Clinical proof of concept for a safe and effective NF-κB-targeting strategy in multiple myeloma

Despite the recent introduction of new treatments and improvements in clinical outcomes, multiple myeloma (MM) remains a medical problem. The majority of patients relapse and die from their disease, underscoring the need for new treatments (Kumar *et al*, 2017). The nuclear factor (NF)-κB pathway is aberrantly activated in virtually all cases of MM, where it enables tumour cell survival, and is therefore considered an effective therapeutic route in MM. Yet, following an aggressive 30-year effort by the pharmaceutical industry, no specific NF-κB inhibitor has been clinically approved, due to the dose-limiting toxicities associated with the general NF-κB suppression. Agents indicated in MM, *e.g.*, proteasome inhibitors and immunomodulatory drugs, inhibit NF-κB, but also many other pathways, and neither specifically target cancer cells nor afford their clinical benefit through NF-κB inhibition (Greten *et al*, 2007; Di Donato *et al*, 2012; Begalli *et al*, 2017; Bennett *et al*, 2018). Therefore, there is a need for a fresh approach to safely block NF-κB signalling in MM.

To overcome the barrier to safe NF-κB inhibition, we developed a novel class of NF-κB-targeting therapeutics. We identified the interaction between the JNK-activating kinase, MKK7, and the NF-κB-regulated antiapoptotic factor, GADD45β (De Smaele *et al*, 2001; Papa *et al*, 2004; Tornatore *et al*, 2014; Capece *et al*, 2018), as an essential, cancer-restricted survival module downstream of NF-κB in MM. Accordingly, GADD45β is selectively highly expressed in MM cells, where it denotes shorter patient overall survival (Tornatore *et al*, 2014). We developed the first-in-class GADD45β/MKK7 inhibitor, DTP3, which selectively kills MM cells by inducing MKK7/JNK-dependent apoptosis, *ex vivo* and *in vivo*, without toxicity to normal tissues (Tornatore *et al*, 2014). Further preclinical investigations demonstrated that DTP3 combines on-target-selective pharmacology and favourable drug-like properties with tolerability and none of the preclusive toxicities of conventional IKKβ/NF-κB-targeting agents (Tornatore *et al* unpublished observations). Due to this unique mode of action, DTP3 represents a significant opportunity for managing MM patients.

The success rate for novel drug candidates in oncology from first-in-human trial to market registration is only ~5%, due to the heterogeneity of tumours and absence of drug-specific biomarkers, clearly demonstrating therapeutic target engagement (Kola & Landis, 2004). Considering that any GADD45β/MKK7-targeting drug would potentially benefit only a discrete subset of patients, we conducted a preclinical proof-of-concept study (REC 11/LO/1628) to develop a companion biomarker platform capable of assessing pharmacodynamic response in the earliest stages of the DTP3 clinical development.

The current *Letter* reports the clinical proof-of-concept for an NF-κB-targeting strategy as a safe and mechanistically effective novel therapy in MM patients. We sought to first verify the cancer-selective mechanistic specificity of DTP3 in primary MM cells (Supplementary Methods). DTP3 was effective in inducing JNK phosphorylation, denoting therapeutic target engagement and caspase-3 cleavage, an apoptosis hallmark, in malignant $CD138^+$ cells, *ex vivo* (Fig 1A). By contrast, DTP3 produced no such effects in healthy $CD20^+$ cells nor in peripheral blood mononuclear cells (PBMCs) from the same patients, even at 30-fold higher concentrations (Fig 1B, C). Given the wide distribution and overall high levels of *GADD45B* expression in MM cells (Fig 1D, Supplementary Figure S1), we evaluated whether the cancer-selective pharmacodynamic response to DTP3 depended on *GADD45B* expression. As shown in Fig 1E, in 13 samples of malignant $CD138^+$ cells which responded to DTP3, *GADD45B* expression was significantly higher than in any of the unresponsive $CD138^+$-cell samples or any sample of normal $CD20^+$ cells or PBMCs. Hence, the capacity of DTP3 to trigger JNK-driven apoptosis in primary MM cells correlates with a high significance with the *GADD45B* expression levels (Fig 1E). These results demonstrate that the on-target-specific therapeutic response to DTP3 can be monitored and potentially predicted by an objective measurement of *GADD45B* expression and JNK-dependent apoptosis in tumour cells. Interestingly, upon clinical validation, this approach could inform a patient stratification platform predictive of objective clinical response to support the clinical development of DTP3.

Accordingly, we initiated the first-in-human phase-I/IIa trial of DTP3 to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of this novel NF-κB-targeting therapeutic in patients with relapsed or refractory MM (EudraCT: 2015-003459-23; Supplementary Methods). Three single-patient, dose-escalation cohorts have been investigated to date, evaluating DTP3 at the dose levels of 0·5, 1 and 2 mg/kg, administered by rapid intravenous infusion three times a week (Fig 2A, Supplementary Figure S2). All patients had progressive disease at the point of entry into the study, despite having received multiple prior lines of therapy. All three patients completed their first 28-day treatment cycle with no complications. No reportable adverse effects, nor

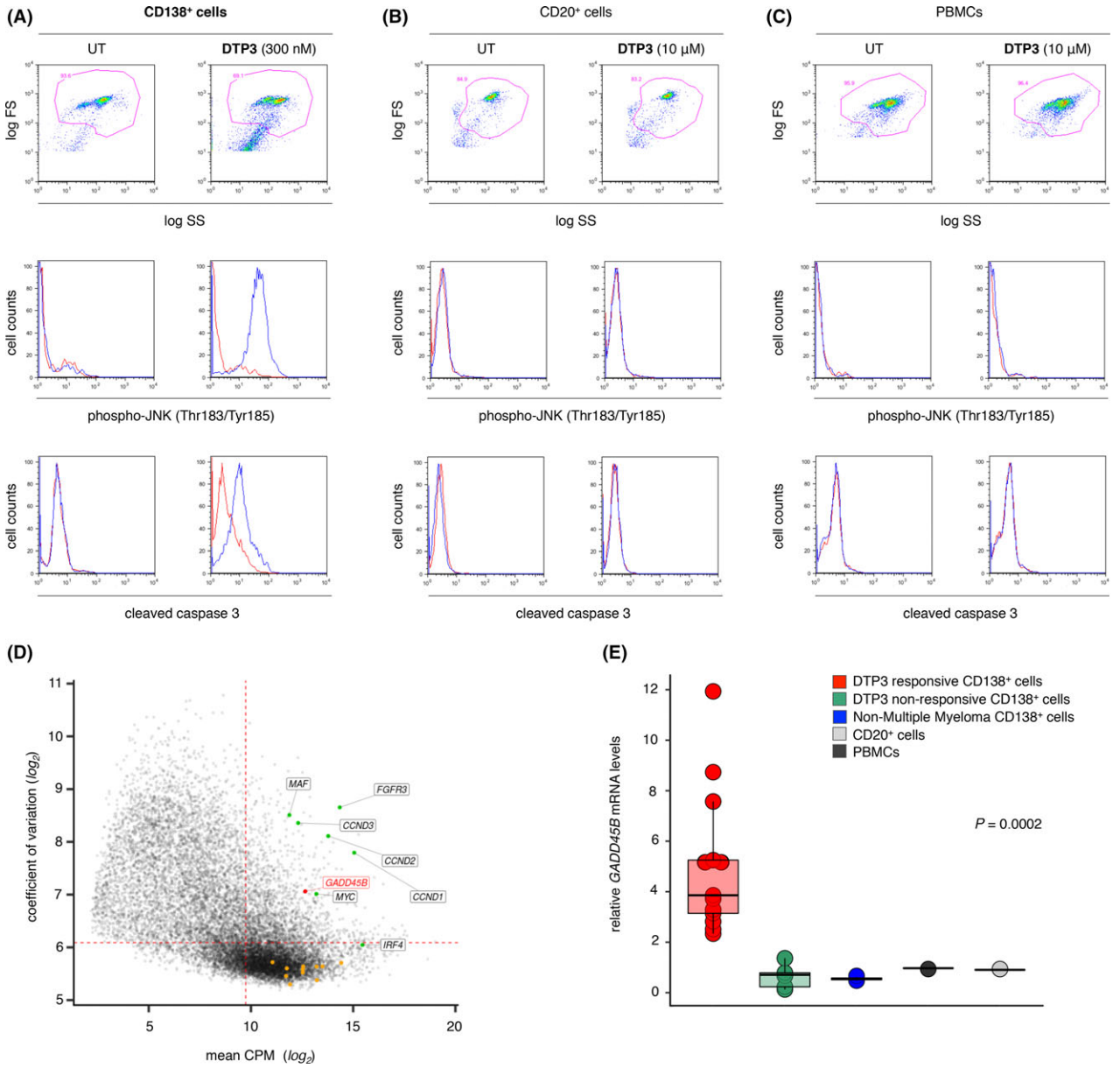

Fig 1. Selective therapeutic target engagement by DTP3 in primary MM cells expressing *GADD45B*. (**A–C**) Dot plots (top) and histograms (middle, bottom) of fluorescence-activated cell sorting (FACS) analysis showing phospho-JNK (middle) and cleaved caspase 3 (bottom) in representative malignant CD138$^+$ cells (**A**), and normal CD20$^+$ B cells (**B**) and peripheral blood mononuclear cells (PBMCs) (**C**) isolated from the same multiple myeloma (MM) patient and treated with DTP3, *ex vivo*, at the indicated concentrations or left untreated (UT) for 24 h, as shown. Depicted are the signals from intracellular staining with phospho-JNK-specific or cleaved caspase 3-specific antibodies (blue) or appropriate immunoglobulin isotype controls (red). FS: forward scatter; SS: sideways scatter. **D**, Analysis of the relationship between mean mRNA expression and coefficient of variation for the 14 351 protein-coding genes annotated in the MM patients reported in the CoMMpass dataset. Coloured are *GADD45B* (red), and select proto-oncogenes (green) and housekeeping genes (orange). Dotted lines denote median values. **E**, qRT-PCR analysis showing the relative *GADD45B* mRNA expression in CD138$^+$ cells from either MM (red or green) or non-MM (blue) patients and normal CD20$^+$ cells (light grey) and PBMCs (dark grey). CD138$^+$ cells that are sensitive (red) or insensitive (green or blue) to treatment with DTP3 *ex vivo* are as shown. Boxes extend from the highest and lowest values of the first and third quartiles, respectively. Whiskers extend to the extreme values within 1·5 interquartile ranges of the upper and lower quartile. Lines within boxes denote medians.

any grade 2 or greater toxicity were recorded at any point, in any patient, indicating that DTP3 was tolerated at all dose levels investigated (Fig 2A). Notably, Patient 002 demonstrated a reduction in serum free light-chain and paraprotein levels in response to DTP3, consistent with stable disease, according to the International Myeloma Working

(A)

Patient ID	Dose level	Reportable Adverse Effects	Full treatment cycles (no.)	Light chain and paraprotein reduction	PD response
001	0.5 mg/kg	None	1	No	Yes
002	1.0 mg/kg	None	3	Yes	Yes
003	2.0 mg/kg	None	1	No	No

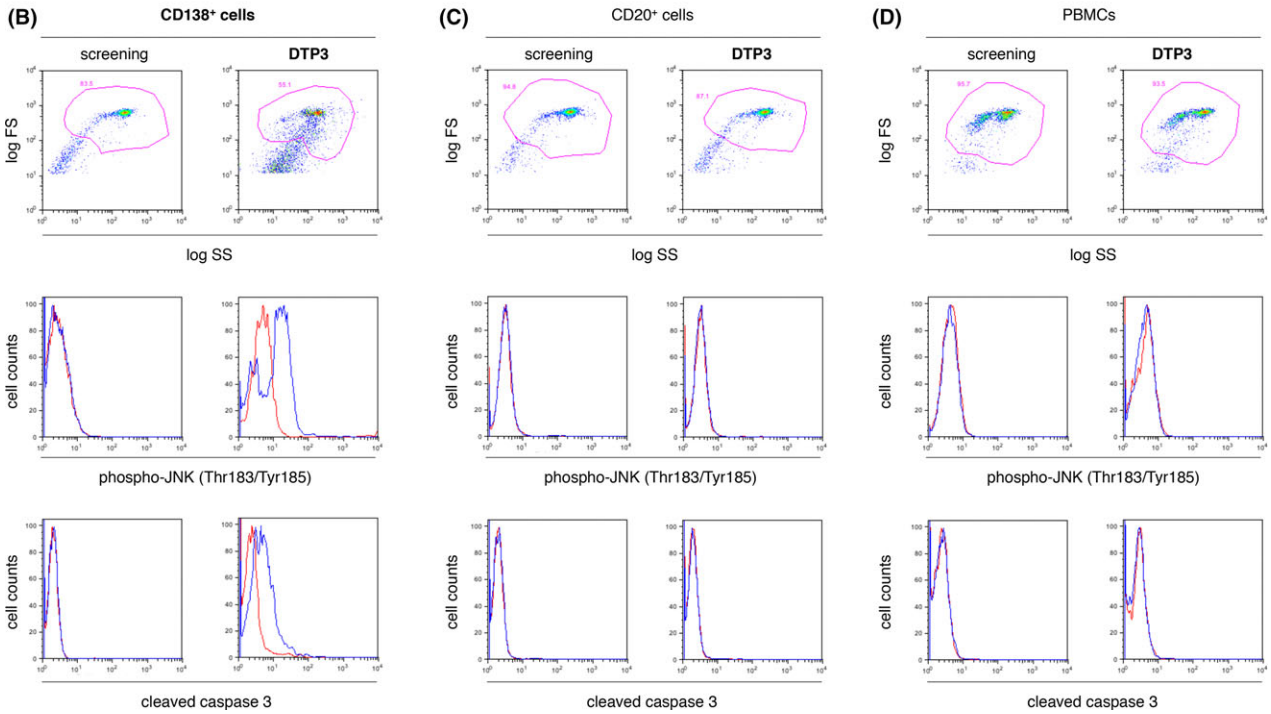

Fig 2. Clinical safety and therapeutic proof-of-mechanism in MM patients from the first-in-human study of DTP3. (**A**) Shown are the main clinical results from the first three patients investigated in the first-in-human study of DTP3. (**B–D**) Dot plots (top) and histograms (middle, bottom) of fluorescence-activated cell sorting (FACS) analysis showing phospho-JNK (middle) and cleaved caspase 3 (bottom) in malignant CD138$^+$ cells (**B**), and normal CD20$^+$ B cells (**C**) and peripheral blood mononuclear cells (PBMCs) (**D**) isolated from Patient 001. Analyses were performed at the screening (left) or 18 to 36 h following the last administration of treatment with DTP3 at the starting dose level of 0·5 mg/kg on days 1, 3, 5 and 8 (post-treatment, right), as shown. Depicted are the signals resulting from intracellular staining with phospho-JNK-specific or cleaved caspase 3-specific antibodies (blue) or appropriate immunoglobulin isotype controls (red). FS: forward scatter; PD: pharmacodynamic; SS: sideways scatter.

Group criteria. This patient was maintained on DTP3 therapy for a total of three 4-week treatment cycles before developing progressive disease, in the absence of any adverse effect (Fig 2A).

To evaluate the pharmacodynamic response to DTP3, we analysed blood and bone marrow samples collected 18–36 h following administration of dose 4, on day 8 of treatment. Strikingly, Patient 001, who had been treated at the starting dose of 0·5 mg/kg, demonstrated a distinct shift in the signals corresponding to JNK phosphorylation and caspase-3 cleavage in MM (CD138$^+$) cells, but, crucially, neither in normal CD20$^+$ cells, nor in PBMCs concurrently isolated from blood (Fig 2B–D). As expected, no such signals denoting JNK activation or apoptosis were detected in MM cells, B lymphocytes or PBMCs isolated at screening, prior to treatment with DTP3 (Fig 2B–D). Hence, consistent with its *ex-vivo* mode of action and on-target-selective pharmacology (Fig 1A–C; Tornatore *et al* unpublished observations), DTP3 demonstrated the clinical capacity to trigger a cancer-specific pharmacodynamic response, inducing JNK-driven apoptosis in MM, but not normal cells (Fig 2B), thereby establishing clinical proof-of-mechanism. Notably, DTP3 produced these clinical effects in refractory MM patients, as a single agent, with a potential 40-fold margin for dose escalation in the trial.

Collectively, the preclinical data and encouraging initial clinical results introduce DTP3 as a first-in-class NF-κB-targeting therapeutic with a novel mode of action in oncology.

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Disclosure of Potential Conflict of Interest

G.F., L.T. and M.R. are named inventors on patents relating to this research.

Authorship

L.T. G.A., R.B., H.E.O., M.F.K., A.W. J.F.A. H.W.A. and G.F. designed experiments. L.T., D.C., F.B., D.V. and J.B. performed experiments. L.T., D.D., G.A., L.E.C., J.K., M.T., N.A., J.F.A.; H.W.A. and G.F. analysed data. L.T. G.A., L.E.C., J.K., M.T., N.A., S.B., J.F.A. H.W.A. and G.F. contributed to the preparation of the regulatory documentation for MHRI approval. A.L., A.S., D.R., M.R., A.C., M.O., M.J.A., R.S. K., I.G., R.B. and H.W.A. contributed clinical samples or key reagents. G.F. and L.T. wrote the paper. D.C., D.D. and H.W.A. contributed to writing the paper.

Laura Tornatore[1,†]
Daria Capece[1,†]
Daniel D'Andrea[1,†]
Federica Begalli[1]
Daniela Verzella[1]
Jason Bennett[1]
Gary Acton[2]
Elizabeth A. Campbell[3]
James Kelly[4]
Michael Tarbit[5]
Nigel Adams[6]
Selina Bannoo[1]
Antonio Leonardi[7]
Annamaria Sandomenico[8]
Domenico Raimondo[9]
Menotti Ruvo[8]
Angela Chambery[10]
Metod Oblak[11]
Magda J. Al-Obaidi[12]
Richard S. Kaczmarski[13]
Ian Gabriel[12]
Heather E. Oakervee[14]
Martin F. Kaiser[15]
Ashutosh Wechalekar[16]
Reuben Benjamin[17]
Jane F. Apperley[18]
Holger W. Auner[18,19,‡]
Guido Franzoso[1,‡]

[1]CCSI, Department of Medicine, Imperial College London, [2]Cancer Research UK Centre for Drug Development, London, [3]C&C Management Consulting Ltd, Exmouth, [4]Alpha Preclinical Consultancy, Halifax, [5]Independent Consultant, Royston, [6]In2Phase Ltd, Welwyn Garden City, UK, [7]Department of Molecular Medicine, University of Naples Federico II, [8]IBB-CNR and CIRPeB, "Federico II" University of Naples, Naples, [9]Department of Molecular Medicine, Sapienza University of Rome, Rome, [10]DiSTABiF, University of Campania "Luigi Vanvitelli", Caserta, Italy, [11]West Middlesex University Hospital, Isleworth, Greater London, [12]Haematology Department, Chelsea and Westminster Hospital, [13]London Haematology Limited, [14]Barts Cancer Centre, St Bartholomew's Hospital London, [15]Division of Molecular Pathology, The Institute of Cancer Research, [16]Royal Free London NHS Foundation Trust, [17]Department of Haematology, King's College Hospital, [18]Centre for Haematology, and [19]Cancer Cell Protein Metabolism, Department of Medicine, Imperial College London, London, UK.
E-mails: g.franzoso@imperial.ac.uk; l.tornatore@imperial.ac.uk

[†]Joint first authors.
[‡]Co-last authors.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig S1. Selective therapeutic target engagement by DTP3 in primary multiple myeloma cells expressing *GADD45B*.

Fig S2. List of inclusion and exclusion criteria of the phase I trial (MHRA reference number: 19174/0369/001-0001).

REFERENCES

Begalli, F., Bennett, J., Capece, D., Verzella, D., D'Andrea, D., Tornatore, L. & Franzoso, G. (2017) Unlocking the NF-κB Conundrum: embracing complexity to achieve specificity. *Biomedicines*, **5**, 50.

Bennett, J., Capece, D., Begalli, F., Verzella, D., D'Andrea, D., Tornatore, L. & Franzoso, G. (2018) NF-κB in the crosshairs: rethinking an old riddle. *The International Journal of Biochemistry & Cell Biology*, **95**, 108–112.

Capece, D., D'Andrea, D., Verzella, D., Tornatore, L., Begalli, F., Bennett, J., Zazzeroni, F. & Franzoso, G. (2018) Turning an old GADDget into a troublemaker. *Cell Death and Differentiation*, **25**, 640–642.

De Smaele, E., Zazzeroni, F., Papa, S., Nguyen, D.U., Jin, R., Jones, J., Cong, R. & Franzoso, G. (2001) Induction of gadd45beta by NF-κB downregulates pro-apoptotic JNK signalling. *Nature*, **414**, 308–313.

Di Donato, J.A., Mercurio, F. & Karin, M. (2012) NF-κB and the link between inflammation and cancer. *Immunological Reviews*, **246**, 379–400.

Greten, F.R., Arkan, M.C., Bollrath, J., Hsu, L.C., Goode, J., Miething, C., Göktuna, S.I., Neuenhahn, M., Fierer, J., Paxian, S., Van Rooijen, N., Xu, Y., O'Cain, T., Jaffee, B.B., Busch, D.H., Duyster, J., Schmid, R.M., Eckmann, L. & Karin,

M. (2007) NF-κB is a negative regulator of IL-1β secretion as revealed by genetic and pharmacological inhibition of IKKβ. *Cell*, **130**, 918–931.
Kola, I. & Landis, J. (2004) Can the pharmaceutical industry reduce attrition rates? *Nature Reviews Drug Discovery*, **3**, 711–715.
Kumar, S.K., Rajkumar, V., Kyle, R.A., van Duin, M., Sonneveld, P., Mateos, M.V., Gay, F. & Anderson, K.C. (2017) Multiple myeloma. *Nature Reviews Disease Primers*, **3**, 17046.
Papa, S., Zazzeroni, F., Bubici, C., Jayawardena, S., Alvarez, K., Matsuda, S., Nguyen, D.U., Pham, C.G., Nelsbach, A.H., Melis, T., De Smaele, E., Tang, W.J., D'Adamio, L. & Franzoso, G. (2004) GADD45 beta mediates the NF-κB suppression of JNK signalling by targeting MKK7/JNKK2. *Nature Cell Biology*, **6**, 146–153.
Tornatore, L., Sandomenico, A., Raimondo, D., Low, C., Rocci, A., Tralau-Stewart, C., Capece, D., D'Andrea, D., Bua, M., Boyle, E., van Duin, M., Zoppoli, P., Jaxa-Chamiec, A., Thotakura, A.K., Dyson, J., Walker, B.A., Leonardi, A., Chambery, A., Driessen, C., Sonneveld, P., Morgan, G., Palumbo, A., Tramontano, A., Rahemtulla, A., Ruvo, M. & Franzoso, G. (2014) Cancer-selective targeting of the NF-κB survival pathway with GADD45β/MKK7 inhibitors. *Cancer Cell*, **26**, 495–508.

Disease site as a prognostic factor for mycosis fungoides: an analysis of 2428 cases from the US National Cancer Database

Mycosis fungoides (MF) is the most common form of cutaneous T-cell lymphoma (CTCL). Studies on various types of CTCL have shown the prognostic significance of primary disease site (Su *et al*, 2017; Nguyen *et al*, 2018), while few studies have investigated the prognostic potential of primary disease site for MF. The primary aim of our study is to compare the survival outcomes of patients with MF among different primary disease sites, while controlling for confounders.

The National Cancer Database (NCDB) was used to ascertain de-identified patients with MF using the International Classification of Disease – Oncology, third edition (ICD-O3) morphological code 9700 diagnosed between 2004 and 2014. Information extracted from the user participant file included demographics, clinical information, the first course of treatment received that contribute to the long-term control of the cancer, length of follow-up and overall survival (OS). Patients with no known primary disease site, follow-up, treatment or income data were excluded.

A Kaplan–Meier estimate was used to evaluate OS, and differences in survival were examined by the log-rank test. Univariate and multivariate Cox proportional hazards regression was used to evaluate independent predictors associated with survival. Pairwise comparisons of different primary sites in OS were performed using propensity score matching to account for confounders (MatchIt package [https://gking.harvard.edu/matchit], R version 2.15.1 [https://www.r-project.org/]). Statistical significance was defined as $P \leq 0{\cdot}05$. Analysis was performed using IBM SPSS Statistics 22 (IBM Corporation, North Castle, NY, USA), and the figure was generated with GraphPad Prism 6 (GraphPad Software, La Jolla, CA, USA).

Survival of the MF patient cohort relative to the expected survival in the age- and gender-matched general US population was analysed with the 'strs' command in STATA/SE 13 (Stata Corporation, College Station, TX, USA) as detailed previously by Dickman *et al* (2015). US Census Bureau and the Centers for Disease Control and Prevention databases (www.census.gov; www.cdc.gov) were used to obtain information on the yearly age- and sex-matched segments of the US population.

A total of 2428 patients with MF identified using the NCDB met the inclusion criteria. Trunk, upper limbs, lower limbs, head and neck and overlapping lesion of skin constituted 36·0% (n = 873), 12·2% (n = 297), 22·0% (n = 535), 10·1% (n = 244) and 19·7% (n = 479) of the entire cohort, with the mean age at diagnosis being 58·2, 57·3, 57·1, 60·5 and 58·5 years, respectively.

The 5-year OS rate of the entire MF cohort was 84·8%. Significant differences in survival outcomes were present among different primary sites, with 5-year survival rates of 85·5%, 82·5%, 88·2%, 79·5%, and 78·3% for patients with disease localized to trunk, upper limbs, lower limbs, head and neck and overlapping lesion of skin (Fig 1). The 5- and 10-year survival relative to the expected survival of an age- and gender-matched US standard population was 90·9% (95% confidence interval [CI] 88·5–93·0%) and 87·8% (95% CI 82·4–92·9%), respectively. Stratified by primary disease sites, 5-year relative survivals to an age- and gender- matched US standard population were 93·0% (95% CI 89·2–96·3%), 87·0% (95% CI 79·6–93·0%), 97·0% (95% CI 92·0–101·0%), 88·5% (95% CI 80·0–95·4%) and 84·1% (95% CI 78·4–89·1%) for patients with disease localized to trunk, upper limbs, lower limbs, head and neck and overlapping lesion of skin.

On multivariate Cox regression analysis, older age ($P < 0{\cdot}001$), male gender ($P < 0{\cdot}001$), African American race ($P = 0{\cdot}044$), Charlson-Deyo score of 1 ($P = 0{\cdot}018$) or >2 ($P = 0{\cdot}019$), higher clinical stage ($P < 0{\cdot}001$), the receipt of chemotherapy ($P < 0{\cdot}001$) and immunotherapy ($P = 0{\cdot}048$) were associated with shorter OS (Table I). Academic facility type ($P = 0{\cdot}006$) and private insurance status ($P < 0{\cdot}001$) were associated with longer OS. Patients with disease localized to the trunk (Hazard ratio [HR] 1·378, 95% CI: 1·045–1·817, $P = 0{\cdot}023$), upper limbs (HR 1·727, 95% CI: