I. ABSTRACT

Background:
Merkel cell carcinoma (MCC) is an aggressive skin cancer with suboptimal therapeutic options. Despite persistent expression of polyomavirus (MCPyV) antigens, MCC has a poor prognosis with a 5-year survival rate of 50%. Effective therapeutic strategies are urgently needed.

Aim:
The aim of this pilot study is to evaluate the immune-modulating properties of intratumoral (IT) G100, an active immunotherapy, in patients with MCC.

Methods:
Eight patients with MCC were enrolled for G100 treatment.

Results:
- Pathologic CR following G100 alone was demonstrated in 2 patients with metastatic disease (Cohort B). All 8 patients experienced tumor reduction (PR or CR) as a result of IT G100. Five patients remain recurrence-free 336+ and 467+ days after starting therapy and both patients with metastatic disease successfully completed G100 monotherapy without surgery, radiation (RT), or chemotherapy.

Conclusions:
G100 monotherapy can alter the local tumor microenvironment and stimulate both the innate and adaptive immune responses against MCC.

II. RATIONALE / BACKGROUND

Merkel Cell Carcinoma: An Uncommon Cancer With Strong Rationale For Immunotherapy

- Aggressive tumor with recurrence and metastasis
- Association with polyomavirus (MCPyV)
- Immunosuppressive local microenvironment, and stimulation of anti-tumor CTLs.

III. MCC TRIAL DESIGN AND RESULTS

Trial Design:
- In Cohort A (N=2), patients with non-metastatic disease received 2 IT G100 injections on Day 0 followed by definitive surgery and/or radiation (RT) starting in week 4.
- In Cohort B (N=6), patients with metastatic disease received 2 IT G100 injections on Day 0, followed by weekly IT G100 up to a total of 6 doses.

Characteristics of MCC Patients
- 80% pts enrolled and analyzed are these patients. 8 with MCC alone and 6 with MCC and metastases.
- 2 additional pts recently started therapy and are not included in these analyses (total 10 MCC pts enrolled, 8 with MCC alone and 2 with MCC and metastases).
- Most patients have had previous chemotherapy and radiation, and 4 of 8 patients received prior surgical therapy.

Clinical Outcome
- Pathologic CR Following G100 Alone
  - Cohort A: 2/7 (29%) with biopsy-proven disease successfully completed G100 monotherapy surgery plus RT (CTG2 Az-D417), both 1 patient had a pathologic CR following the G100 injections alone (before RT).
  - Cohort B: 2/7 (29%) with metastatic disease have ongoing partial response (PR): 1 patient is alive with disease and one (MCPvV+) has a complete response (CR) after 2 months of follow-up (day 46). 2 PRs were seen in patients with melanoma-like progression (MCPvV-).
- 10 patients with PR and >50% regression in the injected tumor following G100 therapy. In Cohort B, 100% of patients with a PR to G100 therapy had a significant increase in median tumor infiltrating lymphocytes (TIL). In Cohort A, the median tumor infiltrating lymphocytes increased significantly after the second cycle, which consisted of RT plus G100. 1 patient presented a FNH following combined G100 and radiation.

IV. IMMUNOLOGIC ACTIVITY

Increased CDDR+ & CDDR+ MCPyV-specific TILs Post-G100

Increased CDDR+ & CDDR+ MCPyV-specific TILs Post-G100:

- Pathologic CR following G100 alone was demonstrated in 2 patients with metastatic disease. Both patients experienced a complete response to G100 monotherapy without surgery, radiation (RT), or chemotherapy. Both patients have remained recurrence-free for >336 days.

- Intratumoral G100 induces increased infiltration of CDDR+ & CDDR+ MCPyV-specific TILs into tumor, which correlates with clinical responses.

- Additional studies of G100 are planned:
  - Further characterization of changes in anti-tumor immune responses, both local (IT) and systemic (PBMCs), as a result of IT G100 treatments are ongoing.
  - Future studies in MCC, NHL or other indications may include checkpoint inhibitors such as anti-PD-1 or other immune modulators.

V. SUMMARY

- G100 is a potent TLR4 agonist that can stimulate both the MyD88 and TRIF/TRAM signaling pathways to potentially stimulate innate immunity via NF-kB and type 1 IFN (Panel A).
- Intratumoral (IT) G100 therapy alone was effective in reducing tumor size in some patients, which correlated with clinical responses.
- Cancer patients treated with G100 showed increased infiltration of CD8+ T-cells post-treatment compared to pretreatment.
- Intratumoral G100 delivery can alter the local tumor microenvironment and stimulate both the innate and adaptive immune responses against MCC.
- G100 induces increased infiltration of CDDR+ & CDDR+ MCPyV-specific TILs into tumor, which correlates with clinical responses.

VI. FUTURE PLANS

- Further characterization of changes in both innate and tumor immunity responses, both local (IT) and systemic (PBMCs), as a result of IT G100 treatments are ongoing.
- Additional studies of G100 are planned:
  - Further characterization of changes in anti-tumor immune responses, both local (IT) and systemic (PBMCs), as a result of IT G100 treatments are ongoing.
  - Future studies in MCC, NHL or other indications may include checkpoint inhibitors such as anti-PD-1 or other immune modulators.

G100: Glucopyranosyl liposome A stable Emulsion

G100 Induces Production of Key Cytokines and Activation of Matured DCs

G100 Targets TLR4 And Potently Activates Genes of the Innate Immune System

- G100 is a TLR4 specific agonist that correlates both the MyD88 and TRIF/TRAM signaling pathways to potentially stimulate innate immunity via NF-kB and type 1 IFN (Panel A).
- As shown in Panel A (TLR4 agonists) and Panel B (IFN-γ), G100 activates an accessible cell surface receptor.
- G100 consists of glucopyranosyl lipid A (GLA)-formulated as a stable emulsion (GLA-SE).
- The GLA-SE formulation maximizes the induction of anti-tumor genes with a greater component dose (GLA is in nontoxic form).
- In addition, the emulsion formulation promotes the ability of the TLR4 agonist against the innate and adaptive immunity responses.
- With the emulsion formulation, the G100 is more potent in stimulating key immune-related genes than MPL, another TLR4 agonist.

- G100 induces increased infiltration of CD8+ T-cells post-treatment compared to pretreatment.
- Infiltration was associated with 28% regression of the injected tumor at week 6 and with increased infiltration of CD8+ T-cells post-treatment (Fig. 3).
- Specific CD8+ T-cells (tetramer pos) in a draining lymph node without any tumor cells.

- MCPyV-specific T cells identified in tumor and draining lymph node (see panel E).
- Pre-treatment biopsy (left panels) demonstrate MCC on H&E and by cytokeratin 20 staining.
- MCPvV-specific T cell identified in tumor and draining lymph node (see panel E).

- The intratumoral therapy in patients with advanced stage MCC is promising.
- Clinical activity was observed in patients with metastatic disease.
- G100 monotherapy can alter the local tumor microenvironment and stimulate both the innate and adaptive immune responses against MCC.
- G100 induces increased infiltration of CDDR+ & CDDR+ MCPyV-specific TILs into tumor, which correlates with clinical responses.
- Additional studies of G100 are planned:
  - Further characterization of changes in anti-tumor immune responses, both local (IT) and systemic (PBMCs), as a result of IT G100 treatments are ongoing.
  - Future studies in MCC, NHL or other indications may include checkpoint inhibitors such as anti-PD-1 or other immune modulators.

- Intratumoral G100 delivery can alter the local tumor microenvironment and stimulate both the innate and adaptive immune responses against MCC.
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  - Further characterization of changes in anti-tumor immune responses, both local (IT) and systemic (PBMCs), as a result of IT G100 treatments are ongoing.
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- G100 is a fully synthetic GLA and nontoxic immunomodulating agent that does not induce toxicity in cancer patients. Intratumoral G100 administration in cancer patients has been shown to stimulate both the innate and adaptive immunity responses against tumor cells.
- G100 induces infiltration of DCs and an upregulation of co-stimulatory molecules on DCs.
- Intratumoral G100 delivery can alter the local tumor microenvironment and stimulate both the innate and adaptive immune responses against MCC.
- G100 targets TLR4 and potently activates genes of the innate immune system.

References:
Lemos, et al. J. American Academy Dermatology 2010
Strong Rationale For Immunotherapy

- GLA at the Core
• GLA is a potent TLR4 agonist
• GLA is a more potent TLR4 agonist compared to MPL
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