Biotechnology Reports 15 (2017) 24–26

Contents lists available at ScienceDirect

Biotechnology Reports

journal homepage: www.elsevier.com/locate/btre

Dusquetide: Reduction in oral mucositis associated with enduring ancillary benefits in tumor resolution and decreased mortality in head and neck cancer patients

Mahesh Kudrimoti[a], Amarinthia Curtis[b], Samar Azawi[c], Francis Worden[d], Sanford Katz[e], Douglas Adkins[f], Marcelo Bonomi[g], Zack Scott[h], Jenna Elder[h], Stephen T. Sonis[i,j], Richard Straube[k], Oreola Donini[k,*]

[a] Radiation Oncology, University of Kentucky, 800 Rose Street, Lexington, KY, 40536, USA
[b] Gibbs Cancer Center, Spartanburg Regional Hospital, 101 E Wood, Spartanburg, SC, 29303, USA
[c] Veteran's Affairs Long Beach Hospital, 5901 E 7th Street, Mail Code 114A, Long Beach, CA, 98022, USA
[d] Department of Medicine, University of Michigan Health System,1500 E Medical Center Drive, Ann Arbor, MI, 48109, USA
[e] Department of Radiation Oncology, Willis-Knighton Cancer Center,2600 Kings Highway, Shreveport, LA, 71103, USA
[f] Division of Hematology and Oncology, Washington University, 660 South Euclid Avenue, Saint Louis, MO, 63110, USA
[g] Department of Hematology and Oncology, Wake Forest Health Sciences Medical Center, 1 Medical Center Blvd., Winston-Salem, NC, 27157, USA
[h] PharPoint Research, 5003 S Miami Blvd #100, Durham, NC, 27703, USA
[i] Oral Medicine and Diagnostic Services, Dana Farber/Harvard Cancer Center, Boston, MA, USA
[j] Biomodels LLC,313 Pleasant Street, Watertown, MA 02472, USA
[k] Soligenix Inc., 29 Emmons Drive, Suite C-10, Princeton, NJ, 08540, USA

ARTICLE INFO

Chemical compounds studied in this article:
dusquetide (PubChem CID: 71722017)

Keywords:
Innate
Immune
Oral mucositis
Head and neck cancer
Cancer supportive care
Dusquetide

ABSTRACT

Innate immunity is a key component in the pathogenesis of oral mucositis, a universal toxicity of chemoradiation therapy (CRT). Dusquetide, a novel Innate Defense Regulator, has demonstrated both nonclinical and clinical efficacy in ameliorating severe oral mucositis (SOM). Long term follow-up studies from the Phase 2 clinical study evaluating dusquetide as a treatment for SOM in head and neck cancer (HNC) patients receiving CRT have now been completed. Extended analysis indicates that dusquetide therapy was well-tolerated and did not contribute to increased infection, tumor growth or mortality. Potential ancillary benefits of duquetide therapy were also identified.

1. Introduction

Interim results from a Phase 2 study evaluating a dose of 1.5 mg/kg of dusquetide as a treatment for severe oral mucositis (SOM) in head and neck cancer (HNC) patients receiving chemoradiation therapy (CRT) demonstrated a 50% decrease in the median duration of severe oral mucositis (SOM) in patients receiving at least 55 Gy irradiation [1]. Patients at higher risk for SOM showed even greater improvements (67%) relative to placebo, particularly in the treatment group receiving the 1.5 mg/kg dose of dusquetide [1]. Over this same treatment period, an increased number of patient classified as having a "complete tumor response" using the RECIST 1.1 tumor status system and a decreased "non-fungal" (i.e. bacterial) infection rate were also observed. Long term follow-up visits were conducted on these same patients for 12 months after the completion of CRT, with the last visits occurring in the fall of 2016.

There are no treatments for SOM approved by the U.S. Food and Drug Administration (FDA) for use in HNC or other cancers with solid tissue tumors. In the case of hematologic tumors, there is only one approved therapy (palifermin), a tissue growth factor which presumably encourages the growth and regrowth of the oral mucosa tissue. Palifermin is specifically approved for use in patients receiving hematopoietic stem cell support for a myelotoxic therapy of a hematologic cancer which lack the receptor for the growth factor [2,3]. Due to its function as a tissue growth factor, palifermin is associated with a potential risk of stimulating/encouraging solid tumor proliferation [4–6]

Abbreviations: CRT, chemoradiation therapy; HNC, head and neck cancer; IDR, innate defense regulator; OM, oral mucositis; SOM, severe oral mucositis

* Corresponding author.
E-mail address: ODonini@Soligenix.com (O. Donini).

http://dx.doi.org/10.1016/j.btre.2017.05.002
Received 23 March 2017; Received in revised form 8 May 2017; Accepted 12 May 2017
Available online 17 May 2017

and is therefore contra-indicated in the case of solid tumors, all of which express the growth factor receptor. Other treatment approaches are under development [7,8] but none have been approved and the risk of interfering with tumor treatment or encouraging tumor growth remains a primary concern [9].

Innate immunity is believed to play a key role in the pathogenesis of oral mucositis [10–13] and indeed the efficacy of dusquetide as an Innate Defense Regulator supports this understanding [14,15,1]. Dusquetide (SGX942) is a first-in-class Innate Defense Regulator (IDR) that modulates the innate immune response downstream of most innate immune receptors, acting at a key adaptor protein known as p62 or sequestosome-1 [14]. Dusquetide modulates innate immune signaling from a pro-inflammatory, pro-macrophage response to an anti-inflammatory and increased pro-macrophage response. This response leads to decreased inflammation, increased bacterial clearance and increased tissue healing [14–16]. Importantly, dusquetide is not an anti-apoptotic or anti-necroptosis agent and cannot directly mitigate the damage done by CRT to the tumor [1].

Although direct interference with tumor therapy is unlikely, and indeed demonstrated not to occur preclinically [1], there are other potential ancillary effects of p62 interactions on tumor biology [17]. Specifically, p62 is a ubiquitous protein that is present in most cells, including aberrant tumor cells, and, through its role in autophagy, has been shown to impact tumorigenesis [18]. Thus, p62 is believed to be important in the tumorigenesis of MCF-7 (breast cancer cell line) where autophagy is otherwise inhibited [19,20]. Again, a xenograft study with MCF-7 cells not only demonstrated a lack of interference with CRT but also demonstrated a lack of tumor enhancement with SGX942 treatment. In fact, reduction in tumor volume was observed preclinically with SGX942 treatment [1].

Innate immunity also plays a role in establishing the microenvironment around a tumor. For example, p62 has been directly implicated in facilitating the stromal cell microenvironment in multiple myeloma via a mechanism involving increased IL-6 signaling [21,22].

To address the remote possibility that dusquetide may protect and/or enhance tumor growth, tumor resolution was monitored in the context of multiple myeloma cell growth in the presence of stromal cells and throughout a recent Phase 2 HNC study, both immediately after treatment and throughout a 12-month follow-up period. Similarly, overall survival of these HNC patients was also monitored through this same period.

2. Results and discussion

Dusquetide was not expected to negatively impact the stromal microenvironment of multiple myeloma cells, since this signaling has been reported to rely on IL-6 signaling [21] and dusquetide has been shown to significantly reduce IL-6 [15]. Nonetheless, a co-culture system with human multiple myeloma cells was previously investigated and demonstrated that when dusquetide was pre-incubated with stromal cells, those same stromal cells provided reduced support for multiple myeloma cell growth (Fig. 1).

As reported previously [1], dusquetide (SGX942) reduced the median duration of SOM between initiation of CRT and the 1-month follow-up visit in a 111-patient double-blind placebo-controlled Phase 2 clinical trial of OM in HNC patients. In the patient population receiving at least 55 Gy irradiation, this reduction was 50% (18 days vs. 9 days in the placebo and SGX942 1.5 mg/kg treatment groups respectively, $p = 0.099$). In higher risk subpopulations, this reduction improved to 67% (30 days vs. 10 days in the placebo and SGX942 1.5 mg/kg group respectively [$p = 0.040$] in those patients receiving the highest doses of cisplatin chemotherapy). Ancillary measures during this initial window between initiation of CRT and the 1-month follow-up visit also demonstrated a decreased rate of non-fungal infection and an increased rate of "complete response" in the tumor assessments at the 1-month follow-up visit. The anti-infective and anti-inflammatory/tissue healing

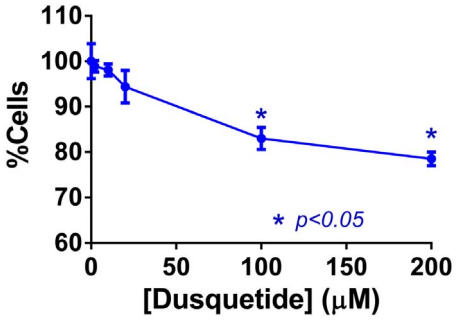

Fig. 1. Dusquetide Pre-Incubated with Stromal Cells inhibits the Future Growth of Co-cultured Multiple Myeloma Cells. Previously defined primary human bone marrow stromal (mesenchymal (MSC)) cells (ReachBio lot# 2221207) were plated at a concentration of 10^4 per well on 12 well plates in a McCoy's based medium (Hyclone, lot# AVE72772) supplemented with 10% FBS (Hyclone, lot# ASF29773) and 2 mM L-Glutamine (Hyclone, lot# AUJ25591). These cells had been characterized by flow cytometry and the standard phenotype (CD45- CD34- CD73 + CD105 + CD90 +) confirmed previously. The cells were used at passage 2 in all experiments. MM1.S cells (lot # 9000068) were purchased from ATCC and were cultured as recommended by the supplier in RPMI medium (Hyclone, lot# AVB62578), supplemented with 10% FBS (Hyclone, lot# ASF29773) and Glutamax (Gibco, lot# 889105) and allowed to expand. The marrow stromal cells were allowed to grow for 3 days. After this time, dusquetide was added at the indicated concentrations. Following 48 h incubation with dusquetide, the medium (and compound) was removed and the wells washed with RPMI containing 10% FBS and 2 mM L-Glutamine. To each well, 3×10^4 MM1.S cells were added, and following an additional 48 h, these cells were removed by pipetting vigorously up and down and collecting the contents of each individual well into 5 mL tubes. Cell counts were performed without dilution using a Neubauer chamber.

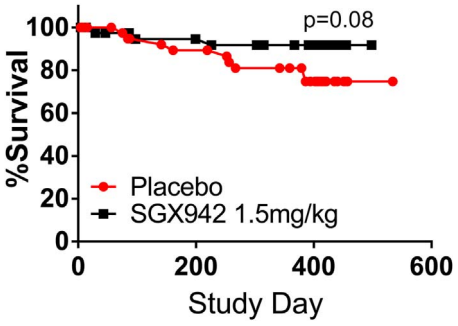

Fig. 2. Kaplan-Meier Survival Curves from the Phase 2 IDR-OM-01 Study. The detailed study design is described in [1]. Survival was monitored for the 12 months following completion of chemoradiation therapy.

aspects of dusquetide were expected on the basis of previous preclinical work [15,1].

The one-year mortality rate in the placebo group was 81% (Fig. 2), consistent with the Surveillance, Epidemiology, and End Results statistics of approximately 80% survival for patients with tumors of the oral cavity, depending on tumor location [23]. In the dusquetide treatment groups, mortality was significantly lower. Using the pre-determined cutoff of $p < 0.1$ for statistical significance in this exploratory study yielded statistically significant improvement in survival using Kaplan-Meier analysis (Fig. 2), compared to placebo. This decreased mortality was primarily observed in the follow-up period (after the 1-month follow-up visit), suggesting it was not related to the decreased infection rate also observed in the dusquetide treated groups in this study prior to the 1-month follow up visit. While the underlying cause of the decreased mortality is unknown, these results certainly support the contention that SGX942 was safe and well-tolerated, without any identifiable negative side-effects in this patient population.

We previously reported a trend towards improved tumor resolution at 1-month post CRT using the RECIST v 1.1 criteria [24]. While it was not previously possible to ascertain if this improvement was transient or enduring, monitoring throughout the 12-month follow-up window demonstrated that the effect was in fact enduring (Table 1). Moreover, the

Table 1
Tumor Progression as a Function of Elapsed Time since Completion of CRT[1].

Timepoint	Placebo	1.5 mg/kg	3.0 mg/kg	6.0 mg/kg
SAFETY POPULATION (N)	41	42	3	23
1-month follow-up	15/32 (47%)	17/27 (63%)	1/3 (33%)	4/16 (25%)
LOCF[2]	26/35 (74%)	28/35 (80%)	1/3 (33%)	13/22 (59%)
mITT [3] POPULATION (N)	38	36	3	19
1-month follow-up	15/32 (47%)	17/27 (63%)	1/3 (33%)	4/16 (25%)
LOCF[2]	26/35 (74%)	28/34 (82%)	1/3 (33%)	12/19 (63%)

[1]Percentage calculation excludes missing/not assessed evaluations.
[2]Last Observation Carried Forward.
[3]modified Intent-to-Treat.

patients in the placebo group continued to improve, eventually having a response rate more similar to the 1.5 mg/kg dusquetide treated group (Table 1). These results may indicate that dusquetide accelerated the tumor resolution. Given the size of this initial Phase 2 trial, it is impossible to ascertain if this improvement was due to increased compliance with CRT therapy or due to a direct effect of dusquetide on the tumor and its microenvironment. Given the known biology of p62 and the previously reported preclinical findings, it is possible that dusquetide had a direct anti-tumor effect in addition to reducing the duration of SOM.

These results demonstrate that not only does dusquetide reduce the duration of a debilitating and burdensome side effect of most cancer treatment regimens, but that it could be associated with significant ancillary benefits including decreased infection rates and accelerated tumor resolution.

Financial disclosure

The authors declare that they have competing financial interests in that two of the authors (RS, OD) are employees of Soligenix Inc., which is developing Innate Defense Regulators as human therapeutics.

Acknowledgments

Funding: A portion of this work was supported by the National Institute of Dental and Craniofacial Research Small Business Innovation Research grant # 1R43 DE024032-01(Soligenix, Inc).

The Spartanburg Regional Healthcare system: Dr. Sharmila Mehta and Dr. Patricia Griffin and to the Dusquetide OM Study Group: Arizona Oncology Services Foundation, Broward Health Medical Center, East Carolina University, Karmanos Cancer Center, Lakeland Regional Cancer Center, Mary Bird Perkins Cancer Center, Martin Health Center, Medstar Franklin Square, Memorial Hospital of Rhode Island, Montefiore Medical Center, Ohio State University, Spartanburg Regional Medical Center, Temple University Hospital, University Hospitals, Seidman Cancer Center, University of Illinois at Chicago, University of Kentucky, University of Michigan Medical Center, University of Rochester, University of Wisconsin-Carbone Cancer, University of Washington Medical Center, VA Long Beach Health System, VA Puget Sound Health Care System, Wake Forest Health Sciences Medical Center, Washington Cancer Institute, Washington University, Willis-Knighton Cancer Center and Virginia Mason Medical Center.

References

[1] M. Kudrimoti, A. Curtis, S. Azawi, F. Worden, S. Katz, D. Adkins, M. Bonomi, J. Elder, S.T. Sonis, R. Straube, O. Donini, Dusquetide A novel innate defense regulator demonstrating a significant and consistent reduction in the duration of oral mucositis in preclinical data and a randomized, placebo-controlled phase 2a clinical study, J. Biotechnol. 239 (2016) 115–125.
[2] D.T. Nguyen, S. Shayani, J. Palmer, A. Dagis, S.J. Forman, J. Epstein, R. Spielberger, Palifermin for prevention of oral mucositis in allogeneic hematopoietic stem cell transplantation: a single-institution retrospective evaluation, Support. Care Cancer 23 (11) (2015) 3141–3147.
[3] A. Lucchese, G. Matarese, M. Manuelli, C. Ciuffreda, L. Bassani, G. Isola, G. Cordasco, E. Gherlone, Reliability and efficacy of palifermin in prevention and management of oral mucositis in patients with acute lymphoblastic leukemia: a randomized, double-blind controlled clinical trial, Minerva Stomatol. 65 (1) (2016) 43–50.
[4] A.M. McDonnell, K.L. Lenz, Palifermin: role in the prevention of chemotherapy- and radiation-induced mucositis, Ann. Pharmacother. 41 (1) (2007) 86–94.
[5] M. Henke, M. Alfonsi, P. Foa, J. Giralt, E. Bardet, L. Cerezo, M. Salzwimmer, R. Lizambri, L. Emmerson, M.G. Chen, D. Berger, Palifermin decreases severe oral mucositis of patients undergoing postoperative radiochemotherapy for head and neck cancer: a randomized, placebo-controlled trial, J. Clin. Oncol. 29 (20) (2011) 2815–2820.
[6] P. Bossi, L.D. Locati, L. Licitra, Palifermin in prevention of head and neck cancer radiation-induced mucositis: not yet a definitive word on safety and efficacy profile, J. Clin. Oncol. 30 (5) (2012) 565–567 (564-5).
[7] R. Fekrazad, N. Chiniforush, Oral mucositis prevention and management by therapeutic laser in head and neck cancers, J. Lasers Med. Sci. 5 (1) (2014) 1–7.
[8] J.E. Raber-Durlacher, I. von Bültzingslöwen, R.M. Logan, J. Bowen, A.R. Al-Azri, H. Everaus, E. Gerber, J.G. Gomez, B.G. Pettersson, Y. Soga, F.K. Spijkervet, W.J. Tissing, J.B. Epstein, S. Elad, R.V. Lalla, Mucositis Study Group of the Multinational Association of Supportive Care in Cancer/International Society of Oral Oncology (MASCC/ISOO), Systematic review of cytokines and growth factors for the management of oral mucositis in cancer patients, Support. Care Cancer 21 (1) (2013) 343–355.
[9] S. Sonis, S. Hashemi, J.B. Epstein, R.G. Nair, J.E. Raber-Durlacher, Could the biological robustness of low level laser therapy (Photobiomodulation) impact its use in the management of mucositis in head and neck cancer patients, Oral Oncol. 54 (2016) 7–14.
[10] S.T. Sonis, A biological approach to mucositis, J. Support. Oncol. 2 (1) (2004) 21–32.
[11] S.T. Sonis, Pathobiology of oral mucositis: novel insights and opportunities, J. Support. Oncol. 5 (2007) 3–11.
[12] S. Sonis, R. Haddad, M. Posner, B. Watkins, E. Fey, T.V. Morgan, L. Mookanamparambil, M. Ramoni, Gene expression changes in peripheral blood cells provide insight into the biological mechanisms associated with regimen-related toxicities in patients being treated for head and neck cancers, Oral Oncol. 43 (2007) 289–300.
[13] R.M. Logan, A.M. Stringer, J.M. Bowen, A.S. Yeoh, R.J. Gibson, S.T. Sonis, D.M. Keefe, The role of pro-inflammatory cytokines in cancer treatment-induced alimentary tract mucositis: pathobiology, animal models and cytotoxic drugs, Cancer Treat. Rev. 33 (2007) 448–460.
[14] H.B. Yu, A. Kielczewska, A. Rozek, S. Takenaka, Y. Li, L. Thorson, R.E. Hancock, M.M. Guarna, J.R. North, L.J. Foster, O. Donin, B.B. Finlay, Sequestosome-1/p62 is the key intracellular target of innate defense regulator peptide, J. Biol. Chem. 284 (52) (2009) 36007–36011.
[15] J.R. North, S. Takenaka, A. Rozek, A. Kielczewska, S. Opal, L.A. Morici, B.B. Finlay, C.J. Schaber, R. Straube, O. Donini, A novel approach for emerging and antibiotic resistant infections: innate defense regulators as an agnostic therapy, J. Biotechnol. 226 (2016) 24–34.
[16] M.G. Scott, E. Dullaghan, N. Mookherjee, N. Glavas, M. Waldbrook, A. Thompson, A. Wang, K. Lee, S. Doria, P. Hamill, J.J. Yu, Y. Li, O. Donini, M.M. Guarna, B.B. Finlay, J.R. North, R.E. Hancock, An anti-infective peptide that selectively modulates the innate immune response, Nat. Biotechnol. 25 (4) (2007) 465–472.
[17] J. Moscat, M. Karin, M.T. Diaz-Meco, P62 in cancer: signaling adaptor beyond autophagy, Cell 167 (3) (2016) 606–609.
[18] M. Komatsu, S. Kageyama, Y. Ichimura, p62/SQSTM1/A170: physiology and pathology, Pharmacol. Res. 66 (6) (2012) 457–462.
[19] V.M. Aita, X.H. Liang, V.V. Murty, D.L. Pincus, W. Yu, E. Cayanis, S. Kalachikov, T.C. Gilliam, B. Levine, Cloning and genomic organization of Beclin-1, a candidate tumor suppressor gene on chromosome 17q21, Genomics 59 (1999) 59–65.
[20] M.T. Rosenfeldt, K.M. Ryan, The role of autophagy in tumour development and cancer therapy, Exp. Rev. Mol. Med. 11 (2009) e36.
[21] Y. Hiruma, T. Honjo, D.F. Jelinek, J.J. Windle, J. Shin, G.D. Roodman, N. Kurihara, Increased signaling through p62 in the marrow microenvironment increases myeloma cell growth and osteoclast formation, Blood 113 (20) (2009) 4894–4902.
[22] J. Teramachi, R. Silbermann, P. Yang, W. Zhao, K.S. Mohammad, J. Guo, J.L. Anderson, D. Zhou, R. Feng, K.Z. Myint, N. Maertz, J.H. Beumer, J.L. Eiseman, J.J. Windle, X.Q. Xie, G.D. Roodman, N. Kurihara, Blocking the ZZ domain of sequestosome1/p62 suppresses myeloma growth and osteoclast formation in vitro and induces dramatic bone formation in myeloma-bearing bones in vivo, Leukemia 30 (2) (2016) 390–398.
[23] SEER Cancer Statistics Review, 1975–2013, in: N. Howlader, A.M. Noone, M. Krapcho, D. Miller, K. Bishop, S.F. Altekruse, C.L. Kosary, M. Yu, J. Ruhl, Z. Tatalovich, A. Mariotto, D.R. Lewis, H.S. Chen, E.J. Feuer, K.A. Cronin (Eds.), National Cancer Institute, Bethesda, MD, 2015(http://seer.cancer.gov/csr/1975_2013/, based on November 2015 SEER data submission, posted to the SEER web site, April 2016).
[24] E.A. Eisenhauer, P. Therasse, J. Bogaerts, L.H. Schwartz, D. Sargent, R. Ford, J. Dancey, S. Arbuck, S. Gwyther, M. Mooney, L. Rubinstein, L. Shankar, L. Dodd, R. Kaplan, D. Lacombe, J. Verweij, New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1), Eur. J. Cancer 45 (2) (2009) 228–247.