# Mechanism of action and translation to the clinic of detalimogene voraplasmid (EG-70) – a novel, investigational, non-viral, gene-based immunotherapy for non-muscle invasive bladder cancer (NMIBC)

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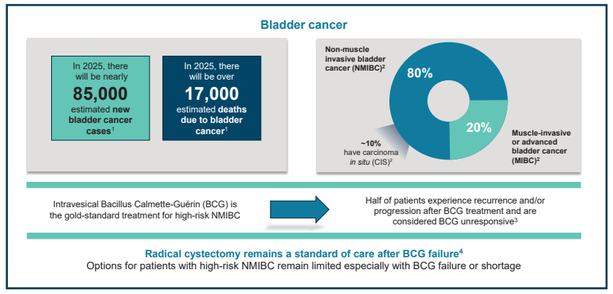
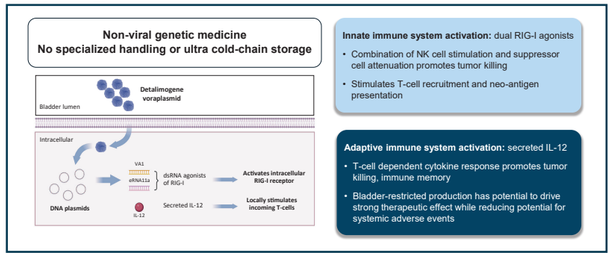
## Introduction

- Bladder-sparing therapies for high-risk non-muscle invasive bladder cancer (NMIBC) address an important unmet need (**Figure 1**).[1–4]
- Detalimogene voraplasmid is a novel, investigational, non-integrating, non-viral, genetic medicine-based immunotherapy carrying a plasmid that simultaneously expresses IL-12 and regulators of RIG-I (**Figure 2**):
  - Stimulates innate and adaptive immunity.
  - Promising efficacy demonstrated in high-risk NMIBC, while minimizing the risk of systemic toxicities.
  - Lyophilized powder easily reconstituted in clinic with sterile water—no special handling and no ultra-cold chain storage required.
  - Intravesical administration via a catheter.
- The ongoing Phase 1/2 LEGEND study (ClinicalTrials.gov: NCT04752722) continues to investigate the safety and efficacy of detalimogene voraplasmid in patients with high-risk NMIBC.
- Here we present preclinical data defining the immunomodulatory mechanism of action of detalimogene voraplasmid, involving immune cell recruitment, tumor microenvironment remodeling and, ultimately, immune training on neoantigens and tumor clearance.

### Figure 1: Bladder-sparing therapies for high-risk NMIBC address an important unmet need




### Figure 2: Detalimogene voraplasmid (EG-70): Designed to bring genetic medicine to any urology clinic




Schematic created using BioRender.com
IL-12, interleukin 12; NK, natural killer; RIG-I, retinoic acid-inducible gene I

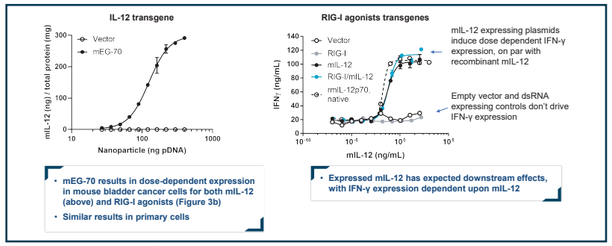
## Preclinical methods

- Preclinical evaluation of detalimogene voraplasmid efficacy was conducted in vitro in relevant cell lines and in vivo in an orthotopic syngeneic mouse model of bladder cancer to recapitulate a physiological tumor microenvironment in immunocompetent C57BL/6 mice.
- Luciferase-expressing MB49 cells were instilled in the bladder on study Day 1; following confirmation of tumor engraftment by in vivo imaging on Day 9, mice received two weekly intravesical instillations of mEG-70 (a murine surrogate of EG-70) on Days 10 and 17.

## Preclinical results

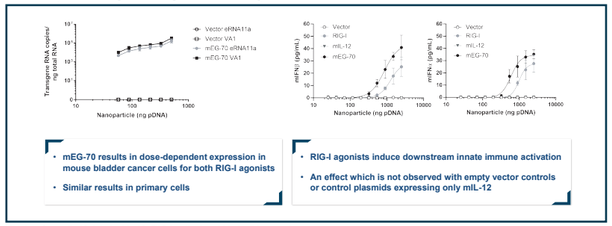
- Immune profiling by flow cytometry, immunoassay, and immunohistochemistry revealed a profound remodeling of the tumor microenvironment from an immunosuppressive phenotype to a pro-inflammatory milieu supportive of tumor clearance (**Figures 3, 4 & 5**).
- Accordingly, administration of mEG-70 was associated with marked reduction in tumor burden and significant improvement of survival (**Figure 6**).
- As demonstrated by either bladder or flank implantation rechallenge, the anti-tumor immune response had resulted in durable protection against subsequent tumor re-challenge, demonstrating systemic immune memory (**Figures 7 & 8**).

### Figure 3a: Detalimogene voraplasmid bioactivity: transfection of bladder cells in vitro, with expected pharmacodynamic effect



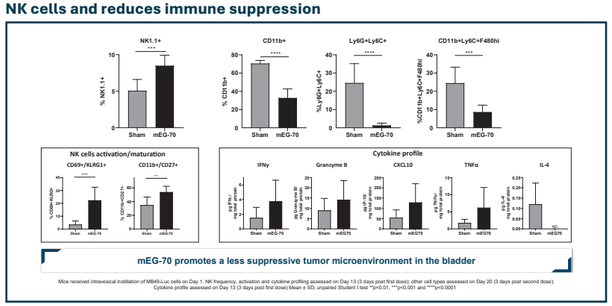

IFN, interferon; IL-12, interleukin 12; RIG-I, retinoic acid-inducible gene I

### Figure 3b: Detalimogene voraplasmid bioactivity: transfection of bladder cells in vitro, with expected pharmacodynamic effect



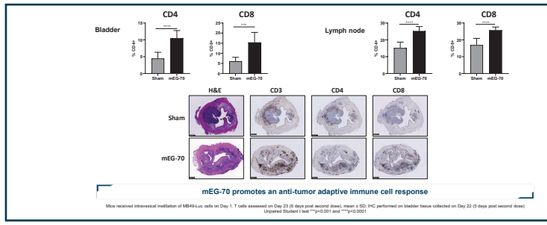

IFN, interferon; IL-12, interleukin 12; RIG-I, retinoic acid-inducible gene I

### Figure 4: Detalimogene voraplasmid mechanism of action: mEG-70 increases NK cells and reduces immune suppression



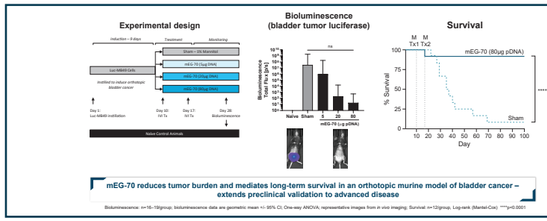

CXCL10, C-X-C motif chemokine ligand 10; IFN, interferon; IL-4, interleukin 4; NK, natural killer; TNF, tumor necrosis factor

### Figure 5: Detalimogene voraplasmid mechanism of action: mEG-70 encourages T-cell recruitment and remodels the tumor microenvironment



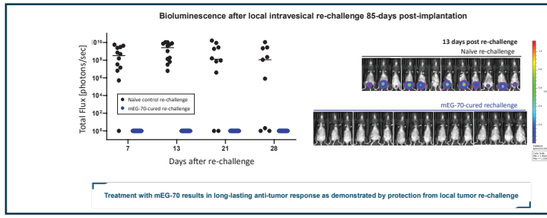

H&E, hematoxylin and eosin; IHC, immunohistochemistry

### Figure 6: Detalimogene voraplasmid's profound antitumor activity: mEG-70 eradicates murine tumors

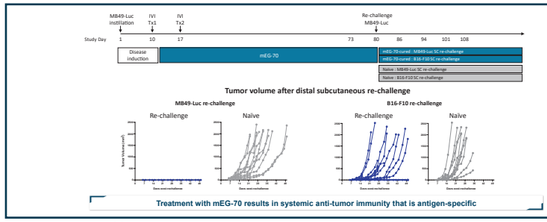



IVI, intravesical instillation

### Figure 7: Detalimogene voraplasmid's mechanism of action generates durable memory: mEG-70 cured animals are protected from rechallenge




### Figure 8: Detalimogene voraplasmid systemic and antigen-specific protection opens opportunities to treat advanced disease: MIBC and metastatic disease




IVI, intravesical instillation; SC, subcutaneous

## LEGEND Phase 1/2 clinical study

- LEGEND (NCT04752722) is an ongoing, multicohort Phase 1/2 study of detalimogene voraplasmid in patients with high-risk NMIBC:
  - Phase 1 patients: BCG-unresponsive NMIBC with CIS.
  - Detalimogene voraplasmid dosing: 2 or 4 doses over 12-week cycle.
  - Cohorts: 3+3 dose escalation: 3 dose levels (0.25 mg/mL, 0.8 mg/mL, 2.5 mg/mL); 2 schedules (2 doses/12-week cycle versus 4 doses/12-week cycle).
  - **Endpoints: 1° – Safety; 2° – Efficacy at 3 months.**
- Phase 1 is now complete (N=24); treatment with detalimogene voraplasmid resulted in a 73% complete response rate at any time and a promising safety and tolerability profile (**Tables 1 & 2**).[5,6]
- The Phase 2 (Cohort 1) portion of LEGEND is registrational and patients are being enrolled.

### Table 1. LEGEND Phase 1 study: efficacy

| | % (n/N)[a] | |
|---|---|---|
| | All doses (N=22) | Selected Phase 2 dose[b] (N=10) |
| **Complete Response** | | |
| 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
BCG, Bacillus Calmette-Guérin; CIS, carcinoma *in situ*; NMIBC, non-muscle invasive bladder cancer

### Table 2. LEGEND Phase 1 study: Treatment-related adverse events (TRAEs)

**All TRAEs**

| Patients with TRAEs, n (%) | N=24 |
|---|---|
| Any | 13 (54.2) |
| Grade 1 | 11 (45.8) |
| Grade 2 | 7 (29.2) |
| Grade 3 | 1 (4.2) |
| Grade 4/5 | 0 (0.0) |

- Promising safety and tolerability profile
- TRAEs reversible and largely consistent with catheterization

| Patients with TRAEs, n (%) | Grade 1 (Mild) | Grade 2 (Moderate) | Grade 3 (Severe) | Grades 4 or 5 (life-threatening or death) |
|---|---|---|---|---|
| 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) |

*Patient had a history of renal failure and recurrent obstructive uropathy with presence of bilateral hydroureteronephrosis at screening – enrollment criteria later modified and excludes patients with a history of unresolved vesicoureteral reflux, indwelling urinary catheter or unresolved hydronephrosis due to ureteral obstruction

## Conclusions

- **Preclinical data indicate that:**
  - mEG-70 treatment results in robust activation of both innate and adaptive immune responses.
  - In tumor-bearing animals, mEG-70 remodels the tumor microenvironment:
    - results in clearance of existing tumors.
    - has a durable, memory-mediated protective effect.
- **Translation to the LEGEND clinical study:**
  - The Phase 1 portion of LEGEND suggests a promising safety, tolerability and efficacy profile:
    - **73% of patients achieved a CR at any time.**
    - **At the dose selected for Phase 2, CR rates were 70% at 3 months and 60% at 6 months.**
    - Treatment-related adverse events reported to date are mostly Grade 1/2 and consistent with catheterization/intravesical administration.
    - **Phase 2 sites are currently being recruited and activated, and patients are being enrolled.**

LEGEND

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