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URL http://hdl.handle.net/20.500.12000/48491
Temozolomide targets and arrests a doxorubicin-resistant follicular dendritic-cell sarcoma patient-derived orthotopic xenograft mouse model

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ARTICLE INFO

Keywords:
Follicular dendritic cell sarcoma
Soft-tissue
Patient-derived orthotopic xenograft
PDOX
Temozolomide
Doxorubicin

ABSTRACT

Follicular dendritic cell sarcoma (FDCS) is a very rare and highly recalcitrant disease. A patient’s doxorubicin-resistant FDCS was previously established orthotopically on the right high thigh into the biceps femoris of mice to establish a patient-derived orthotopic xenograft (PDOX) model. The aim of the present manuscript was to identify an effective drug for this recalcitrant tumor. Here, we evaluated the efficacy of temozolomide (TMZ), trabectedin (TRAB) and pazopanib (PAZ) on the FDCS PDOX model. PDOX mouse models were randomized into five groups of eight to nine mice, respectively. Group 1, untreated control with PBS, i.p.; Group 2, treated with doxorubicin (DOX), 2.4 mg/kg, i.p., weekly for 3 weeks; Group 3, treated with PAZ, 50 mg/kg, oral gavage, daily for 3 weeks; Group 4, treated with TMZ, 25 mg/kg, oral gavage, daily for 3 weeks; Group 5, treated with TRAB, 0.15 mg/kg, i.v., weekly for 3 weeks. Body weight and tumor volume were assessed 2 times per week. TMZ arrested the FDCS PDOX model compared to the control group (p < 0.05). PAZ and TRAB did not have significant efficacy compared to the control group (p = 0.99, p = 0.69 respectively). The PDOX tumor was resistant to DOX (p= 0.99), as was the patient. The present study demonstrates that TMZ is effective for a PDOX model of FDCS established from a patient who failed DOX treatment, further demonstrating the power of PDOX to identify effective therapy including for tumors that failed first line therapy.

1. Introduction

Follicular dendritic cell sarcoma (FDCS) is a very rare and recalcitrant cancer with, which was first reported in 1986 by Monda et al. (1986). FDCS is recognized as a diagnostically-challenging neoplasm and has greater malignant potential than initially thought. FDCS often develops in cervical, mediastinal or axillary lymph nodes. In nearly one-third of cases, FDCS can also occur in extra-nodal sites (Chen and Gopal, 2017; Duan et al., 2010; Biddle et al., 2002; Soriano et al., 2007; Davila et al., 2017; Hassan et al., 2018; Walters et al., 2018). Several markers were suggested as diagnostic marker for FDCS such as CD137 (Anderson et al., 2012), CD23 (Chen and Gopal, 2017), estrogen receptor (ER)-α ( Sapino et al., 2003), clusterin ( Grogg et al., 2004), chemokine (C-X-C motif) ligand 13 (CXCL13), CD21, CD35, follicular dendritic cell secreted protein (FDCSP), serglycin (SRGN) ( Lorenzi et al., 2017), mouse double minute 2 homolog (MDM2) and somatostatin receptor 2A ( SSTR2A) ( Agaimy et al., 2016). FDCS has been linked with extensive chromosomal instability, cell cycle progression defect, with mutations of v-Raf murine sarcoma viral oncogene homolog B ( BRAF), activation of nuclear factor kappa beta (NF-kB), activation of mitogen-activated protein kinase (MAPK), overexpression of epidermal growth factor receptor ( EGFR), neurofibromatosis type 2 ( NF2) mutation, and immune evasion ( Agaimy et al., 2016; Griffin et al., 2016; Sun et al., 2003; Go et al., 2014; Andersen et al., 2017; Karrs et al., 2018). The most frequent reported systemic therapy is C/ EBP homologous protein (CHOP) chemotherapy, which contains cyclophosphamide, doxorubicin (DOX), vincristine, and prednisolone with generally poor outcome ( Conry, 2014a; Dan et al., 2014; Khalid

Received 25 March 2019; Accepted 1 April 2019
Available online 03 April 2019
0040-8166/ Published by Elsevier Ltd.
and Folman, 2005; Shinagare et al., 2011). Therefore, novel approaches for FDCS are needed.

Temozolomide (TMZ) is an imidazo-triazine alkylating agent. TMZ is a small molecule (molecular weight of 194 Daltons). TMZ is easily absorbed in the digestive tract after oral administration, and its bioavailability is almost 100%. TMZ is lipophilic and can easily cross the blood-brain barrier (Simonetti et al., 2014). TMZ is approved for melanoma and refractory anaplastic astrocytoma (Keir et al., 2013; Setty et al., 2018; Kinoshita et al., 2018; Bupathi et al., 2017). TMZ is also a promising target in small cell lung cancer (SCLC) (Lok et al., 2017); melanoma (Kawaguchi et al., 2017a); undifferentiated spindle-cell sarcoma (Igarashi et al., 2018a); myxofibrosarcoma (Kiyuna et al., 2018); Ewing’s sarcoma (Palmerini et al., 2018) alveolar rhabdomyosarcoma (Kinoshita et al., 2018); and rhabdomyosarcoma (Igarashi et al., 2017a).

Trabectedin (TRAB), an alkylating agent, was initially isolated from the marine ascidian Ecteinascidia turbinata (Cuervas and Francesch, 2009). TRAB has been tested on advanced liposarcoma and leiomyosarcoma patient (Demetri et al., 2016; Kawai et al., 2017) and has been marketed for liposarcoma and leiomyosarcoma. TRAB binds to the minor groove of DNA and affects DNA repair pathways, which result in G2-M cell cycle arrest and apoptosis. TRAB showed high efficacy against several soft tissue sarcomas (Le Cesne et al., 2015).

Pazopanib (PAZ) is a small molecule multi-tyrosine kinase inhibitor, blocking various signaling pathways and was tested in a Phase III clinical trial for soft tissue sarcoma (Schoffski, 2012; van der Graaf et al., 2012).

Toward the goal of individualized precision oncology, we have developed the patient-derived orthotopic xenograft (PDOX) nude mouse model for all major cancers (Wang et al., 1992; Yamamoto et al., 2016; Fu et al., 1992; Hiroshima et al., 2014a,b; Fu and Hoffman, 1993; Hiroshima et al., 2015a,b,c; Murakami et al., 2016; 2017; Metildi et al., 2014; Furukawa et al., 1993; Kawaguchi et al., 2017b; Kiyuna et al., 2016; Kawaguchi et al., 2019; Miyake et al., 2017; Igarashi et al., 2018b).

We previously reported that a FDCS PDOX model was resistant to DOX and dactolisib (Kiyuna et al., 2016). However, the FDCS PDOX model was regressed by tumor-targeting Salmonella typhimurium A1-R (Kiyuna et al., 2016).

Recently, we have established PDOX models of pleomorphic rhabdomyosarcoma, undifferentiated spindle-cell sarcoma, and lung metastasis of osteosarcoma (Igarashi et al., 2017a,b,c). TMZ, TRAB, and PAZ agents were previously found to be effective on these PDOX models (Igarashi et al., 2017a,b,c).

The aim of the present manuscript was to identify an effective drug for this recalcitrant tumor. In the present study, we evaluated novel therapeutic strategies, using TMZ, DOX, TRAB, and PAZ on the FDCS PDOX model derived from a patient who failed first line DOX chemotherapy.

2.2. Patient-derived tumor

In this study, we used a tumor surgical specimen from a female patient diagnosed with recurrent extra-nodal FDCS of the left lower extremity who underwent surgical resection (Kiyuna et al., 2016). She previously received adjuvant radiotherapy to the primary tumor in 2014 and four cycles of chemotherapy with DOX and cyclophosphamide for her recurrent disease (Kiyuna et al., 2016). Details of surgical resection and chemotherapy given to this patient have been previously described (Kiyuna et al., 2016). Written informed consent was obtained from the patient as part of a UCLA Institutional Review Board (IRB #10-001857)-approved protocol (Kiyuna et al., 2016).

2.3. Establishment of a PDOX model of FDCS by surgical orthotopic implantation (SOI)

PDOX sample collection from the patient, performing subcutaneous implantation in nude mice, harvesting tumors from the mice, creating a space at the orthotopic site in the biceps femoris to insert tumor fragments in the mice and to establish the PDOX model and wound-closure procedures have been described in detail in our previous publication (Kiyuna et al., 2016).

2.4. Treatment study design in the FDCS PDOX model

All treatment procedures and data collection were performed as previously reported (Kiyuna et al., 2016). PDOX mouse models were randomized into five groups (Fig. 1A) of eight to nine mice, respectively. Group 1, untreated control, i.p. (n = 9); Group 2, treated with DOX, 2.4 mg/kg, i.p., weekly for 3 weeks (n = 9); Group 3, treated with PAZ, 50 mg/kg, oral gavage, daily for 3 weeks (n = 8); Group 4, treated with TRAB, 0.15 mg/kg, i.v., weekly for 3 weeks (n = 8); Group 5, treated with TMZ, 25 mg/kg, oral gavage, daily for 3 weeks (n = 9). Tumor length, width and mouse body weight were measured with calipers and digital balance, respectively twice a week. Mice were treated for 3 weeks (21 days) in each treatment group, and the final tumor measurement and other analysis was done on day 22. Tumor volume was calculated with the following formula: Tumor volume (mm³) = length (mm) × width (mm) × depth (mm) × 1/2. Data are presented as mean ± SD. The tumor volume ratio is defined at the tumor volume at any given time point relative to the initial tumor volume.

2.5. 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 (Murakami et al., 2016).

2.6. Statistical analysis

JMP version 13.0 was used for statistical analyses. Significant differences for continuous variables were determined using the Steel-Dwass test. Line graphs express average values and error bars show ± standard deviation. A probability value of P < 0.05 was considered statistically significant.
from initiation of treatment until end of treatment. Fig. 2A shows the representative tumors in each group. The FDCS PDOX model was resistant to DOX (p = 0.99 at day-22 of treatment, Group 2) and PAZ (p = 0.99 at day-22, Group 3). The FDCS PDOX model was slightly sensitive to TRAB, however, no statistically-significant difference were observed (p = 0.69 at day-22, Group 4). In contrast, the FDCS PDOX model was arrested by TMZ (p < 0.05 at day-22, Group 5). There was a statistically significant difference statistically between TMZ and the untreated control; TMZ and DOX; and TMZ and PAZ.
3.2. Body weight

Body weight was used as a general toxicity marker. The body weights at day-21 for all groups were as follows: Group 1 (untreated control with PBS): 27.55 ± 3.15; Group 2 (DOX): 26.70 ± 3.64; Group 3 (PAZ): 27.19 ± 5.35; Group 4 (TRAB): 27.34 ± 3.06; Group 5 (TMZ): 23.68 ± 4.48, respectively. No animal deaths in any group were observed (Fig. 3). No significant body-weight differences were observed among the groups (Fig. 3).

3.3. Histology

H&E-staining of tumor tissue sections was made from the FDCS PDOX models at the end of the experiment. The untreated-control group showed similar histologic features with the original patient tumor which included relatively uniform spindle-shaped cells present in storiform patterns and admixed chronic inflammatory cells (Fig. 4A). In the PDOX tumor treated with DOX, PAZ or TRAB, cancer cells invading muscle were observed (Fig. 4B, C, D). Necrosis was only detected in PDOX tumors treated with TMZ (Fig. 4E).

4. Discussion

Several studies reported the treatment of FDCS using a combination of gemcitabine and docetaxel (Conry, 2014; Jain et al., 2017), a combination of CHOP, ABVD (DOX, bleomycin, vinblastine, dacarbazine), and ICE (ifosfamide, carboplatin, etopside) (Biddle et al., 2002; Soriano et al., 2007; Shinagare et al., 2011), ridaforolimus, an mTOR (mammalian target of rapamycin) inhibitor (Mita et al., 2013), anthracycline-based regimens (Gounder et al., 2015), and a poly ADP-ribose polymerase (PARP) inhibitor in combination with carboplatin (Lemech et al., 2016), and bendamustine (Sasaki et al., 2017) and imatinib (Azim et al., 2007).

In the present study, DOX, TMZ, TRAB, and PAZ were tested on the FDCS PDOX model. The FDCS PDOX model was resistant to DOX as well as was the patient. In contrast, with regard to arresting tumor growth and causing tumor necrosis, the present results showed that TMZ was effective on the FDCS PDOX model.

TMZ in combination with talazoparib showed highly promising therapy for SCLC (Bupathi et al., 2017). TMZ regressed a DOX-resistant undifferentiated spindle-cell sarcoma PDOX (Igarashi et al., 2018a). TMZ in combination with bevacizumab showed an effective and tolerated treatment for patients with solitary fibrous tumor (de Lemos et al., 2018). TMZ in combination with irinotecan arrested a DOX and TMZ-resistant myxofibrosarcoma PDOX model and sarcoma PDOX model (Kiyuna et al., 2018). TMZ in combination with vincristine and irinotecan was effective for alveolar rhabdomyosarcoma (Setty et al., 2018).

Our previous studies showed that PAZ and TRAB had significant inhibitory efficacy on the PDOX models of undifferentiated spindle cell sarcoma (Igarashi et al., 2017b) and osteosarcoma lung metastasis (Igarashi et al., 2017c). However, TRAB and PAZ did not have significant inhibitory efficacy on the PDOX model in the present study. Some recent studies have demonstrated how TMZ targets different pathways in various tumor systems. TMZ interacts with PD-L1 upstream targets such as the JAK/STAT pathway (Heynckes et al., 2019). TMZ also induces senescence and downregulation of DNA repair pathways in glioma cells via activation of ataxia telangiectasia and Rad3-related protein (ATR)-checkpoint kinase 1 (CHK1), cyclin-dependent kinase inhibitor 1A (p21), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathways (Aasland et al., 2019). TMZ treatment was associated with high expression of pro-apoptotic cleaved caspase-3, PARP, phosphoinositide-3-kinase (PI3K)/protein kinase B (Akt) and extracellular signal-regulated kinase 1/2 (ERK1/2) mitogen-activated protein kinase (MAPK) pathways in musculoskeletal sarcomas (Kusabe et al., 2015). Wang et al. (2016) demonstrated that TMZ suppressed glioma cell growth by downregulating the expression of vascular endothelial growth factor C (VEGF-C) by inhibiting the ERK signaling pathway. TMZ could be delivered to the tumor site using a tumor-targeting immunoliposome nanocomplex formulation delivery system (Kim et al., 2015), and lactoferrin nanoparticles, which enhanced the therapeutic efficacy of TMZ in gliomas (Kumari et al., 2017). The mechanism by which TMZ targets FDCS will be part of our future study.

5. Conclusion

The present study demonstrates that TMZ is effective for a PDOX model of FDCS established from a patient who failed DOX treatment. The PDOX model allowed us to precisely identify TMZ and only TMZ as effective, for this highly recalcitrant disease. The FDCS PDOX model was resistant to DOX as was the patient. The PDOX model is useful as precise individualized oncology which is most valuable for patients who failed the first- or second-line chemotherapy.

Conflict of interests

AntiCancer Inc. uses PDOX models for contract research. H.O., T.K., K.M., K.K., T.H., M.Y., S.R., M.B., S.W. and R.M.H. are or were unsalaried associates of AntiCancer Inc. There are no other competing financial interests.

Author contributions

H.O. and R.M.H. were involved in study conception and design. H.O., Y.T., T.K., KM., T.H., M.Y., Z.Z., S.R., M.B., S.W. and R.M.H. were involved in data acquisition. H.O., Y.T., T.K., KM., T.H., M.Y., Z.Z., S.R., M.B., S.W., S.D.N., T.L., M.B., S.R.S., F.K. and R.M.H. analyzed and interpreted data. H.O. and R.M.H. prepared the manuscript. S.R.S. provided critical revision of the manuscript. All authors reviewed the manuscript.

Ethical approval

All animal studies were conducted 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 Institute of Health Guide for the Care of Laboratory Animals.
Fig. 4. Effect of treatment on tumor histology of the FDACS PDOX. (A, A’ Untreated control (Group 1). (B, B’) DOX (Group 2). (C, C’) PAZ (Group 3). (D, D’) TRAB (Group 4). (E, E’). TMZ (Group 5). Necrotic areas are indicated by yellow broken circles. Scale bars: 50 μm and A-F (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

and Use of Animals under Assurance Number A3873-1. Written informed consent was obtained from the patient as part of a UCLA Institutional Review Board (IRB #10-001857)-approved protocol.

Acknowledgements

This manuscript is dedicated to the memory of Dr. A.R. Moossa and Dr. Sun Lee.

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