Mendonca, A., de Tute, R., Cullen, M., Sedek, L., Vidriales, M.B., Perez, J.J., te Marvelde, J.G., Mejstrikova, E., Hrusak, O., Szczepanski, T., van Dongen, J.J., Orfao, A. & EuroFlow, C. (2012) EuroFlow standardization of flow cytometer instrument settings and immunophenotyping protocols. *Leukemia*, **26**, 1986–2010.

Karawajew, L., Dworzak, M., Ratei, R., Rhein, P., Gaipa, G., Buldini, B., Basso, G., Hrusak, O., Ludwig, W.D., Henze, G., Seeger, K., von Stackelberg, A., Mejstrikova, E. & Eckert, C. (2015) Minimal residual disease analysis by eight-color flow cytometry in relapsed childhood acute lymphoblastic leukemia. *Haematologica*, **100**, 935–944.

Keyhani, A., Huh, Y.O., Jendiroba, D., Pagliaro, L., Cortez, J., Pierce, S., Pearlman, M., Estey, E., Kantarjian, H. & Freireich, E.J. (2000) Increased CD38 expression is associated with favorable prognosis in adult acute leukemia. *Leukemia Research*, **24**, 153–159.

Lonial, S., Weiss, B.M., Usmani, S.Z., Singhal, S., Chari, A., Bahlis, N.J., Belch, A., Krishnan, A., Vescio, R.A., Mateos, M.V., Mazumder, A., Orlowski, R.Z., Sutherland, H.J., Blade, J., Scott, E.C., Oriol, A., Berdeja, J., Gharibo, M., Stevens, D.A., LeBlanc, R., Sebag, M., Callander, N., Jakubowiak, A., White, D., de la Rubia, J., Richardson, P.G., Lisby, S., Feng, H., Uhlar, C.M., Khan, I., Ahmadi, T. & Voorhees, P.M. (2016) Daratumumab monotherapy in patients with treatment-refractory multiple myeloma (SIRIUS): an open-label, randomised, phase 2 trial. *Lancet*, **387**, 1551–1560.

Nijhof, I.S., Casneuf, T., van Velzen, J., van Kessel, B., Axel, A.E., Syed, K., Groen, R.W., van Duin, M., Sonneveld, P., Minnema, M.C., Zweegman, S., Chiu, C., Bloem, A.C., Mutis, T., Lokhorst, H.M., Sasser, A.K. & van de Donk, N.W. (2016) CD38 levels are associated with response and complement inhibitors contribute to resistance in myeloma patients treated with daratumumab. *Blood*, doi:10.1182/blood-2016-03-703439

A phase II trial of TBL-12 sea cucumber extract in patients with untreated asymptomatic myeloma

The sphingolipids/glycosides in sea cucumbers have several antitumour properties (Zou *et al*, 2003) including anti-angiogenesis (Tian *et al*, 2005), direct tumour cytotoxicity (Sugawara *et al*, 2006) and, of particular relevance to multiple myeloma (MM), the inhibition of osteoclastogenesis (Kariya *et al*, 2004). TBL-12, a sea cucumber extract, has been commercially available since 1981 and has been used by human subjects as a food supplement without any reported toxicities (Taiyeb-Ali *et al*, 2003). We investigated the safety and efficacy of TBL-12 in patients with asymptomatic (smouldering) MM (ASMM) in this pilot phase II study.

Inclusion criteria included ≥10% bone marrow plasmacytosis and no associated hypercalcaemia, renal insufficiency, anaemia, lytic bone lesions or recurrent infections. Patients were also required to have a serum monoclonal protein ≥10 g/l and/or urine M-spike ≥200 mg/24 h, Karnofsky performance status ≥80%, no active infections, and be receiving steroids less than the equivalent of 10 mg prednisone daily for other medical conditions.

This study was approved by the St. Vincent's and Mt. Sinai institutional review boards and all subjects provided written informed consent prior to study participation.

Subjects were given open label TBL-12, formulated as a liquid gel (manufactured by Unicorn Pacific Corporation, Port Vila, Vanuatu) and kept frozen until the time of consumption. Patients ingested 2 units of 20 ml twice per day, for a total of 80 ml per day. Disease parameters were monitored monthly and treatment was continued until disease progression as defined by standard International Myeloma Working Group (IMWG) criteria for serological progression or due to end organ symptoms, whichever occurred sooner.

A total of 20 patients participated in this study; baseline characteristics and risk stratification were based on previously published criteria (Kyle *et al*, 2007; Dispenzieri *et al*, 2008; Mateos *et al*, 2013) (Table I).

Based on Kyle *et al* (2007), this population was at high risk for disease progression (PD) with 14 patients having a serum M-spike ≥30 g/l and ≥10% bone marrow plasma cells (BMPC). With the additional high-risk criteria of a serum free light chain (FLC) ratio <0·125 or >8, as defined by Dispenzieri *et al* (2008), 13 patients were high risk. Of the 7 remaining patients, characterized as intermediate risk by the Dispenzieri criteria, 4 had markedly elevated FLC ratios (range 307–12 357) and one had a 9·2 g urine M-spike and, under current criteria, would be defined as a light chain smouldering MM (>0·5 g/24 h urine M-spike and ≥10% BMPC) (Kyle *et al*, 2014). Finally, although immunophenotyping was not performed in this study, 16 patients met the high-risk criteria by serum or urine paraprotein criteria alone and the remaining 4 were intermediate risk, as defined by Mateos *et al* (2013).

Of note, many patients met the criteria for active myeloma based on the new IMWG consensus criteria (Rajkumar *et al*, 2014), including 4 with bone marrow plasmacytosis ≥60% and 10 patients with an involved:uninvolved serum FLC ratio of ≥100, which are associated with 95 and 80% risk of progression at 2 years, respectively. A total of 12 patients had one or both of these criteria.

Compliance was excellent and the treatment was well tolerated with only grade 1 nausea. There was one severe adverse event, a pneumococcal pneumonia requiring hospitalisation, which was felt to be unrelated to study treatment. One subject came off study due to non-compliance at Cycle 6 and 2 subjects came off study at Cycles 8 and 51 because they re-located and were no longer able attend follow-up appointments.

Table I. Baseline demographic, clinical characteristics and risk stratification

Characteristics	TBL-12 (n = 20)
Age	Years
Median (range)	58 (22–75)
Sex	n (%)
Male	11 (55)
Female	9 (45)
Isotype	n (%)
IgG	14 (70)
IgA	5 (25)
Light Chain Only	1 (5)
Immunoparesis	17 (85)
Monoclonal Protein component	
Serum	g/l
Median (range)	34·1 (0–46·3)
IgG M-Spike >30 g/l	13 (65)
IgA M-Spike >20 g/l	2 (10)
Urine	g/24 h
Median (range)	0·04 (0–9·2)
Bence-Jones protein >500 mg/24 h	3 (15)
FLC ratio <0·125 or >8	19 (95)
Level of plasma-cell bone marrow infiltration	%
Median (range)	38 (10–90)
MM-defining Events by 2014 IMWG criteria	n
BMPC ≥60%	4
Serum involved:uninvolved FLC ratio ≥100	10
Either BMPC >60% or involved:uninvolved FLC ratio >100	12
Risk Factors based on Kyle criteria (Kyle *et al*, 2007)	n
High (2)	14
Intermediate (1)	6
Risk Factors based on Dispenzieri criteria (Dispenzieri *et al*, 2008)	
High (3)	13
Intermediate (2)	7
Risk factors based on Mateos criteria (Mateos *et al*, 2013)	
High (2)	16
Intermediate (1)	4

Patients are assigned 1 point for meeting each of the following criteria:
Kyle *et al* (2007): Serum M-spike ≥30 g/l, BMPC ≥10%.
Dispenzieri *et al* (2008): Serum M-spike ≥30 g/l, BMPC ≥10%, and serum FLC ratio either <0·125 or >8.
Mateos *et al* (2013): high risk is defined as BMPC ≥ 10% and serum M-spike ≥30 g/l (IgA ≥20 g/l, urine Bence Jones protein >1 g/24 h) or any one of the four mentioned criteria, plus immunoparesis and >95% phenotypically aberrant plasma cells. Note: flow cytometric detection of aberrant plasma cells was not available for this study.
BMPC, Bone Marrow Plasma Cells; FLC, Free Light Chain.

Patients completed between 2 and 64 cycles. Two patients remained on study at the time of administrative study closure, when they had completed 61 and 64 cycles. The best response to date has been a minimal response (MR) for 5 cycles. A total of 15 patients came off study for PD after a median of 15 cycles (range 2–54). The reasons for PD included hypercalcaemia (n = 1), acute renal insufficiency (n = 1, after 2 cycles with 9·2 g urine M-spike at screening), anaemia (n = 8, 1 after 3 cycles with 90% BMPC at screening), recurrent infections (n = 1), serological PD greater than 25% from baseline (n = 2), and new lytic lesions on magnetic resonance imaging and positron-emission tomography-computed tomography (n = 2).

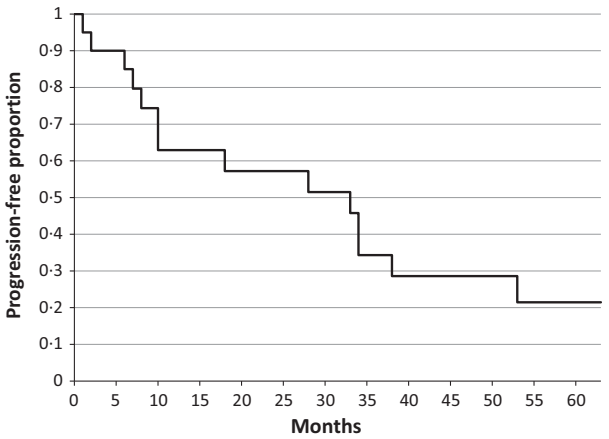

Fig 1. Kaplan–Meier progression-survival curve of patients on TBL-12 (n = 20)

There have been 2 deaths to date, neither while on study treatment. The remaining 18 patients remain alive and therefore the median overall survival has not been reached, with follow-up ranging from 16 to 72 months.

The median progression-free survival (PFS) by Kaplan–Meier survival analysis for all patients was 33 months (Figure 1). The median PFS for the 13 high-risk patients based on the criteria of Dispenzieri *et al* (2008) was 29 months; the median PFS for the 7 intermediate risk patients was not reached. These compare favourably to the expected PFS for these groups of 24 and 60 months respectively (Dispenzieri *et al*, 2008). The median PFS of the 16 high-risk patients as defined according to Mateos *et al* (2013) was 28·5 months, which was better than the 21 months observed for the control arm of that study (Mateos *et al*, 2013). Finally, the median PFS for 12 patients meeting the recent IMWG criteria for active myeloma was 11 months. Four of these patients remain asymptomatic even after a median of 10 years from diagnosis. An additional patient, considered intermediate risk according to Dispenzieri *et al* (2008), has remained asymptomatic for 7·5 years.

TBL-12 was well tolerated in this pilot study of high- and standard-risk ASMM patients, with a median PFS that compares favourably to the expected outcomes of high-risk ASMM patients (Dispenzieri *et al*, 2008) and the observation group reported by Mateos *et al* (2013). Larger prospective studies are required to further define the efficacy of TBL-12 in ASMM. Additionally, the absence of death, including in those four patients defined as having active myeloma by IMWG criteria that remained asymptomatic 6 years after

study enrollment, further highlights the importance of prospective randomized phase 3 studies to determine if there is any overall survival benefit from early intervention in ASMM.

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Author contributions

AC provided conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, original draft creation, review and edits of the manuscript, visualization, supervision and project administration of the project. AM provided conceptualization, methodology, review and edits of the manuscript and research investigation of the project. KL provided formal analysis of the data, provision of study materials, research data management and creation of the tables and figures, review and edits of the manuscript, and preparation of the data presentation. DC provided research and investigation process, resources, review and edits of the manuscript, and visualization of the project. ZG provided formal analysis of the data, resources, data management, review and edits of the manuscript and visualization of the project. SJ provided conceptualization, methodology, research and investigation process, review and edits of the manuscript, supervision and funding acquisition of the project. All co-authors gave final approval of the published version.

Conflict of interest

The following authors have served as paid consultants within the past 2 years for the identified companies: AC for Array BioPharma; AC, JS for Novartis; AC, AM for Millennium/Takeda; AC, AM for Amgen-Onyx; AC, AM for Celgene; AC, JS for Janssen; JS for Bristol Myer Squibb, AM for Teva Pharma; AM for Ipsen Biopharm. The following authors have no conflicts to disclose: KL, DC, and ZG.

Ajai Chari[1]
Amitabha Mazumder[2]
Kenneth Lau[1]
Donna Catamero[1]
Zachary Galitzeck[1]
Sundar Jagannath[1]
[1]Icahn School of Medicine at Mount Sinai, and [2]NYU Langone Medical Center, New York, NY, USA
E-mail: ajai.chari@mssm.edu

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References

Dispenzieri, A., Kyle, R.A., Katzmann, J.A., Therneau, T.M., Larson, D., Benson, J., Clark, R.J., Melton, L.J. III, Gertz, M.A., Kumar, S.K., Fonseca, R., Jelinek, D.F. & Rajkumar, S.V. (2008) Immunoglobulin free light chain ratio is an independent risk factor for progression of smoldering (asymptomatic) multiple myeloma. *Blood*, **111**, 785–789.

Kariya, Y., Mulloy, B., Imai, K., Tominaga, A., Kaneko, T., Asari, A., Suzuki, K., Masuda, H., Kyogashima, M. & Ishii, T. (2004) Isolation and partial characterization of fucan sulfates from the body wall of sea cucumber Stichopus japonicus and their ability to inhibit osteoclastogenesis. *Carbohydrate Research*, **339**, 1339–1346.

Kyle, R.A., Remstein, E.D., Therneau, T.M., Dispenzieri, A., Kurtin, P.J., Hodnefield, J.M., Larson, D.R., Plevak, M.F., Jelinek, D.F., Fonseca, R., Melton, L.J. III & Rajkumar, S.V. (2007) Clinical course and prognosis of smoldering (asymptomatic) multiple myeloma. *New England Journal of Medicine*, **356**, 2582–2590.

Kyle, R.A., Larson, D.R., Therneau, T.M., Dispenzieri, A., Melton, L.J. III, Benson, J.T., Kumar, S. & Rajkumar, S.V. (2014) Clinical course of light-chain smouldering multiple myeloma (idiopathic Bence Jones proteinuria): a retrospective cohort study. *The Lancet. Haematology*, **1**, e28–e36.

Mateos, M.V., Hernandez, M.T., Giraldo, P., de la Rubia, J., de Arriba, F., Lopez Corral, L., Rosinol, L., Paiva, B., Palomera, L., Bargay, J., Oriol, A., Prosper, F., Lopez, J., Olavarria, E., Quintana, N., Garcia, J.L., Blade, J., Lahuerta, J.J. & San Miguel, J.F. (2013) Lenalidomide plus dexamethasone for high-risk smoldering multiple myeloma. *New England Journal of Medicine*, **369**, 438–447.

Rajkumar, S.V., Dimopoulos, M.A., Palumbo, A., Blade, J., Merlini, G., Mateos, M.V., Kumar, S., Hillengass, J., Kastritis, E., Richardson, P., Landgren, O., Paiva, B., Dispenzieri, A., Weiss, B., LeLeu, X., Zweegman, S., Lonial, S., Rosinol, L., Zamagni, E., Jagannath, S., Sezer, O., Kristinsson, S.Y., Caers, J., Usmani, S.Z., Lahuerta, J.J., Johnsen, H.E., Beksac, M., Cavo, M., Goldschmidt, H., Terpos, E., Kyle, R.A., Anderson, K.C., Durie, B.G. & Miguel, J.F. (2014) International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. *The Lancet. Oncology*, **15**, e538–548.

Sugawara, T., Zaima, N., Yamamoto, A., Sakai, S., Noguchi, R. & Hirata, T. (2006) Isolation of sphingoid bases of sea cucumber cerebrosides and their cytotoxicity against human colon cancer cells. *Bioscience, Biotechnology, and Biochemistry*, **70**, 2906–2912.

Taiyeb-Ali, T., Zainuddin, S., Swaminathan, D. & Yaacob, H. (2003) Efficacy of 'Gamadent' toothpaste on the healing of gingival tissues: a preliminary report. *Journal of Oral Science*, **45**, 153–159.

Tian, F., Zhang, X., Tong, Y., Yi, Y., Zhang, S., Li, L., Sun, P., Lin, L. & Ding, J. (2005) PE, a new sulfated saponin from sea cucumber, exhibits anti-angiogenic and anti-tumor activities *in vitro* and *in vivo*. *Cancer Biology & Therapy*, **4**, 874–882.

Zou, Z.R., Yi, Y.H., Wu, H.M., Wu, J.H., Liaw, C.C. & Lee, K.H. (2003) Intercedensides A-C, three new cytotoxic triterpene glycosides from the sea cucumber Mensamaria intercedens Lampert. *Journal of Natural Products*, **66**, 1055–1060.