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Overview of Aduro *Listeria* Platform and Immunotherapy Program Directed Against EGFRvIII and NY-ESO-1

RAC Protocol #1301-1202

RAC Meeting

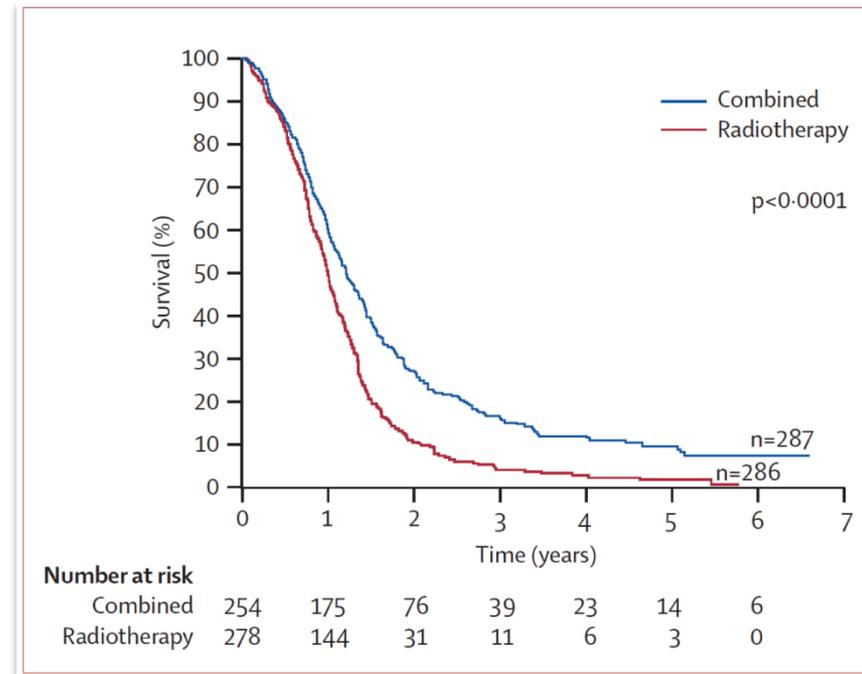
March 12th, 2013

Presentation Outline

- Overview of Glioblastoma multiforme and rationale for targeting EGFRvIII and NY-ESO-1
 - Marka Crittenden, M.D., Ph.D.
 - Director, Translational Radiation Research, Providence Cancer Center
- Live-attenuated *Listeria monocytogenes* (Lm) platform and toxicology program
 - Dirk G. Brockstedt, Ph.D.
 - Senior Vice President, R&D, Aduro BioTech, Inc.
- Clinical experience with Lm-based vaccines
 - Dung Le, M.D.
 - Assistant Professor, Johns Hopkins University
- Proposed Phase 1 clinical trial
 - Marka Crittenden, M.D., Ph.D.

High-grade Astrocytomas

- WHO Grade III/IV astrocytic tumors – Anaplastic Astrocytomas and Glioblastoma Multiforme
- Most common adult primary brain tumor with almost uniformly fatal prognosis
- Standard treatment includes some combination of surgery, radiation therapy and chemotherapy



Stupp et al. The Lancet Oncology 2009

Targeting EGFRvIII

- EGFRvIII is a mutant form of the Epidermal Growth Factor receptor that is found by both IHC and RT-PCR to be expressed in high-grade astrocytomas
- EGFRvIII contains a specific deletion of exons 2-7 that results in a neopeptide that is tumor specific
- EGFRvIII is associated with ligand independent signaling
- EGFRvIII has been identified by immunohistochemistry in approximately 70% of tumors that over express EGFR (40% tumors)
- EGFRvIII has been identified by RT-PCR in approximately 54% of tumors regardless of EGFR overexpression
- EGFRvIII is expressed on CD133 positive cancer stem cells an important therapeutic target
- EGFRvIII is not found expressed on normal cells

Targeting NY-ESO-1

- NY-ESO-1 is a cancer/testis antigen that is not expressed on normal tissues (except testis-an immune privileged site)
- NY-ESO-1 has expression on a wide range of tumor subtypes including melanoma, breast, lung, sarcoma, and GBM CD133 positive stem cells
- NY-ESO-1 has multiple well defined MHC binding epitopes that can provide CD4 help and allow tracking of antigen specific immune responses
- NY-ESO-1 has been shown to be highly immunogenic with minimal toxicity in multiple human studies

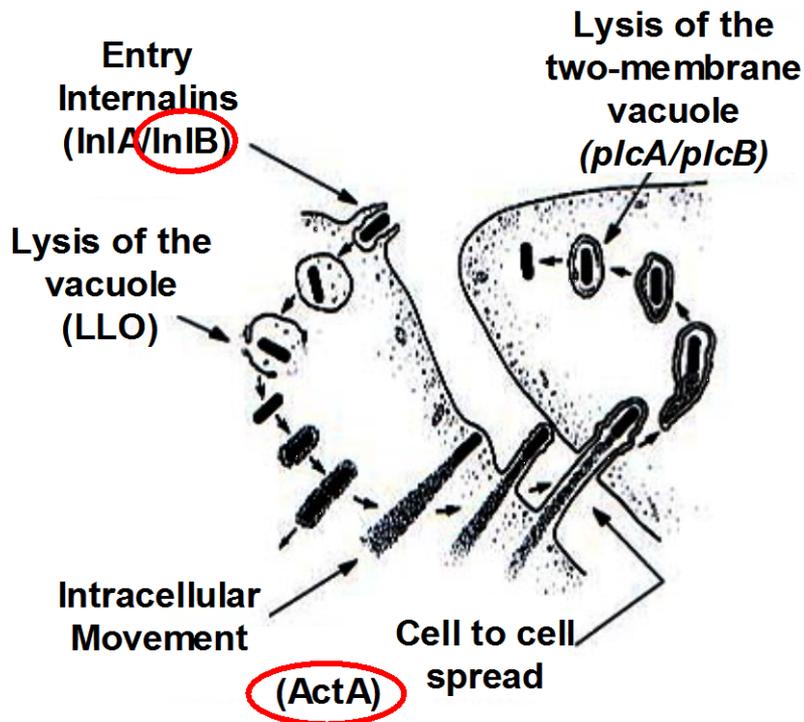
Targeting EGFRvIII and NY-ESO-1

- ADU-623 has the capacity to produce GBM-specific cellular immunity against vaccine encoded EGFRvIII and NY-ESO-1 antigens, and also against additional GBM-specific antigens through cross presentation and antigen spreading mechanisms
- Both vaccine encoded antigens do not have pre-existing tolerance and thus can serve as ideal antigens for generation of robust CTL responses that can enhance epitope spreading

Live-Attenuated
Listeria monocytogenes Platform

L. monocytogenes Lifecycle and Live-Attenuated Double Deleted (LADD) Vaccine Platform Strain

Lifecycle of Live-Attenuated Vaccine



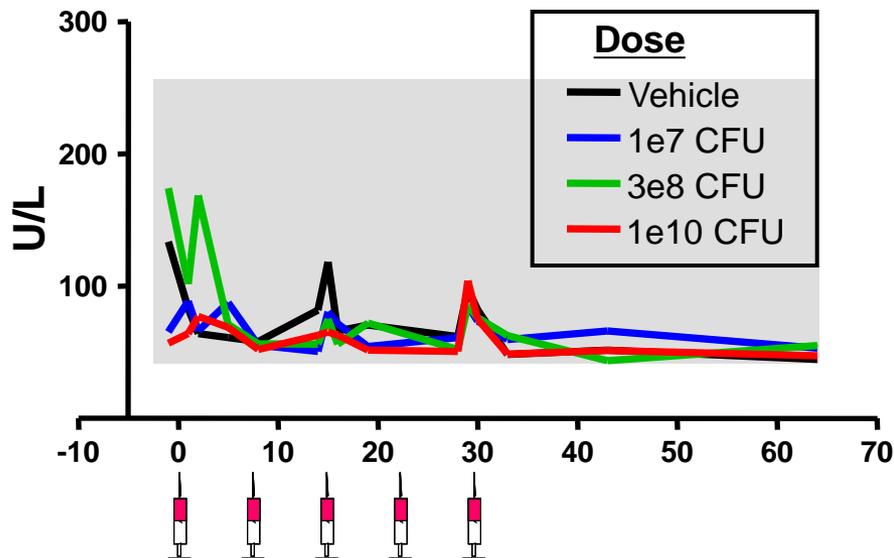
○ : deleted virulence genes in vaccine strain

- Gram-positive bacterium; a food-borne pathogen
- Vaccine strain: complete deletion of 2 virulence genes (ActA and Internalin B)
- >1000-fold reduction in mouse toxicity
- Facultative intracellular lifecycle
 - Targets macrophages and dendritic cells
 - Induces robust innate and adaptive cell-mediated immunity
- Intravenous administration well-tolerated in 79 patients
- 5 clinical trials under US IND, two reviewed by RAC

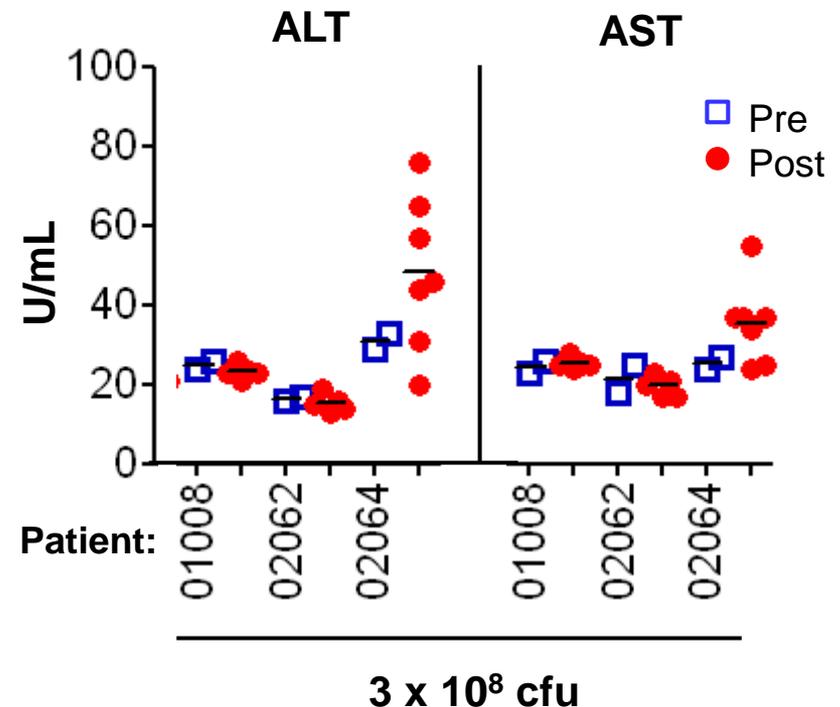
Defined Safety Profile of Live-Attenuated Vaccine Platform Strain (LADD)

Liver Function Test Values Pre- and Post-Dosing

Cynomolgus Monkey

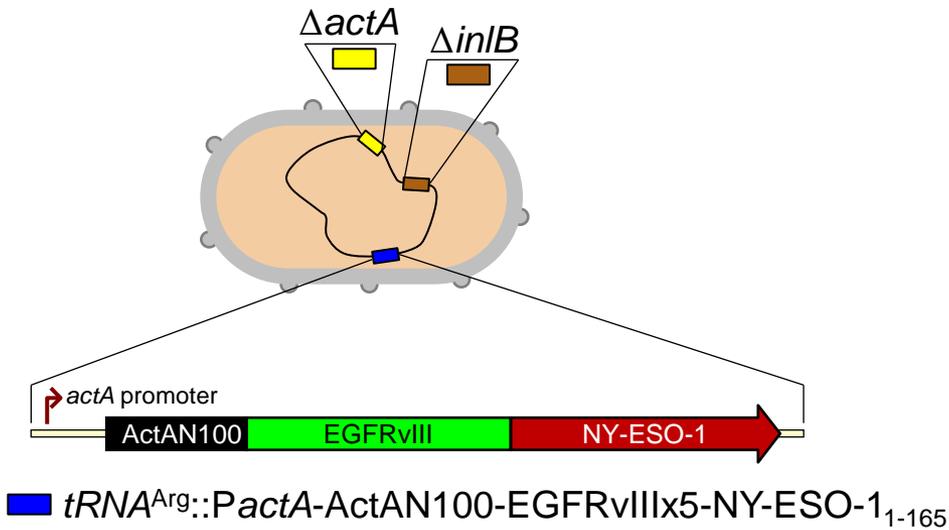


Human

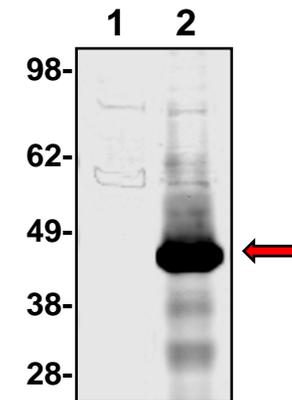


ADU-623 Configuration and Expression

Configuration



Expression in Mouse Dendritic Cells (DC2.4)



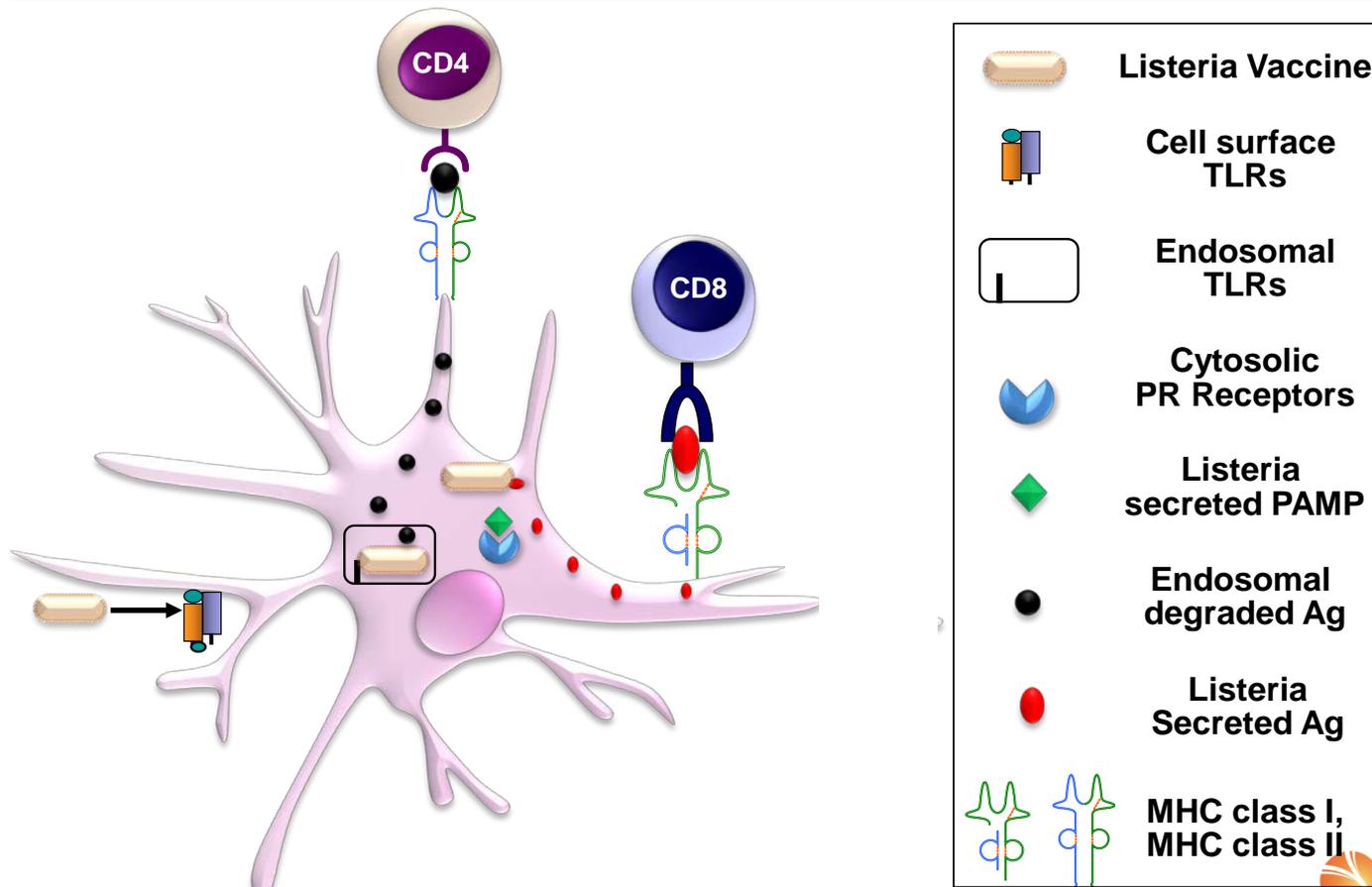
1. Lm $\Delta actA \Delta inlB$
2. Lm $\Delta actA \Delta inlB$ -EGFRvIII-NY-ESO-1

Features

- Based on same vaccine platform as ANZ-100, CRS-207 and ANZ-521
- EGFRvIII-NY-ESO-1 expression cassette integrated in listerial chromosome
- Prokaryotic expression machinery
- Increased antigen expression in the context of infected APCs

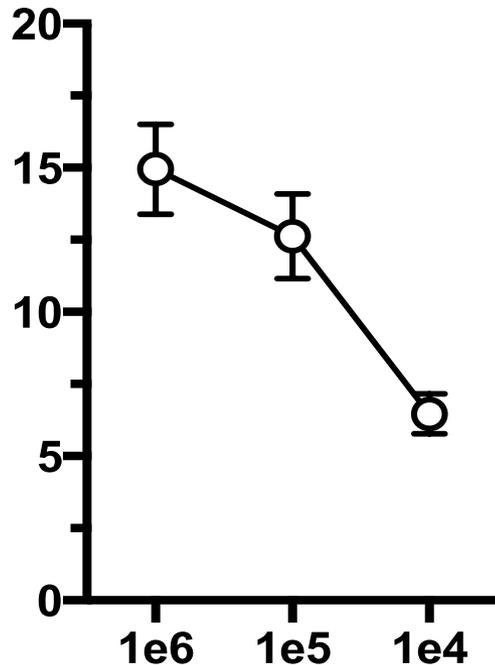
Taking Clues from Listeria: What Innate Immune Triggers are Essential for Vaccine Potency?

Recombinant Attenuated Listeria Vaccine-Infected Dendritic Cell

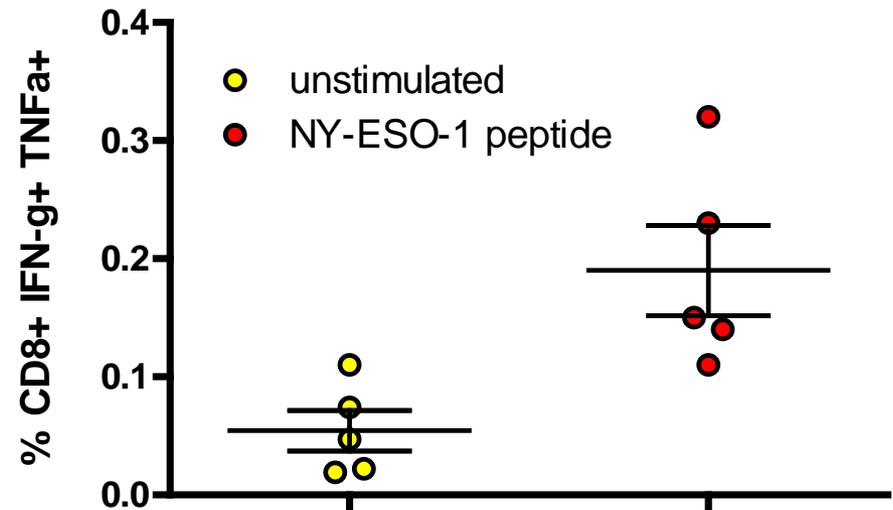


ADU-623 Induces EGFRvIII- and NY-ESO-1 Specific T Cell Immunity

EGFRvIII-Specific Immunity



NY-ESO-1-Specific Immunity



ADU-623 Preclinical Safety Evaluations

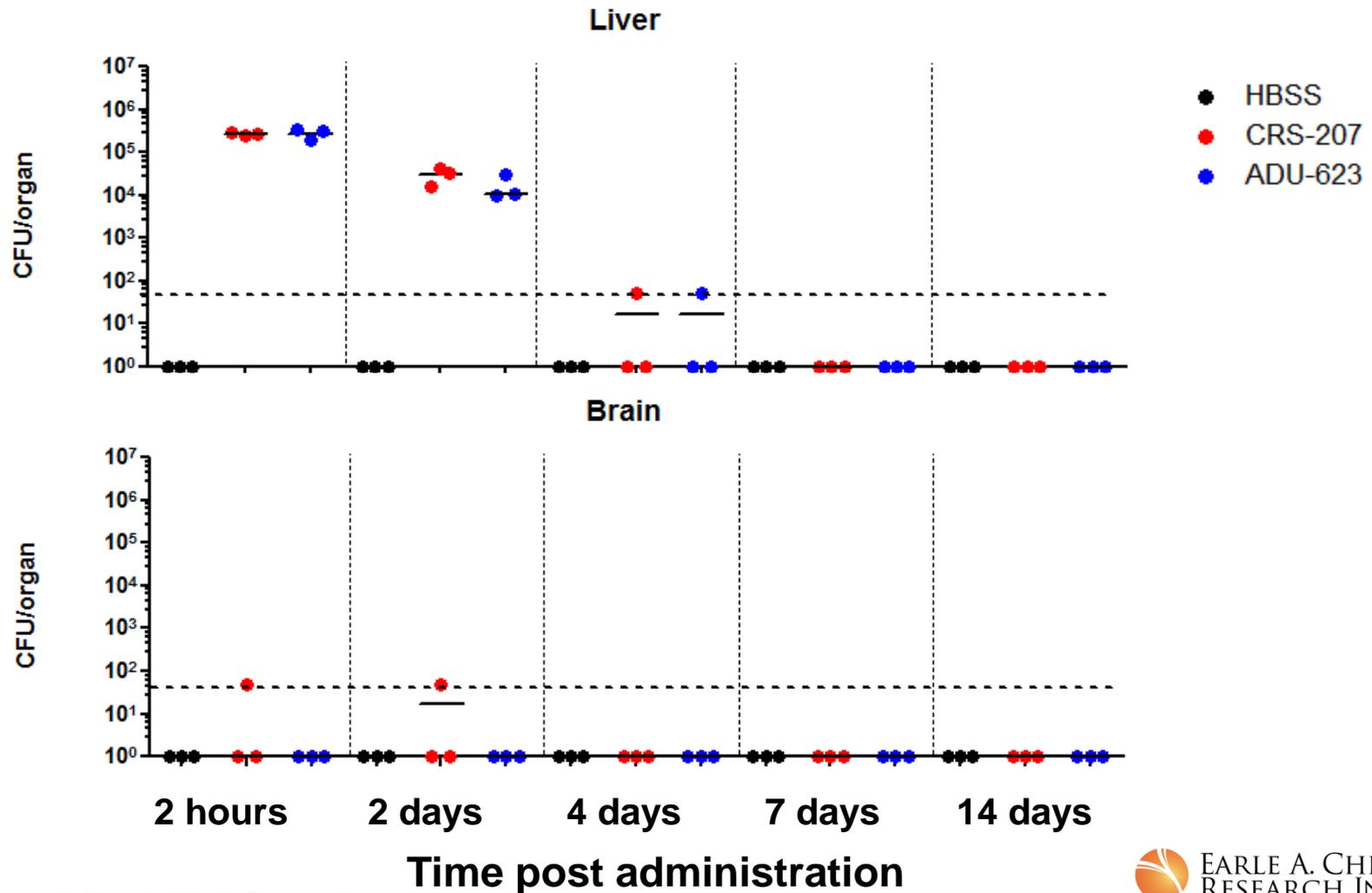
ADU-623 Pre-Clinical Safety Evaluation Strategy - Background

- ADU-623 has the same genetic background as three other investigational agents, known as ANZ-100, CRS-207, and ANZ-521
- ANZ-100 and CRS-207 have been extensively evaluated in cynomolgus monkeys and mice in repeat-dose studies
 - Findings consistent with toxicities caused by a systemic proinflammatory response to bacterial infection
 - Key conclusion: **toxicology profile is independent of antigen**

Safety profile for ADU-623 based upon:

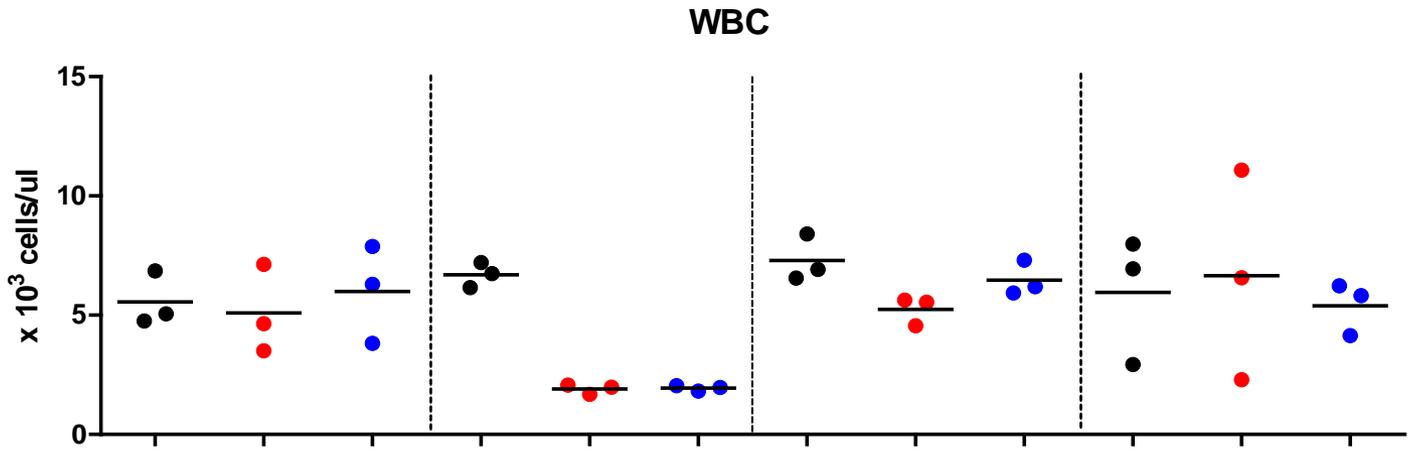
- Demonstration of comparable safety and biodistribution characteristics in mice between ANZ-100, CRS-207 and ADU-623
- FDA has accepted the toxicology plan to support the proposed Phase 1 study

Comparable Biodistribution Between ADU-623 and CRS-207

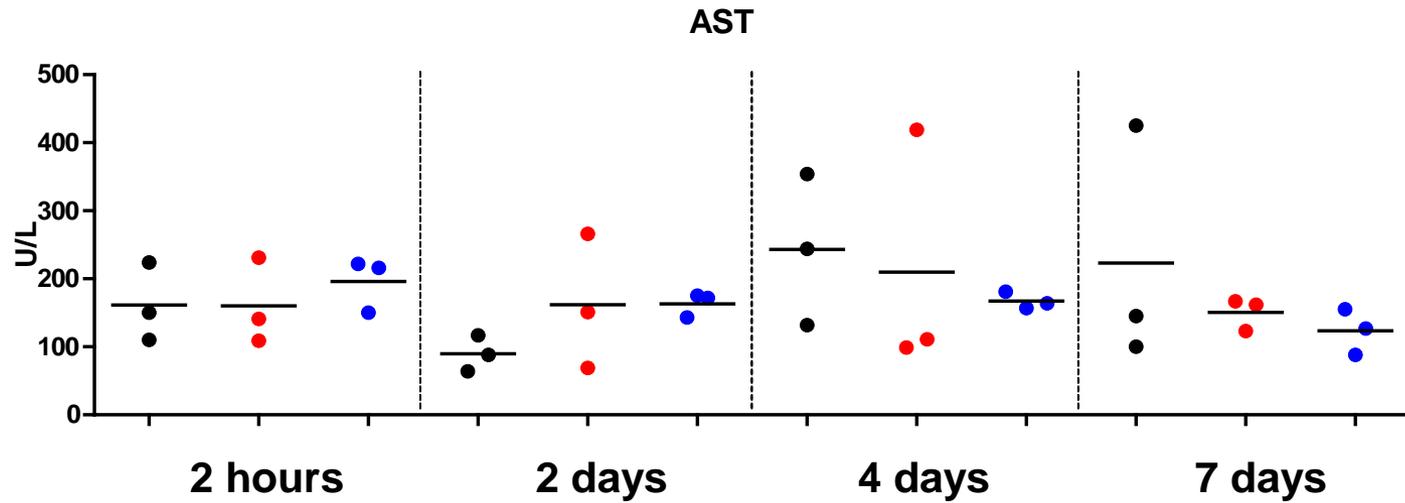


Comparable Changes in Hematologic Parameters and Blood Chemistries Between ADU-623 and CRS-207

Hematology



Clinical Chemistry



Time post administration

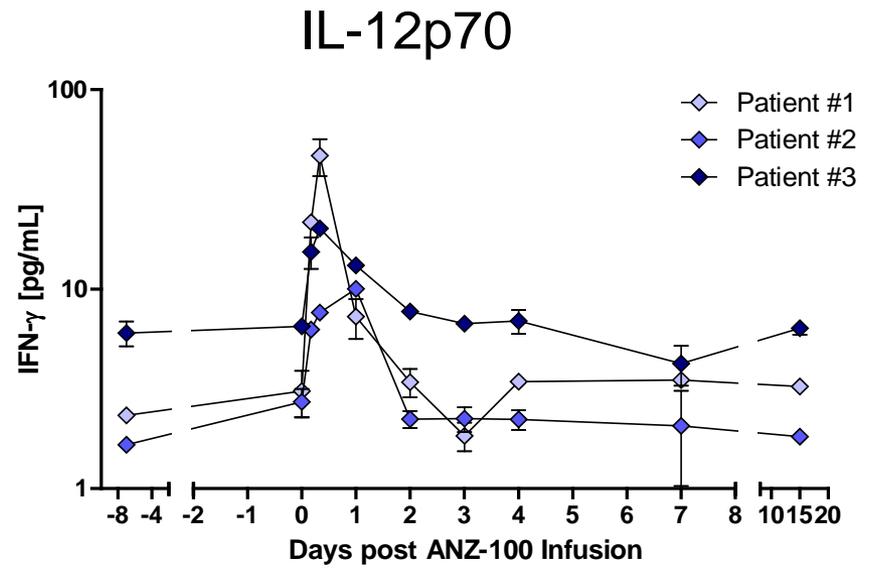
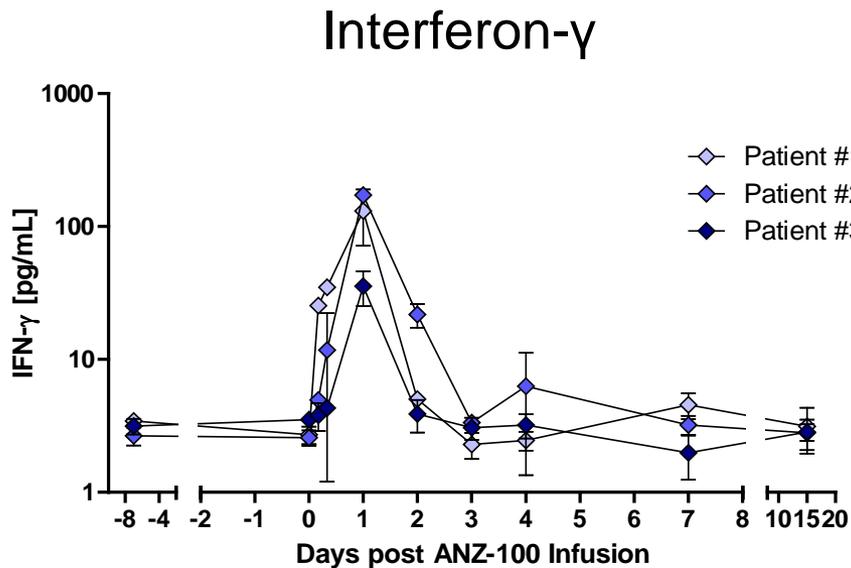
Clinical Experience with Live-Attenuated Listeria Vaccines

Clinical Experience with LADD-Based Therapeutic Vaccines

- A total of 79 subjects have been given LADD vaccine strains
- More than 200 infusions have been administered
- Subjects received up to 10 doses of LADD vaccine
- No related SAEs or unexpected Grade 3 or 4 toxicities in the ongoing 90-subject Phase 2 trial with CRS-207
- Established safety in an out-patient setting

Induction of Th1 Cytokines Following IV Administration of LADD in Cancer Patients

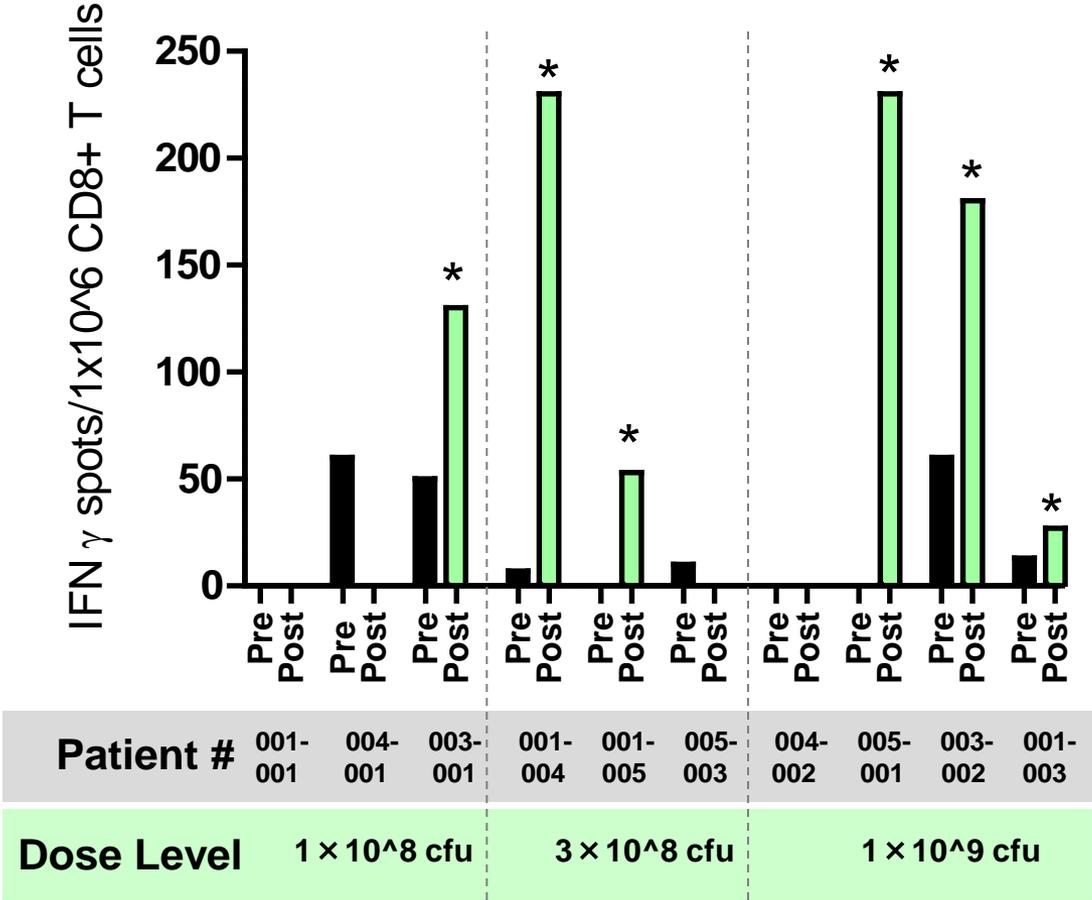
ANZ-100 Induced Th1 Cytokines in 3e8 Dose Cohort



- Induction of IFN- γ and IL-12p70 promotes activation of potent cytotoxic T cells

Le et. al. Clin. Can. Res. 2012

CRS-207 Induced Mesothelin-Specific T Cell Immunity in Cancer Patients



Phase 1 LADD Safety Events

- Phase 1 Study of CRS-207 (LADD expressing mesothelin)
 - Multiple-dose, dose-escalation study in subjects with mesothelioma, NSCLC, ovarian or pancreatic cancer
 - 17 subjects in 4 cohorts (CFU): 1×10^8 , 3×10^8 , 1×10^9 , 1×10^{10}
 - 2 related SAEs reported: constipation which required additional hospitalization (1×10^8); severe hypotension requiring aggressive fluid management/intensive monitoring (1×10^{10})
- Phase 1 Study of ANZ-521 (LADD expressing NS5B-NS3)
 - Placebo-controlled, double-blind, dose-escalation study in subjects with chronic HCV
 - 5 subjects in 1 cohort (CFU): 3×10^7
 - One related SAE reported: severe cough requiring aggressive treatment
 - Reported by original sponsor as anaphylactoid airway event; further review concluded event was inconsistent with anaphylactoid responses

Safety Experience in Phase 2 Study of CRS-207

- Open-label, randomized, controlled Phase 2 study of GVAX pancreas vaccine and CRS-207 in 90 adults with metastatic pancreatic cancer
 - 61 subjects have received between 1-10 doses of CRS-207 at 1×10^9 CFU
- Pre-medication with acetaminophen, additional IV fluids pre- and post-infusion minimize reactions
- No SAEs or deaths reported related to GVAX or CRS-207
- No unexpected Grade 3 or 4 toxicities reported
 - Grade 3/4 non-clinically significant lymphopenia observed in up to 30% of subjects

Grade 3 or Greater Related AEs

| Event | Arm A (GVAX + CRS-207) (N=60) |
|------------------------------------|-------------------------------------|
| Fever | 3 |
| AST, elevated | 2 |
| Hypophosphatemia | 2 |
| ALT, elevated | 1 |
| Lymphopenia | 2 |
| Neutropenia | 1 |
| Fatigue | 2 |
| Rigors/Chills | 1 |
| Hyperglycemia | 1 |
| Pain at injection site (post-GVAX) | 1 |

Phase 1 Study of Safety and
Immunogenicity of ADU-623 in Subjects
with Treated and Recurrent WHO
Grade III/IV Astrocytomas

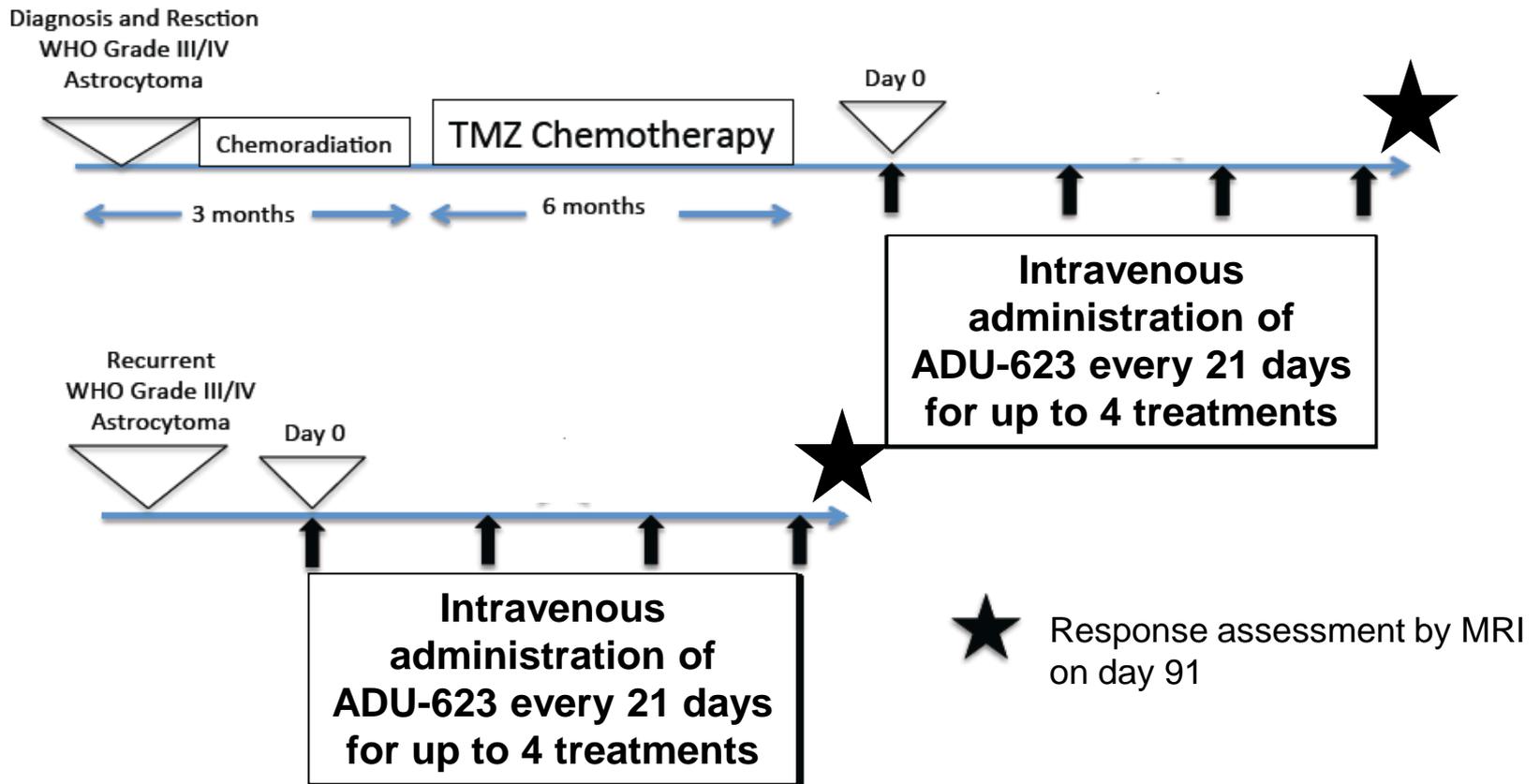
Rationale

- High-grade Astrocytomas are associated with a poor prognosis and universal recurrence and fatality despite the use of combination surgery, radiation and chemotherapy
- EGFRvIII neo-antigen is expressed on high-grade Astrocytomas and is associated with a poor prognosis and resistance to therapies
- NY-ESO-1 is expressed on the CD133 cancer stem cell subset of GBM and can provide helper epitopes for generation of cellular and humoral specific immune responses
- We propose that an *Listeria monocytogenes*-based vaccines targeting EGFRvIII and NY-ESO-1 can generate robust innate, humoral and cellular immune responses capable of targeting high-grade astrocytomas and enhancing survival
- We are proposing a phase 1 dose escalation study of ADU-623 to identify a maximum tolerated dose and characterize the safety profile of this agent in patients with treated and recurrent high-grade astrocytomas

Study Objectives

- Primary objective:
 - To identify the maximally tolerated dose (up to a dose of 1×10^9 CFU IV) and characterize the safety profile of ADU-623 vaccine in patients with treated and recurrent WHO Grade III/IV astrocytomas
- Secondary objectives:
 1. Determine the progression free survival, time to progression and overall survival rates in patients vaccinated with ADU-623.
 2. Characterize EGFRvIII-, NY-ESO-1-, vector-specific and innate immune responses in patients after vaccination.

Study Design



Dose Escalation Schema

- Eligible patients will be registered and assigned consecutively to one of these cohorts
- The dose levels for ADU-623 vaccine will be as follows:
 - Cohort 1: 3×10^7 CFU IV on Day 0, 21, 42, 63
 - Cohort 2: 3×10^8 CFU IV on Day 0, 21, 42, 63
 - Cohort 3: 1×10^9 CFU IV on Day 0, 21, 42, 63

Dose Escalation Schema

| Number of Patients with DLT in a Given Cohort | Escalation Decision Rule |
|--|--|
| 0 out of 3 | Enter 3 patients in the next cohort |
| $\begin{array}{c} > 2 \\ - \end{array}$ | Dose escalation will stop. The dose level of the current cohort will be declared the maximally administered dose (highest dose administered). Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose. |
| 1 out of 3 | Enter 3 more patients at this dose level. <ul style="list-style-type: none"> • If 0 of these 3 patients experience DLT, proceed to the next dose level. • If 1 or more of this group suffer DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose. |
| $\begin{array}{c} < 1 \\ - \end{array}$ out of 6 at highest dose level below the maximally administered dose | This is generally the recommended phase II dose. At least 6 patients must be entered at the recommended phase II dose. |

Inclusion/Exclusion Criteria

- Patients with a pathologic diagnosis of WHO Grade III or Grade IV astrocytic tumors that have completed standard of care external beam radiation therapy and concurrent temozolomide followed by adjuvant temozolomide or with radiographic evidence of progression following standard of care radiation and chemotherapy treatment, including those who have gone on to a second surgical resection. Use of concurrent bevacizumab will be allowed as indicated in recurrent GBM.
- Eastern Cooperative Oncology Group (ECOG) performance status 0 or 1 or KPS performance Status of 70-100.
- Laboratory values (performed within 5 days prior to enrollment) as follows:
 - White blood count (WBC) $\geq 3,500/\mu\text{l}$
 - Absolute neutrophil count (ANC) $\geq 1,500/\mu\text{l}$
 - Hemoglobin (Hgb) $\geq 9\text{g/dl}$ (patients may be transfused to reach this level)
 - Platelets $\geq 90,000$ cells/ μl
 - CD4 $> 0.2 \times 10^9/\text{L}$
 - Creatinine $\leq 2\text{x}$ upper limit of laboratory normal
 - AST/ALT $< 2\text{x}$ upper limit of laboratory normal
 - Alkaline Phosphatase $\leq 2.5\text{x}$ upper limit of laboratory normal
 - Total bilirubin \leq upper limit of laboratory normal
 - Lymphocyte ≥ 500 cc/ml

Inclusion/Exclusion Criteria

- Exclusion
 - Allergy to Sulfa and Penicillin
 - Immunocompromised
 - Significant cardiac or liver disease
 - Ongoing infection or intercurrent illness
 - Unhealed surgical wound
 - Foreign body that is not easily removed
 - Active autoimmunity
 - Requirement for chronic systemic steroid use
 - Pregnant or lactating

Summary and Conclusion

- Improved therapeutic approaches are greatly needed for glioblastoma multiforme
- This tumor as well as the cancer stem cells have been demonstrated to express EGFRvIII and NY-ESO-1
- ADU-623 is a live-attenuated double deleted (LADD) strain of *Listeria monocytogenes* that induces EGFRvIII- and NY-ESO-1-specific immunity
- IV administrations of LADD-based strains have been well-tolerated in other cancers
- The potential safety concerns are addressed in the proposed Phase 1 clinical study and support initiation of the clinical trial