Oncolytic Virus CF33-hNIS Monotherapy for the Treatment of Gastrointestinal Malignancies

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Background

- CF33 is a novel chimeric oncolytic poxvirus, encoding the human Sodium-Iodide Symporter (hNIS) transgene. The transgene is inserted in place of the viral thymidine kinase gene at the J2R locus, resulting in attenuation of viral replication in normal cells. The engineered virus selectively replicates in tumor cells and leads to tumor cell lysis, releasing tumor- and virus-associated antigens and stimulating antitumor immunity.
- The MAST study is an open-label, dose-escalation, multi-center phase I study evaluating the safety of CF33-hNIS administered intratumorally (IT) or intravenously (IV), either as a monotherapy or in combination with pembrolizumab in patients with metastatic or advanced solid tumors.

Methods

Study Design

The MAST study is evaluating the safety of CF33-hNIS administered IT or IV, alone or in combination with pembrolizumab in patients with advanced or metastatic solid tumors with ≥ 2 prior lines of therapy (NCT05346484). CF33-hNIS is administered in 21-day cycles on C1D1 & C1D8, then D1 of each cycle thereafter. Pembrolizumab begins C2D1 for the combination groups and is administered Q3W. The study consists of two parts. Part 1 follows a 3+3 dose escalation scheme independent of each route of CF33-hNIS administration (IT and IV) and for each therapy regimen (monotherapy and combination therapy) with up to 7 dose levels of CF33-hNIS ranging from $8.6x10^5$ to $3.0x10^9$ PFU. Part 2 is a cohort expansion in select indications at the optimal dose (**Figure 1**).

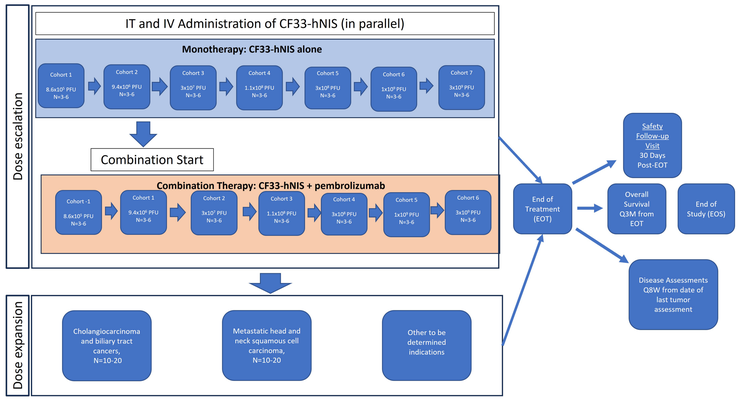

Figure 1. MAST study design

Study Objectives

- The co-primary endpoints are safety and identification of the recommended phase 2 dose.
- Secondary endpoints include objective response rate according to RECIST v1.1 and iRECIST, and assessment of viral replication in tumor lesions via Single-Photon Emission Computerized Tomography (SPECT).
- Evaluation of anti-tumor immune activation

Translational research - Cytometry by Time of Flight (CyToF) analysis of patient immune cells.

- CyTOF analysis was used to characterize changes in immune activation with pre- and post-treatment peripheral blood mononuclear cells (PBMCs) using a 43-marker panel.
- This panel was used to quantify cell frequencies and protein expression levels with "responders and non-responders", across different timepoints post treatment, and to compare any difference between IT and IV administration.

Results

Patient Demographics

- We report results from seven patients from part 1 of the study with gastrointestinal (GI) malignancies, including: colorectal cancer (3), bile duct (2), pancreatic (1) and hepatocellular carcinoma (1) (**Table 1**). All GI patients received monotherapy CF33-hNIS by either IT or IV administration.
- The median age was 61 (range: 35-79), while the median number of prior systemic therapies was 4 (range: 2-8)

Subject	Tumor type	PD-L1 Status	SEX	Age	Prior lines of systemic therapy	Prior treatment with checkpoint blockade
US02-001	Biliary tract	<1%	M	61	3	N
US03-003	Colorectal		F	35	7	N
US03-006	Colorectal		F	58	8	N
US03-007	Colorectal		F	55	7	N
US03-008	Biliary tract		F	79	2	Y
US07-001	Hepatocellular carcinoma	<1%	F	73	3	N
US15-001	Pancreatic		F	62	4	Y

Results (continued)

Efficacy results from the GI-cancer patients treated with CF33-hNIS monotherapy

- The objective response rate was 14% while the disease control rate was 86% (**Figure 2**).

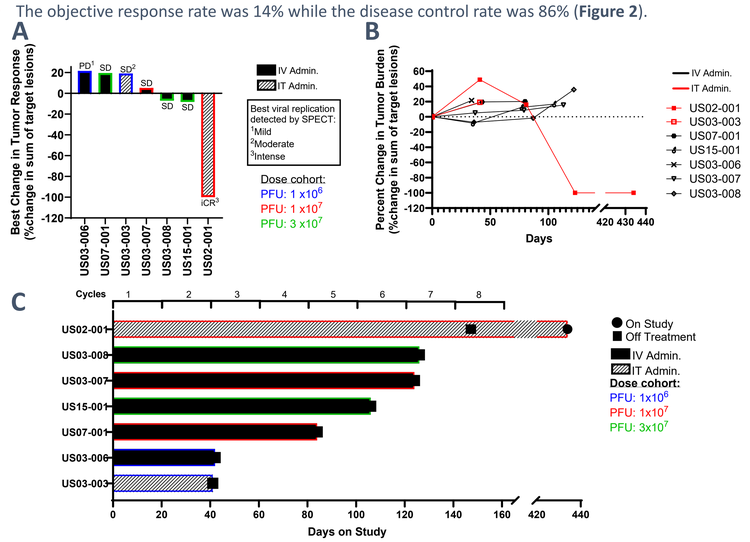

Figure 2. CF33-hNIS monotherapy promotes disease control at a 'low to mid' dose level. (A) waterfall plot depicting the best change in tumor response. The intensity of viral replication assessed via SPECT imaging is also presented. (B) spider plot indicating the percent change in tumor burden over time. (C) swimmers plot indicating the length of study treatment and number of treatment cycles received. Abbreviations: PD, progressive disease; SD, stable disease; iCR, immunological complete response; IV admin, intravenous administration; IT admin, intratumoral administration. Dose levels are rounded up to the nearest whole number.

Patients with biliary tract cancers

- One patient with cholangiocarcinoma, treated IT with $1.0x10^7$ PFU, presented with pseudoprogression with a 49% increase in tumor burden after two cycles of therapy. By the 4th cycle the patient achieved an immunological complete response with no known recurrence after one year (**Figure 3**). A second patient with bile duct cancer, who previously progressed on checkpoint blockade therapy, achieved stable disease for >5 months upon receiving IV-administered CF33-hNIS ($3.0x10^7$ PFU). SPECT analysis of the CR patient reveals intense levels of viral replication within the injected lesion.

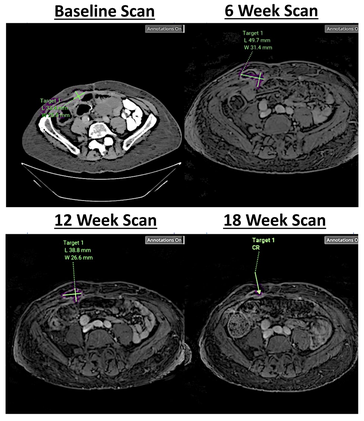

Figure 3. Pseudoprogression followed by immunological complete response in patient with cholangiocarcinoma treated IT with CF33-hNIS monotherapy

Treatment with CF33-hNIS monotherapy in patients with GI cancers is well tolerated

- The most common treatment related adverse events include grade 1 and 2 flu-like symptoms such as pyrexia and fatigue (table 2).
- There were no dose limiting toxicities
- No patients discontinued treatment due to treatment related adverse events

Preferred Term	Grade 1, n (%)	Grade 2, n (%)	≥Grade 3, n (%)
Pyrexia	1 (14.3)	2 (28.6)	0
Fatigue	0	1 (14.3)	0
Injection site pain	1 (14.3)	0	0
Folliculitis	0	1 (14.3)	0
Pneumonia	0	1 (14.3)	0
Rash pustular	1 (14.3)	0	0
Muscle spasms	0	1 (14.3)	0
Myalgia	0	1 (14.3)	0
Lymphadenopathy	1 (14.3)	0	0
Vomiting	1 (14.3)	0	0
Decreased appetite	1 (14.3)	0	0
Headache	0	1 (14.3)	0
Blister	1 (14.3)	0	0
Erythema	1 (14.3)	0	0

Table 2. Treatment related adverse events associated with CF33-hNIS monotherapy (n=7)

Results (continued)

Treatment with CF33-hNIS elicits a robust immune response in patients who respond to treatment

- We divided patents into "responders" and "non-responders" based on an overall increase or decrease in tumor burden, respectively:
  - Responders: US02-001, US15-001, US03-008
  - Non-Responders: US03-007, US03-003, US07-001, US03-006
- Immunological changes in responders are characterized by higher frequencies of T cells, natural killer cells, and monocyte subsets post-treatment. These subsets include gamma delta T cells, central memory CD8 T cells, PD-1+ CD8+ naive T cells, TIGIT+ CD4+ naive and terminal effector memory T cells, as well as KLRG1+ stem cell-like memory T cells (**Figure 4**).

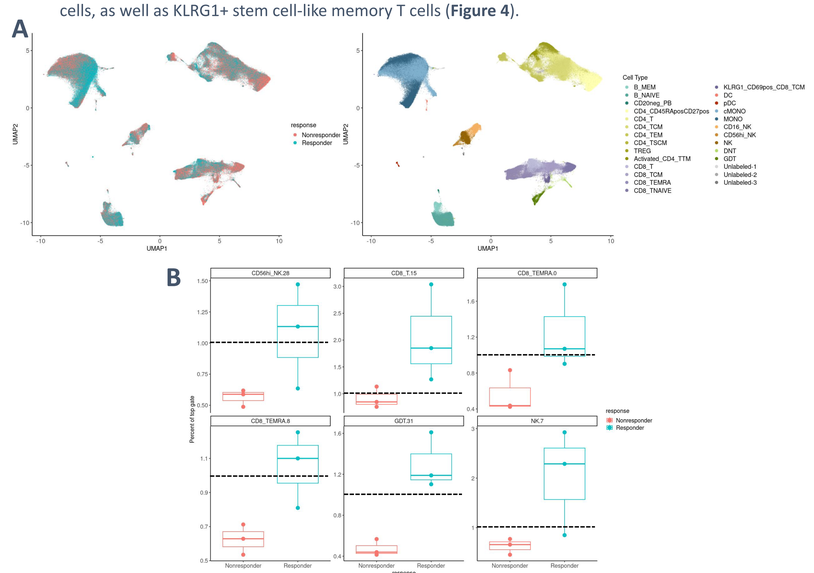

Figure 4. Changes in immune cells between responders and non-responders. (A) Differences in clusters representing different cell populations between responders and non-responders by Uniform Manifold Approximation and Projection (UMAP). (B) Changes in the frequencies of cells in the T and NK cell clusters from C1D1 to C2D2

- Peripheral CD8+ T cells decreased from C1D1 and C2D2 in non-responding patients. Changes in the expression of HLA-DR, CD38, and CD11C in T cell subsets and conventional dendritic cells (DC) were also observed, suggesting diminished DC function and a reduction in the opportunity for antigen cross presentation and the promotion of anti-tumor immunity (**Figure 5**).

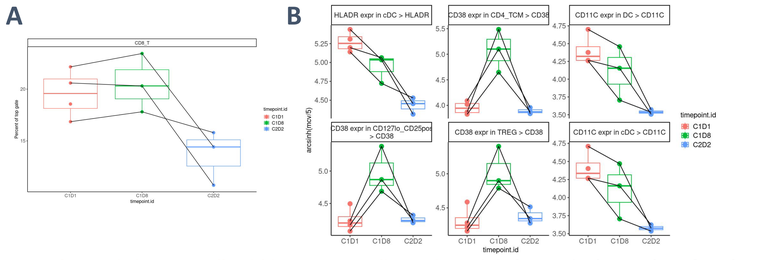

Figure 5. Changes in immune cells within non-responding patients. (A) Changes in the number of total CD8+ T cells in non-responding patients. (B) Changes in the expression of HLA-DR, CD38, and CD11C in T cell subsets and conventional DCs in non-responders.

Conclusions

- This preliminary data from the first three dose levels demonstrates encouraging anti-tumor activity with CF33-hNIS monotherapy. Dose escalation is ongoing.
- CF33-hNIS monotherapy may be an effective and safe treatment option for GI malignancies and warrants further investigation in biliary tract cancer patients.
- Immunological changes in CF33-hNIS responding patients show a robust innate and adaptive immune response known to promote anti-tumor immunity and underscores the immunomodulatory potential of this therapy.