI of detalimogene voraplasmid.
- In the Phase 1 portion of the LEGEND trial, pharmacokinetics/pharmacodynamics were assessed by urine IL-12 and CXCL10 levels.

Results

- In mice, IVI of mEG-70 induced dose- and time-dependent expression of mIL-12 protein and two RIG-I agonists (eRNA11a and VA1) in bladder tissue of mice (**Figure 3**).
- In tumor-bearing mice, mEG-70 treatment activated key antitumor pathways downstream of RIG-I and IL-12, including IFN-γ, type I interferons (IFN-α2 and IFN-β), antiviral effectors (ISG-15, IRF-7), effector cell trafficking chemokines (CXCL10, CCL5), TNFα, IFIT1 and DDX58 (**Figure 4**).
- Elevated IL-4 levels in tumor-bearing mice were abolished, coinciding with reduced myeloid-derived suppressor cells (MDSC) and tumor-associated macrophages (TAM) in the tumor microenvironment (**Figure 5**).
- Dose-dependent transgene expression and downstream activation findings were reproduced in non-human primates (NHP) (**Figure 6**).
- In humans, urinary IL-12 increased dose dependently without detectable elevation in plasma IL-12, and urinary CXCL10 increased after treatment (**Figure 7**).

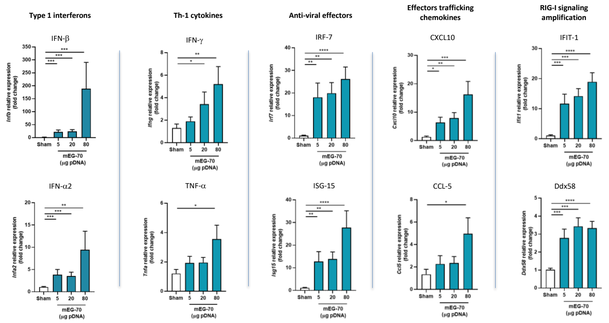
Figure 3. Transgene expression in mouse bladder following single IVI of mEG-70



Each data point represents expression in a single mouse bladder (n=8 per group); data are presented with geometric mean ± 95% CI; *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001; Kruskal Wallis with Dunn's multiple comparisons.

Transgene products show dose- and time-dependent expression in mouse bladders

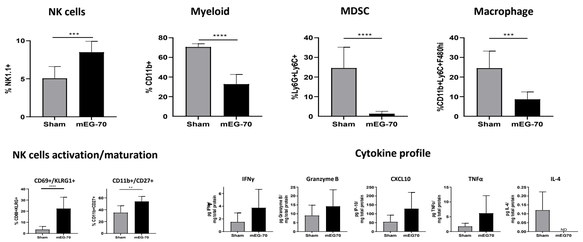
Figure 4. mEG-70 induces dose-dependent target engagement in bladders of tumor-bearing mice



Tumor-bearing mice treated with indicated doses of mEG-70; bladder harvested 24 hours post-administration for custom TaqMan Gene Expression Assays of bladder tissue lysates (n=8 per group); data are mean ± SEM; Mann Whitney t-test; *p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.00001

mEG-70 activates key IL-12 and RIG-I pathways

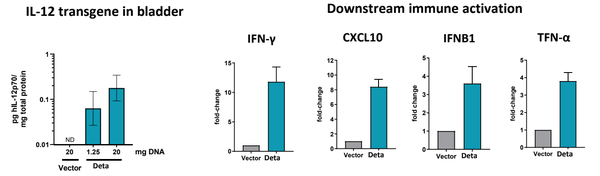
Figure 5. mEG-70 increased NK cell and reduced MDSC and TAM ratios in bladder tissue



NK: natural killer frequency, activation and cytokine profiling assessed on Day 13 (3 days post first dose); mean ± SD.
MDSC: myeloid-derived suppressive cell and TAM: tumor-associated macrophage assessed on Day 20 (3 days post second dose); mean ± SD.
Cytokine profile on Day 13 is shown; mean ± SD.

mEG-70 promotes a less suppressive tumor microenvironment in the bladder

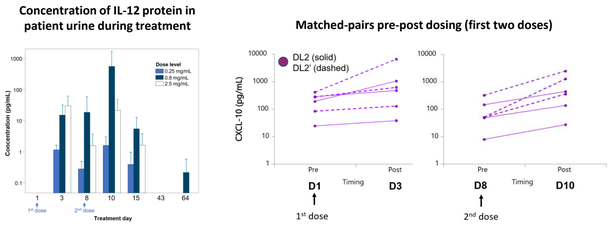
Figure 6. Detalimogene voraplasmid instillation induced dose-dependent transgene expression and downstream activation in non-human primates



Cynomolgus monkeys (n=1/group) received a single administration of detalimogene voraplasmid or empty vector control nanoparticles. Bladder tissue was harvested at 48 hours post-administration, up to 6 fragments of the bladder were sampled from each animal, and (A) hIL-12 protein was quantified by immunoassay; (B) RT-qPCR was performed for the indicated genes. Each fragment was treated as an individual sample and data from all fragments are presented as mean ± SD. Deta = detalimogene voraplasmid

Detalimogene voraplasmid is biologically active in non-human primates

Figure 7. IL-12 and CXCL10 urinalysis demonstrates detalimogene's proof-of-mechanism in humans



DL1 is dose-level 1 (250 µg/mL); DL2 is dose-level 2 (800 µg/mL); DL3 is dose-level 3 (2500 µg/mL). Levels not measured 2 days after third dose to minimize patient burden.

IL-12 protein detected in a dose-responsive manner in urine of all patients

CXCL10 increases after treatment with sustained induction after the 4th dose

LEGEND Phase 1/2 clinical study

Table 1. Efficacy findings

	% (n/N)[a]	
	All doses (N=22)	Selected phase 2 dose[b] (N=10)
Complete response (CR)		
Any time	73 (16/22)	70 (7/10)
3 months	68 (15/22)	70 (7/10)
6 months	45 (10/22)	60 (6/10)
Duration of response		
≥3 months	73 (11/15)	86 (6/7)
≥6 months	60 (6/10)	75 (3/4)

[a]The efficacy evaluable population consisted of 22 patients who received intravesical detalimogene voraplasmid with at least 3-month response assessment available: One patient was dosed but not included in the efficacy evaluable population as they did not meet inclusion criteria. A second patient withdrew consent after a single instillation of detalimogene voraplasmid; CR was defined per FDA guidance on BCG-unresponsive NMIBC [February 2018;August 2024]
[b]The selected Phase 2 dose was determined during the Phase 1 portion of the study

Table 2. Treatment-related adverse events (n=24)

Patients with TRAEs n (%)	Grade 1	Grade 2	Grade 3	Grade 4/5
Hematuria	3 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)
Urinary tract infection	0 (0.0)	3 (12.5)	0 (0.0)	0 (0.0)
Micturition urgency	2 (8.3)	1 (4.2)	0 (0.0)	0 (0.0)
Dysuria	3 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)
Fatigue	2 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)
Nocturia	2 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)
Pyrexia	2 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)
Renal failure	0 (0.0)	0 (0.0)	1 (4.2)	0 (0.0)

Conclusions

- Intravesical detalimogene voraplasmid drives localized, dose-dependent transgene expression, activating key antitumor effectors downstream of IL-12 and RIG-I pathways in preclinical animal models.
- In tumor-bearing mice, mEG-70 reprograms the bladder tumor microenvironment by enhancing natural killer and T-cell infiltration and reducing suppressive MDSC and TAM.
- Dose-dependent transgene expression and downstream activation findings were reproduced in non-human primates.
- Urinary IL-12 and CXCL10 confirm translational pharmacodynamic activity and localized immune activation in patients with NMIBC.
- LEGEND clinical study:
  - Phase 1 portion of LEGEND trial is complete and suggests a promising safety, tolerability and efficacy profile: 73% of patients achieved a CR at any time. Treatment-related adverse events reported to date are mostly Grade 1/2 and consistent with catheterization/intravesical administration.
  - Phase 2 is ongoing, Pivotal Cohort: BCG-unresponsive NMIBC, preliminary results with longer follow-up in 2H 2026.

References & Acknowledgements

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