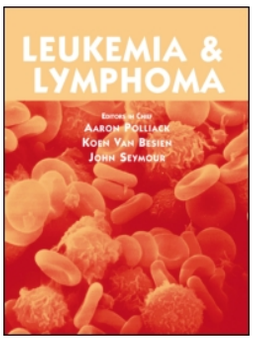

Leukemia & Lymphoma

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ilal20

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To cite this article: Ankita Shahi , Gerald E. Weiss , Saswati Bhattacharya , Dana C. Baiu , Roberta Marino , Taner Pula , Natalie S. Callander , Fotis Asimakopoulos & Mario Otto (2021): Targeted treatment of multiple myeloma with a radioiodinated small molecule radiopharmaceutical, Leukemia & Lymphoma, DOI: 10.1080/10428194.2021.1876858

To link to this article: https://doi.org/10.1080/10428194.2021.1876858

LEUKEMIA & LYMPHOMA
https://doi.org/10.1080/10428194.2021.1876858

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LETTER TO THE EDITOR

Targeted treatment of multiple myeloma with a radioiodinated small molecule radiopharmaceutical

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Despite recent advances in the therapy of multiple myeloma (MM), this blood cancer is characterized by initial response to treatment, but the majority of patients eventually experience refractory relapses [1]. Therefore, new treatment strategies are urgently needed. MM is a very radiosensitive malignancy and ionizing radiation was one of the first therapeutic modalities investigated in the treatment of MM patients [2,3]. However, limited by the infiltrative bone marrow distribution pattern throughout the body and toxicity associated with treatment of large areas, radiotherapy is now primarily reserved for disease palliation and prescribed in the context of emergencies, e.g. for the treatment of spinal cord compression, impending bone fractures, and definitive therapy of isolated plasmacytomas [2]. Molecular-targeted radionuclide therapy could take advantage of the radiosensitive nature of this malignancy and become a new option for the treatment of this often deadly malignancy.

CLR 131 (previously ^{131}I-NM404 or ^{131}I-CLR1404) is a clinical-stage, radioactive isostere of CLR 127 (18-(p-iodophenyl) octadecyl phosphocholine), a tumor-selective alkyl phosphocholine (APC) analog that belongs to the family of anti-tumor alkyl phospholipid (APL) drugs. CLR 127 targets malignant cells across a broad variety of histopathological origins, with little uptake in healthy tissues [4]. The notable tumor selectivity can be attributed for the most part to selective uptake *via* lipid rafts, unique subdomains of the plasma membrane which are much more abundant in malignant compared to normal cells [5]. CLR 127 and its radioactive derivatives have shown almost universal uptake in more than 80 tumor cell lines, in xenograft models of human cancers, cancer stem cells, and most importantly, in primary tumor and metastatic sites in patients [6–8]. Labeled with radioactive, positron-emitting iodine-124 (CLR 124) or the beta/gamma-emitting isotope iodine-131 (CLR 131), the radiopharmaceutical can be utilized as a theranostic agent, i.e. it can be prescribed for diagnostic imaging and molecular radiotherapy, respectively [8,9]. Supplementary Figure 1 (S1) illustrates the various diagnostic and therapeutic analogs of this versatile small molecule APL drug. MM cells contain significant amounts of lipid rafts, providing a rationale for APL-mediated MM targeting [10].

To test the possibility of utilizing CLR 131 for treatment of MM, we first investigated the *in vitro* uptake and retention of the green-fluorescent (BODIPY) analog of CLR 127 (CLR1501) as a surrogate marker in various MM cell lines (MM1.S, MM1.R, U266, RPMI 8226 and KMS-12-BM) by flow cytometry. A detailed description of materials and methods utilized in our experiments can be found in the supplemental data section. Uptake of CLR1501 was greatly increased (up to 3.9-fold) in all tested cell lines, compared to freshly isolated peripheral blood mononuclear cells (PBMC) after 6-hour incubation with 5 μM CLR1501 (Figure 1(A)). The difference was statistically significant ($p \leq 0.05$, three independent repeats) in all but one cell line (KMS-12-BM). However, immortalized cell lines might not accurately reflect uptake in malignant primary cells. Therefore, we determined CLR1501 uptake by flow cytometry in MM cells from 5 separate patient bone marrow aspirate samples and compared the uptake to non-MM bone marrow cell populations from the same sample. MM cells, defined as live $CD38^+$ $CD56^+$ $CD19^{+/-}$ $CD138^+$ cells [11], sequestered and retained significantly higher amounts of CLR1501

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Supplemental data for this article can be accessed here.

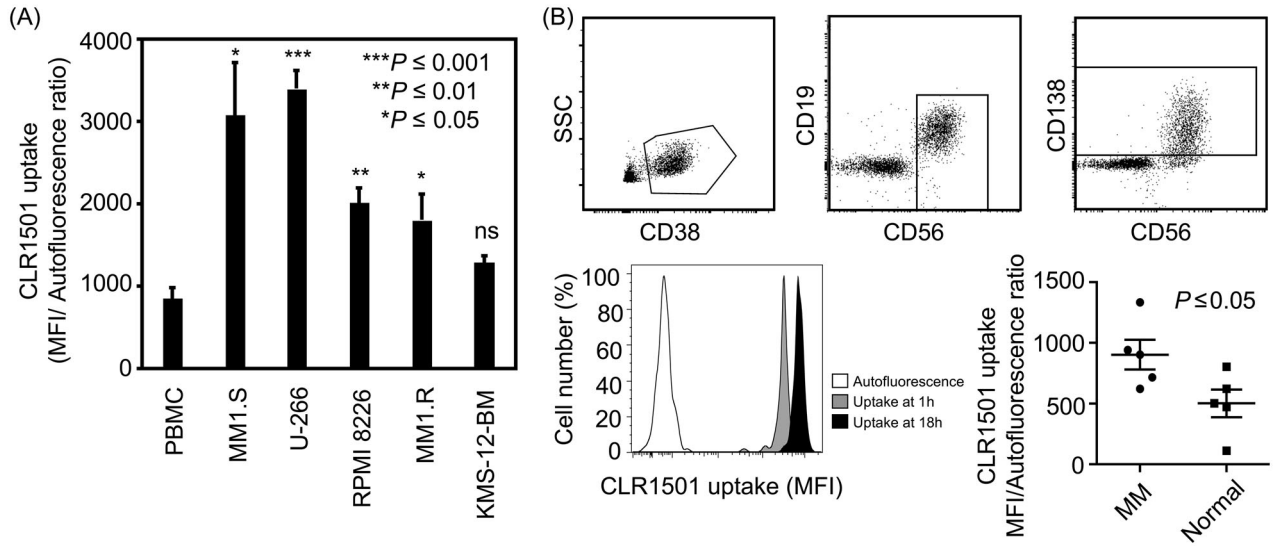

Figure 1. (A) Preferential uptake of CLR 1501 (fluorescent analog of CLR 127) in MM cell lines compared with normal cells (PBMC), by flow cytometry. Data presented as mean ± SE (standard error) from three independent experiments. Indicated *p* values calculated for tumor cells versus peripheral blood mononuclear cells (PBMC). MFI = mean fluorescence intensity, ns = not significant. (B) Patient-derived MM cells from bone marrow sequester a higher amount of CLR1501 than non-MM bone marrow cell populations. Uptake of CLR1501 was assessed in bone marrow aspirate samples of 5 separate patients. Upper panels: Gating strategy of live bone marrow cells. MM cells were defined as $CD38^+$ $CD56^+$ $CD19^{+/-}$ $CD138^+$. Lower panels: Left: CLR1501 uptake in $CD138^+$ cells at 1 h (grey histogram) and 18 h (black histogram), compared to autofluorescence (clear histogram). Right: Average MFI, error bars representing 95% confidence intervals, as a measure of CLR1501 uptake by $CD138^+$ cells (MM) and CD138-cells (normal cells), at 1 h ($n = 3$) and 18 h ($n = 2$).

than the rest of the bone marrow mononuclear cells at 1 and 18 h, respectively ($p < 0.05$, Figure 1(B)). In one patient sample, we used the near-infrared analog CLR1502 as surrogate and detected a 4.2-fold higher uptake in bone marrow MM cells compared to BM plasma cells (Supplementary Figure S2). Incubation times were selected based on studies in solid tumors and leukemias, in which *in vitro* uptake under these conditions predicted *in vivo* sequestration and importantly, tumor-selective drug retention [8].

We then explored the *in vivo* uptake in immunodeficient NSG (NOD-*scid* $IL2Rg^{null}$) mice bearing MM1.S flank xenografts. After tumors reached a minimum volume of 180 mm^3, mice were injected with 5.5 mBq clinical-grade CLR 124 *via* tail vein, and scanned on a microPET/CT instrument, as previously described [12]. We confirmed selective uptake and retention of CLR 124 (previously ^{124}I-CLR1404) in the MM1.S flank xenografts (Supplementary Figure S3), comparable to observations in various adult and pediatric solid tumor flank syngeneic and xenograft models we and others had published previously [8,12]. This was our first proof of concept validating *in vivo* sequestration of the compound in MM and prompted experiments geared toward MM-targeted molecular radiotherapy with the beta-emitting therapeutic radioisostere, CLR 131. MM1.S flank xenografts were established in NSG mice as described above. Once tumors reached an average volume of 500 mm^3, cohorts of mice were randomized to receive a single dose of 3.7 MBq (148 MBq/kg body weight for a 25 g animal, $n = 14$) of clinical-grade CLR 131, injected *via* tail vein. Control mice ($n = 13$) were injected with excipient (vehicle only). Molecular radiotherapy with a single dose of CLR 131 led to significantly lower tumor growth rates in a mixed-linear effects model and afforded a significant survival benefit (Figure 2(A)) while being well-tolerated ($p < 0.0001$). As expected, treatment with CLR 131 led to temporary pancytopenia, with recovering white cell and platelet indices and nadir of hemoglobin documented at day 21 after CLR 131 injection (Supplementary Figure S4). Also, animal weights throughout treatment did not differ between treated and control mice (Supplementary Figure S5). We have previously confirmed that the injected activity of iodine-131 does not induce overt radiotoxicity (direct observation and calculated modified Cooke health scores), nor does it lead to clinically significant hematological abnormalities in NSG or wild-type, immunocompetent mice [12]. Next, we determined treatment efficacy in a clinically relevant murine xenograft model mirroring orthotopic MM disease in the bone marrow, as described previously [13]. NSG mice were injected with 5×10^6 MM1.S cells *via* lateral tail vein. Disease progression was determined by measuring serum human lambda light chain concentrations by ELISA as previously reported [14]. Once lambda light chain production was detected in a range between 10-20 μg/mL in serum samples, mice were randomized to receive either a single dose of CLR 131 (3.3 MBq) or excipient. Serum was collected weekly

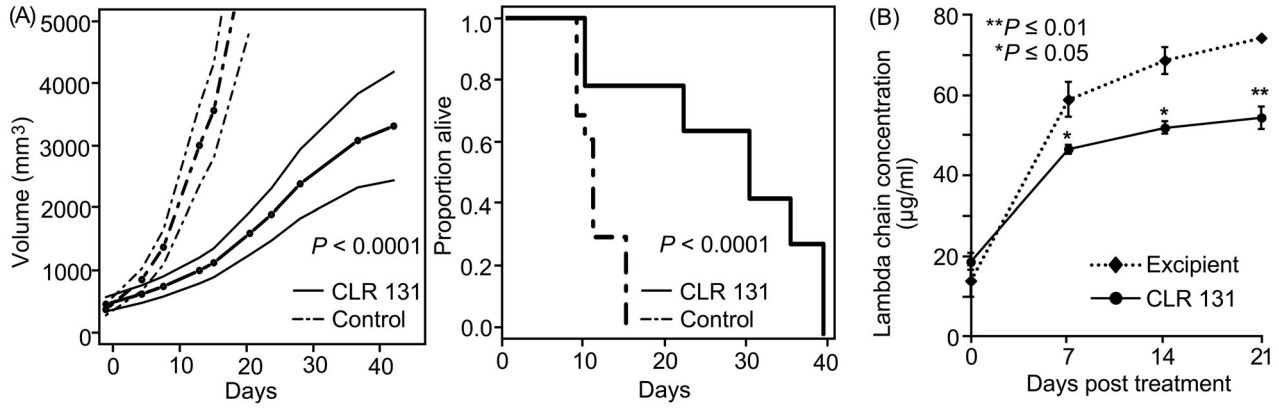

Figure 2. (A) Single-dose treatment of flank-xenograft bearing mice with CLR 131 slows tumor growth and leads to improved survival. NSG mice harboring human MM1.S multiple myeloma xenografts ($n = 14$, approximate volume 500 mm^3) in the flank were administered a single dose (100 µCi) of CLR 131. Tumor-bearing control animals ($n = 13$) were administered a volume equivalent of excipient (vehicle only). Left panel: Mean tumor growth rate of treated mice (solid line) and control animals (broken line) in a mixed linear effects model ($p < 0.0001$) with 95% confidence intervals (fine lines). Right panel: Kaplan–Meier survival analysis demonstrates significant survival benefit in treated (solid line) mice vs. controls ($p < 0.0001$, broken line). (B) Treatment with CLR 131 leads to decreased human lambda light chain production in an orthotopic MM bone marrow xenograft model (MM1.S). Mice with established human MM bone marrow disease ($n = 5$ per cohort) were injected with a single intravenous dose of CLR 131 or excipient, and serum was collected at the indicated time points after injection and analyzed for lambda light chain concentration by ELISA. Data represent mean light chain concentration ± SE (error bars) from 5 mice/cohort per indicated time point. Treated mice exhibited significantly decreased serum light chain concentrations (solid line) at all indicated time points compared to control mice (dotted line).

and cryopreserved until radioactivity had decayed to safe levels prior to analysis of human lambda light chain content. As expected in this well-established model, mice reliably developed MM in the bone marrow. We observed that mice treated with CLR 131 had statistically significantly lower serum concentrations of human lambda light chains, with differences already detectable at the first time point (7 days after start of treatment, $p \leq 0.05$), and flattening of the curve until the endpoint of this experiment was reached (21 days after injection of CLR 131, $p \leq 0.01$, Figure 2(B)).

In summary, MM is a malignancy of plasma cells that displays clinically actionable radiosensitivity. Our data demonstrate uptake of a fluorescent CLR 127 analog across several MM cell lines, selective uptake in patient-derived MM cells from bone marrow samples, and sequestration of the radiolabeled isosteric analog CLR 124 in mouse xenografts. Therapeutic efficacy of CLR 131 can be demonstrated in a heterotopic flank model as well as in an orthotopically engrafted bone marrow xenograft model. These models are well established, allowed us to investigate CLR 131 as a first-in-class radiopharmaceutical agent for MM, and to establish an initial efficacy and adverse effect profile. Lipid rafts are overexpressed in many cancers, as they are intrinsically involved in cell metabolism and cancer-related signaling processes with little to no aptitude for downregulation and hence, tumor escape. Verdonck et al. [15] have shown that even highly drug-resistant acute leukemia lines are still targetable with APC analogs, owing to independent uptake and retention mechanisms. This information is currently lacking for multidrug-resistant, patient-derived MM cells and further investigations would be desirable. CLR 127 and its radioactive derivatives are highly albumin-bound, with slow hepatobiliary excretion [8]. Bone marrow toxicity is therefore an expected, dose-limiting, off-target effect of CLR 131 as is the case with many other radiopharmaceuticals. At the same time, given localization of the tumor cells in the bone marrow, CLR 131 will provide an additional on-tumor effect given the average path length of the beta particles of ^{131}I while circulating through the BM vasculature. In contrast to antibody-mediated radioimmunotherapy approaches, CLR131 does not depend on the expression of tumor-specific surface antigens, which may vary between patients. Therefore, CLR131 could have a distinct advantage over these treatment modalities.

Taken together, our data provide a strong rationale for the clinical evaluation of molecular-targeted radionuclide therapy with CLR 131 in MM patients. Clinical phase I/II studies with CLR 131 in relapsed/refractory MM and other B cell malignancies (including, but not limited to, chronic lymphocytic leukemia and lymphoplasmacytic lymphoma) and in children and adolescents with select lymphomas and other solid tumors have been initiated and are currently ongoing in the United States (clinicaltrials.gov: NCT 02278315, NCT 02952508, NCT 03478462). Given the well-known synergistic effects of radiotherapy with other treatment modalities, the introduction of the first-in-class radiopharmaceutical CLR 131 as a new tool in the armamentarium against MM could allow for future rationally designed combination therapies that might significantly improve patient outcomes.

Disclosure statement

M.O., R.M., and F.A. hold a patent on APC analogs for multiple myeloma imaging and therapy. M.O. and N.S.C. have been past consultants for Cellectar Biosciences, Inc., the license holder of CLR 131. The authors have no other conflict of interest to declare.

Author contributions

M.O. was responsible for the overall design, data analysis, data interpretation, and manuscript preparation. A.S. performed experiments, statistically analyzed and plotted data, and edited the manuscript. G.E.W compiled data and wrote the manuscript. S.B. and R.M. performed animal experiments. D.C.B. and T.P. collected samples and performed experiments. N.S.C. and F.A. provided critical advice, clinical samples, cell lines, and edited the manuscript. All authors read and approved the final manuscript.

Acknowledgments

The authors thank Mohammed Farhoud, Ph.D., and Benjamin Titz, Ph.D., for assistance with animal imaging experiments, Ellen Hebron for expert technical help, and Jens Eickhoff, Ph.D., for help with statistical analysis. The authors also thank James P. Zacny, Ph.D., for his editorial work. The authors thank Cellectar Biosciences Inc., Florham Park, NJ, USA, for providing APC derivatives for our studies.

Funding

This work was supported by NIH/NCI [P30CA014520] (UW Carbone Cancer Center Support Grant), the Midwest Athletes Against Childhood Cancer (MACC) Fund (M.O.), Young Investigator Sub-Award from St. Baldrick's Pediatric Dream Team Translational Research Grant, Stand Up To Cancer [SU2C-AACR-DT1113] (M.O.) and the University of Wisconsin Trillium Fund for Myeloma Research.

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