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Tumor-targeting *Salmonella typhimurium* A1-R arrests a doxorubicin-resistant PDGFRA-amplified patient-derived orthotopic xenograft (PDOX) mouse model of pleomorphic liposarcoma

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Abstract

Pleomorphic liposarcoma (PLPS) is a recalcitrant soft-tissue sarcoma (STS) subtype in need of transformative therapy. We have previously established a patient-derived orthotopic xenograft (PDOX) model, of PLPS with PDGFRA amplification, using surgical orthotopic implantation (SOI). In the present study, the PLPS PDOX model was randomized into three groups of seven mice each: untreated control; doxorubicin (DOX)-treated; and treated with *Salmonella typhimurium* A1-R (*S. typhimurium* A1-R) expressing green fluorescent protein (GFP). Tumor volume and body weight were monitored during the treatment period. The PLPS PDOX was resistant to DOX. In contrast, the PLPS PDOX was highly sensitive to *S. typhimurium* A1-R. There was no significant body-weight loss among these three groups. Fluorescence imaging demonstrated that *S. typhimurium* A1-R-GFP was very effective to target the PLPS PDOX tumor. The present study demonstrates that a PLPS PDOX, resistant to first-line therapy DOX, was highly sensitive to tumor targeting *S. typhimurium* A1-R.

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Keywords

Pleomorphic liposarcoma; soft tissue sarcoma; patient-derived orthotopic xenograft; PDGFRA amplification; cytogenetics; *S. typhimurium* A1-R; tumor-targeting; GFP

Introduction

Pleomorphic liposarcoma (PLPS) is a heterogeneous and recalcitrant disease with a high frequency of local recurrence and distant metastasis.¹ Complete surgical resection is the only known cure for this disease. Both radiation and conventional cytotoxic chemotherapy are largely ineffective for advanced unresectable PLPS.² Therefore, precise individualized therapy is necessary to improve outcome of PLPS patients.

Toward this goal, our laboratory pioneered the patient-derived orthotopic xenograft (PDOX) nude mouse model with the technique of surgical orthotopic implantation (SOI), including breast,³ ovarian,⁴ lung,⁵ cervical,^{6,7} colon,^{8–10} stomach,¹¹ pancreatic,^{12–16} melanoma^{17–22} and sarcoma.^{23–35} The PDOX model, developed by our laboratory over the past 30 years, has many advantages over subcutaneous-transplant models.^{36,37}

A PDOX model of a PDGFRA-amplified PLPS was previously established in the biceps femoris of nude mice by surgical orthotopic implantation (SOI) in order to match the patient.³⁸ The PLPS PDOX was resistant to first-line therapy doxorubicin (DOX). In contrast, trabectedin (TRAB) arrested the PLPS PDOX with no significant body-weight loss. These results suggested that the PLPS PDOX model can precisely identify both effective as well as ineffective drugs for the individual patient. This is of particular importance for a heterogeneous group such as PLPS where standard first-line therapy remains very difficult to determine without individualization.³⁸

In a subsequent study, we determined the efficacy of pazopanib (PAZ) which targets the PDGFRA gene amplification³⁹ of the PLPS compared to temozolomide (TEM) and DOX.⁴⁰ The PLPS PDOX was resistant to DOX as previously observed and responded very well to PAZ as well as TEM. The PLPS PDOX model identified an effective targeted drug (PAZ) as well as effective standard therapy (TEM) at the same time, identified an ineffective drug (DOX), even if it is first-line for this disease.⁴⁰

Materials and Methods

Mice

Athymic *nu/nu* male nude mice (AntiCancer, Inc., San Diego, CA), 4–6 weeks old, were used in this study. All mice were kept in a barrier facility on a high efficiency particulate arrestance (HEPA)-filtered rack under standard conditions of 12-hour light/dark cycles. The animals were fed an autoclaved laboratory rodent diet. All animal experiments were performed with an AntiCancer Institutional Animal Care and Use Committee (IACUC)-protocol specifically approved for this study and in accordance with the principals and procedures outlined in the National Institutes of Health Guide for the Care and Use of Animals under Assurance Number A3873-1. Anesthesia and analgesics were used for all

surgical experiments to avoid excessive suffering of the mice. A ketamine mixture (0.02 ml solution of 20 mg/kg ketamine, 15.2 mg/kg xylazine, and 0.48 mg/kg acepromazine maleate) was used subcutaneously for all mice. The animals were monitored carefully during surgery to keep adequate depth of anesthesia. The animals were observed daily and humanely sacrificed by CO₂ inhalation when they met the following criteria: severe tumor burden (more than 20 mm in diameter), prostration, significant body weight loss, difficulty breathing, rotational motion and body temperature drop.

Patient-derived tumor

A 68-year-old male patient was previously diagnosed as a recurrent PLPS of the right arm and underwent surgical resection. The patient previously received docetaxel, ifosfamide and radiotherapy to the right arm. Surgical resection for the recurrent PLPS was previously performed by FCE on January 8, 2016. Written informed consent was obtained from the patient as part of a UCLA Institutional Review Board (IRB#10-001857) approved protocol.^{38,40}

Establishment of a PDOX model of PLPS by surgical orthotopic implantation (SOI)

A PDOX model of the PLPS was established subcutaneously in nude mice from the patient's resected PLPS as previously described.^{38,40} The subcutaneously-grown tumors were then harvested and cut into 3 mm³ fragments. After the mice were anesthetized with the ketamine solution described above, a 7 mm skin incision was made on the right upper thigh into the biceps femoris, which was split to make space for the tumor fragment. A single tumor fragment was implanted orthotopically into the space to establish the PDOX model, as previously described.^{38,40} The wound was closed with a 6-0 nylon suture (Ethilon, Ethicon, Inc., NJ, U.S.A.).

Treatment study design in the PLPS PDOX model

PDOX mouse models were randomized into three groups of seven mice, respectively: Group 1 (G1), untreated control; Group 2 (G2), treated with DOX (3 mg/kg, i.v., weekly, 2 weeks); Group 3 (G3), treated with *S. typhimurium* A1-R (5×10⁷ CFU/100 ml, i.v., weekly, 2 weeks) (Fig. 1). Tumor length, width, and mouse body weight were measured twice a week. Tumor volume was calculated with the following formula: Tumor volume (mm³) = length (mm) × width (mm) × width (mm) × 1/2. When the tumor volume reached 50 mm³, treatment was started. The tumor volume ratio is defined as the tumor volume at any given time point relative to the initial tumor volume. Body weight was measured throughout the treatment period. Relative body weight is also defined as the body weight at any given time point relative to the initial tumor weight. All mice included in this study were sacrificed on day 15, and tumors were harvested for further histological evaluation. Data are presented as mean ± SD.

Histological examination

Fresh tumor samples were fixed in 10% formalin and embedded in paraffin before sectioning and staining. Tissue sections (5 μm) were deparaffinized in xylene and rehydrated in an ethanol series. Hematoxylin and eosin (H&E) staining was performed according to

standard protocol. Histological examination was performed with a BHS system microscope. Images were acquired with INFINITY ANALYZE software (Lumenera Corporation, Ottawa, Canada).

Statistical analysis

Statistical analyses were performed with JMP pro version 12 for all statistical analyses. The relative tumor volumes and relative body weights of mouse are expressed as mean $\pm$ SD. Significant differences for continuous variables were determined using the Steel-Dwass test for multiple comparison. A probability value of $P < 0.05$ was considered statistically significant.

Results and Discussion

Efficacy of *S. typhimurium* A1-R vs. DOX on the PLPS PDOX

The untreated control group grew 6 times larger than initial tumor volume by day14 (G1: 6.37 ± 2.29). The DOX group did not demonstrate significant tumor growth suppression compared to the control group on day 14 (G2: 4.05 ± 1.21 , $p = 0.1733$). In contrast, tumor-targeting *S. typhimurium* A1-R demonstrated significant tumor growth suppression compared to the control group (G3: 2.03 ± 1.33 , $p = 0.018$) and DOX group on day14 ($p = 0.047$) (Fig. 2). Figure 3 shows representative tumor images at the treatment endpoint for each group.

Tumor targeting in the PLPS PDOX by *S. typhimurium* A1-R

S. typhimurium, expressing green fluorescent protein (GFP), was cultivated in serial dilution from supernatants of tumor homogenates by mincing tumor tissue on agar medium (Fig. 4). Fluorescent bacteria were detected at all dilutions in the tumor obtained from mice treated with by the *S. typhimurium* A1-R. The untreated control group tumors did not contain *S. typhimurium* (Fig. 4).

Effect of treatment on mouse body weight on the PLPS PDOX

Figure 5 shows the relative body weight of all groups. Mice treated with *S. typhimurium* A1-R had slight weight loss at the endpoint. However, there were no significant weight differences among groups, indicating there was not significant adverse effect caused by either agent.

Treatment impact on tumor histology of the PLPS PDOX tumors

Figure 6 represents the tumor histology of all groups and original patient. In both the original patient tumor (Fig. 6A) and control PDOX tumor (Fig. 6B), enlarged and hyperchromatic nuclei with cytoplasm vacuoles were observed. Intact and viable cancer cells were observed in most areas (Fig. 6B). The DOX group tumors did not have significant necrotic areas (Fig. 6C). The *S. typhimurium* A1-R treated tumor had significant necrosis.

We have previously shown that tumor-targeting *S. typhimurium* A1-R has strong efficacy on a PDOX models of Ewing's sarcoma⁷ and follicular dendritic cell sarcoma.³² In a recent study, *S. typhimurium* A1-R was shown to be effective against USTS (2 undifferentiated

pleomorphic sarcoma [UPS], 1 undifferentiated sarcoma not otherwise specified [NOS] and 1 undifferentiated spindle cell sarcoma [USS]) and was significantly more efficacious than DOX in each case, thereby surpassing first-line therapy.⁴¹

The scientific premise for the present study is to determine the efficacy of tumor-targeting *Salmonella typhimurium* A1-R (*S. typhimurium* A1-R) on a patient-derived orthotopic xenograft (PDOX) model of doxorubicin-resistant pleomorphic liposarcoma demonstrating arrest of tumor growth (Figures 2 and 3) and highly-specific tumor targeting of *S. typhimurium* A1-R in the PDOX model (Figure 4). This is the first study that demonstrates efficacy of *S. typhimurium* A1-R on a PDOX model of doxorubicin-resistant pleomorphic liposarcoma and suggests that *S. typhimurium* A1-R can be effective in the clinic in the future in this recalcitrant tumor type.

The present report demonstrates that *S. typhimurium* A1-R is an effective agent for another type of sarcoma, PLPS, and further suggests the generality of *S. typhimurium* A1-R as an effective treatment for sarcoma. Clinical trials of *S. typhimurium* A1-R need to be carried out as soon as possible.

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References

1. Manji GA, Schwartz GK. Managing Liposarcomas: Cutting Through the Fat. *J Oncol Pract.* 2016; 12:221–227. [PubMed: 26962163]
2. Guan Z, Yu X, Wang H, Zhang J, Li G, Cao J, Teng L. Advances in the targeted therapy of liposarcoma. *Onco Targets Ther.* 2015; 8:125–136. [PubMed: 25609980]
3. Fu X, Le P, Hoffman RM. A metastatic-orthotopic transplant nude mouse model of human patient breast cancer. *Anticancer Res.* 1993; 13:901–904. [PubMed: 8352558]
4. Fu X, Hoffman RM. Human ovarian carcinoma metastatic models constructed in nude mice by orthotopic transplantation of histologically-intact patient specimens. *Anticancer Res.* 1993; 13:283–286. [PubMed: 8517640]
5. Wang X, Fu X, Hoffman RM. A new patient-like metastatic model of human lung cancer constructed orthotopically with intact tissue via thoracotomy in immunodeficient mice. *Int J Cancer.* 1992; 51:992–995. [PubMed: 1639545]
6. Hiroshima Y, Zhang Y, Zhang N, et al. Establishment of a patient derived orthotopic xenograph (PDOX) model of HER-2-positive cervical cancer expressing the clinical metastatic pattern. *PLoS ONE.* 2015b; 10:e0117417. [PubMed: 25689852]
7. Murakami T, Kiyuna T, Kawaguchi K, et al. The irony of highly effective bacterial therapy of a patient-derived orthotopic xenograft (PDOX) model of Ewing's sarcoma, which was blocked by Ewing himself 80 years ago. *Cell Cycle.* 2017; 16:1046–1052. [PubMed: 28296559]
8. Hiroshima Y, Maawy A, Metildi CA, et al. Successful fluorescence guided surgery on human colon cancer patient-derived orthotopic xenograft mouse models using a fluorophore-conjugated anti-CEA antibody and a portable imaging system. *J Laparoendosc Adv Surg Tech A.* 2014; 24:241–247. [PubMed: 24494971]
9. Fu X, Besterman JM, Monosov A, Hoffman RM. Models of human metastatic colon cancer in nude mice orthotopically constructed by using histologically intact patient specimens. *Proc Natl Acad Sci USA.* 1991; 88:9345–9349. [PubMed: 1924398]
10. Metildi CA, Kaushal S, Luiken GA, Talamini MA, Hoffman RM, Bouvet M. Fluorescently-labeled chimeric anti-CEA antibody improves detection and resection of human colon cancer in a patient-

derived orthotopic xenograft (PDOX) nude mouse model. *J Surg Oncol*. 2014; 109:451–458. [PubMed: 24249594]

11. Furukawa T, Kubota T, Watanabe M, Kitajima M, Hoffman RM. Orthotopic transplantation of histologically intact clinical specimens of stomach cancer to nude mice: correlation of metastatic sites in mouse and individual patient donors. *Int J Cancer*. 1993; 53:608–612. [PubMed: 8436434]
12. Fu X, Guadagni F, Hoffman RM. A metastatic nude-mouse model of human pancreatic cancer constructed orthotopically with histologically intact patient specimens. *Proc Natl Acad Sci USA*. 1992; 89:5645–5649. [PubMed: 1608975]
13. Kawaguchi K, Igarashi K, Murakami T, et al. MEK inhibitors cobimetinib and trametinib, regressed a gemcitabine-resistant pancreatic cancer patient-derived orthotopic xenograft (PDOX). *Oncotarget*. 2017; 8:47490–47496. [PubMed: 28537897]
14. Hiroshima Y, Zhang Y, Murakami T, et al. Efficacy of tumor targeting *Salmonella typhimurium* A1-R in combination with antiangiogenesis therapy on a pancreatic cancer patient-derived orthotopic xenograft (PDOX) and cell line mouse models. *Oncotarget*. 2014; 5:12346–12357. [PubMed: 25402324]
15. Hiroshima Y, Maawy A, Zhang Y, et al. Metastatic recurrence in a pancreatic cancer patient derived orthotopic xenograft (PDOX) nude mouse model is inhibited by neoadjuvant chemotherapy in combination with fluorescence-guided surgery with an anti-CA 19-9-conjugated fluorophore. *PLoS ONE*. 2014; 9:e114310. [PubMed: 25463150]
16. Hiroshima Y, Maawy AA, Katz MH, et al. Selective efficacy of zoledronic acid on metastasis in a patient-derived orthotopic xenograft (PDOX) nude-mouse model of human pancreatic cancer. *J Surg Oncol*. 2015; 111:311–315. [PubMed: 25394368]
17. Yamamoto M, Zhao M, Hiroshima Y, et al. Efficacy of tumor targeting *Salmonella typhimurium* A1-R on a melanoma patient derived orthotopic xenograft (PDOX) nude-mouse model. *PLoS ONE*. 2016; 11:e0160882. [PubMed: 27500926]
18. Kawaguchi K, Murakami T, Chmielowski B, et al. Vemurafenib-resistant BRAF-V600E mutated melanoma is regressed by MEK targeting drug trametinib, but not cobimetinib in a patient-derived orthotopic xenograft (PDOX) mouse model. *Oncotarget*. 2016; 7:71737–71743. [PubMed: 27690220]
19. Kawaguchi K, Igarashi K, Murakami T, et al. Tumor-targeting *Salmonella typhimurium* A1-R combined with Temozolomide regresses malignant melanoma with a BRAF-V600 mutation in a patient-derived orthotopic xenograft (PDOX) model. *Oncotarget*. 2016; 7:85929–85936. [PubMed: 27835903]
20. Kawaguchi K, Igarashi K, Murakami T, et al. *Salmonella typhimurium* A1-R targeting of a chemotherapy resistant BRAF-V600E melanoma in a patient-derived orthotopic xenograft (PDOX) model is enhanced in combination with either vemurafenib or temozolomide. *Cell Cycle*. 2017; 16:1288–1294. [PubMed: 28622068]
21. Kawaguchi K, Igarashi K, Murakami T, et al. Tumor-targeting *Salmonella typhimurium* A1-R sensitizes melanoma with a BRAF-V600E mutation to vemurafenib in a patient-derived orthotopic xenograft (PDOX) nude mouse model. *J Cell Biochem*. 2017; 118:2314–2319. [PubMed: 28106277]
22. Kawaguchi K, Igarashi K, Li S, et al. Combination treatment with recombinant methioninase enables temozolomide to arrest a BRAF V600E melanoma growth in a patient-derived orthotopic xenograft. *Oncotarget*. 2017; 8:85516–85525. [PubMed: 29156737]
23. Murakami T, Singh AS, Kiyuna T, et al. Effective molecular targeting of CDK4/6 and IGF-1R in a rare FUS-ERG fusion CDKN2A-deletion doxorubicin-resistant Ewing's sarcoma in a patient-derived orthotopic xenograft (PDOX) nude-mouse model. *Oncotarget*. 2016; 7:47556–47564. [PubMed: 27286459]
24. Murakami T, DeLong J, Eilber FC, et al. Tumor-targeting *Salmonella typhimurium* A1-R in combination with doxorubicin eradicate soft tissue sarcoma in a patient-derived orthotopic xenograft PDOX model. *Oncotarget*. 2016; 7:12783–12790. [PubMed: 26859573]
25. Murakami T, Li S, Han Q, et al. Recombinant methioninase effectively targets a Ewing's sarcoma in a patient-derived orthotopic xenograft (PDOX) nude-mouse model. *Oncotarget*. 2017; 8:35630–35638. [PubMed: 28404944]

26. Igarashi K, Kawaguchi K, Murakami T, et al. High efficacy of pazopanib on an undifferentiated spindle-cell sarcoma resistant to first-line therapy is identified with a patient-derived orthotopic xenograft (PDOX) nude mouse model. *J Cell Biochem.* 2017; 118:2739–2743. [PubMed: 28176365]
27. Igarashi K, Kawaguchi K, Kiyuna T, et al. Temozolomide combined with irinotecan caused regression in an adult pleomorphic rhabdomyosarcoma patient-derived orthotopic xenograft (PDOX) nude-mouse model. *Oncotarget.* 2017; 8:75874–75880. [PubMed: 29100276]
28. Igarashi K, Kawaguchi K, Kiyuna T, et al. Patient-derived orthotopic xenograft (PDOX) mouse model of adult rhabdomyosarcoma invades and recurs after resection in contrast to the subcutaneous ectopic model. *Cell Cycle.* 2017; 16:91–94. [PubMed: 27830986]
29. Igarashi K, Kawaguchi K, Murakami T, et al. Intra-arterial administration of tumor-targeting *Salmonella typhimurium* A1-R regresses a cisplatin-resistant relapsed osteosarcoma in a patient-derived orthotopic xenograft (PDOX) mouse model. *Cell Cycle.* 2017; 16:1164–1170. [PubMed: 28494180]
30. Hiroshima Y, Zhang Y, Zhang N, et al. Patient-derived orthotopic xenograft (PDOX) nude mouse model of soft-tissue sarcoma more closely mimics the patient behavior in contrast to the subcutaneous ectopic model. *Anticancer Res.* 2015; 35:697–701. [PubMed: 25667448]
31. Kawaguchi K, Igarashi K, Murakami T, et al. Combination of gemcitabine and docetaxel regresses both gastric leiomyosarcoma proliferation and invasion in an imageable patient-derived orthotopic xenograft (iPDOX) model. *Cell Cycle.* 2017; 16:1063–1069. [PubMed: 28426279]
32. Kiyuna T, Murakami T, Tome Y, et al. High efficacy of tumor-targeting *Salmonella typhimurium* A1-R on a doxorubicin- and dactolisib-resistant follicular dendritic-cell sarcoma in a patient-derived orthotopic xenograft PDOX nude mouse model. *Oncotarget.* 2016; 7:33046–33054. [PubMed: 27105519]
33. Igarashi K, Murakami T, Kawaguchi K, et al. A patient-derived orthotopic xenograft (PDOX) mouse model of an cisplatin-resistant osteosarcoma lung metastasis that was sensitive to temozolomide and trabectedin: implications for precision oncology. *Oncotarget.* 2017; 8:62111–62119. [PubMed: 28977930]
34. Igarashi K, Kawaguchi K, Murakami T, et al. A novel anionic-phosphate-platinum complex effectively targets an undifferentiated pleomorphic sarcoma better than cisplatin and doxorubicin in a patient-derived orthotopic xenograft (PDOX). *Oncotarget.* 2017; 8:63353–63359. [PubMed: 28968995]
35. Miyake K, Murakami T, Kiyuna T, et al. The combination of temozolomide-irinotecan regresses a doxorubicin-resistant patient-derived orthotopic xenograft (PDOX) nude-mouse model of recurrent Ewing's sarcoma with a FUS-ERG fusion and CDKN2A deletion: Direction for third-line patient therapy. *Oncotarget.* 2017; 8:103129–103136. [PubMed: 29262551]
36. Hoffman RM. Patient-derived orthotopic xenografts: better mimic of metastasis than subcutaneous xenografts. *Nature Reviews Cancer.* 2015; 15:451–452.
37. Hoffman RM, Coleman WB, Tsongalis GJ, editors. *Molecular and Translational Medicine.* 2017. Patient-Derived Mouse Models of Cancer.
38. Kiyuna T, Tome Y, Murakami T, et al. Trabectedin arrests a doxorubicin-resistant PDGFRA-activated liposarcoma patient-derived orthotopic xenograft (PDOX) nude mouse model. Submitted.
39. Fu X, Niu W, Li J, et al. Activating mutation of PDGFRB gene in a rare cardiac undifferentiated intimal sarcoma of the left atrium: a case report. *Oncotarget.* 2017; 8:81709–81716. [PubMed: 29113426]
40. Kiyuna T, Murakami T, Tome Y, et al. Doxorubicin-resistant pleomorphic liposarcoma with PDGFRA gene amplification is targeted and regressed by pazopanib in a patient-derived orthotopic xenograft mouse model. Submitted.
41. Igarashi K, Kawaguchi K, Kiyuna T, et al. Tumor-targeting *Salmonella typhimurium* A1-R is a highly effective general therapeutic for undifferentiated soft tissue sarcoma in patient-derived orthotopic xenograft (PDOX) nude-mouse models. *Biochem Biophys Res Comm.* 2018; 497:1055–1061.

Figure 1. Treatment protocol of pleomorphic liposarcoma (PLPS) PDOX

Treatment protocol. Group 1, untreated control (n = 7); Group 2, treated with doxorubicin (DOX) (3 mg/kg, i.v., weekly, 2 weeks, n = 7); Group 3, treated with *S. typhimurium* A1-R (5×10^7 CFU/100 ml, i.v., weekly, 2 weeks, n = 7). All mice included in this study were sacrificed on day 15 to harvest tumor for further histological evaluation.

Figure 2. Efficacy of *S. typhimurium* A1-R vs. DOX on the PLPS PDOX

The line graphs show relative tumor volume on each time point. Relative tumor is the tumor volume at any given time point at the beginning of the treated period divided by the tumor volume. Error bars: $\pm$ SD. * $p < 0.05$.

Figure 3. Macroscopic imaging of control and treated tumor
Images were obtained at the end of the treatment period. Scale bar: 10mm.

Figure 4. Tumor targeting *S. typhimurium* A1-R-GFP of the PLPS PDOX

S. typhimurium, expressing green fluorescent protein (GFP), was cultivated in serial dilution from supernatants of tumor homogenates by mincing tumor tissue on agar medium. Grown colonies of bacterial were imaged by GFP fluorescence. Fluorescent bacteria were detected at all dilutions in the tumor treated with *S. typhimurium* A1-R. Scale bar: 10 mm, 2.5 mm at high magnification. BF: Bright field, GFP: Green fluorescent protein.

Figure 5. Body weight of treated PLPS PDOX mouse models

Bar graphs show the relative body weight of mice treated with each agent. Relative body weight as the body weight at any given time divided by the inhibited body weight. Error bars: $\pm$ SD. N.S.: not significant.

Figure 6. Tumor histology

(A) Control tumor (B) Untreated PLPS PDOX. (C) DOX group. (D) *S. typhimurium* A1-R group. Scale bars: 100µm.