

Preclinical Characterization of MC339, a Novel Radiotherapeutic Agent for DLL3 Expressing Cancers

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KEY FINDINGS & CONCLUSIONS

- **MC339 demonstrated high binding affinity and selectivity for human DLL3, with efficient internalization to DLL3+ cells**
- **²²⁵Ac-MC339 induced cytotoxicity in DLL3+ cell lines in a dose- and target-dependent manner**
- **Biodistribution of radiolabeled MC339 in mice demonstrated rapid radionuclide tumor uptake, persistent tumor retention, a favorable tumor:kidney ratio, and that kidney is the major route of elimination**
- **A single dose of ²²⁵Ac-MC339 resulted in robust tumor regression and prolonged survival in a SCLC mouse cell-derived xenograft model**
- **In summary, preclinical data support further development of ²²⁵Ac-MC339 for DLL3+ solid tumors**

INTRODUCTION

- Neuroendocrine neoplasms (NENs), including small cell lung cancer (SCLC) and treatment-emergent neuroendocrine prostate cancer (NEPC), commonly exhibit upregulated surface expression of the atypical notch Delta-like ligand 3 (DLL3), a protein expressed almost exclusively by tumor cells.
- MC339 is a novel macrocyclic peptide binder to DLL3 designed to achieve high, rapid uptake and retention into tumor tissue and fast clearance of the unbound compound via the renal route.
- MC339 complexed with radioisotopes can be used for therapy or imaging.
- The purpose of these preclinical studies was to characterize in vitro and in vivo properties of MC339.

RESULTS

Binding Characteristics of MC339

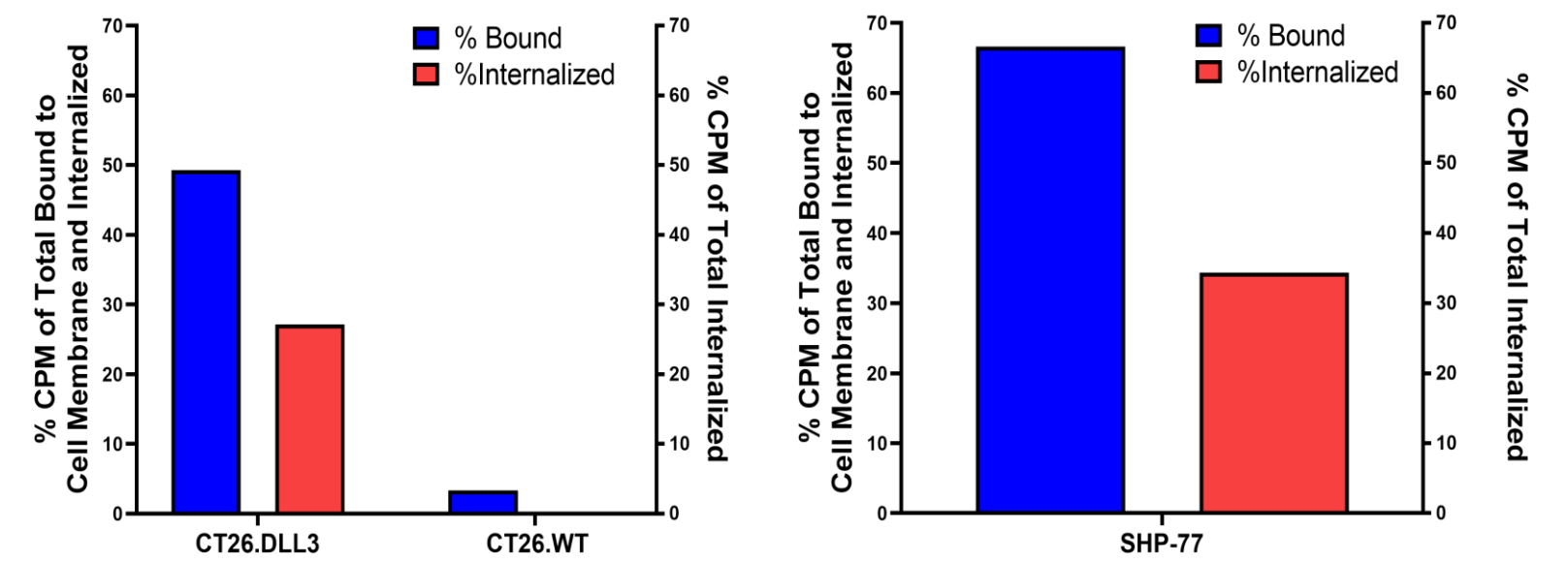
- MC339 binds rapidly and selectively to human DLL3 with picomolar affinity. There was no binding to related DLL1 and DLL4. Binding is similar when labeled with different isotopes (^{nat}Lu and ^{nat}In) (**Table 1**).
- High uptake (67%) and internalization (34%) of Lu-MC339 was observed in SHP-77 cells (**Figure 1**).
- Similarly, there was high uptake (49%) and internalization (27%) in CT26 cells engineered to express DLL3 (CT26.DLL3). Conversely there was minimal binding in CT26.WT cells, indicating specificity of binding to DLL3 (**Figure 1**).

Table 1. Binding Kinetics and Affinity of MC339 to Human DLL3, Human DLL1, and Human DLL4

Ligand	Analyte	(K _a) (1/Ms)	(K _d) (1/s)	(K _D) (pM)
DLL3	MC339	9.5 x 10 ⁵	1.5 x 10 ⁻⁴	160
DLL3	^{nat} Lu-MC339	8.1 x 10 ⁵	1.2 x 10 ⁻⁴	150
DLL3	^{nat} In-MC339	8.1 x 10 ⁵	2.2 x 10 ⁻⁴	270
DLL1	MC339	ND	ND	ND
DLL4	MC339	ND	ND	ND

Binding Affinity was evaluated using SPR (N=4)
ND: Not determined due to no binding at 1.5 μM

Figure 1. ¹⁷⁷Lu-MC339 selectively binds and gets internalized in DLL3+ cells



Binding and internalization was evaluated with gamma counter (% of total cell binding and internalization) 1 hour after incubation in DLL3+ cells (CT26.DLL3 and SHP-77) and in DLL3- cells (CT26.WT)

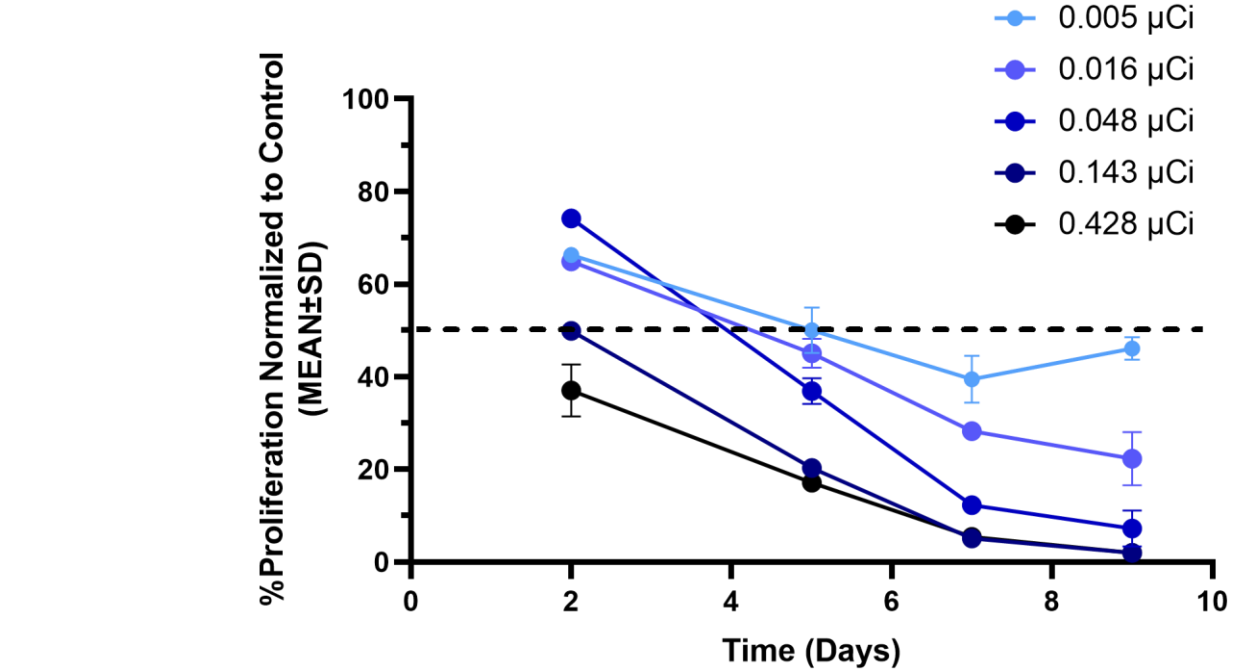
METHODS

- Binding affinity and selectivity were characterized using surface plasmon resonance (SPR)
 - Analytes (MC339, ^{nat}Lu-MC339 and ^{nat}In-MC339) were characterized with a Biacore 8K at 37 °C
- Cell binding and internalization was determined in a cell-based assay in cell lines expressing DLL3 (SHP-77, CT26.DLL3) using ¹⁷⁷Lu-MC339
 - SHP-77 cells express ~3500 DLL3 copies/cell (in range with DLL3 expression in SCLC patients) while CT26.DLL3 is engineered to overexpress DLL3 (~12,000 copies/cell)
 - Cells were incubated with ¹⁷⁷Lu-MC339 for 1 hour at 37 °C before binding and internalization were evaluated using a gamma counter
- Cytotoxicity was determined using ²²⁵Ac-MC339 in SHP-77 cells
 - Cells were incubated with ²²⁵Ac-MC339 for 2.5 hours at 37 °C before proliferation was evaluated using a cell titer glow (CTG) assay

²²⁵Ac-MC339 Induced Cytotoxicity in DLL3+ Cells

- Treatment of DLL3+ cells (SHP-77) with ²²⁵Ac-MC339 ranging from 0.005 – 0.428 uCi resulted in dose-dependent cytotoxicity (**Figure 2**).

Figure 2. Dose Dependent Cytotoxicity in DLL3+ Cells After Incubation with ²²⁵Ac-MC339



Cytotoxicity was evaluated using a CTG assay using DLL3+ cells (SHP-77) after 2.5 hours of incubation with ²²⁵Ac-MC339 (N=5)

¹⁷⁷Lu-MC339 Demonstrated Favorable Biodistribution

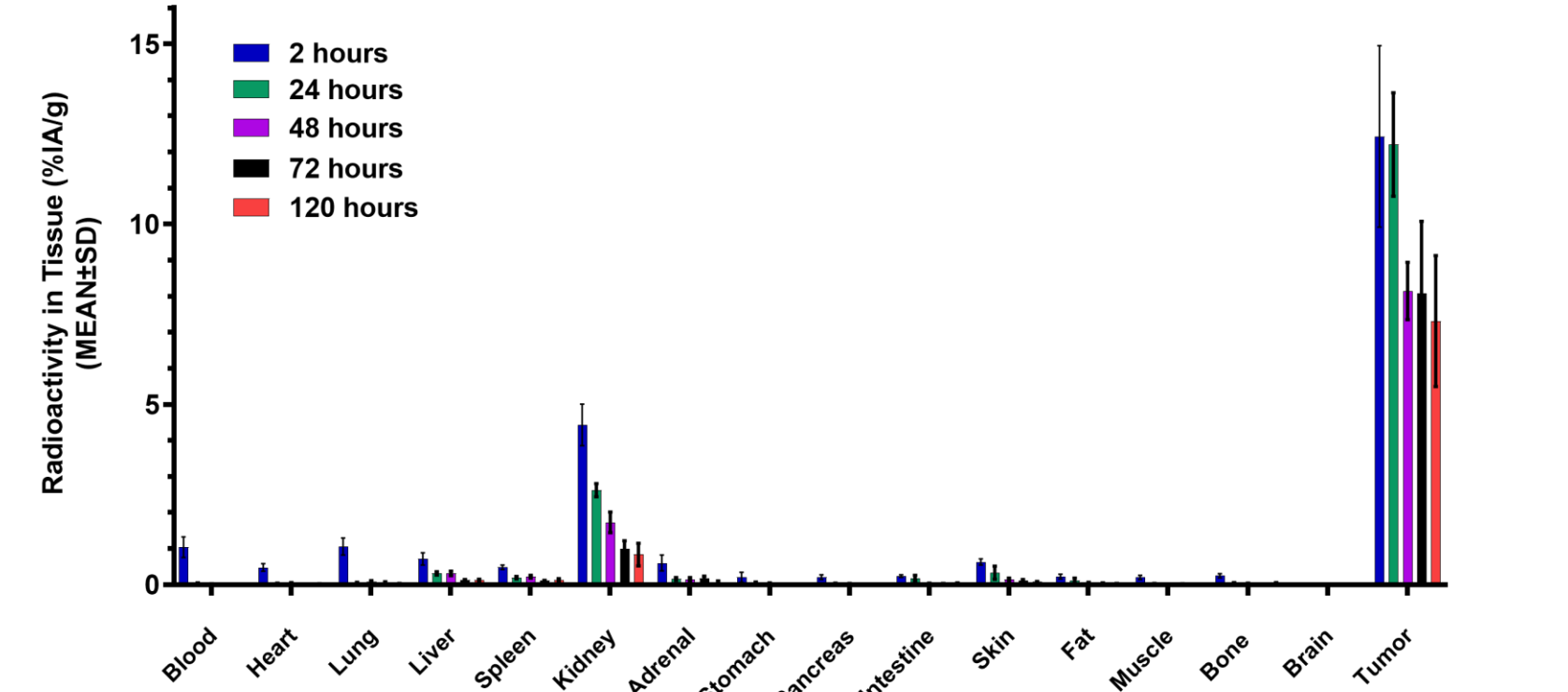
- ¹⁷⁷Lu-MC339 showed stability ≥ 99% in mouse and human serum at 1 and 4 hours measured by reverse phase high performance liquid chromatography (**Table 2**).
- Distribution of ¹⁷⁷Lu-MC339 in a mouse CDX model (SHP-77) occurred primarily to tumor with rapid uptake and persistent retention throughout the study and showed a favorable T:K ratio (5:1) at 24 hours post injection (**Figure 3**).
 - Tumor uptake at 2 h postinjection was 12.4 ± 2.52 %IA/g (mean ± SD, tumor to kidney ratio of 2.8:1) tumor uptake remained stable at 24h (12.2 ± 1.43 %IA/g, T:K ratio of 4.7:1).
 - Uptake in all normal tissues was low (< 0.33 %IA/g at 24 h) with the exception of the kidney tissue (4.43 ± 0.58 %IA/g and 2.62 ± 0.17 %IA/g at 2 h and 24 h respectively), suggesting that the major route of elimination was renal excretion.

Table 2. Summary of the Serum Stability of ¹⁷⁷Lu-MC339

Species	Timepoint (h)	Stability (%)
Mouse	1 and 4	99
Human	1 and 4	99

- In vivo biodistribution was assessed in a subcutaneous cell-derived xenograft (CDX) SCLC mouse model (SHP-77) using ¹⁷⁷Lu-MC339
 - ¹⁷⁷Lu-MC339 was injected intravenously, and tissues were collected at 2, 24, 48, 72, and 120 hours post injection
 - Quantification of radionuclide distribution was done using gamma counting
- In vivo anti tumor activity was evaluated in the same model as was used for biodistribution (SHP-77)
 - ²²⁵Ac-MC339 was administered intravenously as a single dose on Day 13
 - Standard of care (SOC, Cisplatin and Etoposide) was administered intraperitoneally
 - Tumor volume and body weight were measured throughout the study

Figure 3. ¹⁷⁷Lu-MC339 Biodistribution in a mouse CDX model (SHP-77)

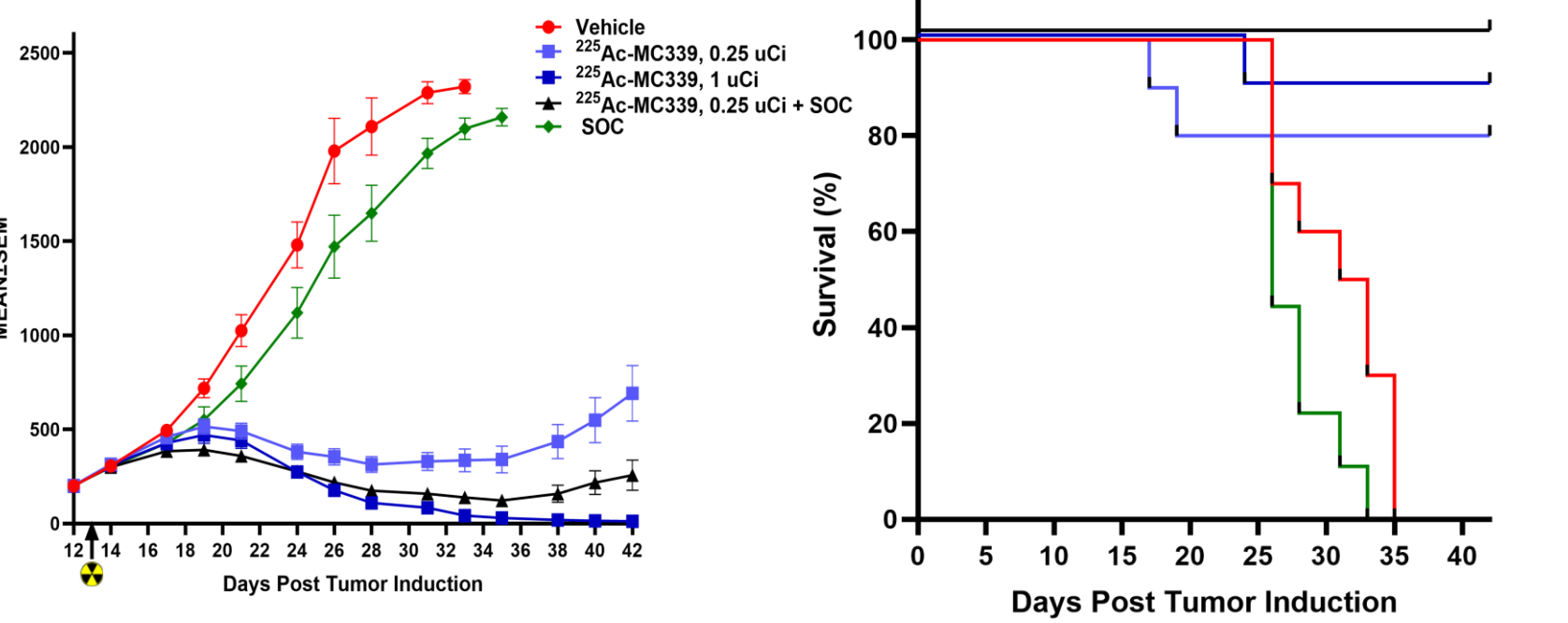


¹⁷⁷Lu-MC339 was injected intravenously, and tissues were collected at different timepoints for ex-vivo biodistribution
%IA/g: percentage of injected activity per gram tissue; SD: standard deviation (N=3 male and 3 female)

²²⁵Ac-MC339 Demonstrated Anti-Tumor Activity

- A single dose of ²²⁵Ac-MC339 induced dose dependent tumor regression and extended survival in a mouse SCLC CDX model (SHP-77) (**Figure 4**).
- When low dose ²²⁵Ac-MC339 was administered in combinations with standard of care (SOC, Cisplatin and Etoposide) the efficacy was improved over either treatment alone.

Figure 4. ²²⁵Ac-MC339 Induced Tumor Regression and Prolonged Survival in a CDX Mouse Model (SHP-77)



²²⁵Ac-MC339 was injected intravenously as a single dose on Day 13 and SOC (Cisplatin and Etoposide) intraperitoneally. Cisplatin, 2.5 mg/kg on Day 13 and Etoposide, 3.5 mg/kg on Day 13, 14 and 15. (N=10/group) . Survival/humane endpoint: Tumor volume>2000 mm³ or bodyweight loss >20% (one animal in 1 μCi group removed for unrelated reason)

Note: Due to the acquisition of Mariana Oncology by Novartis, MC339 is also referred to as ETN029 and ²²⁵Ac-MC339 as ESP359 and will be the nomenclature moving forward.

Disclosures: All authors are employees of Mariana Oncology, a Novartis Company.