Targeting the IL4 Receptor with MDNA55 in Patients with Recurrent Glioblastoma: Results of a Phase 2b Trial

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CONFLICTS OF INTEREST

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Manish K. Aghi: None

Krystof Bankiewiecz: None

Martin Bexon: Employed by Medicenna

Steven Brem: Advisor for Novocure, Northwest Biotherapeutics, and Tocagen

Andrew Brenner: None

Chandtip Chandhasin: Employed by Medicenna

Sajeel Chowdhary: None

Melissa Coello: Employed by Medicenna

Benjamin Ellingson: Paid advisor for Medicenna, MedQIA, Neosoma, Agios, Siemens, Janssen, Imaging Endpoints, Kazia, VBL, Oncoceutics, Boston Biomedical Inc (BBI), and ImmunoGenesis. Grant funding from Siemens, Agios, and Janssen.

John R. Floyd: None

Seunggu Han: None

Santosh Kesari: Advisor and grant funding from Medicenna

Yael Mardor: None

Fahar Merchant: Employed by Medicenna

Nina Merchant: Employed by Medicenna

Dina Randazzo: None

Michael Vogelbaum: Honoraria from Tocagen and Celgene. Indirect equity and royalty rights from Infuseon Therapeutics.

Frank Vrionis: None

Eva Wembacher-Schroeder: None

Miroslaw Zabek: None

Nicholas Butowski: Advisor for Hoffman La-Roche; Nativis; Medicenna; Bristol Meyers Squibb; VBL, Pulse Therapeutics, Lynx Group, Boston Bio. Paid Consultant for Nativis; Hoffman La-Roche; Medicenna, DelMar, QED, Karyopharm. Grant funding: Beigene, BMX, Deciphera, DeNovo, Epicentrix, Oncoceutics, Stellar Orbus, Istari, Kiyatec, Amgen, Tocagen.

ABSTRACT

Background: MDNA55 is an IL4R-targeting toxin in development for recurrent GBM, a universally fatal disease. IL4R is overexpressed in GBM as well as cells of the tumor microenvironment. High expression of IL4R is associated with poor clinical outcome.

Method: MDNA55-05 is an open-label, single-arm Phase 2b study of MDNA55 in recurrent GBM (rGBM) patients with an aggressive form of GBM (*de novo* GBM, *IDH* wild-type, and non-resectable at recurrence) on their 1st or 2nd recurrence. MDNA55 was administered intratumorally as a single dose treatment (dose range of 18 to 240 ug) using convection enhanced delivery (CED) with up to 4 stereo-tactically placed catheters. It was co-infused with a contrast agent (Gd-DTPA, Magnevist®) to assess distribution in and around the tumor margins. The flow rate of each catheter did not exceed 10µL/min to ensure that the infusion duration did not exceed 48 hours. Primary endpoint was mOS, with secondary endpoints determining the effects of IL4R status on mOS and PFS.

Results: MDNA55 showed an acceptable safety profile at doses up to 240 µg. In all evaluable patients (n=44) mOS was 11.64 months (80% one-sided CI 8.62, 15.02) and OS-12 was 46%. A sub-group (n=32) consisting of IL4R High and IL4R Low patients treated with high dose MDNA55 (>180 ug) showed best benefit with mOS of 15 months, OS-12 of 55%. Based on mRANO criteria, tumor control was observed in 81% (26/32), including those patients who exhibited pseudo-progression (15/26).

Conclusions: MDNA55 demonstrated tumor control and promising survival and may benefit rGBM patients when treated at high dose irrespective of IL4R expression level.

TRIAL REGISTRATION: Clinicaltrials.gov NCT02858895

KEYWORDS: MDNA55, IL4R, recurrent glioblastoma, Convection Enhanced Delivery (CED), immunotherapy, treatment outcome

KEY POINTS:

Single treatment with MDNA55 increased mOS by up to 50% and 12-month PFS by almost 100% compared to approved therapies"

Advanced CED (with planning software) for accurate catheter placement, real-time imaging to visualize drug distribution.

IMPORTANCE OF STUDY: Despite considerable efforts over the past 4 decades, outcomes for glioblastoma patients continue to be poor with no standard of care available for rGBM. Approved therapies have shown median survival of only 6-9 months, 1-year survival rate of 0-10%, and 12-month PFS rate of 2-10%. In addition, treatment of rGBM is constrained by the blood brain barrier, its aggressive and infiltrative nature, and presence of an immunosuppressive tumor microenvironment. These challenges are exacerbated in patients with primary *de novo* GBM, tumors not conducive to resection upon relapse, and contain wild-type IDH gene. Evaluation of MDNA55 in this poor prognostic group demonstrated a meaningful benefit after a single treatment resulting in ~50% increase in median survival and almost 100% increase in 12-month PFS when compared to approved therapies. MDNA55 presents a promising treatment option for rGBM patients who otherwise rapidly succumb to this disease.

INTRODUCTION

Recurrent glioblastoma (rGBM) is a condition with bleak outlook as treatment options are very limited with no universally held standard of care[1-2]. Resection is not widely adopted nor regarded as effective since most patients (~70-75%) are not candidates for repeat gross total resection at recurrence, resulting in a large unmet need for this patient population[3-4]. In addition, rGBM treatment is constrained by the blood brain barrier (BBB), its aggressive and infiltrative nature, and the presence of an immunosuppressive tumor microenvironment. These challenges are exacerbated in some patients, including those with initial diagnosis of primary *de novo* GBM [5], tumors that are not conducive to gross total resection upon relapse [1], and presence of wild-type isocitrate dehydrogenase (*IDH*) gene[6].

FDA approved treatment options resulting in prolongation of life have not emerged over the last 25 years. Median overall survival (mOS) following recurrence is 6 to 9 months and 12-month progression-free survival (PFS) rate is 2-10%[7-12]. The most recently approved agent bevacizumab, an anti-vascular endothelial growth factor (VEGF) agent, showed a modest improvement of PFS with maintenance of quality of life. However, neither single agent nor combination trials have led to improved survival [13,8,14,11,15,16]. Recently, a Phase 3 trial of nivolumab (PD-1 inhibitor) compared to bevacizumab also failed to show survival benefit[17].

MDNA55 is an immunotoxin that targets cells expressing the Interleukin-4 receptor (IL4R) in GBM and certain cells of the tumor microenvironment. High IL4R expression is associated with poor outcomes in GBM[18-19] and recent studies have shown that IL4R expression is maintained at the same or higher level upon recurrence[20]. IL4R over-expression has been demonstrated in approximately 75% of cancer biopsies and autopsy samples from adult and paediatric GBM[21], and in tumor infiltrating macrophages and MDSCs[22-23]. Higher levels of IL4R expression inhibits T-cell proliferation in an IL4R-dependent manner[22].

MDNA55 consists of an engineered circularly permuted Interleukin-4 (cpIL4) fused to a truncated and tailored sequence of the *Pseudomonas aeruginosa* exotoxin A (PE) via 5

amino acid linker[24]. Once bound to IL4R, the MDNA55 complex is endocytosed, followed by cleavage and activation by furin-like proteases found in high concentrations in the endosome of cancer cells[25-26]. The catalytic domain of PE is then released into the cytosol where it induces cell death via ADP-ribosylation of Elongation Factor-2 (EF-2) and apoptosis through caspase activation[27]. Cells that do not express the IL4R target do not bind MDNA55 and are not subject to PE-mediated effects [28-29].

MDNA55 was investigated as a single agent in a Phase 2b trial in patients with recurrent *de novo* GBM using Convection-Enhanced Delivery (CED) in order to overcome the Blood Brain Barrier (BBB). CED is a minimally invasive procedure similar to routine biopsy that minimizes systemic exposure, while the image-guided technique enhances exposure of active drug throughout the target region. Earlier studies of MDNA55[30] utilized 1st generation (i.e. non-optimized) CED where MDNA55 was delivered using large uniform diameter ventricular catheters without the use of planning software for surgical catheter placement. The current study employs 2nd generation CED technology consisting of planning software for accurate catheter placement, real-time image guided CED with a surrogate tracer to visualize drug distribution and use of small diameter stepped designed catheters intended to minimize drug leakage and back-flow.

MATERIALS AND METHODS

Study Design

This was a single-arm, non-randomized, open-label, multicenter study designed to test the hypothesis that mOS (primary objective) is improved to a clinically significant degree with MDNA55 administered via CED, as compared to current available treatments for rGBM. Further details on the study design are presented in section A of supplemental methods.

Study Population

Eligible patients included male and female patients ≥18 years of age with histologically confirmed primary (*de novo*) GBM that had recurred or progressed (first or second recurrence, per standard RANO criteria) after treatment(s) including surgery and radiotherapy with or without chemotherapy (according to local practice; Stupp protocol) and following discontinuation of any previous standard or investigational lines of therapy. Patients must have had a life expectancy >12 weeks, Karnofsky performance status (KPS) ≥70, tumor with contrast enhancing diameter of ≥1cm × ≥1cm (minimum) to 4cm (maximum) in any direction, by pre-interventional MRI within 14 days of planned treatment and had no features that made the tumor a poor target for CED (e.g., significant liquefaction or geometric features or location known to cause failure of CED due to impact on drug distribution). Patients had to have adequate bone marrow, liver, and kidney functions. Patients on steroids had to be on a stable or decreasing dose for at least five days prior to screening imaging. Patients with known mutations in either the *IDH1* or *IDH2* gene or history of allergy to gadolinium contrast agent were excluded. Prior investigational or anti-VEGF therapies were permitted following suitable washout.

MDNA55 Dosing and Administration

Patients underwent stereotactic surgery for placement of one to four infusion catheters followed by an intra- and peritumoral infusion of MDNA55 via CED. One subject underwent catheter placement but did not receive MDNA55 treatment. Patients received a single treatment at concentrations ranging from 1.5 to 9.0µg/mL and volumes of up to 66mL with a pre-determined total dose ranging from 18 to 240µg, which is less than or equal to the established MTD of 240µg[30]. The flow rate of each catheter did not exceed 10µL/min and was established to ensure that the infusion duration did not exceed 48 hours. Details on the selection of dose for each subject is presented in section B of supplemental methods.

All sites were required to use the Brainlab iPlan® Flow planning software (version 3.0.6), Brainlab stepped designed catheters and VarioGuide™ frameless image guided stereotactic system to generate a pre-treatment plan for placement of catheters according to specified placement guidelines (see section C of supplemental methods). Co-infusion of a contrast agent (gadolinium diethylenetriamine pentaacetic acid [Gd DTPA], Magnevist®) was applied to assess infusate distribution as well as to determine suitability of the iPlan software, Varioguide, Brainlab catheter and catheter placement guidelines to deliver MDNA55.

Study Assessments

Post-treatment follow-up assessment of safety was performed 14 (±3) days after treatment. Thereafter, safety and efficacy assessments were performed at 30, 60, 90, and 120 (±7) days after treatment and approximately every 8 weeks thereafter until 360 days of active follow-up was completed.

For pharmacokinetic (PK) analysis, samples were collected at 1, 3, 6, 12, 18, and 24 hours post infusion and at Day 14; plasma concentration was measured using an MSD® ligand-binding method. To assess anti-drug antibodies (ADA) titers and presence of neutralizing antibody (NAb), serum samples were collected at screening (baseline) and at Days 14, 30, 120, 240/270, and 360 or at early termination. ADA titers were measured using an MSD® immunoassay and NAbs were determined using a cell based radioactive assay utilizing Daudi B-lymphoblast cell line (ATCC-CCL-213).

Patients who completed Day 360 assessment without progressive disease (PD) or discontinued early without PD continued to be followed for disease status until progression, where possible. After progression (on study or during post-study follow up), patients continued to be followed where possible, for survival until death (or termination of data collection by the Sponsor or withdrawal of consent by the subject).

The expected duration of study participation for each subject was up to 12.5 months, including up to 14 days of screening, up to 3 days planning period and a 12-month follow-up period relative to the day of catheter placement/start of infusion (designated as Day 0).

Response Assessment

All patients had MR images acquired at baseline and at follow up visits according to the international standardized brain tumor imaging protocol in Ellingson et al. (2015) including T2 weighted, T2-weighted FLAIR, diffusion-weighted images, and parameter matched, 1-1.5 mm isotropic 3D T1-weighted scans before and following injection of Gd-DTPA. Advanced imaging such as perfusion MRI and Treatment Response Assessment Maps (TRAMs) were also acquired for some patients.

Since an extended duration of pseudo-progression (PsP) was observed in the Phase 1 study[31- 32], assessment of response in the current study was performed using the modified RANO (mRANO) criteria[33] as it allowed for continuation on study following initial evidence of radiographic progression to confirm either true progression or PsP (see section F of supplemental methods).

IL4 Receptor Expression Analysis

Retrospective analysis of IL4R expression using archival tumor tissue from initial GBM diagnosis and/or tumor tissue sample collected at recurrence was conducted using a validated immunohistochemistry-based assay at a Clinical Laboratory Improvement Amendments (CLIA) compliant reference laboratory. H-score determination method is explained in section E of supplemental methods.

A positivity cut-off of H-score > 60 was determined to provide most predictive value for IL4R status in relation to efficacy endpoints. Applying this cutoff, two subpopulations were identified: IL4R High = H-score >60; IL4R Low = H-score ≤60.

Statistical Analysis

Assessment of safety was based on all patients (safety population; N=47). Evaluation of survival-related efficacy endpoints (OS and effect of IL4R status on OS) was based on the Intent to Treat Population (ITT; N=47) and also on those who received any amount of MDNA55 and had no major protocol violations (Per Protocol Population, PP; N=44). Determination of response-related secondary efficacy endpoints were based on modified intent to treat population (mITT; N=41) comprising of patients with adequate imaging (at least 1 post-treatment scan) and clinical information. Further details on the study endpoints are presented in section D of supplemental methods. All statistical evaluations were performed using SAS® Version 9.3 or above (SAS® Institute, Cary, North Carolina).

RESULTS

Patient Characteristics and Disposition

A total of 47 patients were enrolled in the study in the period between 23 Mar 2017 to 12 Sep 2019 at eight active clinical sites in the US and one site in Poland. As of the study censor date (31 Oct 2019), 11 patients were alive and continued to be followed for survival.

Baseline disease characteristics are summarized in Table 1 for the various analysis populations. Among the ITT population, median age was 56 years (range: 34 to 78); 30 patients (63.8%) were male and 17 (36.2%) were female. Median time between initial diagnosis and start of treatment was 12.72 months (range: 5.15 to 44.23 months); overall mean maximal tumor diameter at baseline was 30.8mm. All 47 (100.0%) patients underwent prior surgery at initial diagnosis and were not suitable for tumor resection at relapse (i.e., first or second relapse). All but one subject (98%) received prior temozolomide treatment and all but one subject (98%) received prior radiotherapy. Thirteen (27.7%) patients had relapsed following failure on an alternate investigational therapy prior to enrolment. Approximately half (49.0% patients) had a KPS score of ≤ 80. Thirty-seven (78.7%) patients had one relapse and 10 (21.3%) patients had two relapses at the time of enrolment. *MGMT* gene promoter

methylation, being a strong indicator for better prognosis in GBM, was observed only in 18 (38.3%) patients. Overall, 24 (51.1%) patients had unmethylated *MGMT* status. Five (10.6%) patients had no *MGMT* data available. With respect to IL4R expression, 23 patients had an H-score >60 (i.e. High expression) and 19 had an H-score ≤ 60 (i.e. Low expression) with 5 unknowns due to lack of tumor tissues.

MDNA55 Dosing

Volume and concentration were adjusted to optimize delivery to the tumor based on infusate distribution and tolerability data obtained in real time from previous patients in this study under the on-going guidance by the Safety Review Committee. During the course of the study, 4 drug concentrations (1.5, 3.0, 6.0 and 9.0 µg/mL) were employed with volume of administration adjusted according to tumor size. Median volume of infusion was 30 mL (range: 12 to 66 mL) for a median duration of 26.57 hours (range: 15.46 to 57.21 hours) at an adjusted median flow rate of 1.095 mL/hour (0.58 to 2.83 mL/hour). Median total dose infused was 180 µg (range: 18.0 to 240.3 µg). Median number of catheters was 2 (range: 1-4 catheters). Nearly all patients received 100% of the planned total dose and infusion volume (median: 100% [range: 88.33% to 100.42%]).

MDNA55 Distribution and Tumor Coverage

A semi-automatic segmentation feature of iPlan Flow was used for volumetric assessment. The volume of Gd-DTPA distribution was analyzed based on pre- and post CED 3D T1-weighted MRI using a customized subtraction algorithm. Based on the tumor volume assessment, Gd-DTPA distribution inside the tumor was considered as coverage. Overall median tumor coverage achieved was 52.66% (range: 0 to 97.8%); median tumor coverage including a 1 cm peritumoral margin was 55.14% (range: 5.4% to 95.2%), and median tumor coverage including a 2 cm peritumoral margin was 37.22% (range: 2.2% to 82.9%). Median Volume of distribution (Vd)/Volume of infusion (Vi) ratio was 1.35 (range: 0.1 to 4.8).

Safety

The most common Adverse Events (AEs) in the total subject population were seizure (N=20, 42.6%), fatigue (N=19, 40.4%), headache (N=18, 38.3%), and muscular weakness (N=15, 31.9%) (see Table G1 in section G of supplemental methods). Of the 47 patients, 32 (68.1%) had AEs considered by the investigator to be at least possibly related to study drug and/or infusion procedure (see Table G2 in section G of supplemental methods). Overall, incidence of AEs between the dose groups was similar and there was with no apparent dose dependent effect. Most frequent possibly related/related AEs were seizure (N=10, 21.3%), fatigue (N=9, 19.1%), headache (N=8, 17.0%), and pyramidal tract syndrome (N=8; 17.0%). Eighteen of 47 patients (38.3%) had a history of seizures prior to enrolment.

AEs were also evaluated based on volume of infusion and total dose administered. In both cases, the incidence of AEs between groups above and below the median was comparable with no clear effect of volume of infusion or total dose administered.

Eight (8) patients experienced Grade 5 AEs, six (6) of which were unrelated to study drug or/ infusion procedure (see Table G3 in section G of supplemental methods). One subject experienced an AE of cerebral hemorrhage after completing infusion and immediately after removal of the catheters and died 14 days post treatment. This AE was thought to be related to catheter removal procedure and was therefore considered to be possibly related to the study drug or the CED procedure. However, the underlying nature of the disease or infectious complications are alternate considerations for this causality.

PK and ADA

Similar to previous MDNA55 trials, pharmacokinetic results were well below the lower limit of quantification (LLOQ) at all timepoints, suggesting that intact MDNA55 did not enter the systemic circulation at measurable levels following intracranial infusion.

Nineteen of 47 patients (40.4%) were confirmed positive for ADA during the study (2 were positive at screening, indicating they were potentially exposed to pseudomonas infection) and all 19 patients had positive samples post-treatment. Sixteen patients tested positive for

neutralizing antibodies (Nabs) where the inhibition of MDNA55 cytotoxic activity ranged between 6.2% to 101.3%. However, these effects observed in plasma may not be representative of effects within the tumor beyond the blood-brain barrier with no clear implications for repeated dosing.

Efficacy

Overall Survival

With a single treatment of MDNA55, the mOS in the ITT population was 10.2 months (one-sided 80% CI: 8.39, 12.75) and in the PP population the mOS was 11.64 months (one-sided 80% CI: 8.62, 15.02). As the lower limit of the CI did not include 8.0 months in both ITT (primary analysis) and PP Populations (supportive analysis), the null hypothesis was rejected using single-sample one-sided log-rank test at one sided 10% significance level and therefore the primary end point was met. Overall survival at 12-month was 43% (95% CI: 29%-57%) in the ITT population and 46% (95% CI: 31%-60%) in the PP population.

Exploratory subgroup analyses were conducted on survival in the PP population for various prognostic and treatment parameters. Results are presented in a Forest Plot (Figure 1). Patients with unmethylated *MGMT* promoters treated with MDNA55 showed a similar survival outcome compared with those with methylated *MGMT* promoters (mOS was 10.20 versus 11.64 months; p=0.632. OS-12 was 46% versus 41%, respectively). There was also no significant difference in survival based on the total dose of MDNA55 administered (either above or below the median dose of 180 µg) or the volume infused (above or below the median infusion volume of 30 mL).

Dose-effect relationship of MDNA55 dose by IL4R status

OS based on IL4R expression of the primary tumor was evaluated as a secondary endpoint in the study. IL4R High patients showed an mOS of 15.02 months and IL4R Low patients showed an mOS of 8.4 months, although this difference did not reach statistical significance (p=0.215) (Figure 1). OS-12 was 57% versus 33%, respectively.

Further analysis of OS evaluating the relationship between MDNA55 dose and IL4R status identified a subpopulation in the IL4R Low group that demonstrated increased survival – those receiving high doses (≥180µg) of MDNA55 (designated as the IL4R Low^{HD} population). These patients demonstrated an OS-12 of 53% (Table 2 and Figure 2) despite not reaching the mOS at the time of study censor. This was roughly equivalent to the IL4R High group receiving any dose (OS-12 of 56% and 58%). In contrast, patients in the IL4R Low group receiving low doses (<180µg; designated as the IL4R Low^{LD} population) demonstrated an mOS of 8.0 months, no different from the literature-derived null assumption of OS for the study. Combination of the IL4R High group (receiving any dose) and the IL4R Low^{HD} groups yielded an mOS of 15.0 months and OS-12 of 55% (Table 2 and Figure 2). These data suggest that MDNA55 treatment may benefit rGBM patients if administered at high dose irrespective of IL4R expression level.

Tumor Control and Progression Free Survival

One of 41 evaluable patients had an objective response (ORR=2.4%), which continued to shrink for more than 358 days until study closure. Twenty of the 41 (48.8%) evaluable patients exhibited PsP on follow-up imaging. Tumor shrinkage or stabilization relative to pre-treatment baseline using mRANO criteria was observed in 31 of 41 patients (Figure 3a), with one patient exhibiting evidence of a durable CR following initial radiographic changes consistent with PsP, resulting in a total Tumor Control Rate (TCR) of 75.6%. In the population comprised of IL4R High + IL4R Low^{HD} patients (Figure 3b), the TCR was 81%. PFS based on radiologic-only assessment using mRANO criteria demonstrated a median PFS of 3.61 months (95% CI: 2.62, 7.70). PFS-6 was 33%, PFS-9 was 27%, and PFS-12 was 27%.

Case studies shown in Figure 4 provide examples of delayed onset response observed after pseudo-progression and confirmed by advanced imaging techniques (perfusion MRI or TRAMs). Figure 4a shows a subject with increase in contrast enhancement in T1-weighted MRI at Day 60 which was suggestive of progression, however, decrease blood uptake by

perfusion MRI suggests decreased active tumor and occurrence of pseudo-progression. Figure 4b shows a subject with increased contrast enhancement at Day 30 and 60, however TRAMs revealed improvement occurring in the lesion area, showing mostly red (necrotic) regions at Day 60.

DISCUSSION

Currently, treatment options following recurrence of primary GBM are very limited and not effective. MDNA55 is a rationally designed targeted therapy with the potential to extend the survival of patients with rGBM. The treatment strategy of this study consisted of intra-tumoral and peritumoral administration of MDNA55 by-passing the BBB using an advanced CED delivery technique thereby targeting the bulk tumor *in situ*. Data from the MDNA55-05 study showed that a single administration of MDNA55 resulted in mOS of up to 15 months and OS-12 of 55% in a population where median survival with approved therapies remained at 6-9 months and 1-year survival rate is less than 35%[7-12].

Though MDNA55 showed promising survival over approved therapies, the single arm (non-randomized) study design and small number of patients are limiting factors. Recognizing these limitations of the study and challenges associated with use of historical controls from published data as a comparator, the survival results of this study were also assessed relative to a well-balanced propensity matched external control arm (ECA). The analysis concluded that the survival benefit observed in the study was attributed to MDNA55[34-36]. We have included the ECA principally to draw attention to this approach for study design given we have recently been approved by the FDA to use this in a registration trial. We believe that this is a significant opportunity for the neuro-oncology community. However, we recognize that these analyses were not part of the pre-specified statistical analysis plan and therefore should be interpreted with some caution.

The mOS benefit of MDNA55 treatment is encouraging, particularly in a population that is known to have poor prognosis. Moreover, unmethylated *MGMT* gene promoter, one

predictive factor of poor prognosis in rGBM, was observed in more than 50% of the MDNA55 study population. Overall survival of this group was similar to that of patients with methylated *MGMT* promoters, suggesting no association between MGMT gene methylation and MDNA55 activity and a potential benefit to this group which comprises nearly 40% to 50% of the GBM population[37].

Overall, the safety profile in this study were consistent with previous MDNA55 studies and no new safety findings were observed. Drug-related AEs were primarily neurological or an exacerbation of pre-existing neurological conditions related to primary disease of GBM. The CED procedure was well tolerated with only two patients having to discontinue infusion due to AEs related to procedure. One subject experienced an AE of cerebral hemorrhage shortly after completion of infusion and immediately after removal of the catheters and died 14 days later. This AE was thought to be related to catheter removal and was therefore considered to be possibly related to the study drug or CED procedure. However the underlying nature of the disease or infectious complications are alternate considerations for this causality.

Patients treated with MDNA55 developed anti-drug antibody, including neutralizing antibodies. These data suggested that there was low systemic exposure of MDNA55 that was sufficient to elicit an immune response. However, ovalbumin injection into the brain or CSF of rats has resulted in immunogenicity related to events occurring with the CNS[38] rather than a result of systemic exposure. This suggests that systemic exposure to MDNA55 may not be necessary to generate an immune response and anti-drug antibody generation.

Standard RANO criteria is the currently accepted benchmark for response assessment in rGBM, however, it was determined not to be the most appropriate tool for determining the therapeutic benefit of MDNA55 due to the high incidence of pseudo-progression (48.8%) following treatment. This confounded the interpretation by standard RANO of objective responses for estimating PFS, as pseudo-progression in these patients could falsely suggest early failure despite a trend toward longer survival[39]. The mRANO criteria allows for continuation on therapy during initial evidence of radiographic progression to distinguish

between true tumor growth from possible PsP[40]. It also allows for use of the initial radiological scan documenting PsP-related “progression” as a new reference baseline, in order to objectively define and document the degree of possible PsP events. In this study, tumor control was observed in a number of patients, particularly after a transient increase in size of the area of enhancement on imaging at the earlier time points.

The engineering of MDNA55 to target the IL4R on tumor cells suggests that patients with high IL4R expression would benefit most from MDNA55 treatment due to the increased probability of toxin exposure. In the MDNA55-05 study, it was observed that the effect of IL4R expression on overall survival could be differentiated when drug dose was low in contrast to when the drug dose was high. Though the IL4R subgroup analysis was not a pre-specified hypothesis and not statistically powered, this observation is consistent with pre-clinical studies showing that tumors expressing low level of the IL4R do respond well to higher doses of MDNA55[41]. These subgroup results are to be interpreted with caution given the small sample size and will be validated in the pivotal trial. Conversely, the apparent lower level of biological effect of MDNA55 at low dose may be attributed to a requirement of higher IL4R expression when drug exposure is low. In patients with high IL4R expression, MDNA55 dose did not have much effect. This could be because once a threshold number of IL4Rs on the target tumor cell bind to MDNA55 and deliver the toxic payload causing cell death, any further increase in dose does not have any incremental effect on the fate of tumor cells[42].

CED is a minimally invasive procedure, similar to a biopsy, that improves drug delivery by utilizing bulk flow, or fluid convection, established as a result of a pressure rather than a concentration gradient[43]. As such, it offers markedly improved distribution of infused therapeutics within the CNS compared to direct injection or via drug eluting polymers, both of which depend on diffusion for parenchymal distribution. Additionally, CED obviates challenges of agents crossing the BBB while minimizing systemic exposure and toxicity[43-45]. In previous studies of MDNA55, it was not possible to assess the distribution in real-time which resulted in leakage and poor tumor coverage. This current study employs state-of-the-art CED techniques with planning software for accurate catheter placement, real-time image

guidance with a surrogate tracer to visualize drug distribution[46-47] resulting in much better drug distribution (~2X the % tumor coverage) compared to earlier studies using 1st generation CED technique[48-49].

In conclusion, MDNA55 treatment showed promising survival compared to currently approved therapies and exhibited an acceptable risk-benefit profile in a population expected to have poor prognosis. Single treatment with MDNA55 increased mOS by up to 50% and 12-month PFS by almost 100% when compared to approved therapies. Combining targeted treatment and advanced drug delivery techniques employed in this study provide opportunities to potentially deliver substantive benefit in patients with rGBM and to explore the efficacy of MDNA55 in a pivotal trial.

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FUNDING

This study was supported by the Cancer Prevention and Research Institute of Texas (CPRIT) and Medicenna Therapeutics, Inc.

ACKNOWLEDGMENTS

We thank all patients and families, and the study teams involved in the study, for making this trial possible. We would also like to dedicate this manuscript to Dr. Dina Randazzo, Assistant Professor of Neurosurgery and Neurology at Duke University, and a key contributor to the MDNA55-05 study. Dr. Randazzo was a respected and admired neuro-oncologist whose life's work was aimed at understanding and treating GBM. She was adored by her patients and has touched so many people with her dedication and passion for finding cure for cancer.

FIGURE LEGENDS

Figure 1: Forest Plot for Median Overall Survival Based on Various Prognostic and Treatment Parameters (PP Population). Abbreviations: CI = confidence interval, HR = hazard's ratio, IL4R = interleukin 4 receptor, N = sample size, KPS = Karnofsky performance status, IDH = isocitrate dehydrogenase, MGMT = O6-methylguanine-methyltransferase, IL4R = interleukin 4 receptor.

Figure 2: Survival curves depicting the dose-effect relationship between MDNA55 dose and IL4R status. (A) IL4R Low group treated with high and low-dose MDNA55. (B) IL4R low and high groups treated with low-dose MDNA55. (C) IL4R high group treated with high and low-dose MDNA55. (D) IL4R low and high groups treated with high-dose MDNA55.

Figure 3: Waterfall Plot of Best Tumor Response Assessed by Modified RANO (mRANO). Bars represent best response in % change in Sum of Product Diameters (SPD) on the basis of contrast-enhanced MRI compared to pre-treatment baseline. Shown are the best tumor response in (A) all evaluable subjects, and (B) Subjects in the IL4R High + IL4R LowHigh Dose sub-population. Asterisks represent subjects that experienced initial pseudo-progression on the first radiographic time point.

Figure 4: Delayed Onset Response After Pseudo-Progression. (A) Increase in contrast enhancement in T1-weighted MRI at Day 60 is suggestive of progression, however, decrease blood uptake by perfusion MRI (arrows) indicate decreased active tumor and suggesting pseudo-progression. Follow up T1-weighted MRI at 90 and 120 days confirm pseudo-progression. (B) TRAMs show improvement occurring between Day 30 and Day 60 in the lesion area, showing mostly red (necrotic) regions at Day 60.

Table 1. Patient Demographics and Baseline Characteristics for MDNA55-05 Analysis Populations

Baseline Characteristics	ITT Population (N=47)	PP Population (N=44)	mITT Population (N=41)
Sex			
Male, n (%)	30 (64%)	27 (61%)	25 (61%)
Female, n (%)	17 (36%)	17 (39%)	16 (39%)
Age			
Median (Range)	56 (34 – 78)	55 (34 – 77)	55 (34 – 77)
Mean (StDev)	57 (± 11.8)	56 (± 11.8)	56 (± 11.7)
KPS, n (%)			
70 and 80	23 (49%)	22 (50%)	19 (46%)
90 and 100	24 (51%)	22 (50%)	22 (54%)
IDH Status			
Wild Type	38 (81%)	37 (84%)	35 (85%)
Mutated	0 (0%)	0 (0%)	0 (0%)
Unknown	9 (19%)	7 (16%)	6 (15%)
MGMT Status			
Pos (methylated)	18 (38%)	17 (39%)	15 (37%)
Neg (unmethylated)	24 (51%)	23 (52%)	22 (54%)
Unknown	5 (11%)	4 (9%)	4 (10%)
IL4 Receptor Status			
High (H-score > 60), n (%)	23 (49%)	21 (48%)	21 (51%)
Low (H-score ≤ 60), n (%)	19 (40%)	19 (43%)	16 (39%)
Unknown	5 (11%)	4 (9%)	4 (10%)
Max Tumor Dimension, mm			
Median (Range)	29.6 (7.8 – 58.5)	29.6 (7.8 – 58.5)	29.7 (12.0 – 8.5)
Mean (St Dev)	30.8 (± 11.08)	30.1 (± 10.8)	31.0 (± 10.5)
Tumor Location			
Parietal	15 (32%)	15 (34%)	14 (34%)
Frontal	14 (30%)	12 (27%)	11 (27%)
Temporal	10 (21%)	9 (21%)	9 (22%)
Other	8 (17%)	8 (18%)	7 (17%)
Extent of Tumor Resection at Diagnosis			
Total Resection	39 (83%)	36 (82%)	33 (80%)
Partial Resection	8 (17%)	8 (18%)	8 (20%)
Time to 1st Relapse, months			
Median (Range)	10.9 (2.6 – 36.4)	11.3 (4.7 – 36.4)	11.1 (4.7 – 36.4)
Mean (St Dev)	13.0 (± 7.7)	13.1 (± 7.5)	13.3 (± 7.5)
Number of prior relapses, n (%):			
1	37 (79%)	35 (80%)	33 (80%)
2	10 (21%)	9 (20%)	8 (20%)
Steroid Use at Baseline			
None	27 (57%)	27 (61%)	25 (61%)
≤ 4 mg/day	14 (30%)	14 (32%)	14 (34%)
> 4mg/day	5 (11%)	3 (7%)	2 (5%)
Unknown	1 (2%)	0 (0%)	0 (0%)
Prior Treatment, n (%)			
Surgery	47 (100%)	44 (100%)	41 (100%)
Temozolomide	46 (98%)	43 (98%)	40 (98%)
Radiation	46 (98%)	43 (98%)	40 (98%)
Exp Therapy	13 (28%)	12 (27%)	11 (27%)

Abbreviations: ITT = intent to treat, PP = per protocol, mITT = modified intent to treat, N = sample size, KPS = Karnofsky performance status, IDH = isocitrate dehydrogenase, MGMT = O^6-methylguanine-methyltransferase, IL4R = interleukin 4 receptor.

Table 2. Comparison of Overall Survival by IL4R Group and MDNA55 Dose

IL4R Groups and MDNA55 Dose	N	Median OS (95% CI)	OS-12	p-value HR (95% CI)
IL4R Low ^{HD}	11	NR (4.39, NR)	53%	0.055 0.35 (0.11, 1.07)
IL4R Low ^{LD}	8	8.00 (0.82, 11.64)	13%	
IL4R High ^{LD}	12	13.52 (6.00, 23.38)	58%	0.009 0.25 (0.08, 0.76)
IL4R Low ^{LD}	8	8.00 (0.82, 11.64)	13%	
IL4R High ^{LD}	12	13.52 (6.00, 23.38)	58%	0.561 1.34 (0.49, 3.65)
IL4R High ^{HD}	9	15.15 (5.74, NR)	56%	
IL4R Low ^{HD}	11	NR (4.39, NR)	53%	0.697 0.79 (0.25, 2.54)
IL4R High ^{HD}	9	15.15 (5.74, NR)	56%	
IL4R High + IL4R Low ^{HD}	32	15.02 (7.70, 16.43)	55%	0.005 0.30 (0.13, 0.73)
IL4R Low ^{LD}	8	8.00 (0.82, 11.64)	13%	

Abbreviations: OS = overall survival, CI = confidence interval, HR = hazard's ratio, IL4R = interleukin 4 receptor, LD = Low Dose, HD = High Dose, N = sample size, NR = not reached, OS-12 = overall survival at 12 months

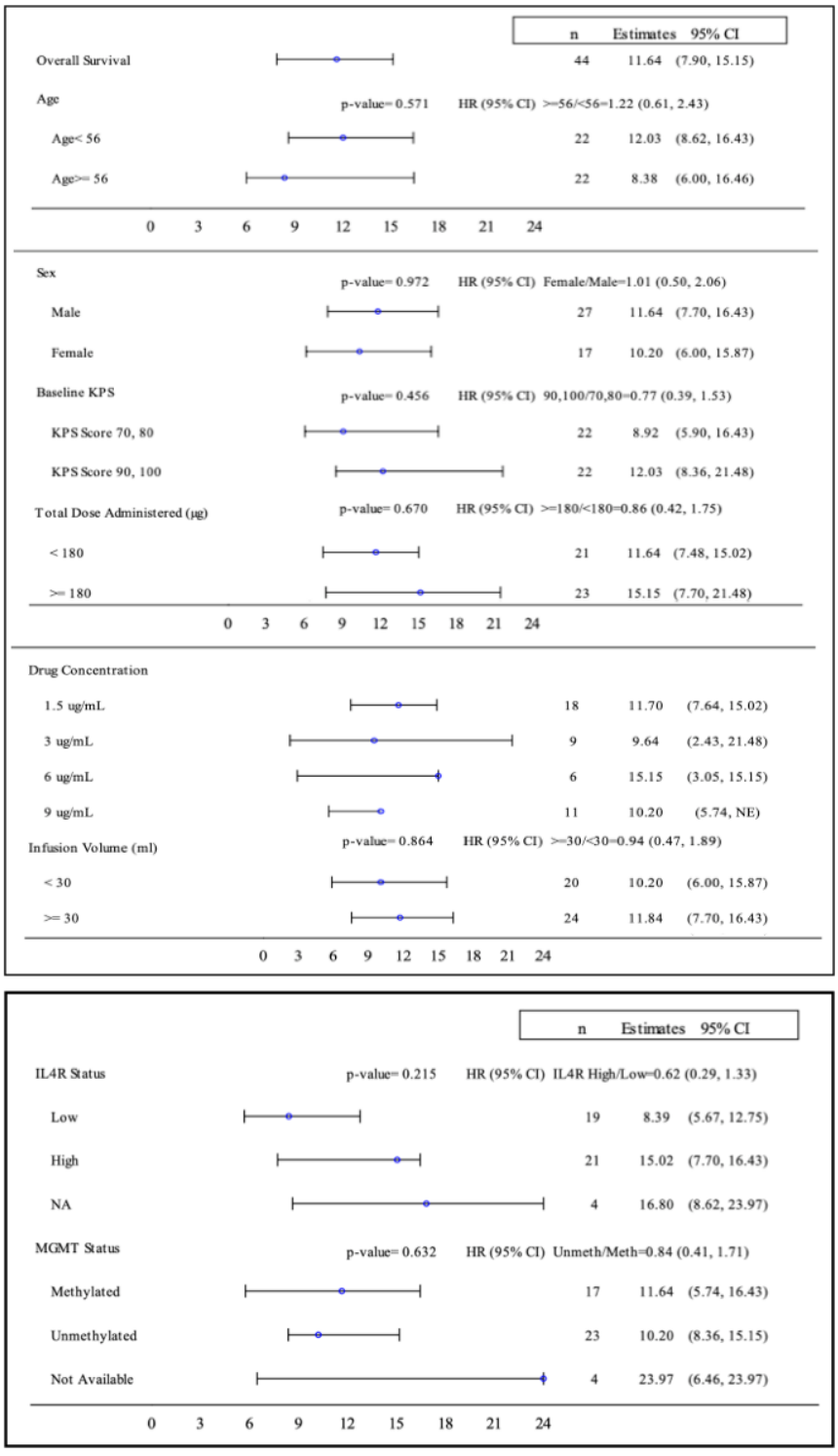

Figure 1

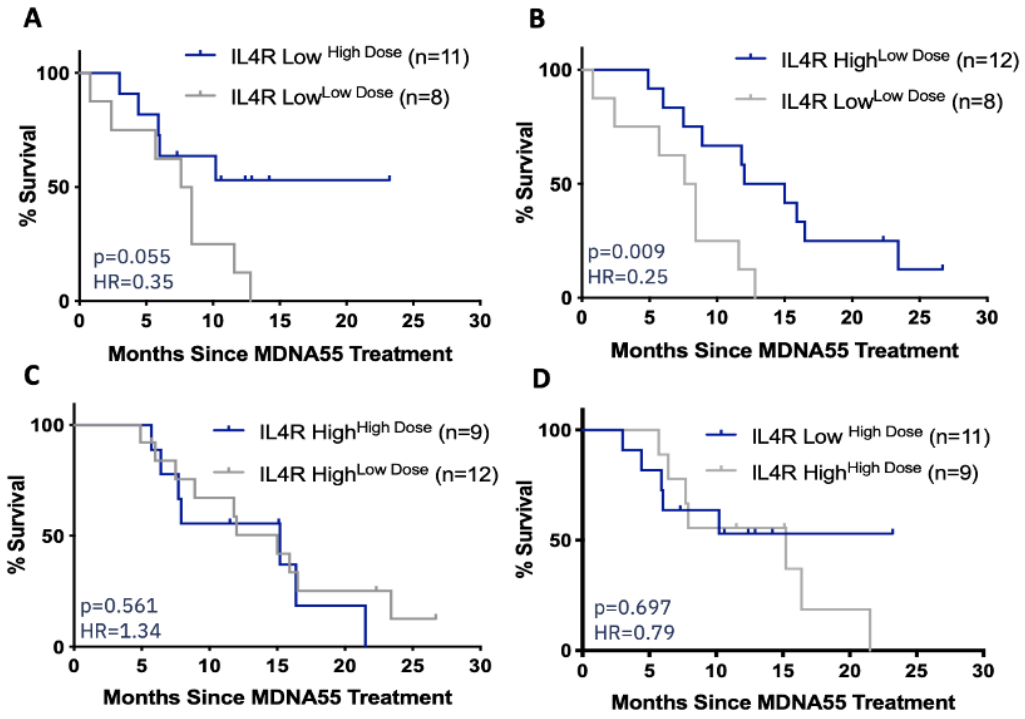

Figure 2

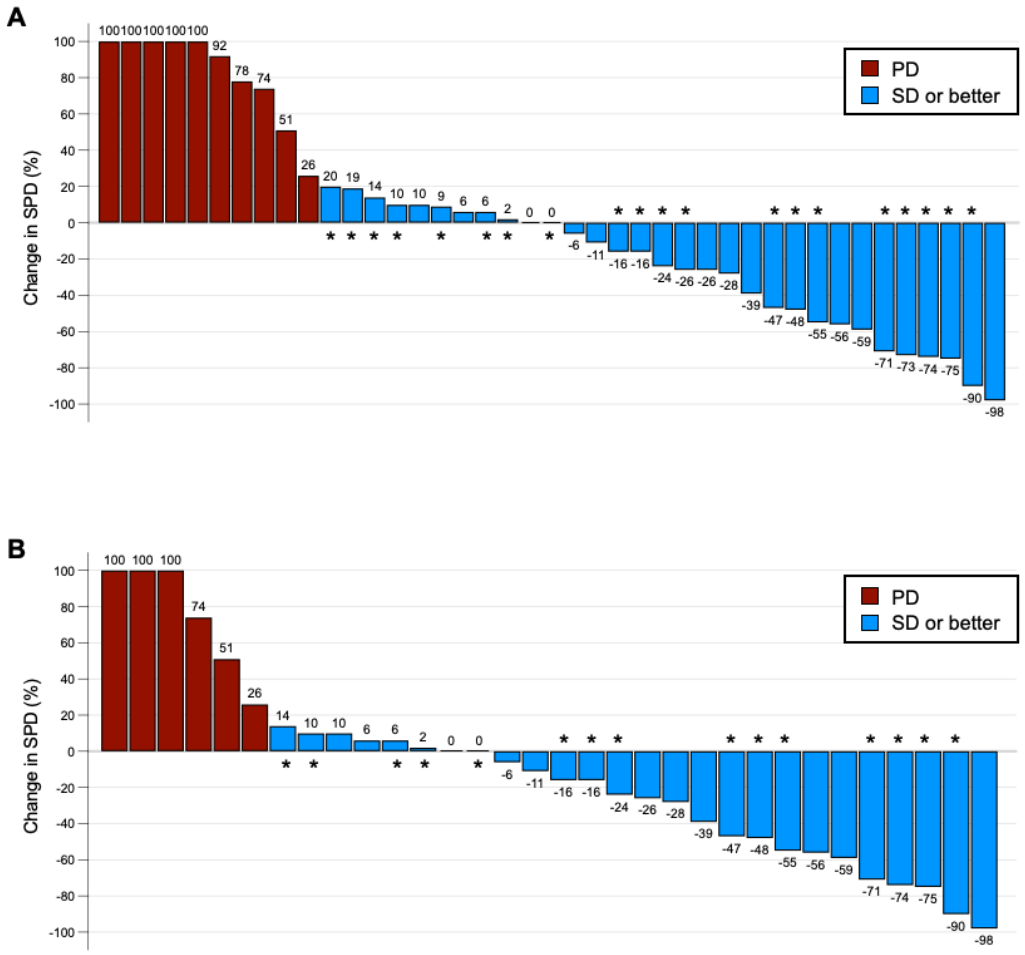

Figure 3

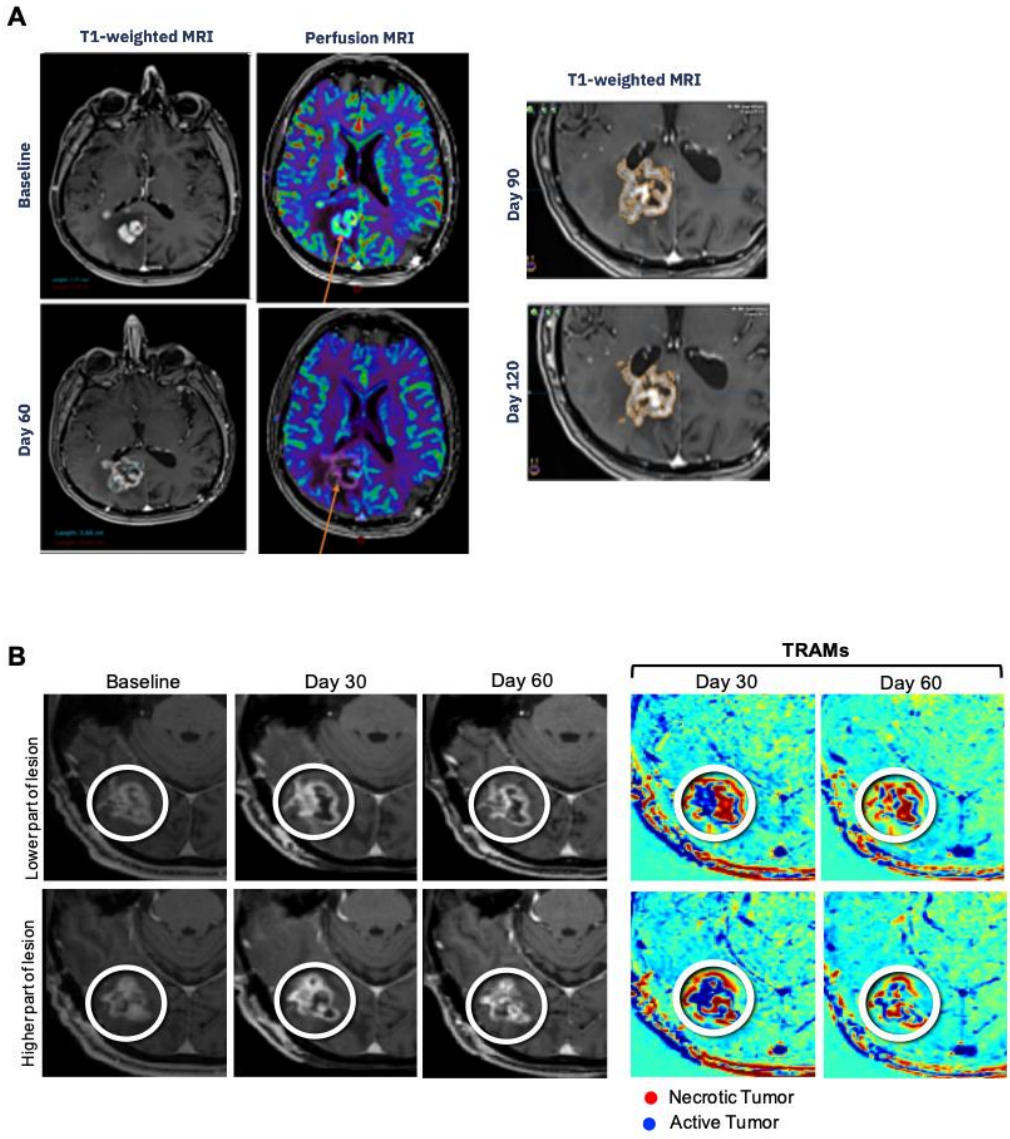

Figure 4