Preliminary immunogenicity results from the dose escalation phase of a first-in-human study of the mRNA-based cancer vaccine CVGBM in patients with newly diagnosed MGMT-unmethylated glioblastoma

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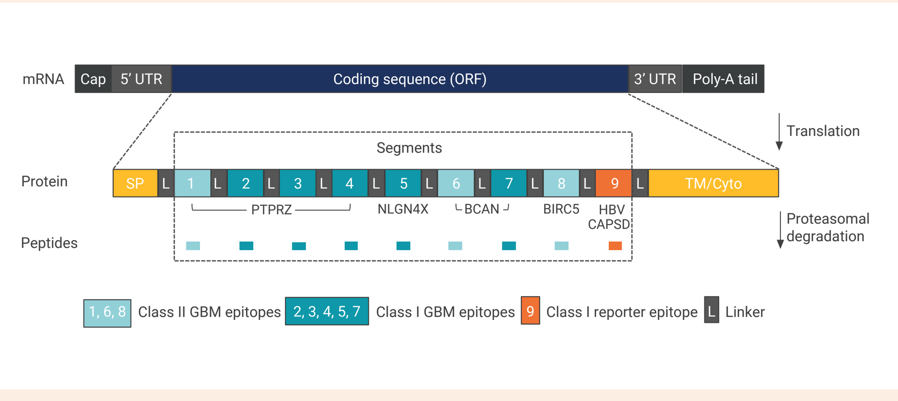
Poster 697

BACKGROUND AND AIMS

- The prognosis in patients with MGMT-unmethylated glioblastoma (GBM) is poor, with a median overall survival of approximately 12 months after initial diagnosis.[1,2]
- Vaccines utilizing various platforms (e.g. peptides, dendritic cells) have demonstrated the ability to induce T cell responses in patients with GBM, with signals of clinical benefit[3,4] and tumor immune infiltration reported.[5]
- mRNA vaccines have been shown to induce CD4+ and CD8+ T cell responses against a variety of cancer antigens,[6] and offer the possibility to encode multiple antigens on a single construct.
- CVGBM is an investigational cancer vaccine based on chemically unmodified mRNA formulated within lipid nanoparticles (LNPs) for treatment of HLA-A*02:01-positive patients with GBM (**Figure 1**).

Figure 1. mRNA vaccine candidate CVGBM



BCAN, brevican; BIRC5, survivin; GBM, glioblastoma; HBV, hepatitis B virus; HBV CAPSD, hepatitis B virus Capsid protein; HLA, human leukocyte antigen; mRNA, messenger ribonucleic acid; NLGN4X, neuroligin; ORF, open reading frame; PTPRZ, receptor-type tyrosine-protein phosphatase zeta B; SP, signal peptide; TM/Cyto, transmembrane and cytosolic domain; UTR, untranslated region.

- The vaccine encodes eight segments derived from four tumor-associated antigens (TAAs) with relevance in GBM, including five HLA-A*02:01 restricted class I, three pan-HLA-DR restricted class II T cell epitopes and one class I reporter epitope (**Figure 1**).
- **The aim of this exploratory analysis was to investigate CVGBM vaccine-induced T cell mediated immunogenicity against four TAAs in the dose escalation part (Part A) of an ongoing, first-in-human study evaluating the safety and immunogenicity of CVGBM in patients with GBM.**

METHODS

STUDY OVERVIEW

- CV-GBLM-001 is an open-label, phase 1 trial consisting of two parts: dose-escalation (Part A) and dose-expansion (Part B).
  - Escalating doses of CVGBM 12–100 µg were investigated in Part A to establish the recommended dose for expansion (RDE) in Part B.
- Eligible patients had newly-diagnosed HLA-A*02:01-positive MGMT-unmethylated GBM (CNS WHO Grade 4) with a gross total or partial resection and completed radiotherapy with or without concomitant temozolomide.
- Patients were to receive seven intramuscular CVGBM vaccinations within ten weeks and six optional maintenance vaccinations in case of non-progression/potential benefit (at the investigator's discretion) (**Figure 2**).

IMMUNOGENICITY ASSESSMENTS

- Patients were evaluable for immunogenicity assessment if baseline samples and ≥1 post-vaccination sample were available for analysis.
- Antigen-specific CD4+ and CD8+ T cell responses in peripheral blood mononuclear cells (PBMC) were assessed by interferon (IFN)-γ ELISpot (*ex vivo* and after *in vitro* stimulation [IVS]; **Figure 3**) at pre-determined timepoints (**Figure 2**).
  - **Immune response criteria**: spot count above in-study threshold; ≥2-fold higher than background; and ≥2-fold above baseline.
- The following chemokines and cytokines were measured in serum samples on Days 1, 2, 8, 43, 44, 71, 72, and 78 using Luminex® and SIMOA® assays: GM-CSF, IFN-γ, IL-2 to IL-8, IL-10, IL-18, MCP-1, MIP-1α, TNF-α, TNF-β, CXCL10 (IP-10) and CXCL9 (MIG), IFN-α, and IFN-β.

RESULTS

PATIENTS

- In total, 16 patients were enrolled in the dose escalation part: three patients each received CVGBM 12 µg, 25 µg, 50 µg, and seven received CVGBM 100 µg.
- Most patients were male (75%), mean age was 54 years, and two patients (12.5%) were using steroids at baseline (**Table 1**).

Table 1. Patient demographics and baseline characteristics

	Dose level 1 12 µg (n=3)	Dose level 2 25 µg (n=3)	Dose level 3 50 µg (n=3)	Dose level 4 100 µg (n=7)	Total (N=16)
Sex, n (%)					
Female	0 (0.0)	2 (66.7)	0 (0.0)	2 (28.6)	**4 (25.0)**
Male	3 (100.0)	1 (33.3)	3 (100.0)	5 (71.4)	**12 (75.0)**
Age, years, mean	59.7	69.7	46.3	48.4	**54.1**
Age group, n (%)					
<65 years	3 (100.0)	1 (33.3)	3 (100.0)	7 (100.0)	**14 (87.5)**
≥65 years	0 (0.0)	2 (66.7)	0 (0.0)	0 (0.0)	**2 (12.5)**
Karnofsky PS (%), mean (SD)	83.3 (15.3)	90.0 (10.0)	90.0 (10.0)	90.0 (8.2)	**88.8 (9.6)**
Resection history, n (%)					
Partial	1 (33.3)	1 (33.3)	2 (66.7)	3 (42.9)	**7 (43.8)**
Complete	2 (66.7)	2 (66.7)	1 (33.3)	4 (57.1)	**9 (56.3)**
Baseline steroid use, n (%)					
Yes	0 (0.0)	1 (33.3)	1 (33.3)	0 (0.0)	**2 (12.5)**
No	3 (100.0)	2 (66.7)	2 (66.7)	7 (100.0)	**14 (87.5)**

PS, performance status; SD, standard deviation.

SAFETY

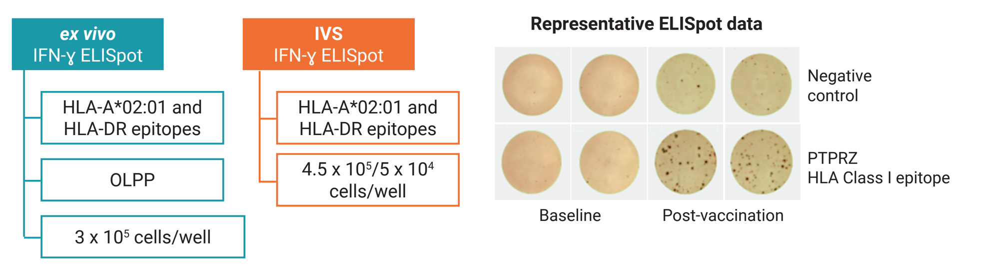
- All patients completed the 2-week dose-limiting toxicity (DLT) evaluation period without any DLT reported.
- The majority of reported TRAEs (90%; 95/105 events) were mild-to-moderate systemic reactions (Grade 1/2), with the most common events reported as fever, chills, fatigue, and headache that resolved within 1–2 days post injection.
- Nine Grade 3 TRAEs were reported in 7/16 patients: 1 tumor pseudoprogression, 2 brain edema, 1 leukoencephalopathy, 1 hypertension, 1 epilepsy, 1 ataxia, 1 malaise, and 1 fever. These events were evenly distributed across dose cohorts.
  - Four of these events were reported as serious adverse events (SAEs; brain edema, leukoencephalopathy, epilepsy, and ataxia).
  - At the data cut-off on 29 February 2024, ataxia Grade 3 was reported associated with worsening of pre-existing leukoencephalopathy (Grade 2), upgraded to Grade 3 on 4 March 2024.
  - From the data cut-off date of 29 February 2024 until 17 October 2024, no additional treatment-related SAEs or Grade 3 TRAEs were reported.
- CVGBM 100 µg was selected as the recommended dose for expansion in Part B of the study.

IMMUNOGENICITY

Antigen-specific T cell responses to CVGBM

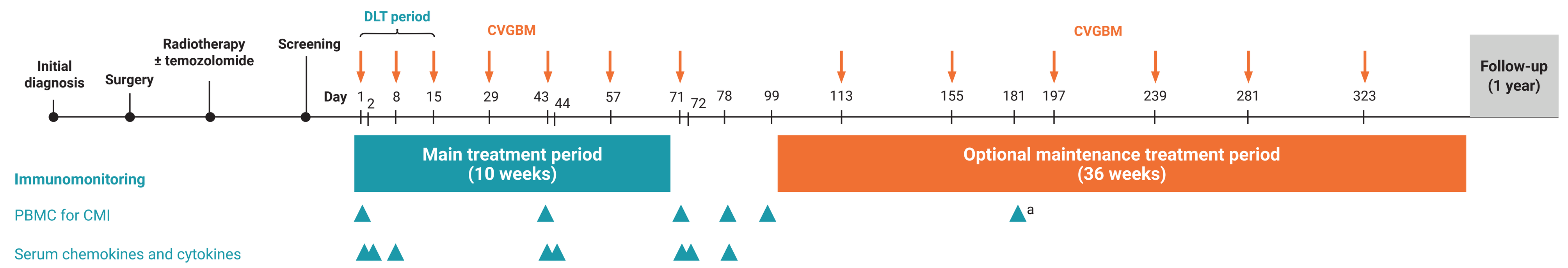
- Overall, 10/13 evaluable patients (77%) had a TAA-specific T cell response (**Figure 4**).
  - 9/13 (69%) showed a CD8+ T cell response, while 31% (4/13) showed a CD4+ T cell response against ≥1 TAA post-baseline.
  - 6/9 (67%) showed a T cell response against ≥2 antigens.

Figure 3. Overview of cell-mediated immunity assessment by IFN-γ ELISpot



ELISpot, enzyme-linked immunosorbent spot; HLA, human leukocyte antigen; IVS, *in vitro* stimulation; PTPRZ, receptor-type tyrosine-protein phosphatase zeta B; OLPP, overlapping peptide pool.

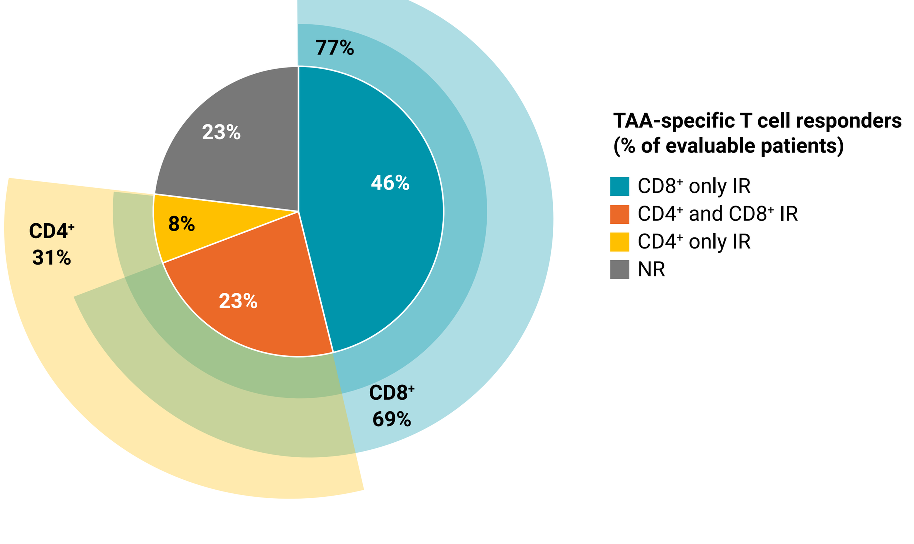
Figure 2. Study design and assessments (Part A)



[a]T cell assessment on Day 181 only performed in patients who did not continue into the optional maintenance treatment period.
CMI, cell-mediated immunity; DLT, dose-limiting toxicity; PBMC, peripheral blood mononuclear cell.

Figure 4. Antigen-specific T cell responses detected by IFNγ ELISpot in PBMC

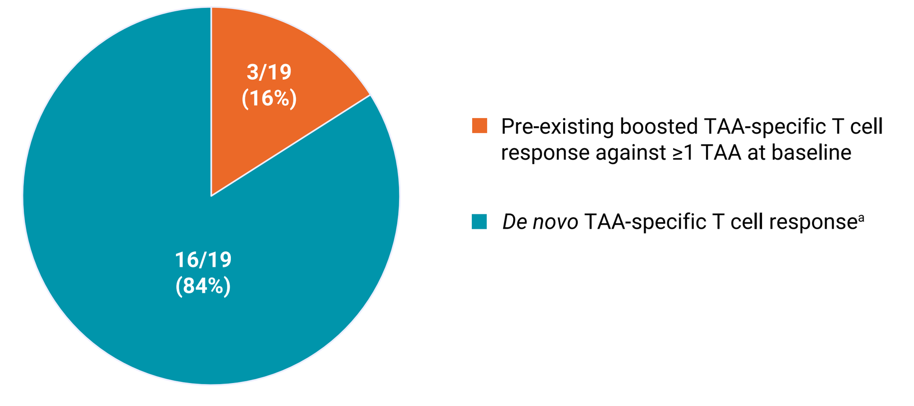
Antigen-specific T cell responses to CVGBM	n (%)
TAA-specific T cell responders (CD4+ and/or CD8+)[a]	**10 (77)**
TAA-specific CD8+ T cell responders[a]	9 (69)
TAA-specific CD4+ T cell responders[a]	4 (31)
TAA-specific CD4+ and CD8+ T cell responders[a]	3 (23)
HBV (reporter antigen) CD8+ T cell responders[b]	6 (46)
TAA-specific T cell responders against ≥2/4 encoded TAAs[c]	6/9 (67)



[a]Patients with immune response against ≥1 TAA; [b]HBV reporter antigen, detected by IFN-γ ELISpot in ≥1 timepoint after baseline; [c]Patients with immune response against ≥2/4 encoded TAAs (only 9 patients could be evaluated for multiple responses).
HBV, hepatitis B virus; IR, immune responder; NR, non-responder; PBMC, peripheral blood mononuclear cell; TAA, tumor-associated antigen.
Data cut-off: 23 July 2024; data are preliminary and partially cleaned.

- A total of 19 T cell responses against individual TAAs were detected in the 10 responders.
  - 84% of the responses were primed and activated *de novo* by CVGBM (**Figure 5**).

Figure 5. Immune responses against individual TAAs (% of antigen responses)



[a]*De novo* post-baseline immune response without detectable pre-existing immune response at baseline.
TAA, tumor-associated antigen. Data cut-off: 23 July 2024; data are preliminary and partially cleaned.

Immunogenicity of TAAs upon CVGBM vaccination

- Immunogenicity was detected against all TAAs encoded in the CVGBM vaccine (**Figure 6**).

Figure 6. Immune response by TAA

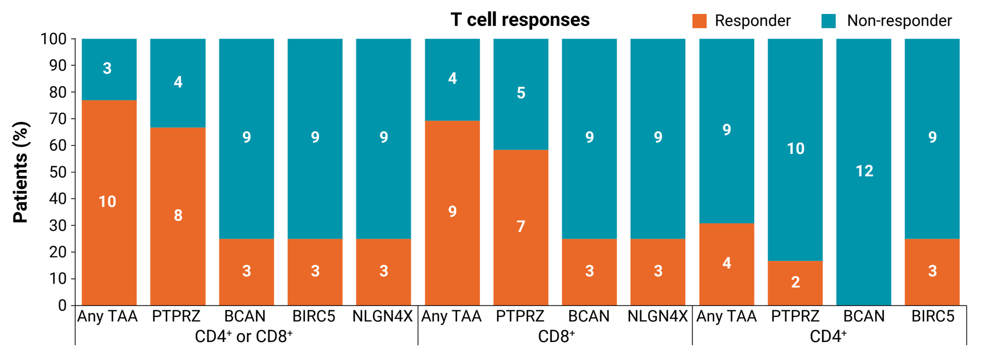
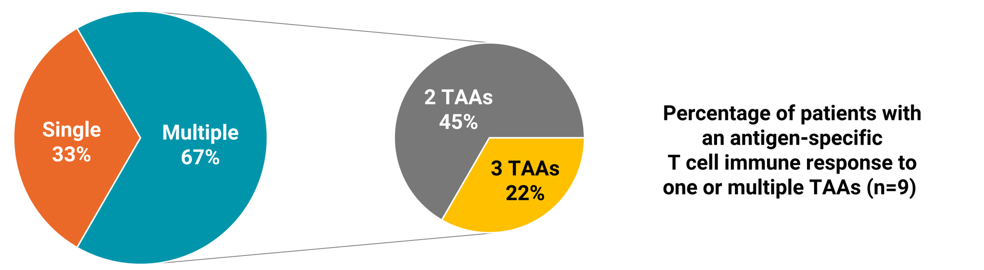


Figure shows the percentage of patients showing an immune response against any, as well as individual, TAAs in three subcategories: CD4+ or CD8+, CD8+, or CD4+ T cell responses. NLGN4X and BIRC5 only included HLA Class I and Class II epitopes, respectively. Responses were measured by ELISpot assay, and analyses were performed on TAAs. For one patient, samples could only be analyzed using Class I and II peptide pools.
BCAN, brevican; BIRC5, survivin; HLA, human leukocyte antigen; NLGN4X, neuroligin; PTPRZ, receptor-type tyrosine-protein phosphatase zeta B; TAA, tumor-associated antigen.

- The majority of immune responders generated CD4+/CD8+ T cell responses to ≥2 TAAs (**Figure 7**):
  - 33% responded to 1 TAA
  - 45% responded to 2 TAAs
  - 22% responded to 3 TAAs
- 5/8 (62%) of CVGBM-encoded known TAA epitopes were immunogenic.

Figure 7. Single and multiple immune responders

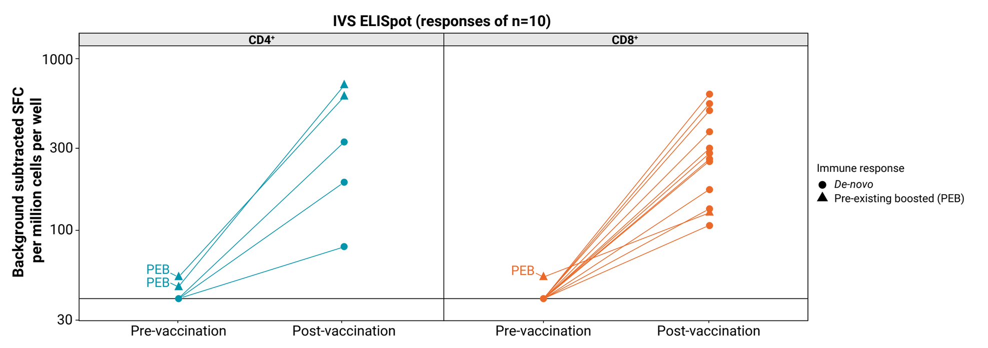


TAA, tumor-associated antigen.

Magnitude of T cell responses detected after vaccination

- Induced T cell responses post CVGBM administration measured by IFN-γ release, in terms of maximal spot forming cells (SFC) after *in vitro* stimulation, are shown in **Figure 8**.
  - Most responses were *de novo* activated.
  - Three pre-existing responses at baseline were boosted post vaccination (NLGN4X, PTPRZ, and BIRC5); these occurred in the same patient.

Figure 8. CVGBM-induced maximum CD4+ and CD8+ T cell responses

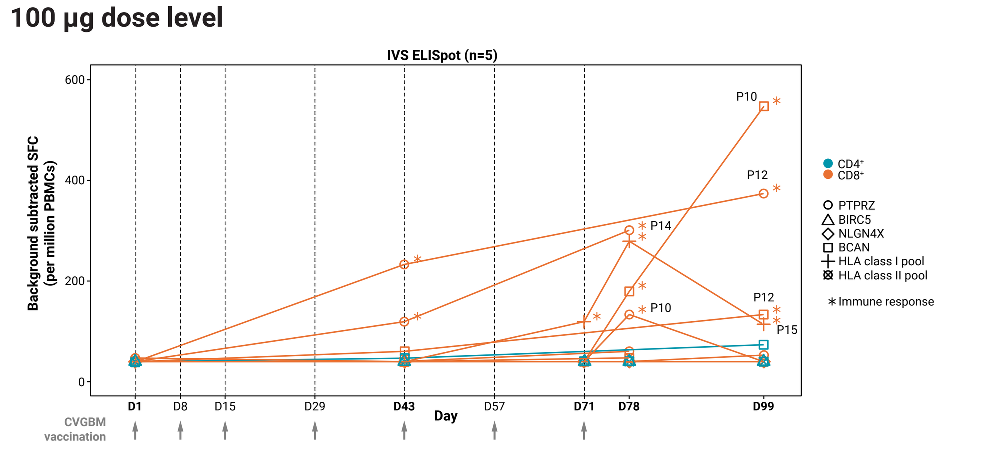


Maximum antigen-specific T cell responses detected in all responders (n=10). Antigen-specific T cell responses measured by IFN-γ ELISpot assay at baseline (pre-vaccination) and post-vaccination in samples from all cohorts. Cells were stimulated using HLA-A*02:01 and/or HLA-DR-restricted CD8+ and/or CD4+ T cell epitopes in culture for 7 days and SFC were normalized per million cells.
Tumor antigen-derived CD4+ T cell epitopes are shown in blue and CD8+ T cell epitopes in orange.
ELISpot, enzyme-linked immunosorbent spot; IVS, *in vitro* stimulation, PEB, pre-existing boosted immune response; SFC, spot forming cells.
Data cut-off 23 July 2024.

TAA-specific immune responses over time after vaccination with CVGBM

- At the recommended dose for expansion (100 µg), 4/5 evaluable patients (80%) showed a TAA-specific CD8+ T cell response against ≥1 TAA (**Figure 9**).

Figure 9. TAA-specific T cell responses over time observed at the 100 µg dose level

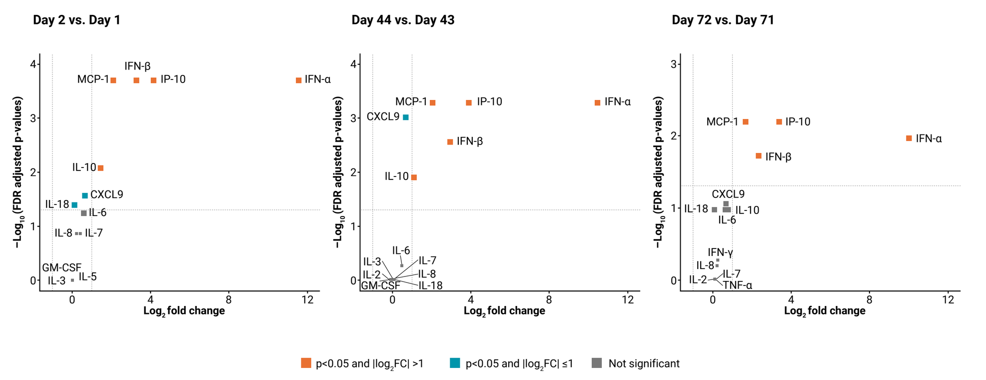


CVGBM vaccine-induced immune responses at baseline (Day 1) and at pre-determined post-vaccination timepoints. Tumor antigen-specific T cell responses were measured by IFN-γ ELISpot using PBMCs at timepoints marked in bold (X axis). Cells were stimulated using HLA-A*02:01 and/or HLA-DR-restricted CD8+ and/or CD4+ T cell epitopes in culture for 7 days, and SFC were normalized per million cells. Responders are represented by coded patient number (Px) and CVGBM-encoded TAAs by different symbols. Not all timepoints could be analyzed for all patients.
BCAN, brevican; BIRC5, survivin; ELISpot, enzyme-linked immunosorbent spot; HLA, human leukocyte antigen; IVS, *in vitro* stimulation; NLGN4X, neuroligin; PBMC, peripheral blood mononuclear cell; PTPRZ, receptor-type tyrosine-protein phosphatase zeta B; SFC, spot forming cells.

Innate immune response induction following vaccination

- A CVGBM-induced increase in systemic cytokine and chemokine levels was observed within 24 hours following the 1st, 5th and 7th vaccinations, including type I interferons, interferon-regulated proteins (IP-10 and MCP-1/CCL2) and immunomodulating IL-10 (**Figure 10**).
- Correlation analysis with reactogenicity data to support the safety profile is ongoing.

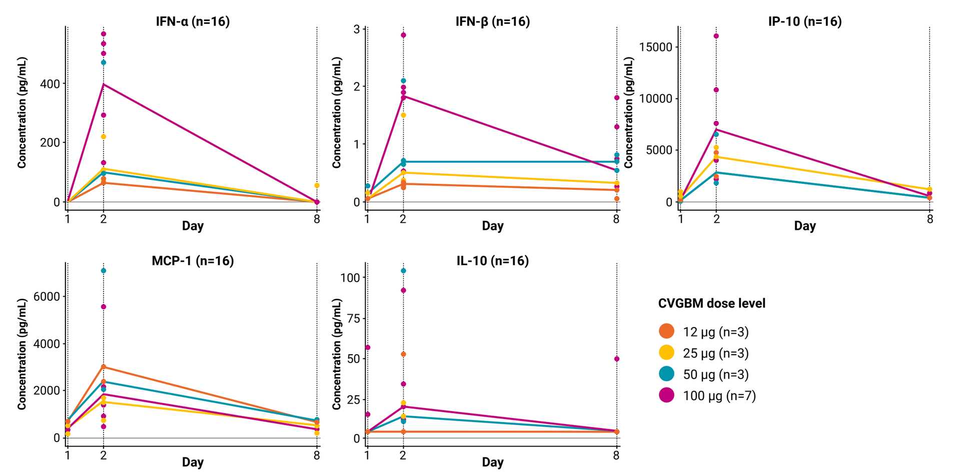
Figure 10. Overall profile of cytokine and chemokine induction



Secretion of innate immune cytokines in patients from all cohorts (n=16). Volcano plots show the magnitude of change (calculated by mean $\log_2$ fold change) versus the statistical significance represented by $-\log_{10}$ of the Benjamini-Hochberg corrected p-values between the time points for the tested cytokines. Orange squares indicate a statistically significant ($p<0.05$ and $|\log_2 FC| >1$) increase compared with baseline; blue squares indicate a statistically significant low magnitude ($p<0.05$ and $|\log_2 FC| \leq 1$) increase compared with baseline; and grey squares indicate a non-statistical significance ($-1 \leq \log_2 FC \leq 1$) versus baseline. High magnitude increases (orange squares) are also supported by the Wilcoxon effect size, which is reflected in the size of the squares (large squares have an effect size ≥0.5).
CXCL9, CXC motif chemokine ligand 9; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; IP, interferon gamma induced protein; MCP, monocyte chemoattractant protein-1; TNF, tumor necrosis factor.

- The induction of cytokines and chemokines upon vaccination is transient; although not statistically assessed, a tendency of higher induction was seen at the 100 µg dose level (**Figure 11**). Data shown include timepoints within the DLT period.

Figure 11. Transient induction of IFN-α, IFN-β, IP-10, MCP-1 and IL-10



Cytokine concentration (pg/mL) before vaccination at baseline (Day 1) and post-vaccination timepoints (Days 2 and 8). Measurements of serum samples performed using human custom MAP and SIMOA technology (IFN-α and IFN-β). Median cytokine/chemokine levels are shown by CVGBM dose level; each CVGBM dose level is represented by different colors (for IP-10, median lines for 12 and 50 µg doses overlap; for IL-10, median lines for 25 and 50 µg doses overlap). Sample size ranges from 3–7 patients, in a total of 16.
Data for subsequent timepoints not shown.
IFN, interferon; IL, interleukin; IP, interferon gamma induced protein; MCP, monocyte chemoattractant protein-1.

KEY TAKEAWAYS

- CVGBM was **generally well tolerated** up to a dose level of 100 µg in patients with newly-diagnosed and surgically resected MGMT-unmethylated glioblastoma.
- Most treatment-related AEs were **mild-to-moderate systemic reactions** such as headache, fatigue, fever and chills that **resolved within 1–2 days** post injection.

- Preliminary immunogenicity results with CVGBM demonstrate:

77% of evaluable patients showed a CD4+ and/or CD8+ T cell response against ≥1 TAA

84% of the detected responses were induced *de novo*

67% of evaluable patients showed an antigen-specific T cell immune response to multiple TAAs

- Immunomonitoring of TAA-specific T cells over time in the 100 µg dose group showed a sustained response profile.
- **IFN-α, IFN-β, IP-10, MCP-1 and IL-10** were transiently induced following CVGBM vaccination.

100 µg

selected as the **recommended dose for the dose expansion phase (Part B)**, which recently started enrollment.

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