

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

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**Background:** Detalimogene voraplasamid (EG-70) is a novel, investigational, non-integrating, non-viral gene therapy designed to elicit local stimulation of anti-tumor immune response in the bladder while mitigating the risk of systemic toxicities from immune stimulation. It is administered by intravesical instillation (IVI) to eligible patients with NMIBC to drive bladder-localized expression of innate (RIG-I agonists) and adaptive (IL-12) immune regulators and remodel the tumor microenvironment. LEGEND is an ongoing Phase 1/2 study (NCT04752722) investigating the safety and efficacy of detalimogene voraplasamid in bacillus Calmette Guerin (BCG)-unresponsive NMIBC. We present preclinical data supporting the mechanism of action of detalimogene voraplasamid, which involves immune cell recruitment, tumor microenvironment remodeling and immune training on neoantigens and tumor clearance.

**Methods:** Preclinical evaluation of detalimogene voraplasamid was conducted *in vitro* in relevant cell lines and *in vivo* in an orthotopic syngeneic model of bladder cancer in immunocompetent C57BL/6 mice. Luciferase-expressing MB49 cells were instilled in the bladder on Day 1; following confirmation of tumor engraftment by *in vivo* bioluminescence imaging on Day 9, mice received two weekly IVIs of mEG-70 (a murine surrogate of EG-70) on Days 10&17. Efficacy of mEG-70 was assessed post-dosing by flow cytometry, MSD immunoassays, immunohistochemistry, bioluminescence *in vivo* imaging, and overall survival. Animal experimentation was approved by the Institutional Animal Care Committee (IACC) and conducted in accordance with Canadian Council on Animal Care (CCAC) guidelines. The Phase 1 component of the LEGEND study evaluated detalimogene voraplasamid in patients with high-risk BCG-unresponsive NMIBC with carcinoma *in situ* (CIS).

**Results:** Immune profiling revealed remodeling of the tumor microenvironment from an immunosuppressive phenotype to a pro-inflammatory milieu supportive of tumor clearance. Accordingly, administration of mEG-70 was associated with marked reduction in tumor burden and improvement in survival in mice. As demonstrated by either bladder or flank tumor cell rechallenge, the anti-tumor immune response in surviving tumor-free mice resulted in durable protection against subsequent tumor re-challenge, and systemic immune memory. In the Phase 1 part of the LEGEND study, detalimogene voraplasamid was generally well tolerated, with an overall complete response rate of 73% in patients with NMIBC with CIS.

**Conclusions:** These preclinical findings demonstrate that detalimogene voraplasamid delivers genetically encoded immunostimulatory payloads locally to the bladder. The mechanism of action described preclinically has been translated into the clinic in the Phase 1 part of the LEGEND study.