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^{212}Pb Alpha-Radioimmunotherapy targeting CD38 in Multiple Myeloma: a preclinical study.

Short running title:

^{212}Pb-anti-CD38 preclinical study in MM

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ABSTRACT

Multiple myeloma (MM) is a plasma cell cancer and represents the second most frequent hematological malignancy. Despite new treatments and protocols including high doses chemotherapy associated with autologous stem cell transplantation, the prognosis of MM patients is still poor. Alpha-radioimmunotherapy (α-RIT) represents an attractive treatment strategy due to the high linear energy transfer and short path length of α-radiation in tissues, resulting in high tumor cell killing and low toxicity to surrounding tissues. In this study, we investigated the potential of α-RIT with ^{212}Pb-Daratumumab (anti-CD38), in both *in vitro* and *in vivo* models, as well as an anti-mouse CD38 antibody using *in vivo* models.

Methods: Inhibition of cell proliferation after incubation of RPMI8226 cell line with increasing activities (0.185-3.7 kBq/ml) of ^{212}Pb-isotypic control or ^{212}Pb-Daratumumab was evaluated. Biodistribution was performed *in vivo* by SPECT-CT imaging and *post-mortem*. Dose range finding (DRF) and acute toxicity studies were conducted. As Daratumumab does not bind the murine CD38, biodistribution and DRF were also determined using an anti-murine CD38 antibody. To evaluate *in vivo* efficacy of ^{212}Pb-Daratumumab, mice were engrafted subcutaneously with 5.10^6 RPMI8226 cells. Mice were treated 13 days post-engraftment with an intravenous injection of ^{212}Pb-Daratumumab or control solutions. Therapeutic efficacy was monitored by tumor volume measurements and overall survival.

Results: Significant inhibition of proliferation of the human myeloma RPMI8226 cell line was observed after three days of incubation with ^{212}Pb-Daratumumab compared to ^{212}Pb-Isotypic Control or cold antibodies. Biodistribution studies showed a specific tumoral accumulation of

Daratumumab. No toxicity was observed with ^{212}Pb-Daratumumab up to 370 kBq due to the lack of cross-reactivity. Nevertheless, acute toxicity experiments with ^{212}Pb-anti-mCD38 established a toxic activity of 277.5 kBq. To remain within realistically safe treatment activities for efficacy studies, mice were treated with 185 kBq or 277.5 kBq of ^{212}Pb-Daratumumab. Marked tumor growth inhibition compared to controls was observed, with a median survival of 55 days for 277.5 kBq of ^{212}Pb-Daratumumab instead of 11 for PBS control groups.

Conclusion: These results showed ^{212}Pb-Daratumumab efficacy on xenografted mice with significant tumor regression and increased survival. This study highlights α-RIT potency in MM treatment.

Key words: ^{212}Pb, Alpha-radioimmunotherapy, Multiple Myeloma, CD38

INTRODUCTION

Multiple myeloma (MM) features monoclonal proliferation of plasma cells in bone marrow. Over the last decade, many advances have been made in MM therapy and the median life expectancy of patients has almost doubled. This improvement was mostly due to the development of proteasome inhibitors, immunomodulatory drugs, histone deacetylase blockers and, more recently, monoclonal antibodies (MAbs) (Daratumumab and Elotuzumab) (*1*). However, the prognosis of myeloma patients remains poor since remission obtained with such treatments is often followed with relapse. Innovative therapies with distinct mechanism of action are therefore needed.

Targeted immunotherapy using MAbs has showed efficacy, nevertheless, strategies to enhance MAb efficiency are necessary and were developed in the form of antibody drug conjugates (ADCs), immunotoxins or radiolabeled antibodies. The efficacy of radioimmunotherapy (RIT) in the treatment of non Hodgkin's lymphoma is well established, with two marketed anti-CD20 MAbs coupled with β-emmiters, ^{90}Y-ibritumomab tiuxetan (Zevalin®) and ^{131}I-tositumomab (Bexxar®) (*2*). Although Zevalin® is highly efficient against tumor cells, it carries severe side effects compared to rituximab, notably bone marrow toxicity. Since α-particles have a short path length of 50–80 μm (compared with a few mm for β-particles), RIT with α-emitting radionuclides is highly attractive and expected to reduce unwanted radiation exposure on normal tissues. α-particle's short path length also explains why α-RIT, by specifically targeting the close environment of each malignant cell, is better suited for micrometastatic and disseminated tumor treatment (*3*). α-particles produce clustered DNA double-strand breaks and highly reactive hydroxyl radicals when hitting biological tissues. Their short path range leads to a high-linear energy transfer of

approximately 50–230 keV/µm compared to 0.1–1.0 keV/µm for β-emitters making α-emitters 100-fold more cytotoxic. Only a few α-emitters are considered suitable for therapeutic use in cancer patients (*4*). ^{212}Pb represents a good candidate. This radioelement is available at high purity through a ^{224}Ra/^{212}Pb generator. After administration, ^{212}Pb (β-emmiter) generates ^{212}Bi (α-emmiter) and so ^{212}Pb, which has a half-life ($t_{1/2}$ = 10.6 h) relatively convenient for mAb pharmacokinetics, serves as an *in vivo* α-emmiter generator (*5*). A macrocyclic bifunctional ligand, TCMC, was designed and synthesized to obtain a greater stability *in vivo* for the chelation of Pb isotopes (*6*). Thus, MAb can be easily functionnalised with TCMC and then radiolabeled with ^{212}Pb. ^{212}Pb was first used in a human trial of ^{212}Pb-TCMC-trastuzumab in patients with HER-2-expressing malignancies (*7*)(*8*).

CD38, a 45-kDa stable transmembrane glycoprotein receptor, is expressed at a high epitope density on 95% to 100% of malignant plasma cells (*9*)(*10*). The CD38 antigen is expressed on activated T cells, monocytes, and NK (natural killer) cells but at much lower levels than found on plasma cells making it a targeting candidate for RIT using anti-CD38 antibodies. Few RIT studies evaluated this target potency and none evaluated ^{212}Pb-anti-CD38 RIT in myeloma. Daratumumab is an anti-human CD38 developped by Janssen and currently used in the clinic (*11*). We have developed a targeted α-therapy where the Daratumumab antibody is coupled to the α-particle-emitting radioisotope ^{212}Pb. The goal of this study was to investigate the potential of ^{212}Pb-Daratumumab in the treatment of plasma cell malignancies. Biodistribution and toxicity studies were performed on tumor-free and RPMI 8226 myeloma tumor-bearing mice. Considering that ^{212}Pb-Daratumumab does not cross-react with the murine CD38, biodistribution and toxicity

studies were also performed with an anti-murine CD38 Ab. The therapeutic efficacy of this treatment was assessed *in vitro* and *in vivo* on subcutaneous xenograft model.

MATERIALS AND METHODS

Cell Lines and Mice

The human myeloma RPMI8226 cell line (ATCC) was maintained in supplemented RPMI 1640 medium. C57BL/6 mice (female, 7 to 10 weeks) were purchased from Janvier Labs (France). $Rag2^{-/-}\gamma C^{-/-}$ mice were kindly provided by Dr James Di Santo (Institut Pasteur, France).

Xenograft models

8-12 weeks-old $Rag2^{-/-}\gamma C^{-/-}$ mice were engrafted subcutaneously in the leg flank with 5.10^6 RPMI8226 cells (in 100 µl of PBS and Matrigel® 50/50, v/v). Mice were monitored daily for signs of pain or discomfort. Tumor volume was measured with a caliper three times a week. Studies were conducted 13 days post-engraftment (tumors between 150 and 400 mm^3), except for SPECT-CT imaging (20 days / 500-800 mm^3). All *in vivo* experiments were performed in accordance with animal ethical rules and all protocols were authorized by the French Ministry of Research according to EU reglementation (APAFIS#15900-201807061621591 v2).

MAbs Conjugation and Radiolabeling

MAbs were obtained from Janssen: IgG anti-human CD38 (hCD38) (Daratumumab), IgG Isotypic Control and IgG anti-murine CD38 (anti-mCD38, which is not a surrogate of Daratumumab as it lacks the *in vitro* and *in vivo* functional activity of the hCD38 antibody). Antibodies were conjugated by Macrocyclics (Plano, TX, USA) with the bifunctional chelating agent TCMC

[(1.4.7.10-tetra-(2-carbamoyl methyl)-cyclododecane] using a proprietary site specific technique, with approximately 1.3–2 TCMC/MAb.

^{212}Pb was produced from ^{224}Ra generators provided by Orano Med SAS (Bessines-sur-Gartempes, France). Chelation was performed by incubating 1 mg mAb-TCMC per 37MBq of ^{212}Pb for 15 minutes at 37°C in 150 mM Ammonium acetate pH 4.5. The labeling yield, assayed by instant thin-layer chromatography, was > 94% and specific activities were approximately 37 MBq/mg for the MAbs at the experiment time. Immunoreactivity of ^{212}Pb-Daratumumab against hCD38 was assessed *in vitro,* by direct binding assays (*12*) on RPMI8226 cells and a Kd of 2.69 ± 1.28 nM was obtained. This value was consistent with known Daratumumab affinity (4.36 nM) (*13*). Furthermore, an Immunoreactive Fraction > 92% was obtained. For SPECT/CT imaging, MAbs were radiolabeled with ^{203}Pb. ^{203}Pb in 0.5M HCl was provided by Lantheus Medical Imaging (North Billerica, MA, USA). After adjusting ^{203}Pb solution pH to 4.5 with 1.5M ammonium acetate, MAbs were incubated for 15 minutes with ^{203}Pb at 37°C. Labeling yield was > 98% and specific activities were approximately 37 MBq/mg.

Cell proliferation analyses

RPMI8226 cells were cultured at 37° C in 96-well plates (25 000 cells in 100 µl/well). Proliferation was assessed in triplicate at Day 1, 2, 3 and 4, under various concentrations of ^{212}Pb-MAb (0.185-3.7 kBq/ml) or cold-MAb (5-100 ng/ml) using Cell Titer Glo (incubation 2h, OD 490 nm) (Promega).

Biodistribution Experiments and SPECT-CT Imaging

Biodistribution experiments were carried out in tumor-free or tumor bearing C57Bl/6 or Rag2$^{-/-}$ γC$^{-/-}$ mice. ^{212}Pb-anti-mCD38, ^{212}Pb-Daratumumab or ^{212}Pb-Isotpypic Control (1.85 MBq) were injected intravenously. At each time point (2, 6, 12, 18, 24, 48h post-injection), 2 to 5 animals/group were euthanised under isoflurane inhalational anesthesia by cervical dislocation. Selected tissues were excised, weighed, and their radioactivity levels were measured with a calibrated gamma-counter (Perkin Elmer) (190-290 keV). The uptake of radioactivity in these organs was expressed as %ID/g after correcting for radioactive decay at each time point.

For SPECT-CT imaging, animals were injected with ^{203}Pb-MAbs (18.5 MBq) and imaged under isoflurane inhalational anesthetics (1,8%, 50% air/ 50% oxygen, 1,4 L/minutes) at 2h, 6h, 24h, 48h and 96h post-injection with a MicroSPECT/CT (U-SPECT4/CT, MILabs, The Netherlands). Image acquisition lasted 30 minutes for earlier time-points to 90 minutes for later time-points. Energy windows were set over the 279 keV peaks (±20%) and GP-RM collimator was used (75 holes of 1.5 mm diameter / iterative reconstruction OS-EM, no filter (16 subsets / 6 iterations / voxel size: 0.8 mm). The SPECT resolution with ^{203}Pb is estimated to be less than 1 mm. Images were analyzed with PMOD Software (PMOD Technologies, Switzerland).

Toxicity studies

Groups of 5-8 tumor-free mice (C57Bl/6 or Rag2$^{-/-}$γC$^{-/-}$) received ^{212}Pb-Daratumumab, ^{212}Pb-anti-mCD38 (185 to 370 kBq) or PBS by intravenous injection. Mice were monitored and weighed daily. At experimental time point, a complete blood count was performed on an automated hematology analyzer (Cell Dyn, Abbott). Biochemical parameters (ASAT, ALAT, urea and creatinine) were measured in blood plasma on an automated biochemistry analyzer (Konelab, Thermo). The

percentage of B220+ positive cells in blood, bone marrow and spleen were determined by flow cytometry (AccuriC6, BD). We took advantage of the coexpression of B220 and CD38 (*14*) to monitor variations in B-lineage using a B220 labelling.

Radioimmunotherapy experiments

Mice were randomly assigned to experimental groups and received a single intravenous injection of ^{212}Pb-Daratumumab (185 kBq or 277.5 kBq), ^{212}Pb-Isotypic Control (277.5 kBq), Daratumumab 10µg, Daratumumab 16mg/kg or PBS. Mice were monitored daily for general appearance. Twice a week, tumor volume was measured and mice were weighed. Mice were euthanized when tumors reached a volume of 1 cm^3, if tumor ulceration occurred or if tumor caused obvious discomfort.

RESULTS

^{212}Pb irradiation induces inhibition of proliferation of MM cells

Effect on cell proliferation of increasing activities (0.185-3.7 kBq/ml) of ^{212}Pb-Daratumumab or ^{212}Pb-Isotypic Control were assessed during 4 days (Fig. 1). Growth inhibition was observed with ^{212}Pb-Daratumumab from Day 3, with a dose-dependent inhibition at Day 4 (37.7$\pm$2.3 % at 0.185 kBq/ml to 80.6$\pm$3.4 % at 3.7 kBq/ml). This inhibition was significantly higher than ^{212}Pb-Isotypic Control inhibition at all activities tested except at 3.7 kBq/ml. The same experiment with increasing concentrations of the two cold MAbs showed no significant effect on growth inhibition (Supplemental Fig. 1). **Biodistribution Experiments and SPECT-CT Imaging**

Biodistribution of Daratumumab was studied in Rag2$^{-/-}\gamma C^{-/-}$ tumor-bearing mice to monitor Daratumumab specific accumulation in the human tumor xenograft and compared to an Isotypic Control (Fig. 2). After ^{212}Pb-Daratumumab injection, radioactivity in tumors increased over time: peak radioactivity was reached after 24 hours (20.8 ± 1.4 % ID/g) and maintained this high level at 48 hours post-injection. From 18h, accumulation of ^{212}Pb-Daratumumab in the tumor was significantly higher than that of ^{212}Pb-Isotypic Control (7.75 ± 2.6 %ID/g at 24h). *In vivo*, imaging data confirmed specific tumoral accumulation of Daratumumab with a peripheral tumoral uptake for larger tumors (Fig. 2C/D). Accumulation of ^{212}Pb-Daratumumab and ^{212}Pb-Isotypic Control in all non-tumor tissues was not significantly different.

As Daratumumab does not bind mCD38, a biodistribution using an anti-mCD38 antibody was performed in tumor-free mice to estimate antibody accumulation in healthy organs and anticipate potential toxicity issues. Biodistribution studies on C57Bl/6 mice showed high

accumulation of radioactivity in three organs as soon as 2h post-injection: liver (58.1 ± 1.9 %ID/g), spleen (25.7 ± 6.9 %ID/g) and lung (30.5 ± 2.4 %ID/g) (Fig. 3). Uptake was then relatively constant over time in these organs, except in the spleen where the radioactivity increased with time (66.8 %ID/g at 24h). Significant radioactivity was also present in the femur (6-8%) but remained low in the blood (<2% after 6h). The biodistribution of anti-mCD38 was also examined in $Rag2^{-/-}\gamma C^{-/-}$ (Supplemental Fig. 2) and a similar pattern was observed, except for a higher uptake in spleen (134.6%ID/g at 24h) due to the splenic hypotrophy of RAG-deficient mice, as observed in SPECT/CT.

Comparatively to ^{212}Pb-anti-mCD38, ^{212}Pb-Daratumumab accumulation in spleen, liver, lung, bone marrow and femur was lower.

Dose Range Finding and Acute Toxicity

Anti-hCD38/Daratumumab

Acute toxicity (7 and 21 days) was studied after injections of 185 kBq, 277.5 kBq or 370 kBq of ^{212}Pb-Daratumumab on $Rag2^{-/-}\gamma C^{-/-}$ healthy mice. For these three activities, no effect was observed on survival (Table 1), body weight, blood cell count and biochemical dosages (data not shown), consistent with Daratumumab lack of binding to mCD38. For the latter reason, toxicity was thus also studied with anti-mCD38.

Anti-mCD38

Acute toxicity of 185 kBq and 370 kBq of ^{212}Pb-anti-mCD38 was studied in C57BL/6 and in $Rag2^{-/-}\gamma C^{-/-}$ healthy mice. Animal behavior and body weight were monitored during 21 days. For both

strains, a significant body weight loss (*p<0.01*) was observed after 185 kBq of ^{212}Pb-anti-mCD38 compared to PBS (Fig. 4 and Supplemental Fig. 3). This loss was dose-dependent and significantly increased with 370 kBq of ^{212}Pb-anti-mCD38 (*p<0.0001*). Survival was not affected after injection of 185 kBq of ^{212}Pb-anti-mCD38, whereas 370 kBq of ^{212}Pb-anti-mCD38 induced 75% lethality for C57BL/6 and 40 % for Rag2$^{-/-}$γC$^{-/-}$ mice at the 21 days time point (Table 1).

In C57BL/6 mice, we investigated kidney and liver toxicity using biochemical dosages of plasma creatinine, urea (kidney toxicity), ASAT and ALAT (liver toxicity). No significant changes in these dosages were observed (Supplemental Fig. 4). Complete blood cell counts revealed hematologic toxicity with a drop in both leukocyte and platelet counts, whereas red blood cell count was not affected by ^{212}Pb-anti-mCD38 injection (Fig. 5). At 7 and 10 days post-injection, the leukocyte counts were reduced to the lower limit of normal ranges for 185 kBq and below normal ranges for 370 kBq (Fig. 5A). The leukocyte decrease was reversible for both activities and values were back to normal 21 days post-injection. In the same way, platelet counts were reduced as early as 7 days but no recovery was observed at day 21 (Fig. 5C). FACS analyses on B220 population in blood, spleen and bone marrow (Fig. 6) confirmed these results and radiation-induced spleen and bone marrow damage.

Acute toxicity manifested at 370 kBq of ^{212}Pb-anti-mCD38 and therapeutic activity should thus remain below.

Efficacy of ^{212}Pb-Daratumumab treatment

Efficacy studies were conducted in Rag2$^{-/-}$γC$^{-/-}$ bearing subcutaneous xenografts of RPMI8226 MM cells. Survival curves and tumoral growth after treatment are presented as Figure 7. In the control

group (PBS), a median survival time (MST) of 11 days was observed (Fig. 7A). All treatments except Daratumumab at 10 µg (equivalent amount used for the radiolabeled antibody) induced a significant MST increase (*p<0.001*): 32, 39.5, 47 and 55.5 days for Daratumumab 16mg/kg (dose used in clinical practice), ^{212}Pb-Isotypic Control at 277.5 kBq and ^{212}Pb-Daratumumab at 185 kBq and 277.5 kBq respectively. Nevertheless, the survival increase induced by ^{212}Pb-Daratumumab at 277.5 kBq was significantly higher than with cold Daratumumab (*p<0.001*). ^{212}Pb-Daratumumab at 277.5 kBq induced a tumoral regression from day 4 to day 25 post-treatment but a resumption of tumoral growth was then observed except for one mouse (Fig 7B).

DISCUSSION

Recent advances in MM therapies, such as immunomodulatory drugs and proteasome inhibitors have significantly prolonged survival of MM patients in the last decade. However, prognosis of relapsed MM patients remains poor. RIT is a therapeutic modality that is not cross-resistant with chemotherapy or other therapeutic agents. This strategy has already shown its value in hematological cancers, but there are few studies of radioimmunotherapy in MM.

Chérel *et al.* have studied RIT efficiency in MM with ^{213}Bi-anti-CD138 and showed significant efficacy in murine myeloma models (*15*). In our study, we targeted CD38 since its expression is high and uniform on malignant plasma cells, but relatively low on normal lymphoid, myeloid cells and non-hematopoietic tissues (*16*). Recent studies confirmed the pertinence of targeting CD38 in the treatment of MM (*17*) (*18*).

Most MM RIT studies used α-emitter. In fact, as MM cells are found either isolated in BM or in small clusters, α particles have a theoretical advantage over beta particles because of their high linear energy transfer and shorter range of action. Cell destruction would be more selective and irradiation less harmful to adjacent cells (*19*). As anticipated, a study of an anti-CD138 coupled with either ^{213}Bi or ^{177}Lu revealed the advantages of α-RIT over β-RIT in the treatment of MM in a preclinical model (*20*). Unfortunately, ^{213}Bi is a very short lived α-emitter (45 min) which hampers its use in therapy. We chose to evaluate the α-emitter ^{212}Pb, selected for its longer half-life (10.6 h), which is more suitable with regards to antibody kinetics.

In this study, *in vitro* experiments showed ^{212}Pb-Daratumumab specific effects on proliferation of cells with high CD38 expression which is consistent with previous observations by *Teiluf et al.* on different MM cell lines, including RPMI8226. Treatment with ^{213}Bi-anti-CD38 for 48h induced an

LD50 of 185 kBq/ml (*21*). In our experiment, the LD50 was 0.370 kBq/ml with 4 days of incubation. ^{212}Pb high superior efficacy when compared to ^{213}Bi is correlated with its longer half-life as observed by *Milenic et al.* which compared ^{212}Pb and ^{213}Bi effects on human carcinoma cell lines growth: concentrations necessary for reaching the same level of efficiency were 30-40 times lower for ^{212}Pb than for ^{213}Bi. (*22*).

^{212}Pb-Daratumumab biodistributions in mice bearing CD38+ tumors are consistent with prior published data with ^{89}Zr-labeled daratumumab (*23*) (*24*). We observed a specific tumor uptake which rapidly increased with time post-injection to reach 15%ID/g at 18h and 20-25%ID/g at 48h. Imaging data underlined a peripherical tumor fixation on large tumors, suggesting that small lesions might be the best indication for ^{212}Pb-RIT due to the slow tumor penetration of full-length antibodies in large tumors. Some uptake was observed in liver, spleen, kidney and lung (around 10%ID/g), while other healthy tissue uptake was minimal. Blood activity rapidly decreased and was around 20%ID/g after 18h.

Due to the low ^{212}Pb activity injected and detection sensitivity, whole-body SPECT/CT images using ^{212}Pb-MAbs could not be acquired and were therefore performed with ^{203}Pb. This radioelement allowed us to consider a theranostic approach for ^{212}Pb α-therapy with a chemically identical radiometal, preventing the need for a radionuclide with different physical-chemical properties that would likely result in different pharmacokinetics. Imaging with ^{203}Pb could provide an effective approach to optimize therapeutic doses using patient-specific dosimetry calculations and monitoring patient response to targeted radionuclide therapy with ^{212}Pb.

As Daratumumab does not bind to mCD38, mCD38 biodistribution and toxicity studies were evaluated with a specific anti-mCD38 MAb. Human CD38 and mouse CD38 share sequence

homology (70%) but display a different expression pattern particulary among lymphocyte subsets and within the B-lineage (*25*). Murine CD38 is expressed abundantly by all murine B-lineage cells; by contrast, human CD38 is highly expressed by germinal center human B cells and less intensely by other human B-lineage cells. This expression pattern explains the high spleen uptake of ^{212}Pb-anti-mCD38 in mice. ^{212}Pb-anti-mCD38 biodistribution studies predicted the spleen and liver to be the dose-limiting normal organs, however the differences in expression patterns limit elementary transposal to human ^{212}Pb-Daratumumab toxicity.

Hematological toxicity was studied through blood cell counts and flow cytometry on lymphoid organs post-mortem. We observed a reversible hematological toxicity similar to the one observed by Boudousq *et al*. with ^{212}Pb-Trastuzumab and ^{212}Pb-irrelevant-MAb in intraperitoneal tumor xenograft-bearing nude mice (*26*). In parallel, we investigated toxicity in liver and kidneys using a chemical approach. Consistent with Chérel *et al.* studies with ^{213}Bi-anti-mCD138, we observed no variations in liver and kidney enzymes in our acute toxicity study (*15*).

No toxicity was observed with ^{212}Pb-Daratumumab up to 370 kBq due to the lack of cross-reactivity with mCD38. Nevertheless, considering the ^{212}Pb-anti-mCD38 toxicity outcome, for this first efficacy study, we have chosen to test an activity of 277.5 kBq to remain under ^{212}Pb-anti-mCD38 toxic activities (370 kBq). In clinical application, injection of cold MAb before RIT could be an attractive option to optimize the therapeutic effect and reduce toxicity.

Efficacy studies were conducted on Rag2$^{-/-}$γC$^{-/-}$ mice bearing RPMI8226 cell line xenografts. *In vitro* results warranted the use of this cell line as a relevant xenograft model for *in vivo* studies. Treatments with ^{212}Pb-MAbs significantly reduced tumor growth and prolonged MST compared to cold-Daratumumab or PBS. A significant partial efficacy was obtained with ^{212}Pb-Isotypic

Control (277.5 kBq) corresponding to non-targeted RIT effect likely due to the enhanced permeability and retention effect in tumors, observed in various studies with ^{212}Pb or other radioelements (*27*). In a single treatment regimen on relatively high tumor volume at treatment time, the significantly greater tumor growth inhibition observed with ^{212}Pb-Daratumumab is encouraging, especially when considering the relatively low activities used compared to other studies (*27*).

These promising results highlight ^{212}Pb-Daratumumab potency in the treatment of MM. The ^{212}Pb half-life of 10.6 hours, its central production and worldwide distribution, provides clinical feasibility. To further optimize ^{212}Pb-Daratumumab effectiveness, fractionated regimen could be tested to improve the long term efficacy of the therapy and prevent tumoral recurrence.

DISCLOSURE

Amal Saidi is an Orano Med employee. No other potential conflicts of interest relevant to this article exist.

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KEY POINTS

Question : What is the potential of ^{212}Pb-Daratumumab in the treatment of plasma cell malignancies?

Petinent finding : In a single treatment regimen, ^{212}Pb-Daratumumab induced a significant tumor growth inhibition.

Implications for patient care : ^{212}Pb-Daratumumab could be promising in MM treatment.

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FIGURE LEGENDS

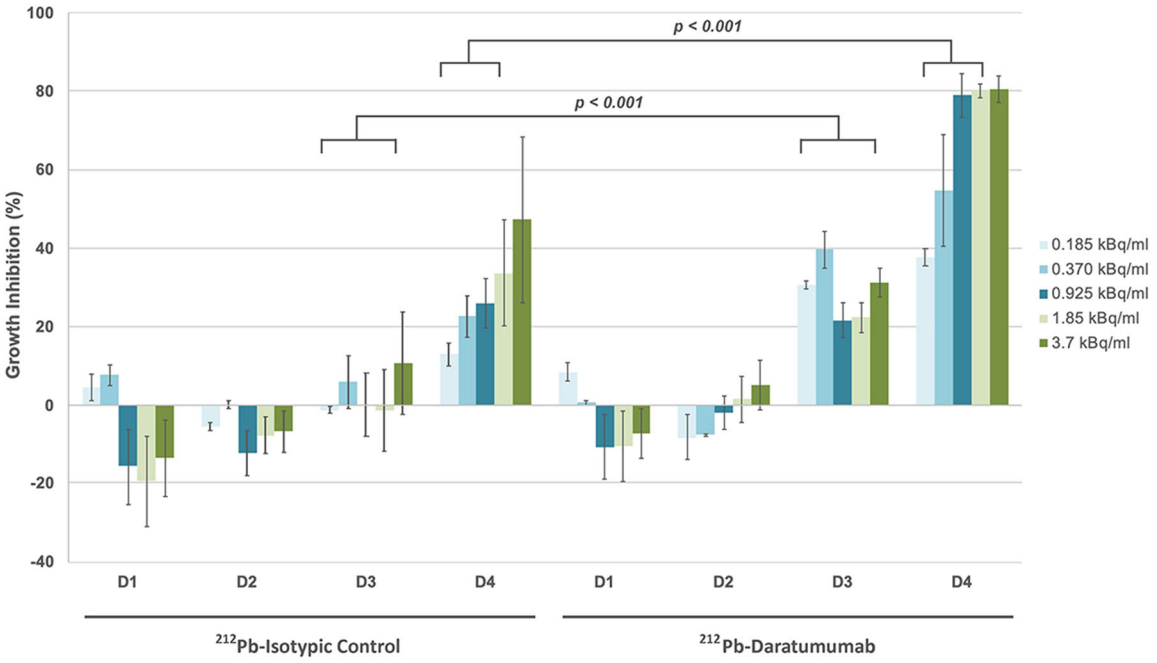


FIGURE 1: Percentage of growth inhibition for increasing activities of ^{212}Pb-MAbs.

The RPMI8226 cell growth inhibition percent was calculated, from Day 1 to Day 4, using untreated cells as controls on the same day. Data are expressed as % growth inhibition ((1 – $OD_{treatment}/OD_{control}$) * 100) ± SEM.

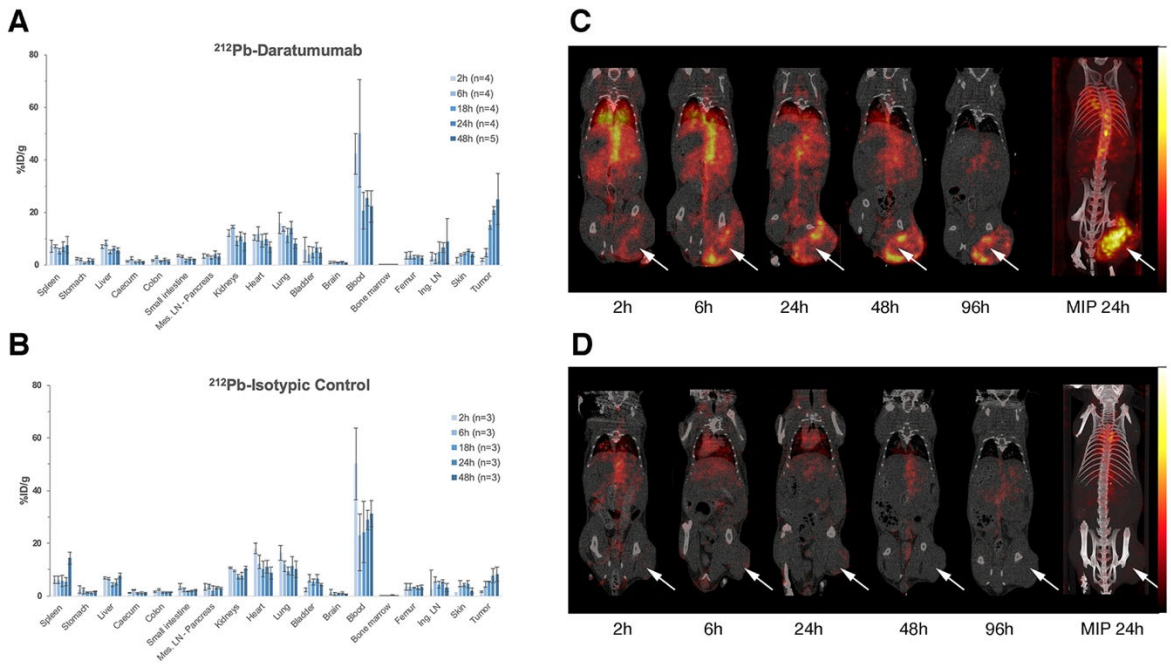


FIGURE 2: Biodistribution of radiolabelled Daratumumab (A,C) and Isotypic Control (B,D) in mice bearing RPMI8226 xenograft. A and B: for *post-mortem* biodistribution studies, mice were injected with 185 kBq of ^{212}Pb-Daratumumab (A) or ^{212}Pb-Isotypic Control (B). The radioactivity in tumor, organs and blood was expressed as the % injected dose per gram of tissue (%ID/g). Radioactivity in the tumor is significantly different between the two MAbs ($p<0.001$). C and D: for *in vivo* imaging studies, 7.4 MBq of ^{203}Pb-Daratumumab (C) or ^{212}Pb-Isotypic Control (D) were injected. Mice were imaged by microSPECT-CT 2h, 6h, 24h, 48h and 96h post-injection. Tumors are indicated by white arrows. MIP: Maximum Intensity Projection.

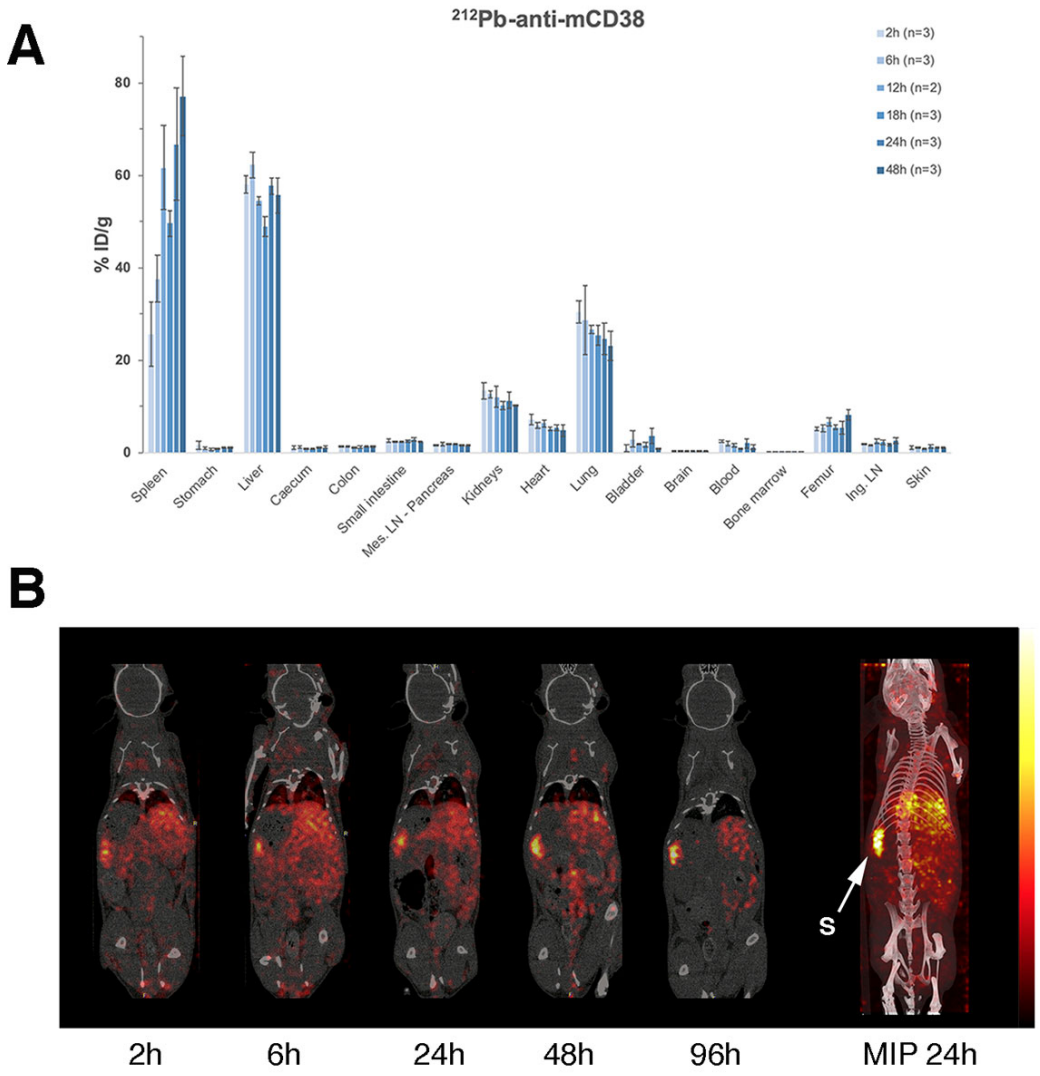


FIGURE 3: Biodistribution of radiolabelled anti-mCD38 on C57BL/6 healthy mice. A: for *post-mortem* biodistribution studies, mice were injected with 185 kBq of ^{212}Pb-anti-mCD38. The radioactivity was expressed as the %ID/g of tissue. B: for *in vivo* studies, mice were imaged, by microSPECT-CT, 2h, 6h, 24h, 48h and 96h post-injection of ^{203}Pb-anti-mCD38 (7.4 MBq). S: Spleen.

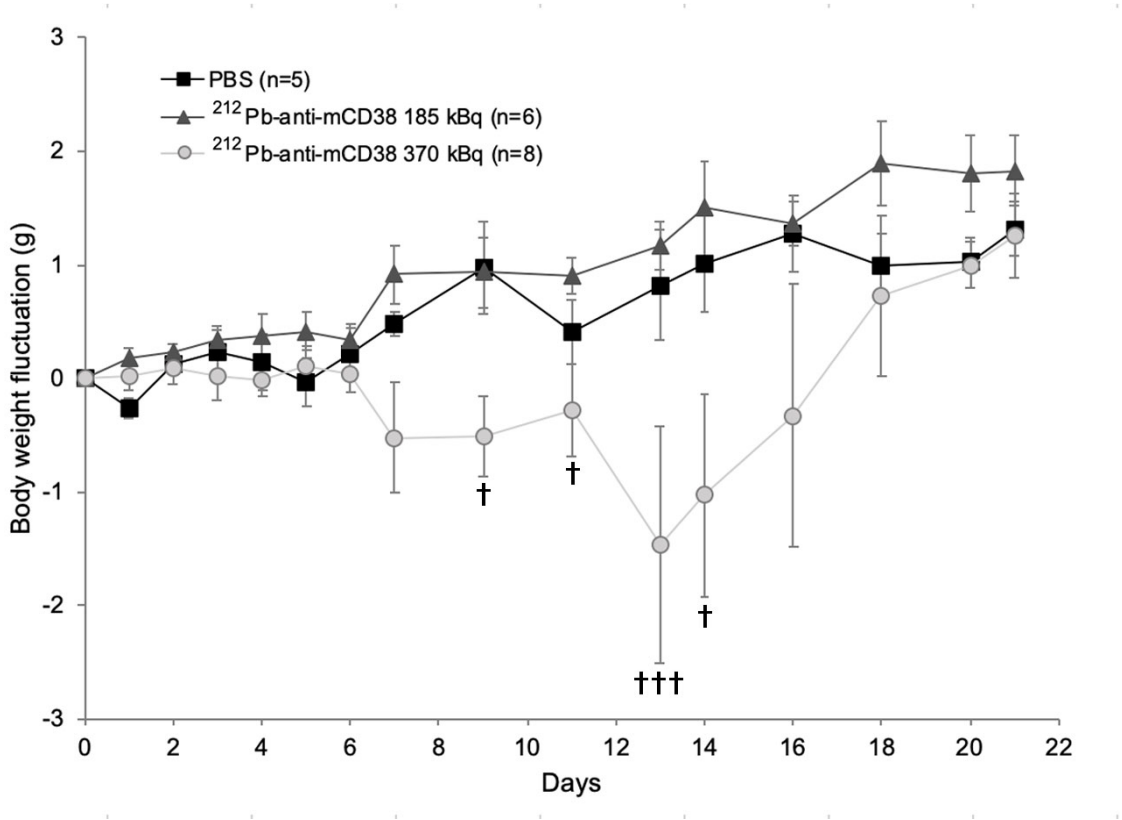


FIGURE 4: Body weight variations of C57Bl/6 mice after acute toxicity study. Mice were injected intravenously with PBS, 185 or 370 kBq of ^{212}Pb-anti-mCD38. Variations of body weight reported relative to Day 0 were represented. Crosses (†): mice euthanazied.

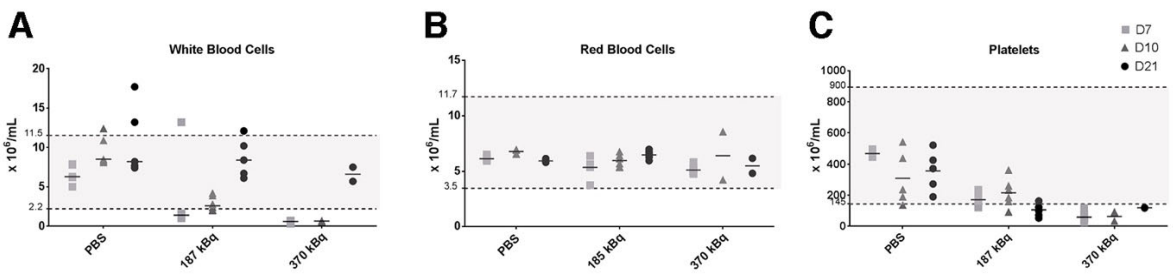


FIGURE 5: Effect of acute toxicity on blood cells counts. C57Bl/6 mice were injected intravenously with PBS, 185 or 370 kBq of ^{212}Pb-anti-mCD38. Mice followed over 7 days were euthanized and blood samples were analyzed. For mice followed over 21 days, blood samples were analyzed on day 10 and day 21. Results for white blood cells (A), red blood cells (B) and platelets (C) are represented.

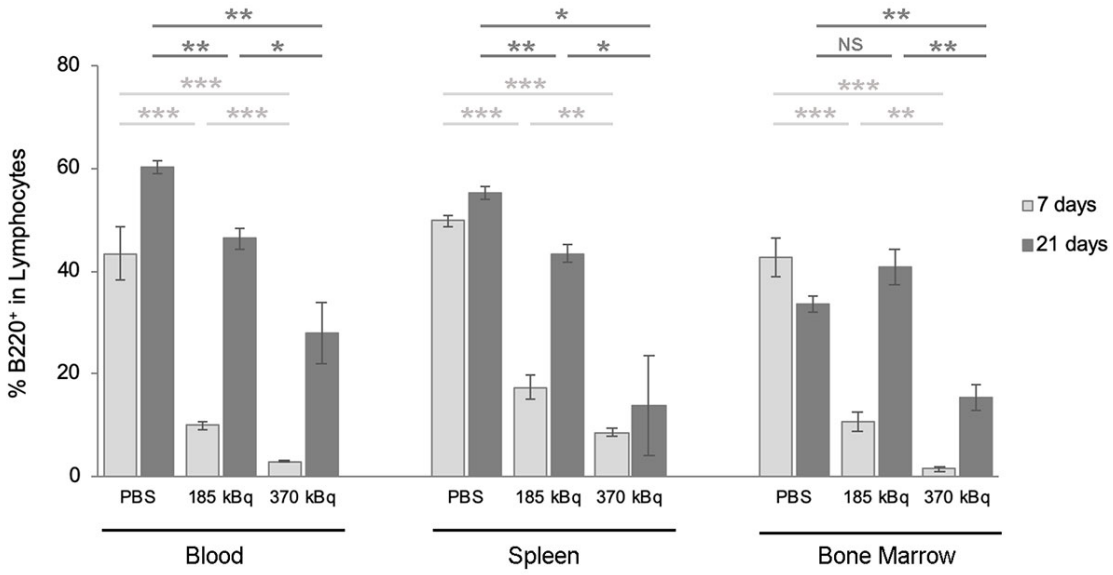


FIGURE 6: Effect of acute toxicity on B220 cells of lymphoid organs. C57Bl/6 mice were injected intravenously with PBS, 185 or 370 kBq of ^{212}Pb-anti-mCD38. At the end-point, 7 or 21 days, blood (left), spleen (middle) and bone marrow (right) were excised and analyzed. NS *p>0.05*, * *p<0.05*, ** *p<0.01* , *** *p<0.001*

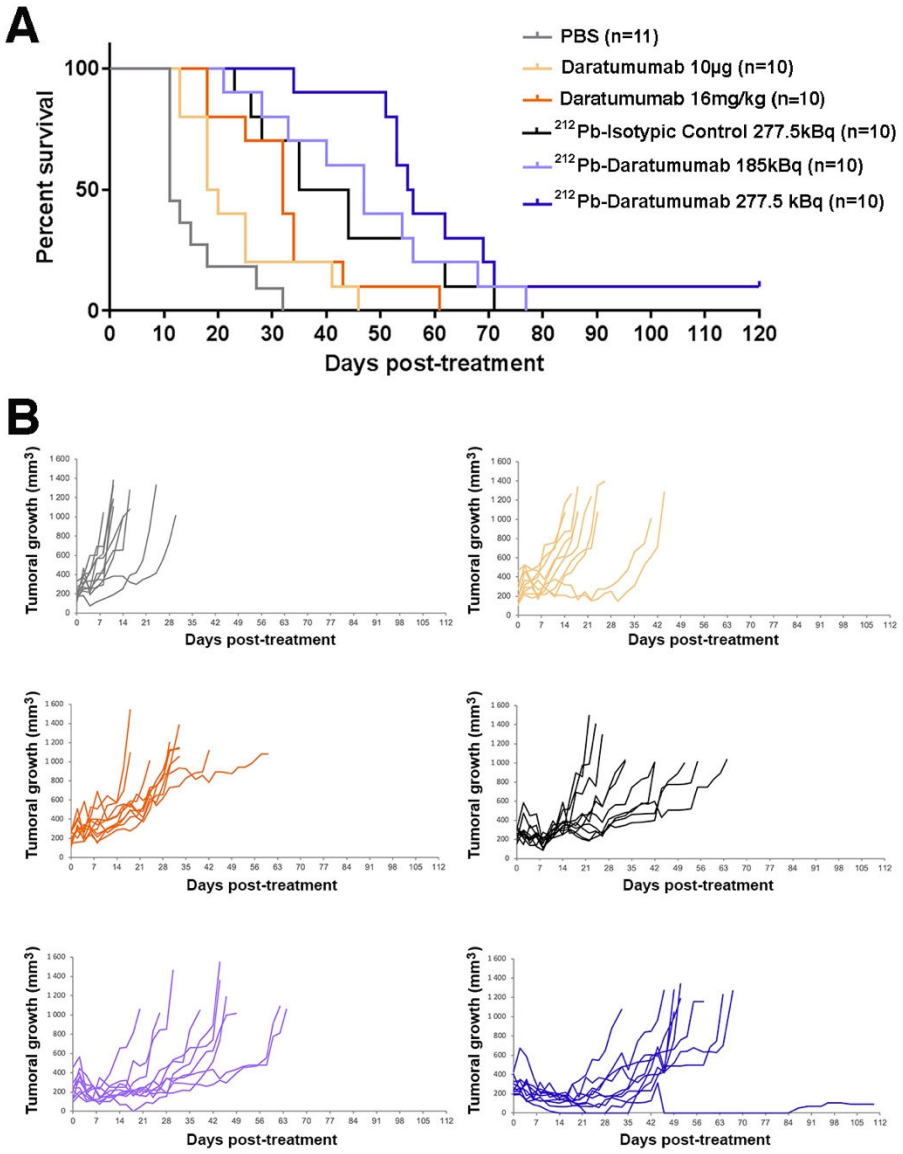


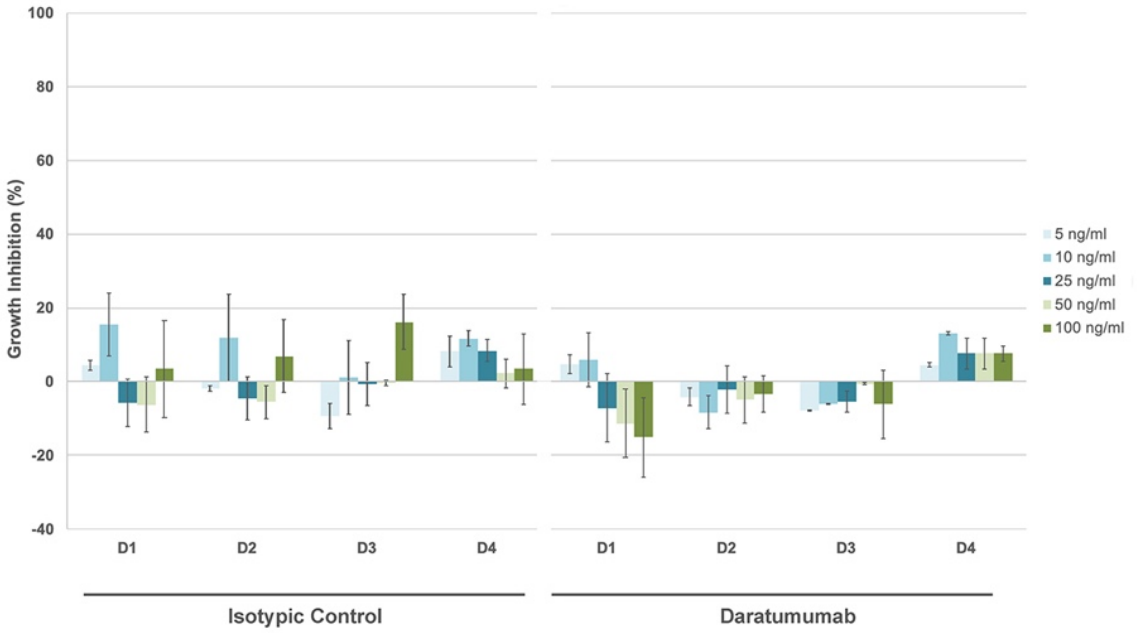
FIGURE 7: Efficacy of ^{212}Pb-Daratumumab treatment on Rag2$^{-/-}$γC$^{-/-}$ bearing s.c. xenograft of MM cells. Thirteen days post-engrafment mice received PBS, Daratumumab (10 µg or 16 mg/kg), ^{212}Pb-Isotypic Control (277.5 kBq), ^{212}Pb-Daratumumab (185 or 277.5 kBq).

(A): Kaplan-Meier survival analysis. Data were analyzed with a logrank test.

(B): Individual tumoral growth evolution. Tumor volume was measured three times a week. Mice were euthanazied when tumors were ≥ 1cm^3 or when 120 days of survival was reached (end of experiment).

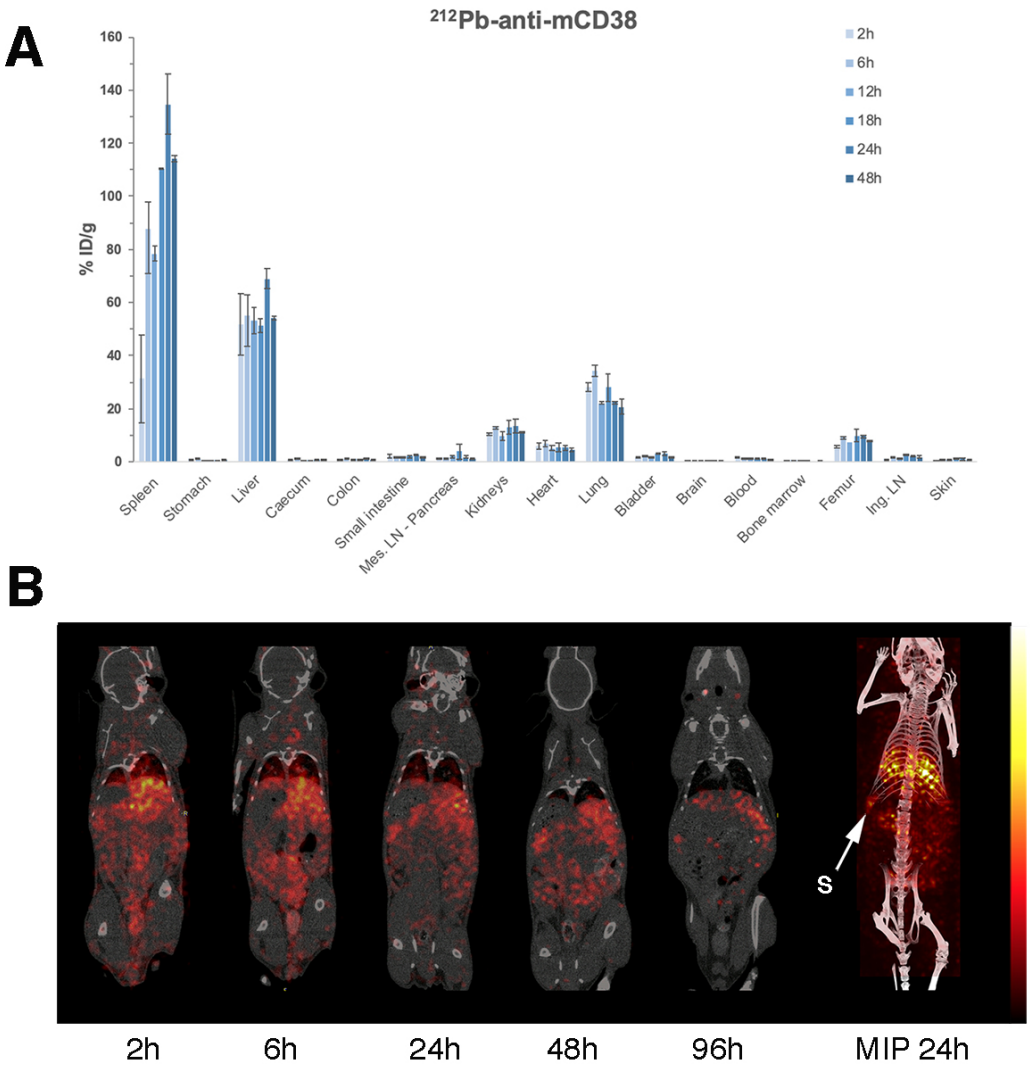
TABLE 1: Mice surviving after acute toxicity study. Two time-points were defined at 7 and 21 days post-injection. Values are in bold when some mice do not reach the time-point defined.

Mice	Treatments	Activity	Survival mice at end-point	
			7D	21D
Rag2$^{-/-}$γC$^{-/-}$	PBS		5/5	5/5
	^{212}Pb-Daratumumab	185 kBq	5/5	5/5
		277.5 kBq	5/5	5/5
		370 kBq	5/5	5/5
	^{212}Pb-anti-mCD38	185 kBq	5/5	5/5
		370 kBq	**4/5**	**3/5**
C57Bl/6	PBS		5/5	5/5
	^{212}Pb-anti-mCD38	185 kBq	6/6	6/6
		370 kBq	8/8	**2/8**

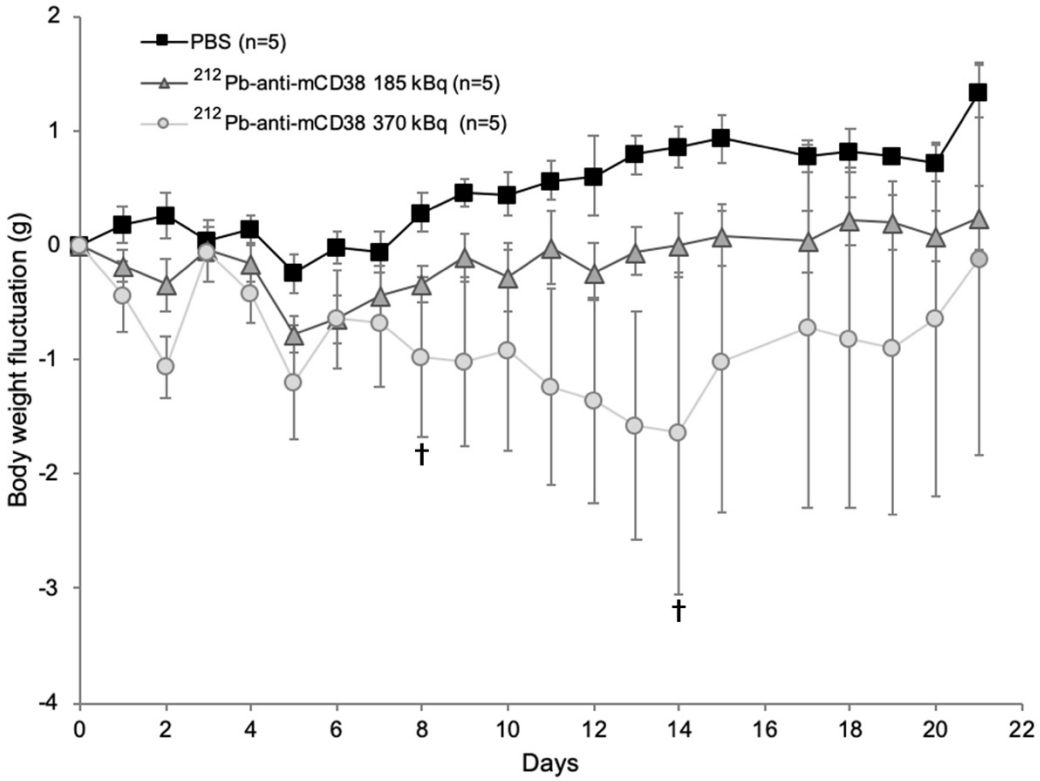


Supplemental FIGURE 1: Percentage of growth inhibition for increasing concentrations of cold-MAb.

Inhibition of cell proliferation, after incubation of RPMI8226 cell line with increasing concentrations of Isotypic Control or Daratumumab (5-100 ng/ml), was measured daily during 4 days by an MTS assay. The growth inhibition percent was calculated, from Day 1 to Day 4, using untreated cells as control on the same day. Datas are expressed as % growth inhibition $((1 - DO_{treatment}/DO_{control}) * 100) \pm$ SEM. No significant effect was observed.

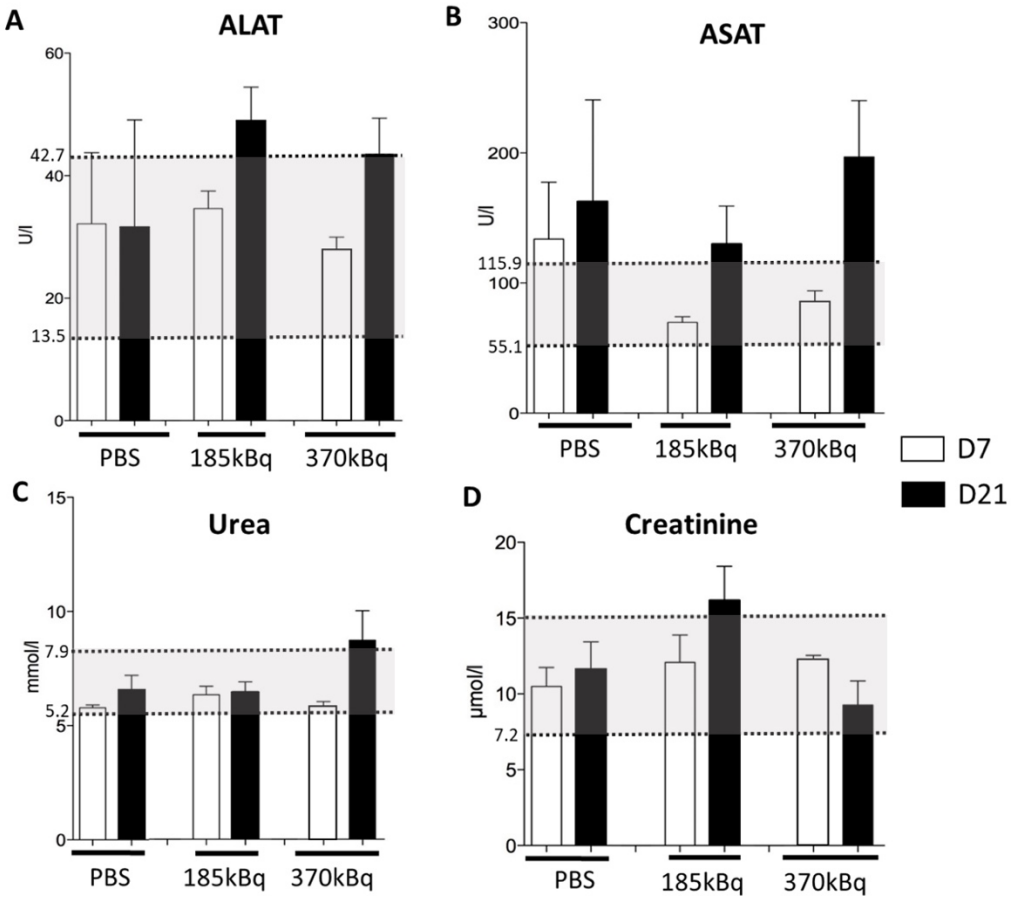


Supplemental FIGURE 2: Biodistribution of radiolabelled anti-mCD38 on Rag2$^{-/-}$γC$^{-/-}$ healthy mice. A: for *post-mortem* biodistribution studies, mice were injected with 185 kBq of ^{212}Pb-anti-mCD38. Groups of 2 mice were euthanized 2, 6, 12, 18, 24 or 48 hours after injection. The radioactivity in normal tissues and blood were quantified by gamma counting, corrected for decay, and expressed as the % ID/g of tissue. B: for *in vivo* studies, mice were imaging, by microSPECT-CT, 2, 6, 24, 48 and 96 hours post-injection of ^{203}Pb-anti-mCD38 (7.4 MBq). MIP: Maximum Intensity Projection; S: Spleen.



Supplemental FIGURE 3: Body weight variations of C57Bl/6 mice after acute toxicity study.

Mice were injected intravenously with PBS, 185 or 370 kBq of ^{212}Pb-anti-mCD38. Variations of body weight reported relative to Day 0 were represented. Crosses (†): mice euthanazied.



Supplemental FIGURE 4: Effect of acute toxicity on biochemical parameters. C57Bl/6 mice were injected intravenously with PBS, 185 or 370 kBq of ^{212}Pb-anti-mCD38. ALAT (A), ASAT (B), urea (C) and creatinine (D) were measured in blood plasma 7 or 21 days post-injection.