Anti-tumor activity of RYZ101 (Ac-225 DOTATATE) in somatostatin receptor-expressing preclinical models of small-cell lung cancer

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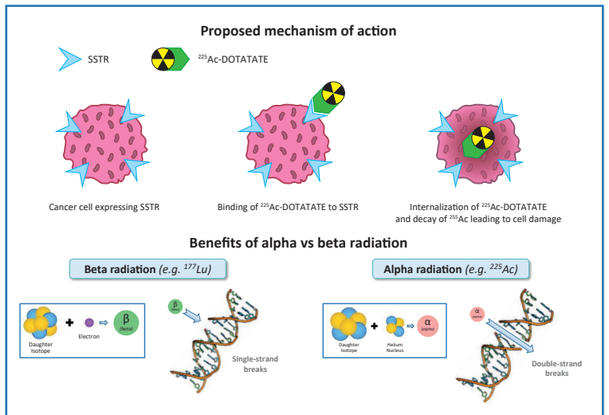
BACKGROUND

- Overexpression of somatostatin receptors (SSTRs), primarily SSTR2, occurs in gastroenteropancreatic neuroendocrine tumors (GEP-NETs), and subsets of other solid tumors such as small-cell lung cancer (SCLC).[1,2]
  - SCLC accounts for approximately 13% of lung cancers and lacks effective therapeutic options.[3,4]
  - Immunohistochemistry indicates that SSTR2 is overexpressed in up to 50% of SCLCs, with a substantial subset showing high and homogenous expression.[5,6]
- β-emitting radiopharmaceutical, Lu-177 DOTATATE, was the first to receive regulatory approval.[7]
  - However, α-emitting radionuclides, such as Ac-225, may offer several advantages over β-emitters for peptide receptor radionuclide therapy (**Figure 1**).
  - α-radiation has a limited range (a few cell lengths) in human tissue, allowing more selective killing of targeted cancer cells while sparing surrounding healthy tissue.
  - α-emitters have linear energy transfer several orders of magnitude greater than β-emitters causing double-stranded DNA breaks and highly effective cancer cell death.[8]

FIGURE 1. RYZ101 (Ac-225 DOTATATE)



- RYZ101 (Ac-225 DOTATATE) is an α-emitting radiopharmaceutical comprised of the radioisotope Ac-225, chemical chelator DOTA, and somatostatin analog octreotate (TATE) (**Figure 1**).
  - RYZ101 is being developed for inoperable SSTR-positive well-differentiated GEP-NETs with disease progression following Lu-177-somatostatin analog therapy (ACTION-1, NCT05477576).
  - The goal of this study was to provide preclinical support for the use of RYZ101 in SSTR+ SCLC administered as either a single-agent or in combination with standard-of-care (SOC) chemotherapy (i.e. carboplatin and etoposide).

METHODS

BINDING AND INTERNALIZATION ASSAYS

- Binding affinity of RAYZ-10001-La (nonradioactive surrogate for RYZ101) and Lu-175-DOTATATE to human SSTR1–5 was determined using a competitive radioligand ([I-125] Tyr-11-somatostatin 14) binding assay in engineered cell lines expressing individual SSTRs.
- Internalization of the RAYZ-10001-La-SSTR2 complex was measured by PathHunter® SSTR2 Activated GPCR Internalization Assay (Eurofins DiscoverX) and expressed as half-maximal rescue concentration (RC_{50}).

BIODISTRIBUTION AND RADIATION DOSIMETRY

- BALB/c mice aged 6–8 weeks received IV RYZ101 (1 uCi, 0.037 MBq, targeted 5 Ci/mmol).
- After RYZ101 administration, animals were sacrificed and selected tissues harvested at 1, 4, 10, 24, 48, and 72 h post-injection (n=3 per timepoint per sex).
- RYZ101 tissue concentrations were quantified (percentage injected dose per gram of tissue, %ID/g).
- Calculated RYZ101 concentrations were used to compute organ and whole-body dosimetry for human adult males and females using OLINDA/EXM 2.0 (HERMES Medical Solutions).

IMMUNOHISTOCHEMISTRY

- Immunohistochemistry (IHC) studies to assess the cell surface expression of SSTR2 were conducted using two human lung cancer tissue microarrays (TMAs; LC2083 and LC802c) and xenograft tumors.
- SSTR2 expression was assessed based on staining intensity using H-Score analysis.

ANTI-TUMOR ACTIVITY

- Studies were performed in cell line-derived (NCI-H524, NCI-H727, NCI-H69) and patient-derived (PDX) xenograft murine models of SSTR2+ SCLC.
- Animals received the following treatments:
  - *NCI-H524 or NCI-H727 xenografts*: single IV injection of RYZ101 4 uCi/0.148 MBq, Lu-177 DOTATATE 3 mCi/111 MBq, or vehicle on day 0.
  - *PDX xenograft*: single IV injection of RYZ101 3 uCi/0.111 MBq or vehicle on day 0 or IP carboplatin 40 mg/kg weekly plus IP etoposide 10 mg/kg QDx3 weekly for 2 weeks.
  - *NCI-H69 xenograft*: single IV injection of RYZ101 1 uCi/0.037 MBq or 4 uCi/0.148 MBq, or RYZ101 4 uCi/0.148 MBq plus IP carboplatin 40 mg/kg weekly plus IP etoposide 10 mg/kg QDx3 weekly for 3 weeks.
- Tumor growth inhibition (TGI) was expressed as mean % Δ inhibition using the formula: [(C-C0)-(T-T0)/(C-C0)]*100%.

RESULTS

BINDING AFFINITY AND INTERNALIZATION

- RAYZ-10001-La (La-DOTATATE) had a high binding affinity (Ki 0.057 nM) to human SSTR2 with more than 600-fold selectivity over SSTR1, 3, 4 and 5 (**Table 1**).
- SSTR binding affinity profile of RAYZ-10001-La was similar to that of Lu-175 DOTATATE.
- RAYZ-10001-La showed efficient internalization in SSTR2+ cells (RC_{50} <0.5 nM vs positive control, mean 1.8 nM).

TABLE 1. Binding affinity of RAYZ-10001-La to human SSTR1 to 5

Peptide	SSTR1	SSTR2	SSTR3	SSTR4	SSTR5
RAYZ-10001-La					
IC_{50} (nM)	>400	0.11 ± 0.002	100 ± 10	>400	200 ± 23
Ki (nM)	n/a	0.057 ± 0.001	35 ± 3	n/a	110 ± 13
Lu-175 DOTATATE					
IC_{50} (nM)	>400	0.066 ± 0.004	140 ± 17	>400	190 ± 15
Ki (nM)	n/a	0.035 ± 0.002	47 ± 6	n/a	110 ± 8

Data expressed as mean ± SEM. All studies performed in triplicate.
Abbreviations: IC_{50}, half-maximal inhibitory concentration; Ki, inhibitory constant; n/a, not applicable; SSTR, somatostatin receptor.

BIODISTRIBUTION AND RADIATION DOSIMETRY

- Biodistribution analysis of RYZ101 showed:
  - *Fast renal clearance*: highest mean (± SEM) concentrations in kidneys at 1 h (15.7 ± 2.2 %ID/g) in female mice, and in the bladder at 10 h (33.6 ± 31.9 %ID/g) followed by kidneys at 1 h (10.4 ± 1.0 %ID/g) in male mice.
  - *Fast clearance/low residence times in whole blood*: mean (± SEM) concentrations at 1 h of 0.22 ± 0.05 %ID/g and 0.105 ± 0.008 %ID/g in female and male mice, respectively.
- Dosimetry estimates for RYZ101 in humans are shown in **Table 2**.

TABLE 2. Estimated Ac-225 human absorbed dose to major organs

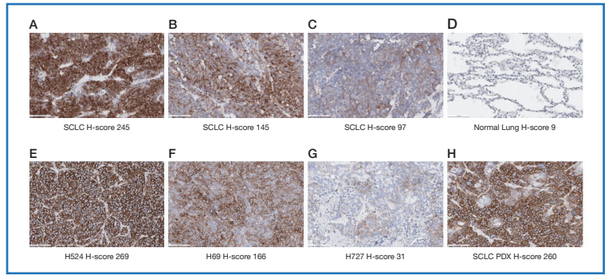
Organ	Ac-225 absorbed dose (Gy/kBq)			
	RBE=1		RBE=5*	
	Female	Male	Female	Male
Adrenals	1.07E-05	1.20E-06	5.33E-05	5.99E-06
Brain	1.52E-07	7.86E-08	7.61E-07	3.93E-07
Esophagus	3.52E-07	1.74E-07	1.76E-06	8.68E-07
Left colon	7.32E-07	5.46E-07	3.66E-06	2.73E-06
Small intestine	3.90E-07	2.08E-07	1.95E-06	1.04E-06
Stomach wall	3.72E-07	1.92E-07	1.86E-06	9.59E-07
Right colon	5.60E-07	3.76E-07	2.80E-06	1.88E-06
Rectum	7.56E-07	5.50E-07	3.78E-06	2.75E-06
Heart wall	2.40E-06	1.17E-06	1.20E-05	5.87E-06
Kidneys	2.48E-05	1.20E-05	1.24E-04	5.99E-05
Liver	4.78E-06	4.70E-06	2.39E-05	2.35E-05
Lungs	4.64E-06	6.98E-06	2.32E-05	3.49E-05
Pancreas	4.18E-06	3.32E-06	2.09E-05	1.66E-05
Red marrow	7.18E-06	7.32E-06	3.59E-05	3.66E-05
Spleen	2.06E-06	1.23E-06	1.03E-05	6.16E-06
Thymus	1.15E-05	1.30E-05	5.77E-05	6.52E-05
Urinary bladder wall	1.17E-06	8.34E-07	5.87E-06	4.17E-06

*for α absorbed dose

SSTR2 EXPRESSION IN SCLC

- Differential SSTR2 expression was observed across human SCLC and xenograft tumors (**Figure 2**).
- The xenograft models tested provide a suitable *in vivo* system with a range of SSTR2 levels and expression patterns for assessment of SSTR2-targeted radiopharmaceuticals.

FIGURE 2. Representative images of SSTR2 IHC staining of human SCLC (A-D) and xenograft tumors (E-H)

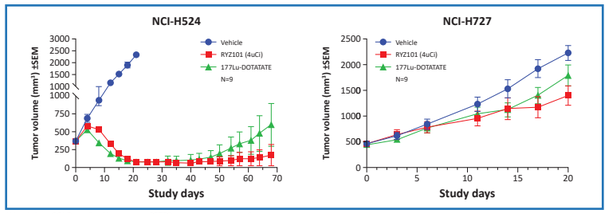


SCLC tissue microarrays and xenografted SCLC sections were stained with an anti-SSTR2 monoclonal antibody and the resulting images were used to generate H-scores.
Abbreviations: IHC, immunohistochemistry; SCLC, small-cell lung cancer; SSTR2, somatostatin receptor 2.

ANTI-TUMOR ACTIVITY

- In SSTR2+ SCLC xenograft models, RYZ101 significantly inhibited tumor growth, with deeper responses and sustained regression observed in the models with higher SSTR2 expression levels (**Figure 3**):
  - Single-dose RYZ101 prolonged tumor regression in the NCI-H524 model, and caused significant TGI (56%; p=0.0003) in the NCI-H727 model relative to vehicle-treated mice.
  - RYZ101 produced a higher level of response in the NCI-H524 model compared with the NCI-H727 model, consistent with higher levels of SSTR2 expression in NCI-H524 xenografts.
  - Compared with Lu-177 DOTATATE, RYZ101 was significantly more active in the NCI-H727 model (TGI, 56% vs 22%; p=0.0319) 20 days post-treatment, and caused more sustained tumor regression in the NCI-H524 model.
  - No significant differences in body weight change or clinical signs of stress were observed, indicating single-dose RYZ101 4 uCi/0.148 MBq was well tolerated.

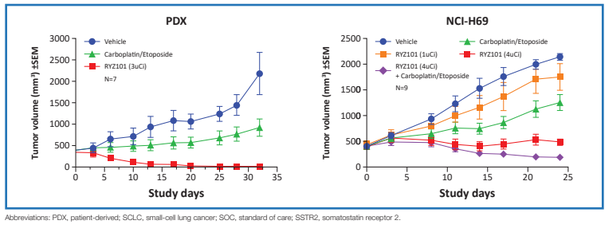
FIGURE 3. RYZ101 treatment of SSTR2+ SCLC xenograft tumors results in target-dependent, durable tumor inhibition



Abbreviations: SCLC; small-cell lung cancer; SSTR2, somatostatin receptor 2.

- RYZ101 had superior anti-tumor activity compared with SOC, and RYZ101 in combination with SOC was superior to SOC alone in SSTR2+ SCLC xenograft models (**Figure 4**).
  - Both single-dose RYZ101 and SOC resulted in significant TGI vs vehicle control (RYZ101, 119%, p=0.0005; SOC, 69% p=0.0287) in an SSTR2+ SCLC PDX model.
  - Comparing the two active therapies, single-dose RYZ101 was significantly superior to SOC in the PDX model (p=0.0008).
  - Single-dose RYZ101 showed dose-dependent anti-tumor activity (TGI:1 uCi/0.037 MBq, 25%, p=0.2745; 4 uCi/0.148 MBq 96%, p<0.0001 vs vehicle control) in the NCI-H69 model.
  - RYZ101 combined with SOC caused sustained tumor regression compared to vehicle control (TGI, 114%, p<0.0001) and significantly greater TGI compared to SOC alone (p<0.0001) in the NCI-H69 model.

FIGURE 4. RYZ101 treatment in SSTR2+ SCLC xenograft tumors results in durable tumor inhibition superior to SOC



Abbreviations: PDX, patient-derived; SCLC, small-cell lung cancer; SOC, standard of care; SSTR2, somatostatin receptor 2.

CONCLUSIONS

- RAYZ-10001-La exhibited high binding affinity and selectivity for human SSTR2, with efficient internalization to SSTR2+ cells.
- In SSTR2+ SCLC xenograft models, RYZ101 demonstrated significant anti-tumor activity versus control as a single agent in a dose- and target-dependent manner.
- RYZ101 showed superior anti-tumor efficacy compared with SOC chemotherapy, and RYZ101 in combination with SOC was superior to SOC alone in an SSTR2+ SCLC model.
- Collectively, these data strongly suggest potential for anti-tumor activity with RYZ101 in patients with SSTR2+ SCLC.
- Based on preclinical modeling and clinical experience in GEP-NETs, a Phase 1b trial of RYZ101 in combination with atezolizumab, carboplatin, and etoposide is underway in patients with extensive-stage SSTR+ SCLC (NCT05595460).

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