Oncolytic virus CF33-hNIS for the treatment of advanced cancer

Daneng Li [1], Anthony F. Shields[2], Hirva Mamdani[2], Patrick Travis[3], Gavin Wright[4], Wallace Akerley[5], Alex Spira[6], Gregory Daniels[7], Jaime Merchan[8], Abhijeet Kumar[9], Emerson Lim[10], Colt A Egelston[1], Oscar Colunga Flores[1], Stanley Hamilton[1], Janet Austria[1], Amanda Seiz[11], Sharon Yavrom[11], Seymour Fein[11], John Byon[11], Paul Woodard[11], Grey Wilkinson[11], Jennifer Leddon[12]

[1]City of Hope Comprehensive Cancer Center, [2]Barbara Ann Karmanos Cancer Institute, [3]Highlands Oncology, [4]St Vincent's Hospital Melbourne, [5]Huntsman Cancer Institute, [6]New Experimental Therapeutics of Virginia-NEXT Oncology, [7]University of California San Diego, [8]University of Miami, [9]University of Arizona, [10]Spectrum Health Medical Group, [11]Imugene Limited, [12]University of Cincinnati Cancer Center.

IMUGENE
Developing Cancer Immunotherapies

Abstract Number: CT182

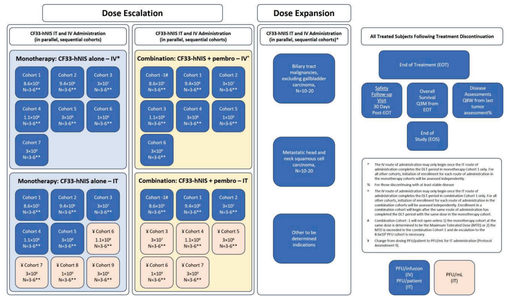
INTRODUCTION

- CF33-hNIS is a novel chimeric oncolytic poxvirus that selectively replicates in tumor cells and leads to tumor cell lysis, releasing tumor- and virus-associated antigens, stimulating anti-tumor immunity.
- CF33-hNIS encodes the human Sodium-Iodide Symporter (hNIS) transgene, which allows for the imaging of viral replication within tumor tissue. The transgene is inserted in place of the viral thymidine kinase gene at the J2R locus, attenuating viral replication in normal cells.
- 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 evaluates 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 dose levels of CF33-hNIS ranging from $8.6x10^5$ to $3.0x10^9$ plaque forming units (PFU). Part 2 is a cohort/dose expansion in select indications (**Figure 1**).



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

- Sequencing of the CDR3 regions of human T-cell receptor-β (TCRβ) chains was performed using Adaptive Immunosequencing (Adaptive Biotechnologies, Seattle, WA). DNA for this assay was isolated from PBMCs collected at C1D1 and C2D2. As previously described, TCRβ CDR3 regions were amplified by a multiplex, bias-controlled PCR with primers targeting the V and J genes of T cells along with primers targeting housekeeping genes to quantitate the total nucleated cells in each sample[1]. PCR products were sequenced on an Illumina NextSeq.
- Multiplex immunofluorescence tumor analysis was performed with formalin-fixed paraffin-embedded tissue (FFPE) collected during screening (pretreatment) or posttreatment (C2D1 or at end or treatment) . FFPE tissue was sequentially staining using primary antibodies: CD8 (Leica, Clone 4B11) Opal 520, PD-L1 (Abcam, Clone SP142) Opal 620, CD3 (Leica, LN10) Opal 540, Granzyme B (Cell Signaling, polyclonal) Opal 650, pan-cytokeratin (Dako, Clone AE1/AE3) or CK20 (Dako, Ks20.8) Opal 690. Tissue slides were scanned using the Vectra 3.0 automated quantitative pathology imaging system (Akoya Biosciences) and image analysis was performed using QuPath software.

RESULTS

Patient Demographics and Enrollment

- As of Dec 2023, 36 patients have been treated in Part 1 of the study with a median age of 59 (range 23-81). Twenty-two (61%) patients were heavily pretreated with ≥3 prior lines of systemic therapy, and 14 (39%) received prior treatment with checkpoint blockade. Tumor types included: colorectal cancer (6), breast (4), sarcomas (4), lung (3), melanoma (3), pancreatic (3), cholangiocarcinoma (2), kidney (2), thyroid (2), bladder (1), hepatocellular carcinoma (1), hidradenocarcinoma (1), mesothelioma (1), neuroendocrine (1), ovarian (1), and testicular (1).
- The following dose levels have cleared the dose limiting toxicity evaluation period as enrollment continues: IT monotherapy ($1.1x10^8$ PFU), IT combination ($9.4x10^6$ PFU), IV monotherapy ($1.1x10^8$ PFU), IV combination ($3.0x10^7$ PFU).

Treatment with CF33-hNIS has been safe and tolerable

- Treatment-related adverse events consisted predominantly of 'flu-like' symptoms, such as grade 1/2: fatigue (31%), pyrexia (22%), and chills (14%). No dose-limiting toxicities have occurred. The highest dose treated to date is $1.1x10^8$ PFU by IT and IV delivery; dose escalation continues.

Preferred Term	Treatment-Related Adverse Events by Severity	
	Grade 1-2	Grade ≥3
Any, n (%)	24 (66.7)	0 (0.0)
Fatigue	11 (30.6)	0 (0.0)
Pyrexia	8 (22.2)	0 (0.0)
Chills	5 (13.9)	0 (0.0)
Cough	5 (13.9)	0 (0.0)
Injection site pain	5 (13.9)	0 (0.0)
Myalgia	5 (13.9)	0 (0.0)
Headache	4 (11.1)	0 (0.0)
Rash pustular	4 (11.1)	0 (0.0)
Decreased appetite	3 (8.3)	0 (0.0)
Erythema	3 (8.3)	0 (0.0)
Nausea	3 (8.3)	0 (0.0)
Vomiting	3 (8.3)	0 (0.0)
Arthralgia	2 (5.6)	0 (0.0)
Injection site erythema	2 (5.6)	0 (0.0)
Pruritus	2 (5.6)	0 (0.0)
Tumor pain	2 (5.6)	0 (0.0)

Note: Only AEs with relationship to CF33-hNIS considered "definite," "possible," or "probable" are included in this table.

Table 1. Summary of Treatment-Related Adverse Events Occurring in >5% of Patients

Efficacy Results

- There are 31 efficacy-evaluable patients in the study. In the IT cohorts, 7 of 17 (41%) injected lesions had a reduction in tumor burden and 3 lesions were completely eradicated. Three IT treated patients had an objective response: 1 complete response by iRECIST in a patient with cholangiocarcinoma and 2 partial responses by RECIST in patients with melanoma. In the IV cohorts, 9 of 17 (53%) patients achieved stable disease as their best response. Patients who received prior checkpoint blockade therapy derived clinical benefit with and without pembrolizumab. Viral replication, assessed by SPECT, was higher in patients who had a reduction in tumor burden.

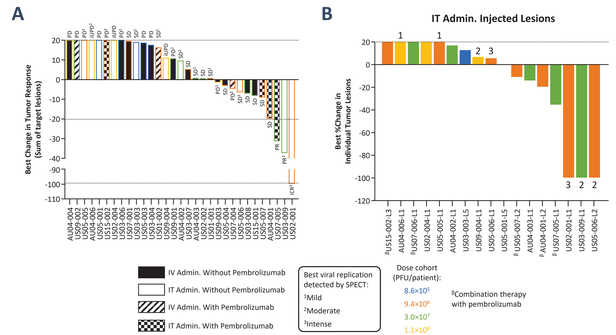


Figure 2. Best Change in Tumor Response. (A) Best Change in Tumor Response by route of administration, monotherapy or combination therapy, dose and levels of viral replication assessed by SPECT. The Best changes in tumor response are listed with either RECIST v1.1 or iRECIST criteria. (B) Best change in individual lesions for IT injected lesions.

High peripheral T cell diversity may serve as a biomarker of response to IT CF33-hNIS

- Peripheral repertoire diversity is higher at baseline in patients that achieved an objective response and after one cycle of therapy, as demonstrated by a low level of Simpson clonality (**Figure 3A**) or a high number of downsampled rearrangements (**Figure 3B**). A hypothetical cut point of 8k downsampled rearrangements (i.e., high diversity) would result in the following response rates from the twenty patients examined: biomarker positive, 3/8 (38%); biomarker negative: 0/12 (0%); overall response: 3/20 (15%). Patients with an objective response also have a greater repertoire turnover within cycle 1, as shown by a lower Morisita index (**Figure 3C**), yet maintained high levels of diversity. Differential abundance analysis of patient US02-001 shows a significant expansion of both new clones (y-axis) and existing clones.

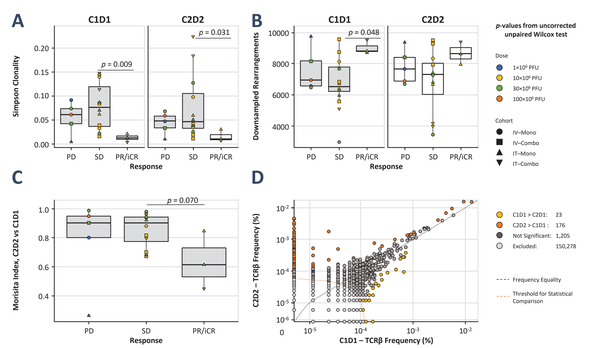


Figure 3. Changes in Peripheral T Cell Repertoire. Differences in Simpson clonality (A) and diversity (B) between patients with progressive disease (PD), stable disease (SD), and partial response/ immunological complete response (PR/iCR). (C) Differences in Morisita index within the above response categories. (D) A representative example of a differential abundance plot of patients who achieved an objective response, from patient US02-001. Orange dots are expanded clones, blue dots are retracted, gray/white dots are clones that have not significantly changed or excluded.

Treatment with CF33-hNIS can transform the tumor microenvironment in both IT and IV treated patients

- A limited number of paired pretreatment and post-treatment tumor biopsies reveals that CF33-hNIS can promote T cell infiltration and upregulate PD-L1 expression within the tumor microenvironment.

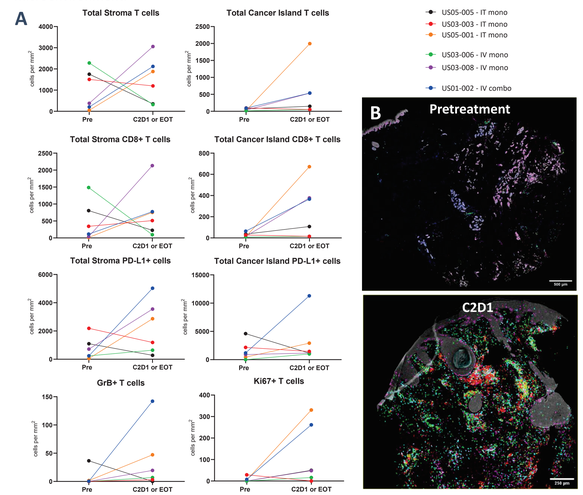


Figure 4. CF33-hNIS can transform the tumor microenvironment. (A) Changes in T cell infiltration, PD-L1, Ki67, and Granzyme B (GrB) expression within the tumor stroma and cancer cell 'islands' in CF33-hNIS treated patients. (B) Representative example of pre- and post-treatment images from patient US05-001. CD3 green, CD8 cyan, Ki-67 magenta, Granzyme B orange, PD-L1 red, pan-CK gray.

CONCLUSIONS

- **CF33-hNIS alone or in combination with pembrolizumab is a safe treatment option for advanced cancer patients. Encouraging efficacy has warranted advancement to part 2 of this study with a cohort expansion in biliary tract cancer and other tumor types.**
- **We have previously shown that CF33-hNIS responding patients show a robust innate and adaptive immune response that promotes anti-tumor immunity[2]. Further analysis of the T cell repertoire reveals that T cell diversity may serve a predictive biomarker, as previously shown for other oncolytic virus-based therapies and may require confirmation in a prospective trial[3,4].**

REFERENCES

1. Carlson CS, et al., Using synthetic templates to design an unbiased multiplex PCR assay. *Nat Commun* 2013;4:2680.
2. Li et al. ASCO-GI Poster (2024), *Journal of Clinical Oncology*, Volume 42, Number 3_suppl.
3. Mahalingam D et al., *Clin Cancer Res*. 2020 Jan 1;26(1):71-81.
4. Samson A et al., *Cancer Immunol Res*. 2022 Jun 3;10(6):745-756

Digital copy

© Poster Template by Genigraphics® 1.800.790.4001 www.genigraphics.com