§ Department of Periodontics, Virginia Commonwealth University School of Dentistry, Richmond, VA.
‖ Private practice, Park Ridge and Oakbrook Terrace, IL.
¶ Department of Oral and Maxillofacial Surgery, School of Dentistry, Louisiana State University, New Orleans, LA.
# Department of Graduate Periodontics, College of Dentistry, University of Illinois at Chicago, Chicago, IL.
** Private practice, Tigard, OR.
†† Department of Periodontology, Oregon Health and Science University, Portland, OR.
‡‡ Department of Periodontology, Graduate School of Dentistry, Osaka University, Osaka, Japan.

Members of the Emerging Regenerative Approaches for Periodontal Reconstruction consensus group met and began with individual introductions and provision of appropriate disclosures. The authors of the written review provided a summary of the construction and contents of the review paper.[1] Each member of this consensus group provided comments on the review.

As an overview, one of the issues arising was the question of defining what constitutes an emerging technology. The group considered two broad categories: 1) products and components of products approved by the US Food and Drug Administration (FDA) and 2) non-approved therapeutic modalities. For example, components of FDA-approved products are being examined for periodontal regeneration and thus were considered an emerging therapeutic approach. In addition, currently available therapeutics that have limited data supporting their use in periodontal regeneration were also considered an emerging technology.

We discussed the contents of the review paper and made suggestions for additions. The consensus group agreed with the contents of the review paper and the scope of products and technologies that were covered in the paper with the additions and comments noted below.

Most of the emerging approaches discussed in the review paper were focused on the concepts of tissue engineering[2] and also included other approaches. The topics discussed included the following: 1) protein and peptide therapy;[3-8] 2) cell-based therapy;[9] 3) genetic therapy; 4) scaffolds;[10] 5) bone anabolics; and 6) lasers. FDA-approved products evaluated included the following: 1) enamel matrix derivative; 2) recombinant human platelet-derived growth factor; and 3) anorganic bone matrix. Non-approved therapeutic modalities included the following: 1) recombinant human fibroblast growth factor-2; 2) recombinant human growth differentiation factor-5; 3) bone morphogenetic proteins (BMP-2, BMP-7, BMP-6, and BMP-12); 4) parathyroid hormone/teriparatide; 5) brain-derived neurotrophic factor; and 6) sclerostin antibodies.[11] Cell-based therapies included the following: 1) mesenchymal stem cells; 2) bone marrow stromal cells; 3) periodontal ligament cells; 4) embryonic stem cells; and 5) induced pluripotent stem cells. Gene therapies included viral and non-viral vectors. Scaffolds are also promising for delivery of growth factors and gene therapy and may be composed of either natural or synthetic polymeric materials.[12,13] Finally, various types of lasers were discussed.

In addition to the approaches highlighted in the review, the consensus group recognized that many of the therapeutics in current use are based on fundamental knowledge and understanding of the development of the periodontium.[14-16] Furthermore, the host inflammatory response should also be considered, as well as such interactions between the host genome, epigenetics, and the microbiome.[17-20] Areas of future interest might include inflammatory regulators such as resolvins[21] and interleukin-17 antibodies, as well as phosphate/pyrophosphate local regulation.[22]

## IMPLICATIONS OF REVIEW TO PATIENT-RELATED CLINICAL OUTCOMES

The review did not identify any reports addressing patient-reported outcomes. However, the review did reveal clinical parameter-based outcomes of several individualized approaches over various time periods, with the longest follow-up being 3 years in one report of 83 patients.[23] Although there are multiple papers focused on various emerging technologies, there are no studies that allow for direct comparison of clinical outcomes.

When dealing with emerging technologies, there can be both positive and negative issues of clinician adoption and patient acceptance of treatment.

Barriers to adopting this technology include limited evidence supporting efficacy and indications for use. With emerging technologies, safety issues include unknown long-term effects along with known risk for rare but serious side effects, such as sarcoma. This is, in part, attributable to evaluation of such emerging technologies in populations defined by selected inclusion/exclusion criteria, further limiting outcome assessments.

With any emerging technology, the cost-to-benefit ratio for clinicians and patients must be determined. For the clinician, peer and market pressures, surgical time, technical complexity, healing times, predictability, liability, and cost must be considered.

For patients, experience of pain and morbidity, adverse events, both short and long term, cost, time, material (ethical and religious concerns), esthetic perceptions, and satisfaction with treatment outcomes should be considered.

## RESEARCH PRIORITIES FOR THE FUTURE

With all emerging therapies, the prevalence, predictability, and efficacy of outcomes and safety should be well defined. Future research should

promote the goal of emerging technologies to regenerate the periodontium as a functional organ system. The review made a number of recommendations, and the consensus group highlighted that future studies should do the following: 1) develop a non-invasive assessment of clinical periodontal regeneration; 2) evaluate the efficacy and safety of combining emerging and/or current therapies; 3) validate existing and/or emerging therapies being used "off label"; 4) explore therapies developed for other purposes for their application to periodontal regeneration; 5) define the individual's genetic and epigenetic profile so that it can be used to personalize the choice of therapy; 6) assess the effect of individual disease pathogenesis, etiology, and healing potential on therapeutic treatment selection; 7) optimize the understanding of risk factors to aid in the selection of appropriate therapy and the achievement of enhanced outcomes to restore the structure and function of the periodontium; 8) define molecular and cellular mechanisms of the emerging therapy using in vitro and in vivo models; 9) identify developmental pathways of the periodontium for potential application in regenerative therapy; 10) focus on developing minimally invasive technologies to minimize pain and morbidity without compromising outcomes; 11) define what constitutes clinical success; and 12) characterize the effect of the selected therapy on the patient's quality of life.

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## REFERENCES

1. Lin Z, Rios HF, Cochran DL. Emerging regenerative approaches for periodontal reconstruction: A systematic review from the AAP regeneration workshop. *J Periodontol* 2015;86(Suppl.):S134-S152.
2. Somerman M. Growth factors and periodontal engineering: Where next? *J Dent Res* 2011;90:7-8.
3. Amin HD, Olsen I, Knowles J, Dard M, Donos N. A tyrosine-rich amelogenin peptide promotes neovasculogenesis in vitro and ex vivo. *Acta Biomater* 2014;10:1930-1939.
4. Amin HD, Olsen I, Knowles JC, Donos N. Differential effect of amelogenin peptides on osteogenic differentiation in vitro: Identification of possible new drugs for bone repair and regeneration. *Tissue Eng Part A* 2012;18:1193-1202.
5. Foster BL, Nagatomo KJ, Nociti FH Jr., et al. Central role of pyrophosphate in acellular cementum formation. *PLoS One* 2012;7:e38393.
6. Iizuka S, Kudo Y, Yoshida M, et al. Ameloblastin regulates osteogenic differentiation by inhibiting Src kinase via cross talk between integrin beta1 and CD63. *Mol Cell Biol* 2011;31:783-792.
7. Kakegawa A, Oida S, Gomi K, et al. Cytodifferentiation activity of synthetic human enamel sheath protein peptides. *J Periodontal Res* 2010;45:643-649.
8. Kanazashi M, Gomi K, Nagano T, Tanabe T, Arai T, Fukae M. The 17-kDa sheath protein in enamel proteins induces cementum regeneration in experimental cavities created in a buccal dehiscence model of dogs. *J Periodontal Res* 2006;41:193-199.
9. Arany PR, Cho A, Hunt TD, et al. Photoactivation of endogenous latent transforming growth factor-beta1 directs dental stem cell differentiation for regeneration. *Sci Transl Med* 2014;6:238ra69.
10. Costa PF, Vaquette C, Zhang Q, Reis RL, Ivanovski S, Hutmacher DW. Advanced tissue engineering scaffold design for regeneration of the complex hierarchical periodontal structure. *J Clin Periodontol* 2014;41:283-294.
11. Das S, Sakthiswary R. Bone metabolism and histomorphometric changes in murine models treated with sclerostin antibody: A systematic review. *Curr Drug Targets* 2013;14:1667-1674.
12. Oortgiesen DA, Walboomers XF, Bronckers AL, Meijer GJ, Jansen JA. Periodontal regeneration using an injectable bone cement combined with BMP-2 or FGF-2. *J Tissue Eng Regen Med* 2014;8:202-209.
13. Yamano S, Haku K, Yamanaka T, et al. The effect of a bioactive collagen membrane releasing PDGF or GDF-5 on bone regeneration. *Biomaterials* 2014;35:2446-2453.
14. Cortellini P, Pini-Prato G, Nieri M, Tonetti MS. Minimally invasive surgical technique and enamel matrix derivative in intrabony defects: 2. Factors associated with

healing outcomes. *Int J Periodontics Restorative Dent* 2009;29:257-265.
15. Fukae M, Kanazashi M, Nagano T, Tanabe T, Oida S, Gomi K. Porcine sheath proteins show periodontal ligament regeneration activity. *Eur J Oral Sci* 2006;114 (Suppl. 1):212-218; discussion 254-256, 381-382.
16. Warotayanont R, Frenkel B, Snead ML, Zhou Y. Leucine-rich amelogenin peptide induces osteogenesis by activation of the Wnt pathway. *Biochem Biophys Res Commun* 2009;387:558-563.
17. Hajishengallis G. Aging and its impact on innate immunity and inflammation: Implications for periodontitis. *J Oral Biosci* 2014;56:30-37.
18. Hajishengallis G, Sahingur SE. Novel inflammatory pathways in periodontitis. *Adv Dent Res* 2014;26: 23-29.
19. Maekawa T, Abe T, Hajishengallis E, et al. Genetic and intervention studies implicating complement C3 as a major target for the treatment of periodontitis. *J Immunol* 2014;192:6020-6027.
20. Moutsopoulos NM, Konkel J, Sarmadi M, et al. Defective neutrophil recruitment in leukocyte adhesion deficiency type I disease causes local IL-17-driven inflammatory bone loss. *Sci Transl Med* 2014;6: 229ra40.
21. Mustafa M, Zarrough A, Bolstad AI, et al. Resolvin D1 protects periodontal ligament. *Am J Physiol Cell Physiol* 2013;305:C673-C679.
22. Rodrigues TL, Nagatomo KJ, Foster BL, Nociti FH, Somerman MJ. Modulation of phosphate/pyrophosphate metabolism to regenerate the periodontium: A novel in vivo approach. *J Periodontol* 2011;82:1757-1766.
23. Nevins M, Kao RT, McGuire MK, et al. Platelet-derived growth factor promotes periodontal regeneration in localized osseous defects: 36-month extension results from a randomized, controlled, double-masked clinical trial. *J Periodontol* 2013;84:456-464.

Correspondence: Dr. David L. Cochran, Department of Periodontics, University of Texas Health Science Center at San Antonio Dental School, 7703 Floyd Curl Dr., San Antonio, TX 78240. Fax: 210/567-3393; e-mail: cochran@uthscsa.edu.

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# Cyclic depsipeptides as potential cancer therapeutics

Jirouta Kitagaki[a,b], Genbin Shi[c], Shizuka Miyauchi[a], Shinya Murakami[a] and Yili Yang[d]

**Cyclic depsipeptides are polypeptides in which one or more amino acid is replaced by a hydroxy acid, resulting in the formation of at least one ester bond in the core ring structure. Many natural cyclic depsipeptides possessing intriguing structural and biological properties, including antitumor, antifungal, antiviral, antibacterial, anthelmintic, and anti-inflammatory activities, have been identified from fungi, plants, and marine organisms. In particular, the potent effects of cyclic depsipeptides on tumor cells have led to a number of clinical trials evaluating their potential as chemotherapeutic agents. Although many of the trials have not achieved the desired results, romidepsin (FK228), a bicyclic depsipeptide that inhibits histone deacetylase, has been shown to have clinical efficacy in patients with refractory cutaneous T-cell lymphoma and has received Food and Drug Administration approval for use in treatment. In this review, we discuss antitumor cyclic depsipeptides that have undergone clinical trials and focus on their structural features, mechanisms, potential applications in chemotherapy, and pharmacokinetic and toxicity data. The results of this study indicate that cyclic depsipeptides could be a rich source of new cancer therapeutics. *Anti-Cancer Drugs* 00:000–000 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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[a]Department of Periodontology, Division of Oral Biology and Disease Control, Osaka University Graduate School of Dentistry, [b]Challenge to Intractable Oral Diseases, Center for Translational Dental Research, Osaka University Dental Hospital, Suita, Osaka, Japan, [c]Macromolecular Crystallography Laboratory, National Cancer Institute, National Institutes of Health, Frederick, Maryland, USA and [d]Center for Translational Medicine, Shanghai Changzheng Hospital, Second Military Medical University, Shanghai, People's Republic of China

Correspondence to Yili Yang, PhD, Center for Translational Medicine, Shanghai Changzheng Hospital, 800 Xiangyin Road, Yangpu District, Shanghai, 200433, P. R. China
Tel/fax: +86 21 8187 1908; e-mail: nathanyang@hotmail.com

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## Introduction

Cyclic depsipeptides are polypeptides in which one or more amino acid is replaced by a hydroxy acid, resulting in the formation of at least one ester bond in the core ring structure. Many cyclic depsipeptides are natural products initially isolated from fungi, plants, and marine organisms (Table 1). As cyclic depsipeptides possess a number of biological functions, including antitumor, antibacterial, antifungal, and anti-inflammatory effects, several cyclic depsipeptides have been identified [20,21]. For example, the cyclic dodecadepsipeptide valinomycin was discovered around 50 years ago and was isolated from the genus *Streptomyces* [12]. Valinomycin is an ionophore specific for potassium that induces cell death by mitochondrial swelling and autophagy [22]. Valinomycin possesses antitumor activity against several tumor cell lines, including rat C6 glioma cells, human breast carcinoma cells MCF-7, human ovarian carcinoma cells A2780, and liver hepatocellular carcinoma cells HepG2, with $IC_{50}$ values of 0.0004, 2.18, 1.77, and 0.0008 μmol/l, respectively [23]. Cyclic hexadepsipeptide enniatins were first reported over 60 years ago and were isolated from *Fusarium* spp. fungi [1]. Because enniatins have diverse ionophoric activities specific for sodium, potassium, and calcium, they have a wide range of biological activities, including antifungal, antibiotic, and antitumor activities [24,25]. Sansalvamide A is a cyclic pentadepsipeptide isolated from marine *Fusarium* spp. fungi [2]. As sansalvamide A inhibits topoisomerase I, it is believed to possess marked antitumor activity against the 60 cancer cell lines in the National Cancer Institute panel, such as human prostate cancer PC3, human breast cancer MDA-MB-231, and human melanoma WM-115 [2,26]. More recently, Zhang *et al.* [27] identified that the sansalvamide A derivative H-10 exhibits antiproliferative effects against murine melanoma B16 cells.

Interestingly, with the development and application of high-performance liquid chromatography coupled with tandem mass spectrometry, bioactivity screening has often identified a group of cyclic depsipeptides differing only in a few substitutions. For example, in the search for potential antitumor compounds, an active fraction from the Australian sponge *Neamphius huxleyi* was found to contain three novel cyclic depsipeptides, namely, neamphamides B, C, and D, which differ only in $R_1$ ($NH_2$, OH, and $NH_2$, respectively) and $R_2$ ($CH_3$, $CH_3$, and $C_2H_5$, respectively; Fig. 1a) [11]. A minor structural difference in cyclic depsipeptides could result in a significant difference in biological activity. The substitution of a lactyl-proline residue in didemnin B with a pyruvoyl-proline residue forms aplidine, which has significantly more potent antiproliferative activity than didemnin B [8]. These interesting features are continuing to stimulate active research in medicinal chemistry, cell biology, and oncology.

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**Table 1 Fungus, plant, marine source, and bacteria derived cyclic depsipeptides**

| Compound | Source organism | Biological activity | References |
|---|---|---|---|
| Fungus-derived cyclic depsipeptides | | | |
| Enniatins | *Fusarium* spp. | Ionophore | Gaumann *et al.* [1] |
| Sansalvamide A | *Fusarium* spp. | Topoisomerase I inhibitor | Hwang *et al.* [2] |
| Paecilodepsipeptide | *Paecilomyces cinnamomeus* | Antitumor and antimalarial activity | Isaka *et al.* [3] |
| Beauvericin | *Beauveria bassiana* | Ionophore | Tonshin *et al.* [4] |
| Plant-derived cyclic depsipeptides | | | |
| Celogentins | *Celosia argentea* | Tumor growth inhibition | Kobayashi and colleagues [5,6] |
| Cyclolinopeptides F-1 | *Linum usitatissimum* | Immunosuppressive activity | Matsumoto *et al.* [7] |
| Marine organism-derived cyclic depsipeptides | | | |
| Aplidine | *Aplidium albicans* | Oxidative stress | Urdiales *et al.* [8] |
| Kahalalide F | *Elysia rufescens* | ErbB pathway | Hamann and Scheuer [9] |
| Didemnin B | *Trididemnum* genus | eEF1A | Rinehart *et al.* [10] |
| Neamphamide | *Neamphius huxleyi* | Antitumor activity | Tran *et al.* [11] |
| Bacteria-derived cyclic depsipeptides | | | |
| Valinomycin | *Streptomyces* spp. | Ionophore | Azzi and Azzone [12] |
| Korkormicin A | *Micromonospora* spp. | DNA intercalator | Lam *et al.* [13] |
| Luzopeptin | *Actinomycete* spp. | DNA intercalator | Huang and Crooke [14] |
| Thiocoraline | *Actinomycete* spp. | DNA intercalator | Zolova *et al.* [15] |
| Romidepsin | *Chromobacterium violaceum* | HDAC | Ueda *et al.* [16] |
| Cryptophycin 1 | Genus *Nostoc* | Tubulin | Schwartz *et al.* [17] |
| LY355703 | Genus *Nostoc* | Tubulin | Chen *et al.* [18] |
| Echinomycin | *Streptomyces* spp. | DNA intercalator | Mackedonski [19] |

HDAC, histone deacetylase.

**Fig. 1**

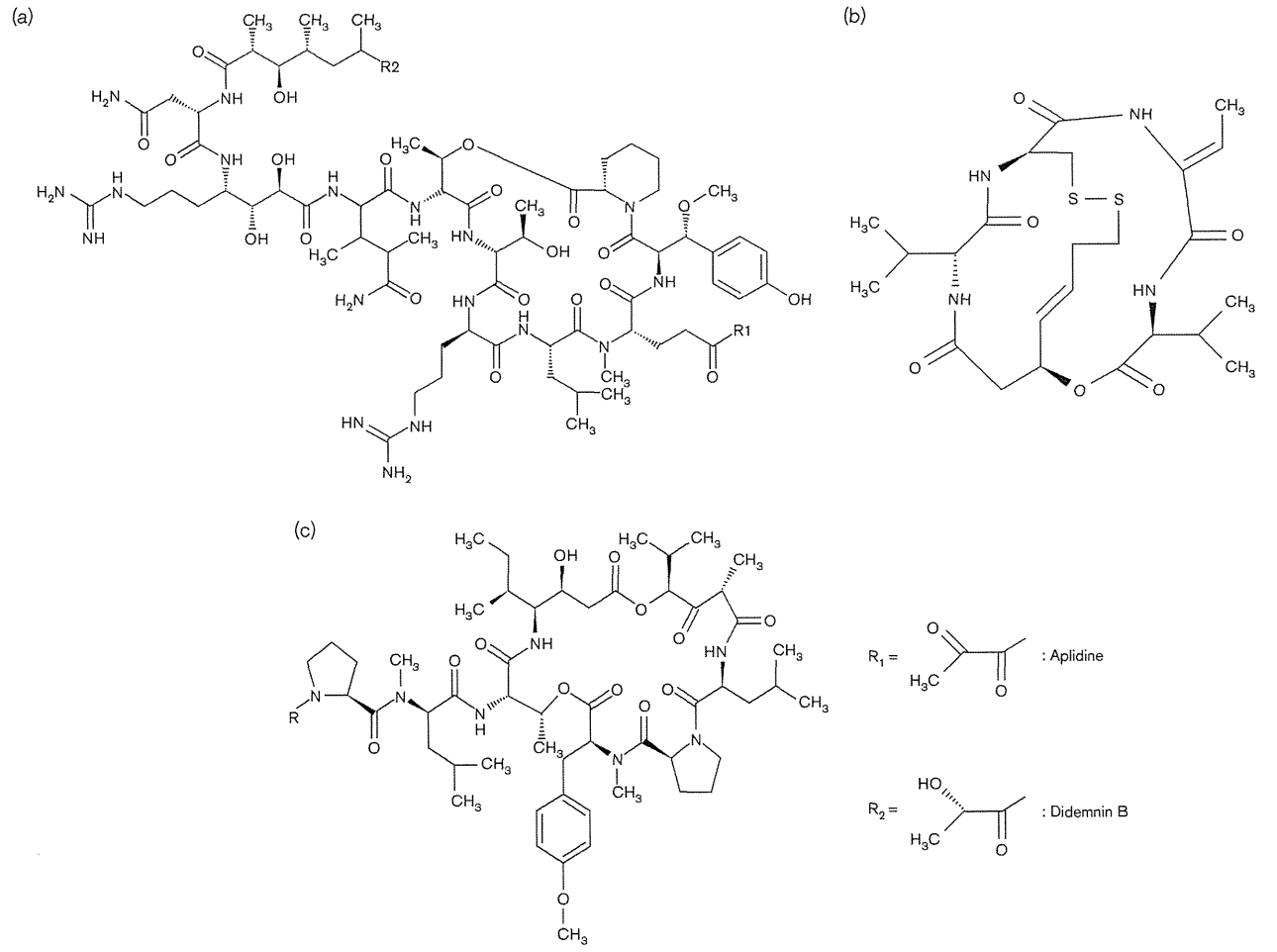
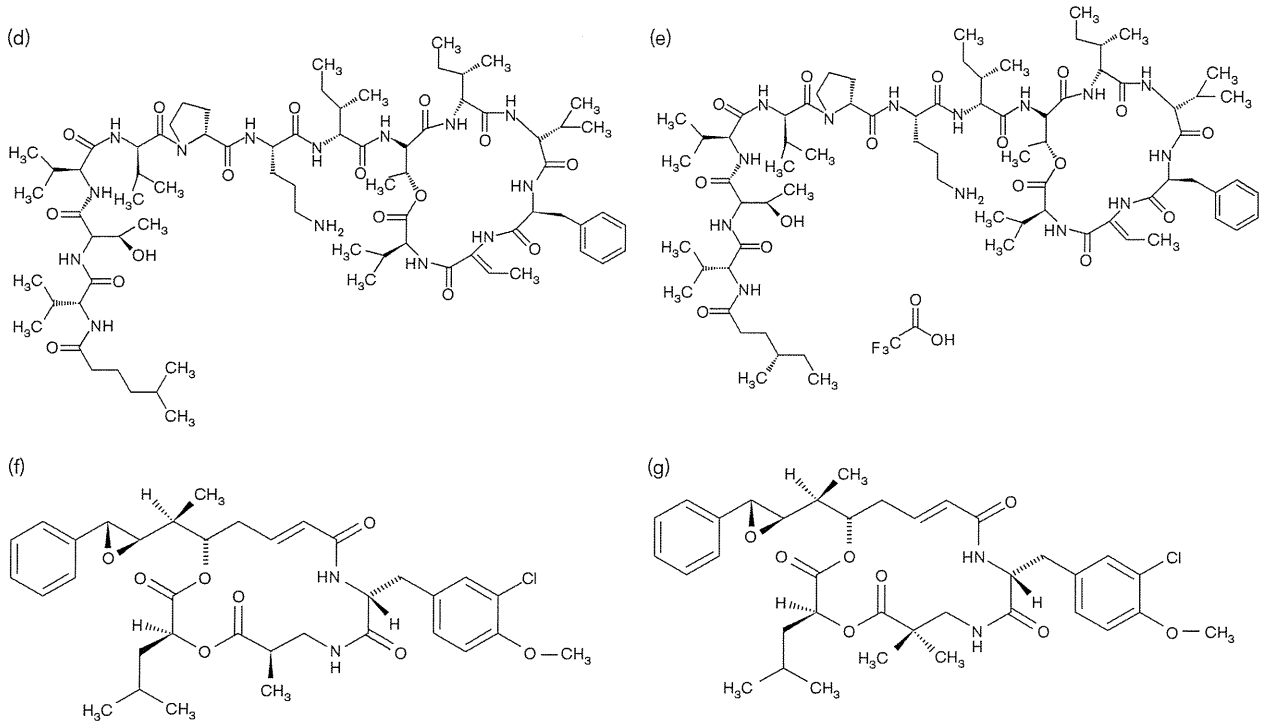


**Fig. 1 (*Continued*)**




Structure of cyclic depsipeptides. Structure of (a) neamphamides, (b) romidepsin, (c) aplidine and didemnin B, (d) kahalalide F, (e) PM02734, (f) cryptophycin 1, and (g) LY335703.

Many different cyclic depsipeptides with antitumor activity have been identified since the 1950s. They often have potent cytotoxic effects on tumor cells in culture and in xenografted mice. Korkormicin A, a cyclic depsipeptide from microorganisms, kills transformed cells with an $IC_{50}$ of ~ 2 nmol/l [28], as well as inhibits the growth of inoculated tumor cells and significantly prolongs the lifespan of tumor-bearing mice at a concentration of 0.05–0.2 mg/kg [13]. The core ring structure of cyclic depsipeptides appears to be critical for their activity, because luzopeptin, which has core ring structure similar to that of korkormicin A, has similar antitumor activity to korkormicin A [13,14]. It has been shown that fungus-derived paecilodepsipeptide A exhibits potent cytotoxic activity against several cancer cell lines ($IC_{50}$, ~ 6 µmol/l), whereas its linear analogs paecilodepsipeptide B and C are inactive [3]. It has also been found that at least some cyclic depsipeptides, such as enniatins and beauvericin, possess ionophoric properties and can increase the permeability of the cell membrane toward various ions. The ability of enniatins and beauvericin to affect the mitochondrial membrane likely contributes to their cytotoxic activity [4]. Another group of cyclic depsipeptides, including echinomycin, luzopeptins, sandramycin, thiocoraline, quinoxapeptin, and korkormicin A, characterized by the cyclic depsipeptide backbone and two planar chromophores, has the ability to intercalate into DNA [15]. There is substantial evidence indicating that the cytotoxicity of these compounds results from their ability to interfere with DNA replication, repair, and/or transcription [28,29]. It is conceivable that their selective antitumor activity is related to their altered chromatin structure and to epigenetic changes in various tumor cells. The effects of some cyclic depsipeptides may also be related to their ability to inhibit various proteases [30]. It was found recently that a group of cyclic depsipeptides from marine cyanobacteria, namely, grassypeptolides, are able to inhibit dipeptidyl peptidase 8 and block T-cell activation [31]. Interestingly, the bicyclic depsipeptide romidepsin is an inhibitor of histone deacetylase (HDAC), a generally acknowledged target of chemotherapeutics [32], and has received Food and Drug Administration (FDA) approval for the treatment of patients with refractory cutaneous T-cell lymphoma [33,34].

In this review, we will discuss cyclic depsipeptides that are undergoing clinical evaluation for their antitumor activities and focus on their structural features, possible mechanisms of action, and potential applications in the chemotherapeutic treatment of various cancers. With the clinical success of romidepsin, it is conceivable that these cyclic depsipeptides may be hopeful leads for more effective chemotherapeutic agents.

## Romidepsin

### In-vitro and animal studies

Romidepsin (also NSC-630176, depsipeptide, FK228, FR901228, and Istodax) was first isolated from *Chromobacterium violaceum* as a novel compound that could induce morphological reversion in Ha-*ras*-transformed NIH3T3 cells. It is a bicyclic depsipeptide with the molecular formula $C_{24}H_{36}N_4O_6S_2$ [molecular weight (MW): 540; Fig. 1b; Table 2] [16]. Further studies showed that intraperitoneal administration of romidepsin prolonged the lifespan of mice implanted with murine ascetic tumors, such as P388, L1210 leukemia, and B16 melanoma [35]. It was also demonstrated that intravenous injection (i.v.) of the compound inhibited the growth of xenografted solid tumors, including both murine (Colon 38 carcinoma, M5076 reticulum cell sarcoma, Meth A fibrosarcoma) and human (Lu-65 and LC-6-JCK lung large cell carcinomas, and SC-6 stomach adenocarcinoma) cancer cells. Moreover, this agent exhibited potent antitumor activity against the P388 cells that were resistant to multiple drugs, including mitomycin C, cyclophosphamide, vincristine, and 5-fluorouracil [16].

### Clinical trials

As romidepsin possesses both in-vitro and in-vivo antitumor activity, the compound was entered into phase I clinical trials. Four patients with T-cell lymphoma received 12.7 or 17.8 mg/m$^2$ romidepsin as a 4-h infusion on days 1 and 5 of a 21-day cycle. Three patients with cutaneous T-cell lymphoma (CTCL) showed a partial response, whereas one patient with peripheral T-cell lymphoma (PTCL) had a complete response [32]. As the first trial was successful for T-cell lymphoma, a number of phase I clinical studies were subsequently performed. The maximum tolerated dose (MTD) in phase II against advanced refractory neoplasms was 17.8 mg/m$^2$ with a 4-h i.v. administration on days 1 and 5 of a 21-day cycle [36] and 13.3 mg/m$^2$ against advanced cancer with a 4-h i.v. administration for 3 weeks and a 1-week break during week 4 [37]. On the basis of these results, a number of phase II clinical trials of romidepsin were performed. Seventy-one CTCL patients were entered into a phase II multi-institutional clinical trial and received a 4-h i.v. infusion of romidepsin at 14 mg/m$^2$ on days 1, 8, and 15 of a 28-day cycle; complete responses were observed in four patients and partial responses in 20. The overall response rate was 34%, with a median duration of response of 13.7 months [33]. Another international, pivotal, single-arm, open-label phase II study was conducted on 96 patients with refractory CTCL, in whom romidepsin was administered as a 4-h i.v. infusion at 14 mg/m$^2$ on days 1, 8, and 15 of each 28-day cycle for up to six cycles. Of the patients, 27 had a partial response and six had a complete response, yielding an overall response rate of 34% and a median duration of response of 15 months [34]. Next, 47 patients with PTCL were enrolled in this trial and received romidepsin as a 4-h i.v. infusion on days 1, 8, and 15 of a 28-day cycle with a straight dose of 14 mg/m$^2$. Complete response was seen in eight patients and partial response in nine, whereas the overall response rate was 38% and the median duration of overall response was 8.9 months [38]. On the basis of these encouraging phase II results, romidepsin was approved by the FDA in 2009 for the treatment of patients with relapsed and/or refractory CTCL [39]. Unfortunately, romidepsin has not shown promising activity against most solid tumors thus far [40–42].

Interestingly, a number of studies have demonstrated that romidepsin synergistically increases the antitumor activity of other agents. The proteasome inhibitor bortezomib (Velcade), approved by the FDA in 2008 for the treatment of patients with multiple myeloma, showed synergistic activity with romidepsin toward oxidative injury and cell apoptosis [43]. A phase I study of romidepsin and bortezomib was conducted on 25 patients with relapsed or refractory multiple myeloma. Bortezomib (1.3 mg/m$^2$), dexamethasone (20 mg/m$^2$), and romidepsin (10 mg/m$^2$) were injected i.v. over a 4-h period every 28 days. Two patients showed complete remission, 13 showed partial response, and three showed minor responses; the overall response rate was 72%, with a median time to progression of 7.2 months [44]. On the basis of these results, phase I/II clinical trials are ongoing.

**Table 2 Summary of romidepsin**

| |
|---|
| Indications |
| Approved by the FDA in 2009 for the treatment of CTCL |
| Mode of action |
| HDAC inhibitor |
| Recommended initial dosage |
| 14 mg/m$^2$ as an intravenous infusion over 4 h on days 1, 8, and 15 of a 28-day cycle |
| Pivotal trials |
| Phase II multi-institutional trial [33] |
| 71 patients with CTCL |
| Overall response rate 34% |
| Median duration of response 13.7 months |
| Phase II multicenter international pivotal trial [34] |
| 96 patients with CTCL |
| Overall response rate 34% |
| Median duration of response 15 months |

CTCL, cutaneous T-cell lymphoma; HDAC, histone deacetylase.

### Pharmacokinetics and toxicity

Pharmacokinetic data on romidepsin were documented in a number of phase I and II trials [45]. The phase I dose-escalation study evaluated pharmacokinetics in patients with refractory neoplasms. Thirty-seven patients received romidepsin as a 4-h i.v. infusion on days 1 and 5 of a 21-days treatment cycle, and the MTD was defined at 17.8 mg/m$^2$. The mean values of volume of distribution and clearance (CL) at the 17.8 mg/m$^2$ dose were 8.6 l/m$^2$ and 11.6 l/h/m$^2$, respectively. The distribution half-life ($t_{1/2}$) and elimination $t_{1/2}$ were 0.42 and 8.1 h, respectively. The mean volume of maximum plasma

concentration ($C_{max}$) was 473 ng/ml. Toxicity at the MTD included anorexia, fatigue, fever, nausea, and vomiting [36].

Two phase II studies assessed pharmacokinetics in 71 patients with CTCL and 36 patients with PTCL. Romidepsin was administered as a 4-h i.v. infusion at 14 mg/m$^2$ on days 1, 8, and 15 of a 28-day cycle. The half-life and $C_{max}$ at a dose of 14 mg/m$^2$ were 2.95–3.04 h and 362–427 ng/ml, respectively. The area under the curve extrapolated to infinity ($AUC_{inf}$) was 1457–1899 h ng/ml. The volume of distribution during the terminal phase was 19–41 l/m$^2$. The systemic clearance was 7.37–9.61 l/h/m$^2$ [33,38]. Nonhematologic side effects included fatigue, nausea, vomiting, and anorexia. Hematologic adverse events included leukopenia, granulocytopenia, lymphopenia, thrombocytopenia, and anemia [33,38]. The overall response rate for CTCL was 34%, with a median duration of response of 13.7 months [38], whereas the overall response rate for PTCL was 38%, with a median duration of response of 8.9 months in phase II clinical trials [33].

### Mode of action

Romidepsin belongs to a class of drugs known as inhibitors of HDAC [46]. This agent is known to possess activity restricted to class 1 HDAC. However, it was recently reported that whereas a low dose of romidepsin inhibited activity of class I HDAC, high doses of romidepsin (>2 nmol/l) possessed activity against both class 1 and 2 HDACs [47]. Similar to other HDAC inhibitors, romidepsin regulates the expression of several genes linked to the cell cycle and apoptosis. A comprehensive analysis of the gene expression profile regulated by romidepsin with regard to human esophageal squamous cancer cell lines showed that the drug upregulated 11 genes and downregulated four. One of the highly upregulated genes is the cyclin dependent kinase inhibitor p21 [48]. p21 is a key regulator that promotes cell cycle arrest by inducing growth arrest in the G2 phase of the cell cycle [49]. Romidepsin was found to upregulate p21 expression in a p53-dependent or p53-independent manner and to inhibit cyclin dependent kinase activity, leading to the progression of cell cycle arrest [50–52].

Romidepsin was also shown to induce apoptosis by depletion of proto-oncogene epidermal growth factor receptor (EGFR). This depletion leads to the inhibition of several EGFR-related pathways including Src, extracellular signal-regulated kinase (ERK) 1/2, and phosphatidylinositol-3 kinase (PI3K)/AKT, leading to downregulation of the antiapoptotic proteins myeloid cell leukemia-1 (Mcl-1), B-cell lymphoma-2 (bcl-2), and B-cell lymphoma-extra large (Bcl-xL), and upregulation of the proapoptotic protein bcl-associated x (Bax) [53,54]. However, romidepsin upregulates the proapoptotic bcl-2 homology 3 (BH3)-only proteins bcl-2 interacting mediator of cell death (Bim) and bcl-2 modifying factor (Bmf) in a broad range of cancer cells, leading to a distinct loss of mitochondrial membrane potential ($\Delta\Psi m$) [55,56].

## Didemnin B/Aplidine

### Didemnin B

Didemnin B is a cyclic depsipeptide that was initially isolated from a Caribbean tunicate (*Tridemnum* genus), and it belongs to the 'didemnidae' family, with the molecular formula $C_{57}H_{89}N_7O_{15}$ (Fig. 1c). Preclinical studies have shown that it effectively inhibits the growth of L1210 leukemia cells, with an $IC_{50}$ value of 0.001 μg/ml *in vitro*, and increases the survival of P388 leukemia-bearing mice [10]. Didemnin B has been tested in phase I and II clinical trials against a number of human tumors, including epithelial ovarian cancer [57], renal cell carcinoma [58], breast cancer [59], melanoma [60], small-cell lung cancer [61], myeloma [62], prostate cancer [63], and lymphoma [64]. However, because of severe secondary effects, it failed in those trials, and clinical studies were stopped in order to identify an analog of didemnin B harboring more effective antitumor activity. One promising analog of didemnin B is aplidine. Aplidine has a pyruvyl group instead of a lactyl group in the linear peptide moiety of didemnin B (Fig. 1c) [8].

### Aplidine

#### *In-vitro and animal studies*

Aplidine (also plitidepsin, dehydrodidemnin B, or Aplidin) was first isolated from the Mediterranean tunicate *Aplidium albicans*, although it is currently being produced by chemical synthesis. Aplidine is a cyclic depsipeptide with the molecular formula $C_{57}H_{87}N_7O_{15}$ (MW: 1110; Fig. 1c; Table 3) and belongs to the 'didemnidae' family, similar to didemnin B [8]. This agent possesses a pyruvoyl-proline residue instead of the lactyl-proline residue seen in didemnin B. In addition, aplidine is more sensitive than didemnin B in terms of its antiproliferative activity against Ehrlich carcinoma cells *in vitro* [8].

Aplidine has been shown to have potent antitumor activity against several freshly explanted human tumors, including melanoma, lymphoma, and breast, ovarian,

**Table 3 Summary of aplidine**

| |
|---|
| Indications |
| Currently, aplidine is in a phase III clinical trial for treatment of refractory/relapsed multiple myeloma in combination with dexamethasone |
| Mode of action |
| Interferes with DNA and protein synthesis and induces cell cycle arrest |
| Recommended initial dosage |
| 5 mg/m$^2$ as a 3-h intravenous infusion every 2 weeks, with an addition of 20 mg/day of oral dexamethasone on days 1–4 of every cycle |
| Trials |
| Phase II prospective multicenter open-label single-arm trial [65] |
| 47 patients with refractory/relapsed multiple myeloma |
| Overall response rate 22% |
| Duration of response 2.8–5.7 months |

lung, colorectal, and gastric carcinomas, as determined by a soft agar cloning assay *in vitro*, with an $IC_{50}$ value of 0.001 μmol/l [66]. This agent also possesses in-vitro cytotoxic activity against several human tumor cell lines, including murine lymphoma (P388 cells), melanoma (MEL-28 cells), and lung (A549 cells) and colon (HT-29 cells) carcinomas, with $IC_{50}$ values of 0.2, 0.5, 0.2, and 0.5 ng/ml, respectively [67,68]. However, human normal hematopoietic progenitors (bone marrow and cord blood) were found to be resistant to aplidine, with $IC_{50}$ values ranging from 150 to 2250 nmol/l [69]. In a mouse xenograft model, this agent exhibited potent antitumor activity against melanoma (B16 cells), leukemia (P388 cells), Lewis lung carcinoma [67], anaplastic thyroid cancer [70], and multiple myeloma (5T33MM cells) [71].

### *Clinical trials*

As aplidine showed in-vitro and in-vivo antitumor activities in a mouse xenograft model, it was entered into a phase I clinical trial to examine its effects on solid tumors. Thirty-seven patients with refractory solid tumors received a 1-h i.v. infusion daily for 5 days every 3 weeks [72], whereas another 48 received a 1-h weekly i.v. infusion for three continuous weeks of a 4-week treatment cycle [73]. On the basis of the findings of the study, it was concluded that the recommended dose for a phase II clinical trial should be 1.2 mg/m$^2$/day or 3.2 mg/m$^2$/week. Sixty-six patients with advanced malignancies (such as colon cancer and non-Hodgkin's lymphoma) were entered into another phase I clinical trial. They received aplidine as a 24-h i.v. infusion every 2 weeks. The recommended dose for phase II clinical trials was shown to be between 5 and 7 mg/m$^2$ with and without L-carnitine to prevent aplidine-induced side effects on muscle tissues [74]. Another group performed a phase I clinical trial of children (between 2 and 17 years old). Thirty-eight patients were enrolled in this trial to investigate the effects of aplidine on advanced tumors, and they were treated with a 3-h i.v. infusion every 2 weeks. The recommended dose for phase II trials was found to be 5 mg/m$^2$ [75].

On the basis of the results of this study, aplidine was entered into phase II clinical trials, and several trials were performed against advanced malignant melanoma [76], advanced medullary thyroid carcinoma [77], advanced renal cell carcinoma [78], and small-cell lung cancer [79]. However, aplidine showed only limited antitumor activity in those studies. Most recently, a multicenter, open-label, single-arm phase II clinical trial of aplidine was performed in 47 patients with relapsed/refractory multiple myeloma. They were given aplidine at 5 mg/m$^2$ as a 3-h i.v. infusion every 2 weeks with or without 20 mg daily of oral dexamethasone (days 1–4 of every cycle). The overall response rate was 22% with and 13% without dexamethasone, and the duration of response was 1.8–6.2 and 2.8–5.7 months with and without dexamethasone, respectively [65]. Furthermore, 67 patients with relapsed/refractory non-Hodgkin's lymphoma were entered into another phase II trial of aplidine and administered a dose of 3.2 mg/m$^2$ aplidine as a 1-h i.v. infusion weekly on days 1, 8, and 15 every 4 weeks. Six patients had a response, with an overall response rate of 20.7%, including two complete responses and four partial responses [80]. These results suggest that aplidine has limited but reproducible antitumor activity against multiple myeloma or non-Hodgkin's lymphoma. Currently, aplidine is in a phase III clinical trial for relapsed/refractory multiple myeloma in combination with dexamethasone.

### *Pharmacokinetic and toxicity data*

Several studies of phase I and II clinical trials have described the pharmacokinetics of aplidine. Forty-nine patients with solid tumors were enrolled in this dose-escalating phase I trial and received 1-h weekly i.v. infusion for three consecutive weeks during a 4-week treatment cycle. The mean values of $C_{max}$ and $AUC_{inf}$ at an MTD of 3.2 mg/m$^2$ are 46.8 ng/ml and 209.6 ng h/ml, respectively. The mean values of $t_{1/2}$, CL, and volume of distribution in steady state ($V_{ss}$) are 16.8 h, 34 l/h, and 525 l, respectively [73]. Sixty-seven patients with non-Hodgkin's lymphoma in a phase II trial received a dose of 3.2 mg/m$^2$ aplidine as a 1-h i.v. infusion weekly on days 1, 8, and 15 every 4 weeks. The mean $t_{1/2}$, CL, and $V_{ss}$ were 36.5 h, 7.45 l/h, and 355 l, respectively [80]. The most common nonhematologic adverse events were nausea, fatigue, vomiting, anorexia, and myalgia. Some patients had grade 3/4 aplidine-related adverse events [73,80]. Hematologic abnormalities included grade 3 anemia and grade 3/4 lymphopenia, leukopenia, neutropenia, and thrombocytopenia. The most common biochemical abnormalities were grade 3 aspartate transaminase/alanine transaminase (ALT/AST) increase and grade 3/4 creatine phosphokinase increase [80].

### *Mode of action*

The primary mode of action of aplidine has not been fully explained, although several studies have been published. The agent induces early oxidative stress, leading to depletion of glutathione, which then activates EGFR, Src, c-Jun $NH_2$-terminal kinase (JNK), and p38 mitogen-activated protein kinase. Aplidine induces tumor cell apoptosis by inducing mitochondrial dysfunction, cytochrome *c* release, and caspase-3 or caspase-9 activation in several cell lines, including human breast MDA-MB-31, cervical HeLa, renal cancer, and multiple myeloma 5T33MM cell lines [71,81,82]. Notably, JNK activation through Rac1 GTPase activation and MKP-1 phosphatase downregulation is the primary target related to its sensitivity, as JNK1-deficient and JNK2-deficient mouse embryo fibroblasts were found to be much less sensitive to aplidine [83,84]. In addition to its effect on cell apoptosis, aplidine possesses antiproliferative effects [85–87]. Studies have shown that it causes blockage

of the cell cycle by inducing $G_1$ arrest and $G_2$/M blockade in acute lymphoblastic leukemia and human Molt-4 leukemia [85–87]. Interestingly, a lower concentration (< 45 nmol/l) of aplidine inhibited the cell cycle in SK-MEL-28 and UACC-257 melanoma cells by inducing $G_1$ arrest and $G_2$/M blockade, whereas a higher concentration induced cell apoptosis in melanoma cells [88]. Another mechanism of action for its antitumor activity is its antiangiogenic function. Aplidine inhibited the secretion of the angiogenic factor vascular endothelial growth factor (VEGF) in human leukemia cells (MOLT-4), leading to blockage of the VEGF/VEGF receptor-1 autocrine loop [89]. Moreover, it inhibited spontaneous angiogenesis induced by exogenous VEGF and FGF-2, assessed by a cell invasion assay *in vitro* and a chick embryo allantoic membrane assay *in vivo* [9].

## Kahalalide F/PM02734

### Kahalalide F

#### *In-vitro and animal studies*

Kahalalide F, a cyclic depsipeptide with the molecular formula $C_{75}H_{124}N_{14}O_{16}$ (MW: 1477) (Fig. 1d), was first isolated from the Hawaiian sacoglossan mollusk *Elysia rufescens*, which feeds on a green alga *Bryopsis* spp. [9]. Kahalalide F belongs to the kahalalide family of compounds, whose members include kahalalides A–H, J, K, and O–Q [90]. Kahalalide F is the largest compound in the family and possesses potent antitumor activities *in vitro* against several cell lines, including prostate (PC3, DU145, LNCaP), breast (SKBR-3, BT474, MCF-7, MDA-MB-231), and colon (LoVo) cancer cell lines, with $IC_{50}$ values of 0.07, 0.18, 0.26, 0.23, 0.28, 0.39, and 0.16 μmol/l, respectively. However, kahalalide F is much less sensitive to human nontumor cell lines, such as the mammary epithelial (MCF10A), endothelial umbilical cord (HUVEC), endothelial dermal microvascular (HMEC-1), and human diploid fibroblast (IMR90) cell lines, with $IC_{50}$ values of 2.44, 1.62, 1.88, and 3.13 μmol/l, respectively [82]. Another study investigated a number of cell lines, including breast (HS578T), colon (HCC-2998, HCT-15, HT-29, KM12), non-small-cell lung (NSCLC; A549, NCI-H322M), central nervous system (SNB-75), and ovarian (SK-OV-3) cancer cell lines, and reported $IC_{50}$ values for those ranging from 0.162 to 0.288 μmol/l [91]. In addition, kahalalide F showed antitumor activity against human breast cancer, NSCLC, and colon cancer cell lines *in vivo* in an animal xenograft model [92]. Structurally, L-Val(3) and D-Val(4) of kahalalide F are crucial for its antitumor activities [93].

#### *Clinical trials*

On the basis of these observations, kahalalide F has been selected for at least two phase I clinical trials. Thirty-two patients with advanced androgen refractory prostate cancer received kahalalide F as a 1-h i.v. infusion for five consecutive days every 3 weeks at nine different doses (20–930 μg/m$^2$/day). The recommended dose for a phase II trial was 560 μg/m$^2$/day as a 1-h i.v. infusion daily for 5 days in cycles of 3 weeks. Importantly, one patient who received the agent at 80 μg/m$^2$/day showed a significant decrease (at least 50%) in prostate specific antigen levels over 4 weeks [94]. In another trial, 38 patients with advanced solid tumors received kahalalide F as an i.v. infusion once weekly at several different doses between 266 and 1200 mg/m$^2$. One patient with a metastatic malignant melanoma who received kahalalide F for up to 80 cycles of weekly administration at 600 μg/m$^2$ had an unconfirmed partial response. The recommended dose for a phase II trial from those findings was 650 μg/m$^2$ administered as a 1-h i.v. infusion once weekly [95]. A total of 106 patients with advanced solid tumors entered another phase I trial and received a 3-h or 24-h weekly i. v. infusion at several different doses. The MTD for the 3-h and 24-h weekly schedules was 1200 and 6650 μg/m$^2$, respectively [96]. In a phase II clinical trial, 24 patients with advanced malignant melanoma received 650 μg/m$^2$ of kahalalide F as a 1-h weekly i.v. infusion. However, because of the lack of an objective response, this trial was closed [97].

#### *Pharmacokinetic and toxicity data*

Patients with advanced solid tumors received kahalalide F at a recommended dose of 650 μg/m$^2$ in a phase I clinical trial. The mean pharmacokinetic parameters $V_{ss}$ and $t_{1/2}$ after the first infusion of kahalalide F were 5.6 l and 0.5 h, respectively. The $C_{max}$, $AUC_{inf}$, and CL were 124.5 ng/ml, 170 ng h/ml, and 7.4 l/h, respectively [95]. In another trial, patients with advanced androgen refractory prostate cancer received kahalalide F at a recommended dose of 560 μg/m$^2$. The median pharmacokinetic variables $V_{ss}$ and $t_{1/2}$ were 7.1 l and 0.5 h, respectively. The $C_{max}$, $AUC_{inf}$, and CL were 86.3 ng/ml, 105.1 ng h/ml, and 11.1 l/h, respectively [94]. The most common non-hematologic kahalalide F-related adverse events were grade 1/2 pruritus, paresthesia, and fatigue. The most frequent biochemical abnormalities were grade 3/4 AST/ALT and γ-glutamyl transferase increases [97].

#### *Mode of action*

A number of studies on the mechanism of antitumor activity of kahalalide F have also been performed. Kahalalide F-treated COS-1 cells became significantly swollen and developed large vacuoles, suggesting that the agent may change the lysosomal membrane [98]. Moreover, kahalalide F primarily induced cell death through oncosis in several types of tumor cells, as it induced disruption of mitochondrial membrane potential ($\Delta\Psi m$) and altered lysosomal membrane permeability [82]. The oncosis induced by kahalalide F seemed to be dependent on receptor tyrosine kinase ErbB3 protein levels, because kahalalide F-sensitive tumor cells were shown to be positively correlated with ErbB3 protein expression. It also decreased the expression of Erb3B

protein, leading to inhibition of the PI3K-Akt downstream signaling cascade [99].

### PM02734

#### *In-vitro studies*

Unfortunately, because of the unavailability of a natural source of kahalalide F, its development as a therapeutic agent was halted. PM02734 (also elisidepsin and Irvalec) is a novel compound related to kahalalide F and produced by solid-phase synthesis [100]. PM02734 is a cyclic depsipeptide with the molecular formula $C_{75}H_{125}F_3N_{14}O_{18}$ (MW: 1591; Fig. 1e; Table 4). It possesses in-vitro antitumor activities against a number of human cell lines, including prostate, pancreatic, ovarian, lung, liver, leukemia, kidney, stomach, colon, and breast cancer cell lines, with a mean $IC_{50}$ of 2.3 μmol/l. Whereas prostate cancer cell lines (PC3, 22RV1) were shown to be sensitive to PM02734, pancreatic cancer cell lines (PANC-1, MiaPaCa-2) were relatively resistant [102]. *In vivo*, PM02734 administered in combination with the EGFR inhibitor erlotinib prolonged the lifespan of mice implanted with NSCLC A549 cells [103].

#### *Clinical trials, pharmacokinetics, and toxicity data*

Forty-two patients with advanced solid tumors were enrolled in a phase I clinical trial and administered a 24-h i.v. infusion every 3 weeks at doses ranging from 0.5 to 6.8 mg/m$^2$. Of those patients, one with metastatic esophageal adenocarcinoma achieved a complete response. The recommended dose for a phase II study was 5.5 mg/m$^2$. The $C_{max}$ and AUC were 32.3 ng/ml and 772 h ng/ml, respectively. The $t_{1/2}$, CL, and $V_{ss}$ were 100 h, 15.4 l/h, and 502 l, respectively. The most frequent nonhematologic adverse events included grade 1/2 fatigue, headache, rash, vomiting, and alopecia. The most common hematologic and biochemical abnormalities were anemia, leukopenia, lymphopenia, and ALT/AST increase [101].

**Table 4 Summary of PM02734**

| |
|---|
| Indications |
| Currently, PM02734 is in phase I/II clinical trials for treatment of advanced solid tumors |
| Mode of action |
| Induces necrotic cell death through severe plasma membrane damage |
| In-vitro activity |
| Possess antiproliferative activity against a number of tumor cell lines, including breast, colon, lung, neuroblastoma, prostate, sarcoma, and thyroid cancer cell lines, with $IC_{50}$ values ranging from $10^{-6}$ to $10^{-8}$ mol/l |
| Recommended initial dosage |
| 10 mg/m$^2$ as a 24-h intravenous infusion every 3 weeks |
| Trials |
| Phase I trial against advanced solid tumors [101] |
| 42 patients with advanced solid tumors |
| One patient with esophageal adenocarcinoma achieved complete response |

#### *Mode of action*

Similar to kahalalide F, PM02734 induces necrotic cell death by severe plasma membrane damage [102]. In contrast, it has also been shown that PM02734-induced antitumor activity is associated with autophagy. This agent inhibited the Akt/mTOR signaling pathway and activated death-associated protein kinase in a mouse xenograft model [104]. Whereas kahalalide F decreased ErbB3 protein levels, PM02734 also induced the distribution of ErbB3 from the plasma membrane to the intracellular space or the nucleus [105]. The antitumor activity of PM02734 appeared to depend on the hydroxylase FA2H. In another study, overexpression of FA2H was found to increase the sensitivity of PM02734 against human colon cancer HCT116 cells, whereas knockdown of FA2H was found to relatively increase the resistance of those cells [106].

## Cryptophycin 1/LY355703

### Cryptophycin 1

#### *In-vitro and animal studies*

Cryptophycin 1 (also named cryptophycin A) is a cyclic depsipeptide that was originally isolated from cyanobacteria of the genus *Nostoc*, with a molecular formula of $C_{35}H_{43}ClN_2O_8$ (MW: 655; Fig. 1f) [17], and is currently produced by chemical synthesis [107]. *In vitro*, cryptophycin 1 inhibited the proliferation of the L1210 murine leukemia cell line, the human nasopharyngeal carcinoma cell line KB, and the human colorectal adenocarcinoma cell line LoVo, with $IC_{50}$ values of 4 pmol/l, and 3 and 5 pg/ml, respectively [108,109]. In a mouse xenograft model, cryptophycin 1 injected i.v. was found to exhibit antitumor activity against a number of cell lines, including colon adenocarcinoma, mammary adenocarcinoma M16, MX-1 human breast carcinoma, and pancreatic ductal adenocarcinoma cell lines [109,110]. However, when administered by intraperitoneal or subcutaneous injection, or by oral gavage, cryptophycin 1 was found to be inactive or only modestly active [110]. Because of these in-vivo results, efforts were undertaken to develop a number of analogs in the cryptophycin series. Cryptophycin 8 and cryptophycin 55 are chlorohydrin analogs of cryptophycin 1 and LY355703 (also named cryptophycin 52) that have markedly improved antitumor activity. Unfortunately, however, cryptophycin 8 and cryptophycin 55 were not sufficiently stable in solution to be a clinical candidate [111]. Cryptophycin 24 (also named arenastatin A) is another analog that possesses potent antitumor activity against epidermal carcinoma KB cells [112]. However, cryptophycin 24 showed only marginal in-vivo antitumor activity, making it ineligible as a clinical candidate [113].

### LY355703

#### *In-vitro studies*

One of the most promising candidates is LY355703 (also named cryptophycin 52), with a molecular formula of

$C_{36}H_{45}ClN_2O_8$ (MW: 669; Fig. 1g; Table 5). In-vitro studies have shown its antiproliferative activity against the human leukemia cell line THP-1, the human lung cancer cell line H-125, and the human leukemia cell line CCRF-CEM, with $IC_{50}$ values of 0.1 ng/ml, 20 μg/ml, and 22 pmol/l, respectively [18,114]. Another study also reported antiproliferative effects against a number of cell lines, including leukemia (U937, CCRF-CEM, HL60), colon carcinoma (HT-29, GC3, Caco-2), mammary carcinoma (MCF-7, MDA-MB-231), and cervical carcinoma (HeLa) cell lines, with $IC_{50}$ values in the range of 0.013–0.232 nmol/l [115].

### *Clinical trials, pharmacokinetics, and toxicity data*

In a phase I clinical trial study, 25 patients with NSCLC received LY355703 as a 2-h i.v. infusion on days 1 and 8 every 3 weeks, and the recommended dose for phase II evaluation was shown to be 1.5 mg/m$^2$. The mean values of the pharmacokinetic parameters CL and volume of distribution were 51.5 l/h and 131 l/m$^2$, respectively [116]. In another phase I study, 35 patients with solid tumors received LY355703 as a 2-h i.v. infusion on days 1, 8, and 15 every 4 weeks, and the recommended dose for a phase II trial was 1.48 mg/m$^2$. The mean values of the pharmacokinetic parameter CL and volume of distribution were 54.9 l/h and 139 l/m$^2$, respectively [117].

On the basis of those phase I studies, phase II clinical studies were conducted. Twenty-five patients with NSCLC [118] and 24 with platinum-resistant ovarian cancer [119] were enrolled and received 1.5 mg/m$^2$ LY355703 as a 2-h i.v. infusion on days 1 and 8 every 3 weeks. Unfortunately, the results of those studies were disappointing. It seems that LY355703 has limited activity against NSCLC and platinum-resistant ovarian cancer, and no other clinical trials have been conducted to date. The most frequent nonhematologic adverse events included neuropathy, constipation, fatigue, and nausea [118,119].

**Table 5 Summary of LY355703**

| |
|---|
| Indications |
| Phase I/II trials were performed. However, LY355703 has shown no antitumor activity clinically thus far |
| Mode of action |
| Interacts with microtubules, leading to the induction of cell cycle arrest at the $G_2$/M phase and to cell apoptosis |
| In-vitro activity |
| Possess antiproliferative effects against leukemia, lung cancer, and colon, mammary, and cervical carcinoma |
| Recommended initial dosage |
| 1.5 mg/m$^2$ as a 2-h intravenous infusion on days 1 and 8 every 3 weeks |
| Trials |
| Phase II trials |
| 25 patients with non-small-cell lung cancer |
| 24 patients with platinum-resistant ovarian cancer |
| LY355703 has shown no antitumor activity |

### *Mode of action*

Cryptophycins interact with microtubules at the vinca alkaloid-binding domain and are more potent than other vinca alkaloids [120,121]; they also potently induce bcl-2 phosphorylation, leading to cell cycle arrest and apoptosis [122]. LY355703 was found to inhibit the proliferation of HeLa cells at mitosis, with an $IC_{50}$ value of 11 pmol/l. This agent exerts a cytotoxic effect by depolymerizing spindle microtubules and disrupting chromosome organization at high concentrations (30–300 pmol/l), and it also inhibits HeLa cell proliferation without depolymerization of microtubules at low concentrations (3–30 pmol/l). LY355703 at low concentrations also appears to reduce the rate and extent of growth and shorting of the microtubule ends, which is termed as dynamic instability. On using [$^3$H]LY355703, the binding of five to six molecules of LY355703 to microtubules was sufficient to reduce those dynamics by 50% [123]. Similar to other cryptophycins, LY355703 induces $G_2$/M cell cycle arrest, phosphorylation of bcl-2, and activation of caspase-3, leading to apoptosis [124–126].

## Conclusion

In this review, we have summarized seven cyclic depsipeptides that have been found to possess antitumor activity in clinical trials. One compound, romidepsin, was approved by the FDA for the treatment of relapsed/refractory CTCL in 2009 (Table 2). Aplidine is currently under phase III clinical trial for the treatment of relapsed/refractory multiple myeloma in combination with dexamethasone (Table 3). PM02734 is currently under phase I/II clinical trial for the treatment of advanced solid tumors (Table 4). Didemnin B, kahalalide F, cryptophycin 1, and LY355703 are under phase I/II clinical trials. However, because of the lack of promising

**Table 6 List of cyclic depsipeptides that have entered phase II clinical trials and beyond**

| Compound | Source organism | Molecular target | Current status | Oncological use |
|---|---|---|---|---|
| Romidepsin | Bacteria (*Chromobacterium violaceum*) | HDAC | Available for clinical use | CTCL/PTCL |
| Aplidine | Marine animal (*Aplidium albicans*) | Oxidative stress | Phase III (NCT01102426) | Multiple myeloma |
| PM02734 | Synthesis (kahalalide F derivative) | ErbB pathway | Phase II | Non-small-cell lung, esophageal, and gastric cancers |
| Didemnin B | Marine animal (*Tridemnum* genus) | eEF1A | Phase II (discontinued) | Solid tumors |
| Kahalalide F | Marine animal (*Elysia rufescens*) | ErbB pathway | Phase II (discontinued) | Psoriasis |
| Cryptophycin 1 | Bacteria (genus *Nostoc*) | Tubulin | Phase II (discontinued) | Solid tumors |
| LY355703 | Bacteria (genus *Nostoc*) | Tubulin | Phase II (discontinued) | Solid tumors |

CTCL, cutaneous T-cell lymphoma; HDAC, histone deacetylase; PTCL, peripheral T-cell lymphoma.

responses or because of severe secondary effects, these trials have currently been closed (Tables 5 and 6).

Natural products have been the major source of antitumor drugs, and ~80% of the chemotherapeutic agents are classified as natural products or mimicked natural products [127], propelling continued efforts to discover and isolate new active components from various biological sources. A large number of cyclic depsipeptides have been identified from fungi, plants, and marine organisms. The unique structures, diversified cellular targets, and potent biological activities of these compounds make them attractive candidates as novel therapeutic agents. With the success of romidepsin and the lessons learnt from other clinically failed compounds, it is conceivable that more natural or semisynthetic cyclic depsipeptides will be evaluated and hopefully succeed in preclinical and clinical studies.

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### Conflicts of interest

There are no conflicts of interest.

## References

1 Gaumann E, Roth S, Ettlinger L, Plattner P, Nager U. Ionophore antibiotics produced by the fungus *Fusarium orthoceras* var. ennitum and other. *Experientia* 1947; **3**:202.
2 Hwang Y, Rowley D, Rhodes D, Gertsch J, Fenical W, Bushman F. Mechanism of inhibition of a poxvirus topoisomerase by the marine natural product sansalvamide A. *Mol Pharmacol* 1999; **55**:1049–1053.
3 Isaka M, Palasarn S, Lapanun S, Sriklung K. Paecilodepsipeptide A, an antimalarial and antitumor cyclohexadepsipeptide from the insect pathogenic fungus *Paecilomyces cinnamomeus* BCC 9616. *J Nat Prod* 2007; **70**:675–678.
4 Tonshin AA, Teplova VV, Andersson MA, Salkinoja-Salonen MS. The *Fusarium* mycotoxins enniatins and beauvericin cause mitochondrial dysfunction by affecting the mitochondrial volume regulation, oxidative phosphorylation and ion homeostasis. *Toxicology* 2010; **276**:49–57.
5 Kobayashi J, Suzuki H, Shimbo K, Takeya K, Morita H. Celogentins A–C, new antimitotic bicyclic peptides from the seeds of *Celosia argentea*. *J Org Chem* 2001; **66**:6626–6633.
6 Ma B, Banerjee B, Litvinov DN, He L, Castle SL. Total synthesis of the antimitotic bicyclic peptide celogentin C. *J Am Chem Soc* 2010; **132**:1159–1171.
7 Matsumoto T, Shishido A, Morita H, Itokawa H, Takeya K. Cyclolinopeptides F–I, cyclic peptides from linseed. *Phytochemistry* 2001; **57**:251–260.
8 Urdiales JL, Morata P, Núñez De Castro I, Sánchez-Jiménez F. Antiproliferative effect of dehydrodidemnin B (DDB), a depsipeptide isolated from Mediterranean tunicates. *Cancer Lett* 1996; **102**:31–37.
9 Hamann MT, Scheuer PJ. Kahalalide F: a bioactive depsipeptide from the sacoglossan mollusk *Elysia rufescens* and the green alga *Bryopsis* sp. *J Am Chem Soc* 1993; **115**:5825–5826.
10 Rinehart KL, Gloer JB, Hughes RG, Renis HE, McGovren JP, Swynenberg EB, *et al.* Didemnins: antiviral and antitumor depsipeptides from a Caribbean tunicate. *Science* 1981; **212**:933–935.
11 Tran TD, Pham NB, Fechner G, Zencak D, Vu HT, Hooper JN, *et al.* Cytotoxic cyclic depsipeptides from the Australian marine sponge *Neamphius huxleyi*. *J Nat Prod* 2012; **75**:2200–2208.
12 Azzi A, Azzone GF. Potassium-transport-linked swelling induced by valinomycin in liver mitochondria. *Biochem J* 1965; **96**:1C–2C.
13 Lam KS, Gustavson DR, Hesler GA, Dabrah TT, Matson JA, Berry RL, *et al.* Korkormicins, novel depsipeptide antitumor antibiotics from Micromonospora sp C39500: fermentation, precursor directed biosynthesis and biological activities. *J Ind Microbiol* 1995; **15**:60–65.
14 Huang CH, Crooke ST. Effects of structural modifications of antitumor antibiotics (luzopeptins) on the interactions with deoxyribonucleic acid. *Cancer Res* 1985; **45**:3768–3773.
15 Zolova OE, Mady AS, Garneau-Tsodikova S. Recent developments in bisintercalator natural products. *Biopolymers* 2010; **93**:777–790.
16 Ueda H, Nakajima H, Hori Y, Fujita T, Nishimura M, Goto T, *et al.* FR901228, a novel antitumor bicyclic depsipeptide produced by *Chromobacterium violaceum* No. 968. I. Taxonomy, fermentation, isolation, physico-chemical and biological properties, and antitumor activity. *J Antibiot (Tokyo)* 1994; **47**:301–310.
17 Schwartz RE, Hirsch CF, Sesin DF, Flor JE, Chartrain M, Fromtling RE, *et al.* Pharmaceuticals from cultured algae. *J Ind Microbiol* 1990; **5**:113–124.
18 Chen BD, Nakeff A, Valeriote F. Cellular uptake of a novel cytotoxic agent, cryptophycin-52, by human THP-1 leukemia cells and H-125 lung tumor cells. *Int J Cancer* 1998; **77**:869–873.
19 Mackedonski VV. Effect of echinomycin and olivomycin on RNA synthesis in ehrlich ascites tumour cells. *FEBS Lett* 1969; **5**:73–76.
20 Ballard CE, Yu H, Wang B. Recent developments in depsipeptide research. *Curr Med Chem* 2002; **9**:471–498.
21 Lemmens-Gruber R, Kamyar MR, Dornetshuber R. Cyclodepsipeptides – potential drugs and lead compounds in the drug development process. *Curr Med Chem* 2009; **16**:1122–1137.
22 Klein B, Wörndl K, Lütz-Meindl U, Kerschbaum HH. Perturbation of intracellular K(+) homeostasis with valinomycin promotes cell death by mitochondrial swelling and autophagic processes. *Apoptosis* 2011; **16**:1101–1117.
23 Iacobazzi RM, Annese C, Azzariti A, D'Accolti L, Franco M, Fusco C, *et al.* Antitumor potential of conjugable valinomycins bearing hydroxyl sites: in vitro studies. *ACS Med Chem Lett* 2013; **4**:1189–1192.
24 Kamyar M, Rawnduzi P, Studenik CR, Kouri K, Lemmens-Gruber R. Investigation of the electrophysiological properties of enniatins. *Arch Biochem Biophys* 2004; **429**:215–223.
25 Sy-Cordero AA, Pearce CJ, Oberlies NH. Revisiting the enniatins: a review of their isolation, biosynthesis, structure determination and biological activities. *J Antibiot (Tokyo)* 2012; **65**:541–549.
26 Liu S, Gu W, Lo D, Ujiki M, Adrian TE, Soff GA, *et al.* *N*-methylsansalvamide a peptide analogues. Potent new antitumor agents. *J Med Chem* 2005; **48**:3630–3638.
27 Zhang G, Liu S, Liu Y, Wang F, Ren J, Gu J, *et al.* A novel cyclic pentapeptide, H-10, inhibits B16 cancer cell growth and induces cell apoptosis. *Oncol Lett* 2014; **8**:248–252.
28 Kitagaki J, Yang Y. DNA intercalator korkormicin A preferentially kills tumor cells expressing wild type p53. *Biochem Biophys Res Commun* 2011; **414**:186–191.
29 Dawson S, Malkinson JP, Paumier D, Searcey M. Bisintercalator natural products with potential therapeutic applications: isolation, structure determination, synthetic and biological studies. *Nat Prod Rep* 2007; **24**:109–126.
30 Viehrig K, Surup F, Harmrolfs K, Jansen R, Kunze B, Müller R. Concerted action of P450 plus helper protein to form the amino-hydroxy-piperidone moiety of the potent protease inhibitor crocapeptin. *J Am Chem Soc* 2013; **135**:16885–16894.
31 Kwan JC, Liu Y, Ratnayake R, Hatano R, Kuribara A, Morimoto C, *et al.* Grassypeptolides as natural inhibitors of dipeptidyl peptidase 8 and T-cell activation. *Chembiochem* 2014; **15**:799–804.
32 Piekarz RL, Robey R, Sandor V, Bakke S, Wilson WH, Dahmoush L, *et al.* Inhibitor of histone deacetylation, depsipeptide (FR901228), in the treatment of peripheral and cutaneous T-cell lymphoma: a case report. *Blood* 2001; **98**:2865–2868.
33 Piekarz RL, Frye R, Turner M, Wright JJ, Allen SL, Kirschbaum MH, *et al.* Phase II multi-institutional trial of the histone deacetylase inhibitor romidepsin as monotherapy for patients with cutaneous T-cell lymphoma. *J Clin Oncol* 2009; **27**:5410–5417.
34 Whittaker SJ, Demierre MF, Kim EJ, Rook AH, Lerner A, Duvic M, *et al.* Final results from a multicenter, international, pivotal study of romidepsin in refractory cutaneous T-cell lymphoma. *J Clin Oncol* 2010; **28**:4485–4491.

35 Ueda H, Manda T, Matsumoto S, Mukumoto S, Nishigaki F, Kawamura I, *et al.* FR901228, a novel antitumor bicyclic depsipeptide produced by *Chromobacterium violaceum* No. 968. III. Antitumor activities on experimental tumors in mice. *J Antibiot (Tokyo)* 1994; **47**:315–323.

36 Sandor V, Bakke S, Robey RW, Kang MH, Blagosklonny MV, Bender J, *et al.* Phase I trial of the histone deacetylase inhibitor, depsipeptide (FR901228, NSC 630176), in patients with refractory neoplasms. *Clin Cancer Res* 2002; **8**:718–728.

37 Marshall JL, Rizvi N, Kauh J, Dahut W, Figuera M, Kang MH, *et al.* A phase I trial of depsipeptide (FR901228) in patients with advanced cancer. *J Exp Ther Oncol* 2002; **2**:325–332.

38 Piekarz RL, Frye R, Prince HM, Kirschbaum MH, Zain J, Allen SL, *et al.* Phase 2 trial of romidepsin in patients with peripheral T-cell lymphoma. *Blood* 2011; **117**:5827–5834.

39 Mercurio C, Minucci S, Pelicci PG. Histone deacetylases and epigenetic therapies of hematological malignancies. *Pharmacol Res* 2010; **62**:18–34.

40 Stadler WM, Margolin K, Ferber S, McCulloch W, Thompson JA. A phase II study of depsipeptide in refractory metastatic renal cell cancer. *Clin Genitourin Cancer* 2006; **5**:57–60.

41 Whitehead RP, Rankin C, Hoff PM, Gold PJ, Billingsley KG, Chapman RA, *et al.* Phase II trial of romidepsin (NSC-630176) in previously treated colorectal cancer patients with advanced disease: a Southwest Oncology Group study (S0336). *Invest New Drugs* 2009; **27**:469–475.

42 Schrump DS, Fischette MR, Nguyen DM, Zhao M, Li X, Kunst TF, *et al.* Clinical and molecular responses in lung cancer patients receiving romidepsin. *Clin Cancer Res* 2008; **14**:188–198.

43 Paoluzzi L, Scotto L, Marchi E, Zain J, Seshan VE, O'Connor OA. Romidepsin and belinostat synergize the antineoplastic effect of bortezomib in mantle cell lymphoma. *Clin Cancer Res* 2010; **16**:554–565.

44 Harrison SJ, Quach H, Link E, Seymour JF, Ritchie DS, Ruell S, *et al.* A high rate of durable responses with romidepsin, bortezomib, and dexamethasone in relapsed or refractory multiple myeloma. *Blood* 2011; **118**:6274–6283.

45 Bertino EM, Otterson GA. Romidepsin: a novel histone deacetylase inhibitor for cancer. *Expert Opin Investig Drugs* 2011; **20**:1151–1158.

46 Nakajima H, Kim YB, Terano H, Yoshida M, Horinouchi S. FR901228, a potent antitumor antibiotic, is a novel histone deacetylase inhibitor. *Exp Cell Res* 1998; **241**:126–133.

47 Kikuchi J, Wada T, Shimizu R, Izumi T, Akutsu M, Mitsunaga K, *et al.* Histone deacetylases are critical targets of bortezomib-induced cytotoxicity in multiple myeloma. *Blood* 2010; **116**:406–417.

48 Hoshino I, Matsubara H, Akutsu Y, Nishimori T, Yoneyama Y, Murakami K, *et al.* Gene expression profiling induced by histone deacetylase inhibitor, FK228, in human esophageal squamous cancer cells. *Oncol Rep* 2007; **18**:585–592.

49 Abbas T, Dutta A. p21 in cancer: intricate networks and multiple activities. *Nat Rev Cancer* 2009; **9**:400–414.

50 Rajgolikar G, Chan KK, Wang HC. Effects of a novel antitumor depsipeptide, FR901228, on human breast cancer cells. *Breast Cancer Res Treat* 1998; **51**:29–38.

51 Sandor V, Senderowicz A, Mertins S, Sackett D, Sausville E, Blagosklonny MV, *et al.* P21-dependent g(1)arrest with downregulation of cyclin D1 and upregulation of cyclin E by the histone deacetylase inhibitor FR901228. *Br J Cancer* 2000; **83**:817–825.

52 Zhao Y, Lu S, Wu L, Chai G, Wang H, Chen Y, *et al.* Acetylation of p53 at lysine 373/382 by the histone deacetylase inhibitor depsipeptide induces expression of p21(Waf1/Cip1). *Mol Cell Biol* 2006; **26**:2782–2790.

53 Khan SB, Maududi T, Barton K, Ayers J, Alkan S. Analysis of histone deacetylase inhibitor, depsipeptide (FR901228), effect on multiple myeloma. *Br J Haematol* 2004; **125**:156–161.

54 Yu XD, Wang SY, Chen GA, Hou CM, Zhao M, Hong JA, *et al.* Apoptosis induced by depsipeptide FK228 coincides with inhibition of survival signaling in lung cancer cells. *Cancer J* 2007; **13**:105–113.

55 Zhang Y, Adachi M, Kawamura R, Imai K. Bmf is a possible mediator in histone deacetylase inhibitors FK228 and CBHA-induced apoptosis. *Cell Death Differ* 2006; **13**:129–140.

56 Zhang Y, Adachi M, Zhao X, Kawamura R, Imai K. Histone deacetylase inhibitors FK228, *N*-(2-aminophenyl)-4-[*N*-(pyridin-3-yl-methoxycarbonyl) amino-methyl]benzamide and m-carboxycinnamic acid bis-hydroxamide augment radiation-induced cell death in gastrointestinal adenocarcinoma cells. *Int J Cancer* 2004; **110**:301–308.

57 Cain JM, Liu PY, Alberts DE, Gallion HH, Laufman L, O'Sullivan J, *et al.* Phase II trial of didemnin-B in advanced epithelial ovarian cancer. A Southwest Oncology Group study. *Invest New Drugs* 1992; **10**:23–24.

58 Taylor SA, Goodman P, Crawford ED, Stuckey WJ, Stephens RL, Gaynor ER. Phase II evaluation of didemnin B in advanced adenocarcinoma of the kidney. A Southwest Oncology Group study. *Invest New Drugs* 1992; **10**:55–56.

59 Benvenuto JA, Newman RA, Bignami GS, Raybould TJ, Raber MN, Esparza L, *et al.* Phase II clinical and pharmacological study of didemnin B in patients with metastatic breast cancer. *Invest New Drugs* 1992; **10**:113–117.

60 Sondak VK, Kopecky KJ, Liu PY, Fletcher WS, Harvey WH, Laufman LR. Didemnin B in metastatic malignant melanoma: a phase II trial of the Southwest Oncology Group. *Anticancer Drugs* 1994; **5**:147–150.

61 Shin DM, Holoye PY, Forman A, Winn R, Perez-Soler R, Dakhil S, *et al.* Phase II clinical trial of didemnin B in previously treated small cell lung cancer. *Invest New Drugs* 1994; **12**:243–249.

62 Weiss RB, Peterson BL, Allen SL, Browning SM, Duggan DB, Schiffer CA. A phase II trial of didemnin B in myeloma. A Cancer and Leukemia Group B (CALGB) study. *Invest New Drugs* 1994; **12**:41–43.

63 Williamson SK, Wolf MK, Eisenberger MA, O'Rourke M, Brannon W, Crawford ED. Phase II evaluation of didemnin B in hormonally refractory metastatic prostate cancer. A Southwest Oncology Group study. *Invest New Drugs* 1995; **13**:167–170.

64 Goss G, Muldal A, Lohmann R, Taylor M, Lopez P, Armitage G, *et al.* Didemnin B in favourable histology non-Hodgkin's lymphoma. A phase II study of the National Cancer Institute of Canada Clinical Trials Group. *Invest New Drugs* 1995; **13**:257–260.

65 Mateos MV, Cibeira MT, Richardson PG, Prosper F, Oriol A, de la Rubia J, *et al.* Phase II clinical and pharmacokinetic study of plitidepsin 3-hour infusion every two weeks alone or with dexamethasone in relapsed and refractory multiple myeloma. *Clin Cancer Res* 2010; **16**:3260–3269.

66 Depenbrock H, Peter R, Faircloth GT, Manzanares I, Jimeno J, Hanauske AR. In vitro activity of aplidine, a new marine-derived anti-cancer compound, on freshly explanted clonogenic human tumour cells and haematopoietic precursor cells. *Br J Cancer* 1998; **78**:739–744.

67 Sakai R, Rinehart KL, Kishore V, Kundu B, Faircloth G, Gloer JB, *et al.* Structure–activity relationships of the didemnins. *J Med Chem* 1996; **39**:2819–2834.

68 Lobo C, García-Pozo SG, Núñez de Castro I, Alonso FJ. Effect of dehydrodidemnin B on human colon carcinoma cell lines. *Anticancer Res* 1997; **17**:333–336.

69 Gómez SG, Faircloth G, López-Lázaro L, Jimeno J, Bueren JA, Albella B. In vitro hematotoxicity of Aplidine on human bone marrow and cord blood progenitor cells. *Toxicol In Vitro* 2001; **15**:347–350.

70 Straight AM, Oakley K, Moores R, Bauer AJ, Patel A, Tuttle RM, *et al.* Aplidin reduces growth of anaplastic thyroid cancer xenografts and the expression of several angiogenic genes. *Cancer Chemother Pharmacol* 2006; **57**:7–14.

71 Caers J, Menu E, De Raeve H, Lepage D, Van Valckenborgh E, Van Camp B, *et al.* Antitumour and antiangiogenic effects of Aplidin in the 5TMM syngeneic models of multiple myeloma. *Br J Cancer* 2008; **98**:1966–1974.

72 Maroun JA, Belanger K, Seymour L, Matthews S, Roach J, Dionne J, *et al.* Phase I study of aplidine in a dailyx5 one-hour infusion every 3 weeks in patients with solid tumors refractory to standard therapy. A National Cancer Institute of Canada Clinical Trials Group study: NCIC CTG IND 115. *Ann Oncol* 2006; **17**:1371–1378.

73 Izquierdo MA, Bowman A, García M, Jodrell D, Martinez M, Pardo B, *et al.* Phase I clinical and pharmacokinetic study of plitidepsin as a 1-hour weekly intravenous infusion in patients with advanced solid tumors. *Clin Cancer Res* 2008; **14**:3105–3112.

74 Faivre S, Chièze S, Delbaldo C, Ady-Vago N, Guzman C, Lopez-Lazaro L, *et al.* Phase I and pharmacokinetic study of aplidine, a new marine cyclodepsipeptide in patients with advanced malignancies. *J Clin Oncol* 2005; **23**:7871–7880.

75 Geoerger B, Estlin EJ, Aerts I, Kearns P, Gibson B, Corradini N, *et al.* A phase I and pharmacokinetic study of plitidepsin in children with advanced solid tumours: an Innovative Therapies for Children with Cancer (ITCC) study. *Eur J Cancer* 2012; **48**:289–296.

76 Eisen T, Thomas J, Miller WH, Gore M, Wolter P, Kavan P, *et al.* Phase II study of biweekly plitidepsin as second-line therapy in patients with advanced malignant melanoma. *Melanoma Res* 2009; **19**:185–192.

77 Baudin E, Droz JP, Paz-Ares L, van Oosterom AT, Cullell-Young M, Schlumberger M. Phase II study of plitidepsin 3-hour infusion every 2 weeks in patients with unresectable advanced medullary thyroid carcinoma. *Am J Clin Oncol* 2010; **33**:83–88.

78 Schöffski P, Guillem V, Garcia M, Rivera F, Tabernero J, Cullell M, *et al.* Phase II randomized study of Plitidepsin (Aplidin), alone or in association with L-carnitine, in patients with unresectable advanced renal cell carcinoma. *Mar Drugs* 2009; **7**:57–70.

79 Eisen T, Thatcher N, Leyvraz S, Miller WH, Couture F, Lorigan P, *et al.* Phase II study of weekly plitidepsin as second-line therapy for small cell lung cancer. *Lung Cancer* 2009; **64**:60–65.

80 Ribrag V, Caballero D, Fermé C, Zucca E, Arranz R, Briones J, *et al.* Multicenter phase II study of plitidepsin in patients with relapsed/refractory non-Hodgkin's lymphoma. *Haematologica* 2013; **98**:357–363.

81 García-Fernández LF, Losada A, Alcaide V, Alvarez AM, Cuadrado A, González L, *et al.* Aplidin induces the mitochondrial apoptotic pathway via oxidative stress-mediated JNK and p38 activation and protein kinase C delta. *Oncogene* 2002; **21**:7533–7544.

82 Cuadrado A, Garcia-Fernandez LF, Gonzalez L, Suarez Y, Losada A, Alcaide V, *et al.* Aplidin induces apoptosis in human cancer cells via glutathione depletion and sustained activation of the epidermal growth factor receptor, Src, JNK, and p38 MAPK. *J Biol Chem* 2003; **278**:241–250.

83 Cuadrado A, González L, Suárez Y, Martínez T, Muñoz A. JNK activation is critical for aplidin-induced apoptosis. *Oncogene* 2004; **23**:4673–4680.

84 González-Santiago L, Suárez Y, Zarich N, Muñoz-Alonso MJ, Cuadrado A, Martínez T, *et al.* Aplidin induces JNK-dependent apoptosis in human breast cancer cells via alteration of glutathione homeostasis, Rac1 GTPase activation, and MKP-1 phosphatase downregulation. *Cell Death Differ* 2006; **13**:1968–1981.

85 Erba E, Bassano L, Di Liberti G, Muradore I, Chiorino G, Ubezio P, *et al.* Cell cycle phase perturbations and apoptosis in tumour cells induced by aplidine. *Br J Cancer* 2002; **86**:1510–1517.

86 Erba E, Serafini M, Gaipa G, Tognon G, Marchini S, Celli N, *et al.* Effect of aplidin in acute lymphoblastic leukaemia cells. *Br J Cancer* 2003; **89**:763–773.

87 Biscardi M, Caporale R, Balestri F, Gavazzi S, Jimeno J, Grossi A. VEGF inhibition and cytotoxic effect of aplidin in leukemia cell lines and cells from acute myeloid leukemia. *Ann Oncol* 2005; **16**:1667–1674.

88 Muñoz-Alonso MJ, González-Santiago L, Zarich N, Martínez T, Alvarez E, Rojas JM, *et al.* Plitidepsin has a dual effect inhibiting cell cycle and inducing apoptosis via Rac1/c-Jun NH2-terminal kinase activation in human melanoma cells. *J Pharmacol Exp Ther* 2008; **324**:1093–1101.

89 Broggini M, Marchini SV, Galliera E, Borsotti P, Taraboletti G, Erba E, *et al.* Aplidine, a new anticancer agent of marine origin, inhibits vascular endothelial growth factor (VEGF) secretion and blocks VEGF-VEGFR-1 (flt-1) autocrine loop in human leukemia cells MOLT-4. *Leukemia* 2003; **17**:52–59.

90 Gao J, Hamann MT. Chemistry and biology of kahalalides. *Chem Rev* 2011; **111**:3208–3235.

91 Shilabin AG, Kasanah N, Wedge DE, Hamann MT. Lysosome and HER3 (ErbB3) selective anticancer agent kahalalide F: semisynthetic modifications and antifungal lead-exploration studies. *J Med Chem* 2007; **50**:4340–4350.

92 Faircloth G, Cuevas C. Kahalalide F and ES285: potent anticancer agents from marine molluscs. *Prog Mol Subcell Biol* 2006; **43**:363–379.

93 Bonnard I, Manzanares I, Rinehart KL. Stereochemistry of kahalalide F. *J Nat Prod* 2003; **66**:1466–1470.

94 Rademaker-Lakhai JM, Horenblas S, Meinhardt W, Stokvis E, de Reijke TM, Jimeno JM, *et al.* Phase I clinical and pharmacokinetic study of kahalalide F in patients with advanced androgen refractory prostate cancer. *Clin Cancer Res* 2005; **11**:1854–1862.

95 Pardo B, Paz-Ares L, Tabernero J, Ciruelos E, García M, Salazar R, *et al.* Phase I clinical and pharmacokinetic study of kahalalide F administered weekly as a 1-hour infusion to patients with advanced solid tumors. *Clin Cancer Res* 2008; **14**:1116–1123.

96 Salazar R, Cortés-Funes H, Casado E, Pardo B, López-Martín A, Cuadra C, *et al.* Phase I study of weekly kahalalide F as prolonged infusion in patients with advanced solid tumors. *Cancer Chemother Pharmacol* 2013; **72**:75–83.

97 Martín-Algarra S, Espinosa E, Rubió J, López López JJ, Manzano JL, Carrión LA, *et al.* Phase II study of weekly Kahalalide F in patients with advanced malignant melanoma. *Eur J Cancer* 2009; **45**:732–735.

98 García-Rocha M, Bonay P, Avila J. The antitumoral compound Kahalalide F acts on cell lysosomes. *Cancer Lett* 1996; **99**:43–50.

99 Janmaat ML, Rodriguez JA, Jimeno J, Kruyt FA, Giaccone G. Kahalalide F induces necrosis-like cell death that involves depletion of ErbB3 and inhibition of Akt signaling. *Mol Pharmacol* 2005; **68**:502–510.

100 Provencio M, Sánchez A, Gasent J, Gómez P, Rosell R. Cancer treatments: can we find treasures at the bottom of the sea? *Clin Lung Cancer* 2009; **10**:295–300.

101 Salazar R, Jones RJ, Oaknin A, Crawford D, Cuadra C, Hopkins C, *et al.* A phase I and pharmacokinetic study of elisidepsin (PM02734) in patients with advanced solid tumors. *Cancer Chemother Pharmacol* 2012; **70**:673–681.

102 Molina-Guijarro JM, Macías Á, García C, Muñoz E, García-Fernández LF, David M, *et al.* Irvalec inserts into the plasma membrane causing rapid loss of integrity and necrotic cell death in tumor cells. *PLoS One* 2011; **6**: e19042.

103 Ling YH, Aracil M, Jimeno J, Perez-Soler R, Zou Y. Molecular pharmacodynamics of PM02734 (elisidepsin) as single agent and in combination with erlotinib; synergistic activity in human non-small cell lung cancer cell lines and xenograft models. *Eur J Cancer* 2009; **45**:1855–1864.

104 Ling YH, Aracil M, Zou Y, Yuan Z, Lu B, Jimeno J, *et al.* PM02734 (elisidepsin) induces caspase-independent cell death associated with features of autophagy, inhibition of the Akt/mTOR signaling pathway, and activation of death-associated protein kinase. *Clin Cancer Res* 2011; **17**:5353–5366.

105 Váradi T, Roszik J, Lisboa D, Vereb G, Molina-Guijarro JM, Galmarini CM, *et al.* ErbB protein modifications are secondary to severe cell membrane alterations induced by elisidepsin treatment. *Eur J Pharmacol* 2011; **667**:91–99.

106 Herrero AB, Astudillo AM, Balboa MA, Cuevas C, Balsinde J, Moreno S. Levels of SCS7/FA2H-mediated fatty acid 2-hydroxylation determine the sensitivity of cells to antitumor PM02734. *Cancer Res* 2008; **68**:9779–9787.

107 Barrow RA, Hemscheidt T, Liang J, Paik S, Moore RE, Tius MA. Total Synthesis of cryptophycins. Revision of the structures of cryptophycins A and C. *J Am Chem Soc* 1995; **117**:2479–2490.

108 Smith CD, Zhang X, Mooberry SL, Patterson GM, Moore RE. Cryptophycin: a new antimicrotubule agent active against drug-resistant cells. *Cancer Res* 1994; **54**:3779–3784.

109 Trimurtulu G, Ohtani I, Patterson GML, Moore RE, Corbett TH, Valeriote FA, *et al.* Total structures of cryptophycins, potent antitumor depsipeptides from the blue-green alga *Nostoc* sp. Strain GSV 224t. *J Am Chem Soc* 1994; **116**:4729–4737.

110 Corbett TH, Valeriote FA, Demchik L, Lowichik N, Polin L, Panchapor C, *et al.* Discovery of cryptophycin-1 and BCN-183577: examples of strategies and problems in the detection of antitumor activity in mice. *Invest New Drugs* 1997; **15**:207–218.

111 Liang J, Moore RE, Moher ED, Munroe JE, Al-awar RS, Hay D, *et al.* Cryptophycins-309, 249 and other cryptophycin analogs: preclinical efficacy studies with mouse and human tumors. *Invest New Drugs* 2005; **23**:213–224.

112 Kobayashi M, Wang W, Ohyabu N, Kurosu M, Kitagawa I. Improved total synthesis and structure-activity relationship of arenastatin A, a potent cytotoxic spongean depsipeptide. *Chem Pharm Bull (Tokyo)* 1995; **43**:1598–1600.

113 Andavan GS, Lemmens-Gruber R. Cyclodepsipeptides from marine sponges: natural agents for drug research. *Mar Drugs* 2010; **8**:810–834.

114 Patel VF, Andis SL, Kennedy JH, Ray JE, Schultz RM. Novel cryptophycin antitumor agents: synthesis and cytotoxicity of fragment "B" analogues. *J Med Chem* 1999; **42**:2588–2603.

115 Wagner MM, Paul DC, Shih C, Jordan MA, Wilson L, Williams DC. In vitro pharmacology of cryptophycin 52 (LY355703) in human tumor cell lines. *Cancer Chemother Pharmacol* 1999; **43**:115–125.

116 Stevenson JP, Sun W, Gallagher M, Johnson R, Vaughn D, Schuchter L, *et al.* Phase I trial of the cryptophycin analogue LY355703 administered as an intravenous infusion on a day 1 and 8 schedule every 21 days. *Clin Cancer Res* 2002; **8**:2524–2529.

117 Sessa C, Weigang-Köhler K, Pagani O, Greim G, Mora O, De Pas T, *et al.* Phase I and pharmacological studies of the cryptophycin analogue LY355703 administered on a single intermittent or weekly schedule. *Eur J Cancer* 2002; **38**:2388–2396.

118 Edelman MJ, Gandara DR, Hausner P, Israel V, Thornton D, DeSanto J, *et al.* Phase 2 study of cryptophycin 52 (LY355703) in patients previously treated with platinum based chemotherapy for advanced non-small cell lung cancer. *Lung Cancer* 2003; **39**:197–199.

119 D'Agostino G, del Campo J, Mellado B, Izquierdo MA, Minarik T, Cirri L, *et al.* A multicenter phase II study of the cryptophycin analog LY355703 in patients with platinum-resistant ovarian cancer. *Int J Gynecol Cancer* 2006; **16**:71–76.

120 Bai R, Schwartz RE, Kepler JA, Pettit GR, Hamel E. Characterization of the interaction of cryptophycin 1 with tubulin: binding in the Vinca domain, competitive inhibition of dolastatin 10 binding, and an unusual aggregation reaction. *Cancer Res* 1996; **56**:4398–4406.

121 Smith CD, Zhang X. Mechanism of action cryptophycin. Interaction with the Vinca alkaloid domain of tubulin. *J Biol Chem* 1996; **271**:6192–6198.
122 Lu K, Dempsey J, Schultz RM, Teicher BA. Cryptophycin-induced hyperphosphorylation of Bcl-2, cell cycle arrest and growth inhibition in human H460 NSCLC cells. *Cancer Chemother Pharmacol* 2001; **47**:170–178.
123 Panda D, DeLuca K, Williams D, Jordan MA, Wilson L. Antiproliferative mechanism of action of cryptophycin-52: kinetic stabilization of microtubule dynamics by high-affinity binding to microtubule ends. *Proc Natl Acad Sci USA* 1998; **95**:9313–9318.
124 Shih C, Teicher BA. Cryptophycins: a novel class of potent antimitotic antitumor depsipeptides. *Curr Pharm Des* 2001; **7**: 1259–1276.
125 Kessel D, Luo Y. Cells in cryptophycin-induced cell-cycle arrest are susceptible to apoptosis. *Cancer Lett* 2000; **151**:25–29.
126 Subramanian B, Nakeff A, Media JE, Wiegand RA, Valeriote FA. Inhibition of macromolecular synthesis by cryptophycin-52. *Anticancer Drugs* 2002; **13**:1061–1068.
127 Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J Nat Prod* 2012; **75**:311–335.

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# Emerging innovation towards safety in the clinical application of ESCs and iPSCs

*Shigeo Masuda, Shigeru Miyagawa, Satsuki Fukushima, Nagako Sougawa, Emiko Ito, Maki Takeda, Atsuhiro Saito and Yoshiki Sawa*

The Review by Behfar and colleagues (Cell therapy for cardiac repair—lessons from clinical trials. *Nat. Rev. Cardiol.* **11**, 232–246; 2014)[1] summarized that 'first-generation' cell therapies for heart failure[2] using autologous cells are safe for use in humans. Conversely, 'next-generation' cell therapies, which include pluripotent stem cells such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), have major safety concerns, because contamination of undifferentiated cells might lead to teratoma formation.[3] However, novel and efficient protocols for selective shutdown of tumour formation in these cells have been reported in several studies, which merit discussion (Table 1).

Firstly, chemical inhibitors of survivin potently induce selective and complete cell death of undifferentiated human ESCs or iPSCs.[4,5] A single pretreatment exposure to survivin inhibitors is sufficient to completely inhibit teratoma formation after transplantation.[4] Importantly, differentiated cells derived from human ESCs or iPSCs maintain their functionality after treatment with survivin inhibitors.[4] The survivin inhibitor QC has been widely used as nutritional supplement and no adverse effects have been reported.[4] Secondly, chemical inhibitors of oleate synthesis have been identified as compounds for selective elimination of human ESCs or iPSCs.[6,7] Oleate synthesis inhibitors lead to apoptosis in human ESCs or iPSCs through lipid metabolism, revealing a dependence of these cells on oleate. At present, application of oleate synthesis inhibitors is limited to *in vitro* culture before transplantation; whether these inhibitors might be applied *in vivo* remains to be determined. Thirdly, the diabetes mellitus drug metformin[8] can reduce tumour forming potential of iPSCs without affecting pluripotency;[9] however, in this study only mouse iPSCs were investigated. Metformin, an agonist of AMP-activated protein kinase, suppresses the expression of Oct4 and survivin thereby showing previously unrecognized stem-cell toxicity.[10] Finally, an antibody against stage-specific embryonic antigen-5 (a newly identified PSC-specific surface antigen) can be used to remove undifferentiated cells by fluorescence-activated cell sorting.[11] However, because this method depends on cell sorting, which includes *ex vivo* manipulation (such as single-cell dissociation and cell-staining techniques), cells might lose viability. New synthesized small molecules (such as JC011), which selectively induce a cytotoxic endoplasmic reticulum stress response in ESCs and iPSCs, have also been reported, but further studies should reveal the precise mechanisms of this pathway.[12]

We believe that two issues relating to the use of ESC or iPSC therapies need to be addressed. After treating cells with chemical inhibitors to prevent teratoma, these cells should be tested to ensure that they have maintained functional properties, including differentiation capacity[13] and engraftment potential. Efficiency, as well as safety, is required for ideal cell transplantation. A second problem is that malignant cell transformation, other than teratoma formation, after transplantation of PSC-derived cells might also exist. Pluripotent tumour forming potential can be divided into two categories: malignant transformation of differentiated PSCs, and benign teratoma formation from residual undifferentiated PSCs.[14,15] The former should be also investigated for safety. For example, CD30, which is a biomarker for transformed human ESCs, is correlated with karyotype abnormalities such as partial duplication of chromosome.[16] Further elucidation of this issue is needed before a judgement on iPSC clinical safety can be made.

**Table 1** | Novel strategies for selective elimination of teratoma

| Reference | Chemical or antibody | Mode of action | Drug |
|---|---|---|---|
| Lee *et al.*[4] | Chemical inhibitor | Survivin inhibition | QC; YM155 |
| Ben-David *et al.*[6] | Chemical inhibitor | Oleate synthesis inhibition | PluriSln #1 |
| Vazquez-Martin *et al.*[9] | Chemical inhibitor | AMP-activated protein kinase activation | Metformin |
| Tang *et al.*[11] | Antibody | SSEA-5 purging | Anti-SSEA-5 monoclonal antibody |
| Richards *et al.*[12] | Chemical | Endoplasmic reticulum stress | JC011 |

Abbreviations: PluriSln, pluripotent cell-specific inhibitor; SSEA-5, stage-specific embryonic antigen-5.

*Department of Cardiovascular Surgery, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan (**S.Masuda, S.Miyagawa, S.F., N.S., E.I., M.T. A.S., Y.S.**).*
*Correspondence to: Y.S.*
*sawa@surg1.med.osaka-u.ac.jp*

**Competing interests**
The authors declare no competing interests.

1. Behfar, A. *et al.* Cell therapy for cardiac repair—lessons from clinical trials. *Nat. Rev. Cardiol.* **11**, 232–246 (2014).
2. Sawa, Y. & Miyagawa, S. Present and future perspectives on cell sheet-based myocardial regeneration therapy. *Biomed. Res Int.* http://dx.doi.org/10.1155/2013/583912.
3. Masuda, S. & Hanazono, Y. Induced pluripotent stem cells in long-QT syndrome. *N. Engl. J. Med.* **364**, 181–182 (2011).
4. Lee, M. O. *et al.* Inhibition of pluripotent stem cell-derived teratoma formation by small molecules. *Proc. Natl Acad. Sci. USA* **110**, E3281–E3290 (2013).
5. Blum, B. *et al.* The anti-apoptotic gene survivin contributes to teratoma formation by human embryonic stem cells. *Nat. Biotechnol.* **27**, 281–287 (2009).
6. Ben-David, U. *et al.* Selective elimination of human pluripotent stem cells by an oleate synthesis inhibitor discovered in a high-throughput screen. *Cell Stem Cell* **12**, 167–179 (2013).

7. Li, W., Li, K., Wei, W. & Ding, S. Chemical approaches to stem cell biology and therapeutics. *Cell Stem Cell* **13**, 270–283 (2013).
8. Pernicova, I. & Korbonits, M. Metformin—mode of action and clinical implications for diabetes and cancer. *Nat. Rev. Endocrinol.* **10**, 143–156 (2014).
9. Vazquez-Martin, A. *et al.* Metformin limits the tumourigenicity of iPS cells without affecting their pluripotency. *Sci. Rep.* **2**, 964 (2012).
10. Vazquez-Martin, A. *et al.* Activation of AMP-activated protein kinase (AMPK) provides a metabolic barrier to reprogramming somatic cells into stem cells. *Cell Cycle* **11**, 974–989 (2012).
11. Tang, C. *et al.* An antibody against SSEA-5 glycan on human pluripotent stem cells enables removal of teratoma-forming cells. *Nat. Biotechnol.* **29**, 829–834 (2011).
12. Richards, M. *et al.* A new class of pluripotent stem cell cytotoxic small molecules. *PLoS ONE* **9**, e85039 (2014).
13. Masuda, S. *et al.* A simplified *in vitro* teratoma assay for pluripotent stem cells injected into rodent fetal organs. *Cell Med.* **3**, 103–112 (2012).
14. Lee, A. S. *et al.* Tumorigenicity as a clinical hurdle for pluripotent stem cell therapies. *Nat. Med.* **19**, 998–1004 (2013).
15. Ben-David, U. & Benvenisty, N. The tumorigenicity of human embryonic and induced pluripotent stem cells. *Nat. Rev. Cancer* **11**, 268–277 (2011).
16. Herszfeld, D. *et al.* CD30 is a survival factor and a biomarker for transformed human pluripotent stem cells. *Nat. Biotechnol.* **24**, 351–357 (2006).

# Regulating ES or Induced Pluripotent Stem Cells by Innate Lymphoid Cells

*To The Editor:*

Natural killer (NK) cells are innate lymphocytes that are able to be stem cell (ESC) rejection; they show that, after injecting anti–asialo-GM-1 or anti-Ly49G2 into mice, ESC rejection rate was remarkably decreased, suggesting that a subset of Ly49G2$^+$ NK cells would play a crucial role in killing ESCs (*1*).

Recently, innate lymphoid cells (ILCs) are emerging as novel modulators of innate immunity, enabling early immune responses (*2*). Actually, conventional NK cell is a member of ILCs. It has been proposed that ILCs should be classified into three distinct groups based on functional characteristics and cytokines that they can produce. Group 1 ILCs are defined by production of Th1 cell-associated cytokine interferon-γ, and include NK cells and ILC1s. Group 2 ILCs produce Th2 cell-associated cytokines (interleukin [IL]-5 and IL-13), and include ILC2s. Group 3 ILCs secrete IL-17 and/or IL-22, and include lymphoid tissue inducer (LTi) cells and natural cytotoxicity receptor (NCR)$^+$ ILC3s (*2*).

Are there any cells that express NK cell receptors other than conventional NK cells? Recent evidence suggests that an NK cell receptor-expressing innate lymphocyte subset has been identified as intraepithelial ILC1-like cells (*3*). Moreover, NCR+ ILC3s could be converted to ILC1s under the influence of IL-12 (ref. *4*). These cells are expressing NK cell receptors, but their functions remain poorly understood.

In the present study by Perez-Cunningham et al. (*1*), it would be critical to explore whether not only conventional NK cells but also ILC1s (if expressing NK cell receptors) can be depleted by treatment with anti-Ly49G2; whether a subset of mouse ILC1s express Ly49G2 NK receptor would be interesting. One hypothesis is that interferon-γ–secreting ILC1s would also have a pivotal role in regulating immune responses in transplantation, although cytotoxic via perforin and granzyme B to cells with low expression of major histocompatibility complex class I molecules. ILC1s lack perforin and granzyme B. It would be meaningful if some ILCs might have a novel role in immunity during allogeneic transplantation, such as rejecting ES cells. Interestingly, it has been reported that RORγt-NKR-LTi cells express perforin and granzyme B, leading to cytotoxicity (*5*). Regarding specificity of antibodies, for example, anti–asialo-GM-1, which is well known to be capable of depleting NK cell subsets, has been revealed to also deplete basophils as off-target effect (*6*). It would be essential to understand expression patterns exactly.

When we transplant human ES-derived or induced pluripotent stem (iPS)-derived cells into patients in allogeneic settings in clinical trials, we will, under treatment with immunosuppressants, use differentiated cells (expressing major histocompatibility complex class I molecules) but not undifferentiated cells. Indeed, recent studies have shown that terminally differentiated cells derived from ES or iPS cells elicit negligible immune rejection in their host, although recipients are syngeneic (*7–9*). Therefore, condition in the present study (*1*), where undifferentiated cells were used, would be quite different from that of clinical settings. However, in view of removal of undifferentiated cells, we can apply a strategy of regulating immune responses as shown in the present study (*1*). Collectively, it could be rational hypothesis to modulate ILC function in transplantation immunity, thereby providing principle of concept.

**Shigeo Masuda**
**Shigeru Miyagawa**
**Satsuki Fukushima**
**Takuji Kawamura**
**Noriyuki Kashiyama**
**Atsuhiro Saito**
**Yoshiki Sawa**

In a study by Perez-Cunningham et al. (*1*), the authors demonstrate that a subset of NK cells is responsible for embryonic

Department of Cardiovascular Surgery
Osaka University Graduate School of Medicine
Osaka, Japan, 2-2 Yamadaoka
Suita, Osaka, Japan

The authors declare no funding and conflicts of interest.

Address correspondence to: Yoshiki Sawa, M.D., Ph.D., Department of Cardiovascular Surgery, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan.
E-mail: sawa-p@surg1.med.osaka-u.ac.jp

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## REFERENCES

1. Perez-Cunningham J, Ames E, Smith RC, et al. Natural killer cell subsets differentially reject embryonic stem cells based on licensing. *Transplantation* 2014; 97: 992.
2. Spits H, Artis D, Colonna M, et al. Innate lymphoid cells—a proposal for uniform nomenclature. *Nat Rev Immunol.* 2013; 13: 145.
3. Fuchs A, Vermi W, Lee JS, et al. Intraepithelial type 1 innate lymphoid cells are a unique subset of IL-12- and IL-15-responsive IFN-γ-producing cells. *Immunity.* 2013; 38: 769.
4. Bernink JH, Peters CP, Munneke M, et al. Human type 1 innate lymphoid cells accumulate in inflamed mucosal tissues. *Nat Immunol.* 2013; 14: 221.
5. Vonarbourg C, Mortha A, Bui VL, et al. Regulated expression of nuclear receptor RORγt confers distinct functional fates to NK cell receptor-expressing RORγt(+) innate lymphocytes. *Immunity.* 2010; 33: 736.
6. Nishikado H, Mukai K, Kawano Y, et al. NK cell-depleting anti-asialo GM1 antibody exhibits a lethal off-target effect on basophils in vivo. *J Immunol.* 2011; 186: 5766.
7. Araki R, Uda M, Hoki Y, et al. Negligible immunogenicity of terminally differentiated cells derived from induced pluripotent or embryonic stem cells. *Nature.* 2013; 494: 100.
8. Guha P, Morgan JW, Mostoslavsky G, et al. Lack of immune response to differentiated cells derived from syngeneic induced pluripotent stem cells. *Cell Stem Cell.* 2013; 12: 407.
9. Kaneko S, Yamanaka S. To be immunogenic, or not to be: that's the iPSC question. *Cell Stem Cell.* 2013; 12: 385.

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# *N*-Glycans: Phenotypic Homology and Structural Differences between Myocardial Cells and Induced Pluripotent Stem Cell-Derived Cardiomyocytes

**Takuji Kawamura[1], Shigeru Miyagawa[1], Satsuki Fukushima[1], Akira Yoshida[2], Noriyuki Kashiyama[1], Ai Kawamura[1], Emiko Ito[1], Atsuhiro Saito[1], Akira Maeda[3], Hiroshi Eguchi[3], Koichi Toda[1], Jong-Kook Lee[2], Shuji Miyagawa[3], Yoshiki Sawa[1]***

**1** Department of Cardiovascular Surgery, Osaka University Graduate School of Medicine, Suita, Osaka, Japan, **2** Department of Cardiovascular Regenerative Medicine, Osaka University Graduate School of Medicine, Suita, Osaka, Japan, **3** Division of Organ Transplantation, Department of Surgery, Osaka University Graduate School of Medicine, Suita, Osaka, Japan

## Abstract

Cell surface glycans vary widely, depending on cell properties. We hypothesized that glycan expression on induced pluripotent stem cells (iPSCs) might change during cardiomyogenic differentiation toward the myocardial phenotype. *N*-glycans were isolated from iPSCs, iPSC-derived cardiomyocytes (iPSC-CM), and original C57BL/6 mouse myocardium (Heart). Their structures were analyzed by a mapping technique based on HPLC elution times and MALDI-TOF/MS spectra. Sixty-eight different *N*-glycans were isolated; the structures of 60 of these *N*-glycans were identified. The quantity of high-mannose type (immature) *N*-glycans on the iPSCs decreased with cardiomyogenic differentiation, but did not reach the low levels observed in the heart. We observed a similar reduction in neutral *N*-glycans and an increase in fucosylated or sialyl *N*-glycans. Some structural differences were detected between iPSC-CM and Heart. No *N*-glycolyl neuraminic acid (NeuGc) structures were detected in iPSC-CM, whereas the heart contained numerous NeuGc structures, corresponding to the expression of cytidine monophosphate-*N*-acetylneuraminic acid hydroxylase. Furthermore, several glycans containing Galα1-6 Gal, rarely identified in the other cells, were detected in the iPSC-CM. The expression of *N*-glycan on murine iPSCs changed toward the myocardial phenotype during cardiomyogenic differentiation, leaving the structural differences of NeuGc content or Galα1-6 Gal structures. Further studies will be warranted to reveal the meaning of the difference of *N*-glycans between the iPSC-CM and the myocardium.

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* Email: sawa-p@surg1.med.osaka-u.ac.jp

## Introduction

*In vitro* generation of cardiac myocytes by reprogramming is a promising technology in developing cell-transplant therapy for advanced cardiac failure [1] and drug discovery for a variety of cardiac diseases [2]. For both purposes, induced pluripotent stem cells (iPSCs) are most useful, since generation and cardiomyogenic differentiation of iPSCs has been standardized in human and a number of animals [3,4]. In fact, derivatives of iPSCs have been developed to the pre-clinical stage for cell transplantation therapy [5], while cardiac myocytes generated from patient-specific iPSCs have been studied to explore pathologic mechanisms and guide drug discovery [6,7]. However, cardiac myocyte preparations from iPSCs contain immature phenotypes, observed by electrophysiology, electron microscopy, and immunohistochemistry [8,9]; this may limit the safety and efficacy of cell transplantation therapy or reduce the accuracy and efficiency of drug discovery. The maturity of iPSC-derived cardiac myocytes (iPSC-CMs) has not been comprehensively or quantitatively evaluated.

Cell surface glycans have several important functions interacting with numerous proteins, including growth factors, morphogens and adhesion molecules, modulating dynamic cellular mechanisms such as cell-cell adhesion, cell activation, and malignant alterations [10–12]. In early mammalian embryos, associated with fertilization, some *N*-glycans play important roles of cell-cell adhesion [13–15]. In addition, cellular responsiveness to growth or arrest depends on total *N*-glycan number and the degree of branching of cell surface glycoproteins [16]. Furthermore, heparan sulfate, a kind of glycans, is required for embryonic stem cell (ESC) pluripotency, in particular lineage specification into mesoderm through facilitation of FGF and BMP signaling by stabilizing BMP ligand [17], leading the evidence that the expression patterns of cell surface glycans on ESCs changes during differentiation [18]. Thus, we hypothesized that cell surface glycan expression may