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Supplementary appendix

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Supplement to: Rajan A, Carter CA, Berman A, et al. Cixutumumab for patients with recurrent or refractory advanced thymic epithelial tumours: a multicentre, open-label, phase 2 trial. *Lancet Oncol* 2014; published online January 15. http://dx.doi.org/10.1016/S1470-2045(13)70596-5.

Supplementary appendix

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Additional methods

Patients

Adequate organ and bone marrow function was defined as: leucocytes $>3\times10^9$ cells per L, absolute neutrophil count $>1{\cdot}5\times10^9$ cells per L, haemoglobin >90 g/L, platelets $>100\times10^9$ cells per L, total bilirubin ≤1·5 times institutional upper limit of normal (ULN), aspartate aminotransferase or alanine aminotransferase <3 times institutional ULN (5 times if liver function test elevations are due to liver metastases), and creatinine ≤1·5 times institutional ULN (or creatinine clearance >1 mL per s/m^2 for patients with creatinine levels above institutional normal).

Patients with symptomatic brain metastases, fasting blood glucose ≥6·7 mmol/L or above the institutional ULN, major surgery, radiation therapy, chemotherapy, biological therapy, or hormonal therapy within 4 weeks of enrolling, and unresolved residual toxic effects related to previous treatment were ineligible for this study. Concurrent steroids for myasthenia gravis or other thymic epithelial tumour-related paraneoplastic conditions were permitted provided the dose was stable for ≥8 weeks prior to enrolment.

Procedures

Baseline evaluation consisted of a complete history and physical examination, tumour imaging with a CT scan of the chest, abdomen and pelvis and PET scan, and laboratory assessment including a complete blood count, prothrombin time, partial thromboplastin time, serum chemistries, lipid profile, amylase, lipase, HbA1c, IGF-1 levels, quantitative immunoglobulin assessment, urinalysis, and electrocardiogram.

Although pharmacokinetic analysis was proposed initially, it was not done after completion of the study since pharmacokinetic data were available for every 3-week dosing of cixutumumab by the time enrolment was completed to this trial.

Pharmacodynamic analysis

For multiparameter flow cytometric analysis, mononuclear cells were obtained by centrifugation and the cells were viably frozen until analysis. For assessment of interferon γ expression in CD4+ T cells, mononuclear cells were incubated with phorbol myristate acetate, ionomycin, and monensin for 4 h, stained for CD4 and interferon γ, and analysed in a MACSQuant flow cytometer (Miltenyi Biotec, Bergisch Gladbach, Germany). Peripheral blood populations were defined as central memory CD4 T cells (CD4+ CCR7+, CD27+, CD45RA-), naive CD4 T cells (CD4+ CCR7+, CD27+, CD45RA+), regulatory T cells (Tregs; CD4+CD25hiFoxp3+), and analysed in an LSR II (BD Biosciences). Circulating endothelial cells (CECs; CD45–, CD31+, CD146+ CD133–) and circulating endothelial progenitor cells (CEPs; CD45–, CD31+, CD146– CD133+) were analysed in a MACSQuant flow cytometer. All flow cytometric data were analysed using FlowJo software (Tree Star, Ashland, OR, USA).

Statistical analysis

The association between a variety of clinical and laboratory parameters and response and survival was determined using the Kaplan-Meier method and a log-rank test. As a total of 44 survival analyses were performed, p values for these analyses need to be interpreted in that context. For parameters obtained at multiple timepoints, the association with survival according to values at cycle 1, day 1 (CID1), cycle 2, day 1 (C2D1), and the difference between the two timepoints were all evaluated. Paired differences in total and free IGF-1, and in IGF-1R, between baseline and values at later timepoints were evaluated for significance using a Wilcoxon signed rank test.

Separately by histology, responses were divided into three categories as partial response, stable disease, and progressive disease, or into two response groups: partial response and no response. A Jonckheere-Terpstra test for trend was used to assess the significance of the trends in the changes in CEPs and CECs over the three response categories, whereas a Wilcoxon rank sum test was used to compare the changes in CEPs and CECs when only two response groups were compared.

All p values are two-tailed and presented without any adjustment for multiple comparisons.

	Ethnic origin	WHO subtype	Cycles (n)	New or worsening* autoimmune disease during treatment	Previous regimens of systemic treatment (n)	Previous radiation therapy	Previous surgery	Presence of extra-thoracic metastases	Time to response (months)
57-year-old man	White	B3	46	No	3	Yes	Yes	No	19·5
52-year-old man	White	B2	18	No	4	No	Yes	Yes	8·5
39-year-old woman	White	B1	6	Yes	3	Yes	No	Yes	3
42-year-old man	Black	AB	24	Yes	2	No	Yes	No	4·5
59-year-old woman	White	B2	15	Yes	2	No	Yes	No	7·5

*Details of worsening autoimmune disease during treatment are provided in table 4.

Supplemental Table 1: **Details of partial responders**

	Response or disease stabilisation	Duration of response or disease stabilisation (months)
Patient description		
57-year-old man, B3 subtype		
PAC	No	NA
Ifosfamide	Yes	4
Belinostat*	Yes	11
52-year-old man, B2 subtype		
Saracatinib*	No	NA
Belinostat*	No	NA
Paclitaxel	Yes	4
39-year-old woman, B1 subtype		
ADOC	Yes	35
Pemetrexed	Yes	9
Pemetrexed	Yes	7
42-year-old man, AB subtype		
Pemetrexed	Yes	23
59-year-old woman, B2 subtype		
ADOC	Yes	16
Carboplatin plus paclitaxel	Yes	4

NA=not applicable; PAC=cisplatin, doxorubicin, cyclophosphamide. ADOC=cisplatin, doxorubicin, cyclophosphamide, vincristine. *Clinical trial.

Supplemental Table 2: **Previous systemic treatment for recurrent disease in patients responding to cixutumumab**

	Patients (n)	Median (IQR) concentration	p (paired)*
Free IGF-1 in serum (nmol/L)			
Baseline, day 1	24	0·1 (0·02–0·11)	NA
Cycle 1, day 3	23	0·3 (0·22–0·41)	<0·0001
Cycle 1, day 7	22	0·5 (0·32–0·63)	<0·0001
Cycle 1, day 14	21	0·4 (0·25–0·57)	<0·0001
Total IGF-1 in serum (nmol/L)			
Baseline, day 1	24	7·4 (2·3–13·0)	NA
Cycle 1, day 3	23	13·0 (6·5–20·5)	0·0001
Cycle 1, day 7	22	16·1 (11·0–28·6)	<0·0001
Cycle 1, day 14	21	18·3 (10·3–29·0)	<0·0001
IGF-1R in PBMC (pmol/g)			
Baseline, day 1	13	8·1 (6·2–13·0)	NA
Cycle 1, day 3	13	6·3 (4·9–9·1)	0·006
Cycle 1, day 7	13	6·3 (3·9–8·6)	0·006
Cycle 2, day 1	13	5·0 (3·5–7·0)	0·001

NA=not applicable. PBMC=peripheral blood mononuclear cells. *Wilcoxon signed rank test.

Supplemental Table 3: **Concentrations of IGF-1 in serum and IGF-1R in PBMCs before and after treatment with cixutumumab**

	Patients (n)	Median (IQR) amount of phospho-AKT in PBMCs (RU)	p (paired)*
Baseline, day 1	13	8·1 (5·9–12·3)	NA
Cycle 1, day 3	13	7·0 (6·3–10·4)	0·69
Cycle 1, day 7	13	8·4 (6·1–13·4)	0·60
Cycle 2, day 1	13	8·3 (5·9–14·1)	0·61

NA=not applicable. PBMCs=peripheral blood mononuclear cells. RU=relative unit. *Student's *t* test.

Supplemental Table 4: **Concentrations of phospho-AKT, normalised against total protein in PBMCs before and after treatment with cixutumumab**

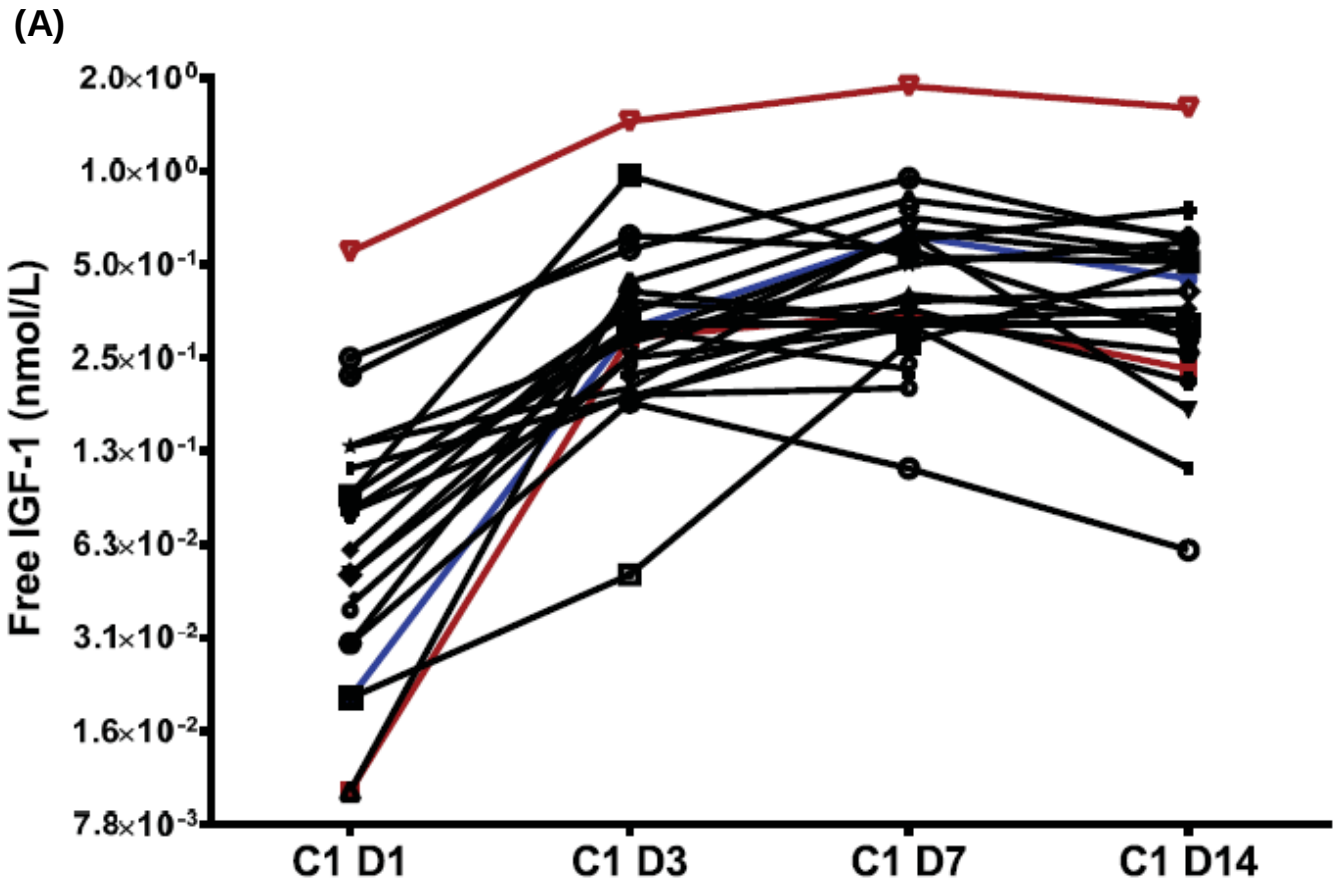
	Timepoint	Samples analysed (n)	Median (range) value	p
Interferon γ-expressing CD4+ T cells	..	..	..	0·0017
Partial response	C2D1 to C1D1	5	1·31 (0·32 to 2·04)	..
Stable disease	C2D1 to C1D1	31	1·11 (0·42 to 2·21)	..
Progressive disease	C2D1 to C1D1	11	0·93 (0·48 to 1·16)	..
Circulating endothelial progenitors	..	..	..	0·049
Responders (thymoma)	C2D1 to C1D1	5	–8·13 (–30·97 to 119·28)	..
Non-responders (thymoma)	C2D1 to C1D1	31	–63·29 (–1604·22 to 259·69)	..
Circulating endothelial cells	..	..	..	0·29
Responders (thymoma)	C2D1 to C1D1	5	–28·11 (–180·18 to 12·73)	
Non-responders (thymoma)	C2D1 to C1D1	31	10·91 (–533·46 to 451·20)	
Anti-interferon α antibody	..	..	..	0·021
Responders	Pretreatment	5	609 (60·50 to 10 044)	..
Non-responders	Pretreatment	30	15 356 (7 to 22 381)	..
Anti-interleukin 22 antibody	..	..	..	0·0048
Responders	Pretreatment	5	100 (32 to 309)	..
Non-responders	Pretreatment	30	516·75 (16 to 10 097)	..
Anti-GMCSF antibody	..	..	..	0·014
Responders	Pretreatment	5	212·50 (43·50 to 526)	..
Non-responders	Pretreatment	29	28 (19 to 247·50)	..
Anti-interferon γ antibody	..	..	..	0·0091
New or worse autoimmune condition	Pretreatment	8	108 (46 to 405·50)	..
No new or worse autoimmune condition	Pretreatment	38	49·75 (3 to 1414)	..
Interferon γ-expressing CD4+ T cells	..	..	..	0·011
New or worse autoimmune condition	Pretreatment	8	4·80 (1·21 to 24·60)	..
No new or worse autoimmune condition	Pretreatment	39	14·20 (1·40 to 48·60)	..
Interleukin 17-expressing CD4+ T cells	..	..	..	0·033
New or worse autoimmune condition	Pretreatment	8	0·14 (0·06 to 1·15)	..
No new or worse autoimmune condition	Pretreatment	39	0·46 (0·04 to 2·51)	..
Interleukin 4-expressing CD4+ T cells	..	..	..	0·021
New or worse autoimmune condition	Pretreatment	8	0·55 (0·18 to 2·61)	..
No new or worse autoimmune condition	Pretreatment	39	2·34 (0·14 to 17·30)	..
Interleukin 17-expressing CD4+ T cells	..	..	..	0·0058
New or worse autoimmune condition	C2D1 to C1D1	8	0·13 (0·06 to 0·58)	..
No new or worse autoimmune condition	C2D1 to C1D1	39	0·56 (0·04 to 3·13)	..

Interferon γ-expressing CD4+ T cells	..	..	..	0·016
New or worse autoimmune condition	C2D1 to C1D1	8	4·34 (1·49 to 24·50)	..
No new or worse autoimmune condition	C2D1 to C1D1	39	14·80 (1·62 to 54)	..
Interleukin 4-expressing CD4+ T cells	..	..	..	0·043
New or worse autoimmune condition	C2D1 to C1D1	8	0·63 (0·23 to 4·74)	..
No new or worse autoimmune condition	C2D1 to C1D1	39	2·38 (0·16 to 11·50)	..

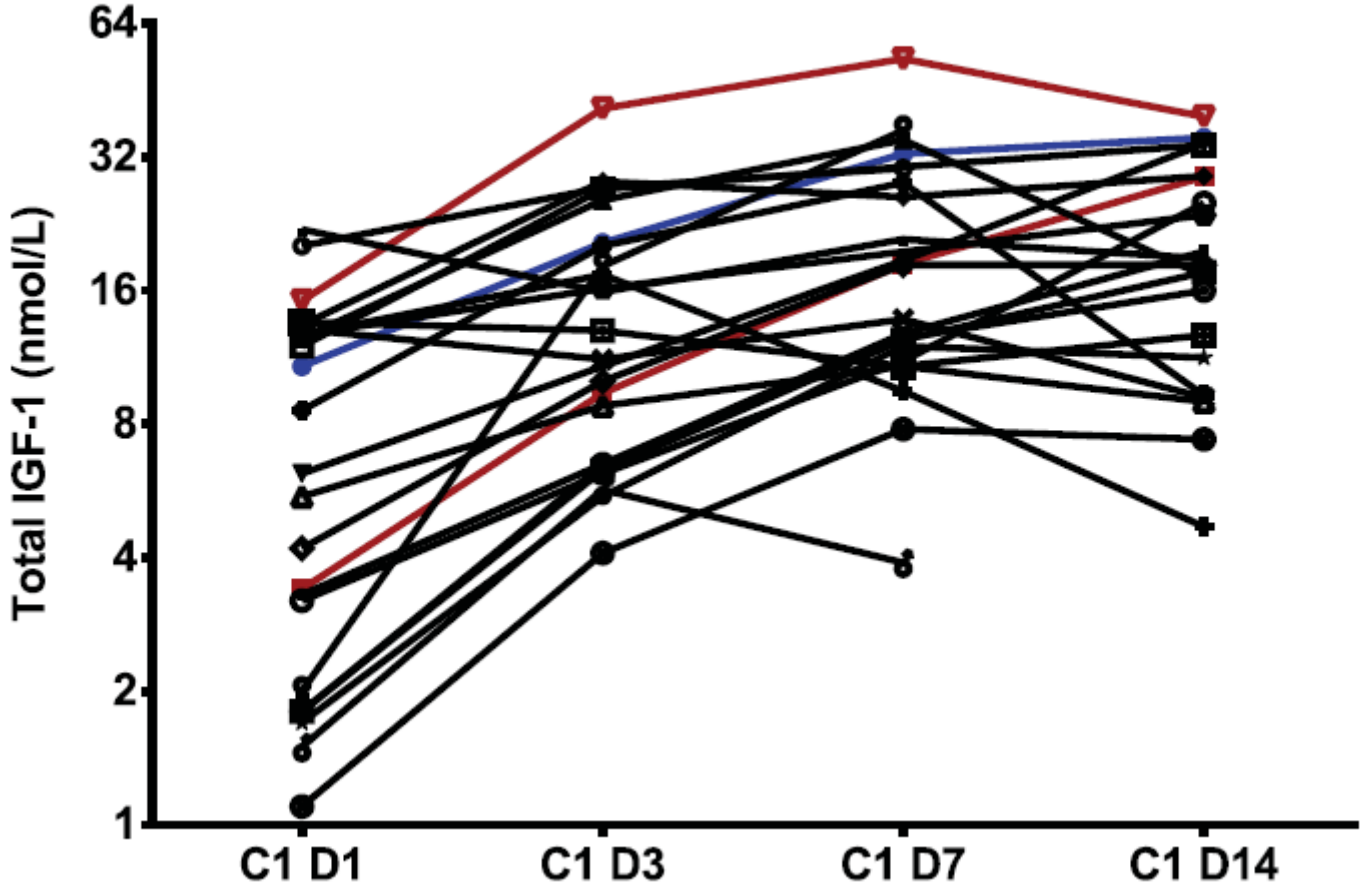
C2D1=cycle 2, day 1. C1D1=cycle 1, day 1. GMCSF=granulocyte macrophage colony-stimulating factor.

Supplemental Table 5: **Results of selected immune correlative studies**

Supplemental Figure 1: **Change in amounts of (A) free IGF-1 and (B) total IGF-1 after treatment with cixutumumab**
C1D1=cycle 1, day 1. C1D3=cycle 1, day 3. C1D7=cycle 1, day 7. C1D14=cycle 1, day 14.

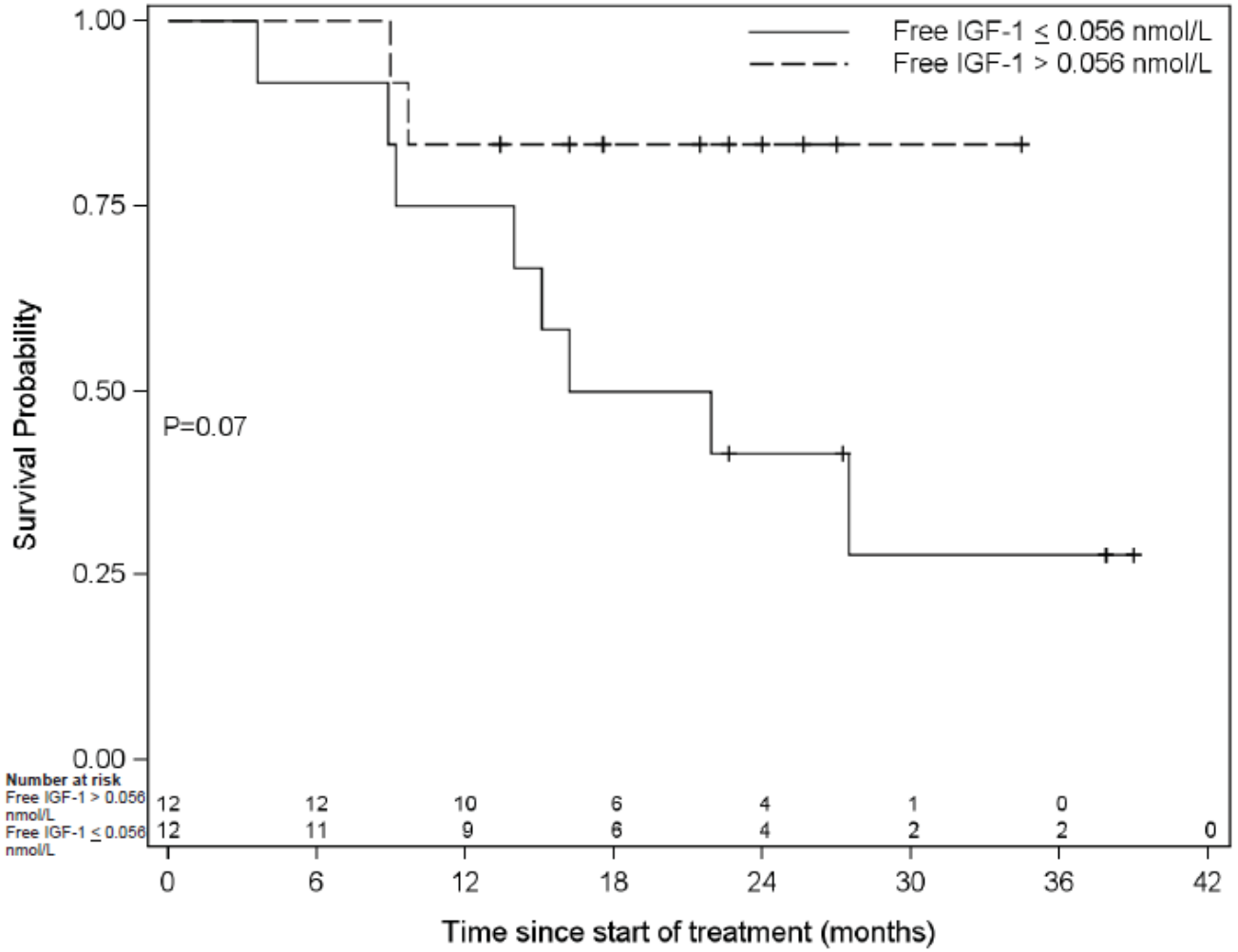


(B)

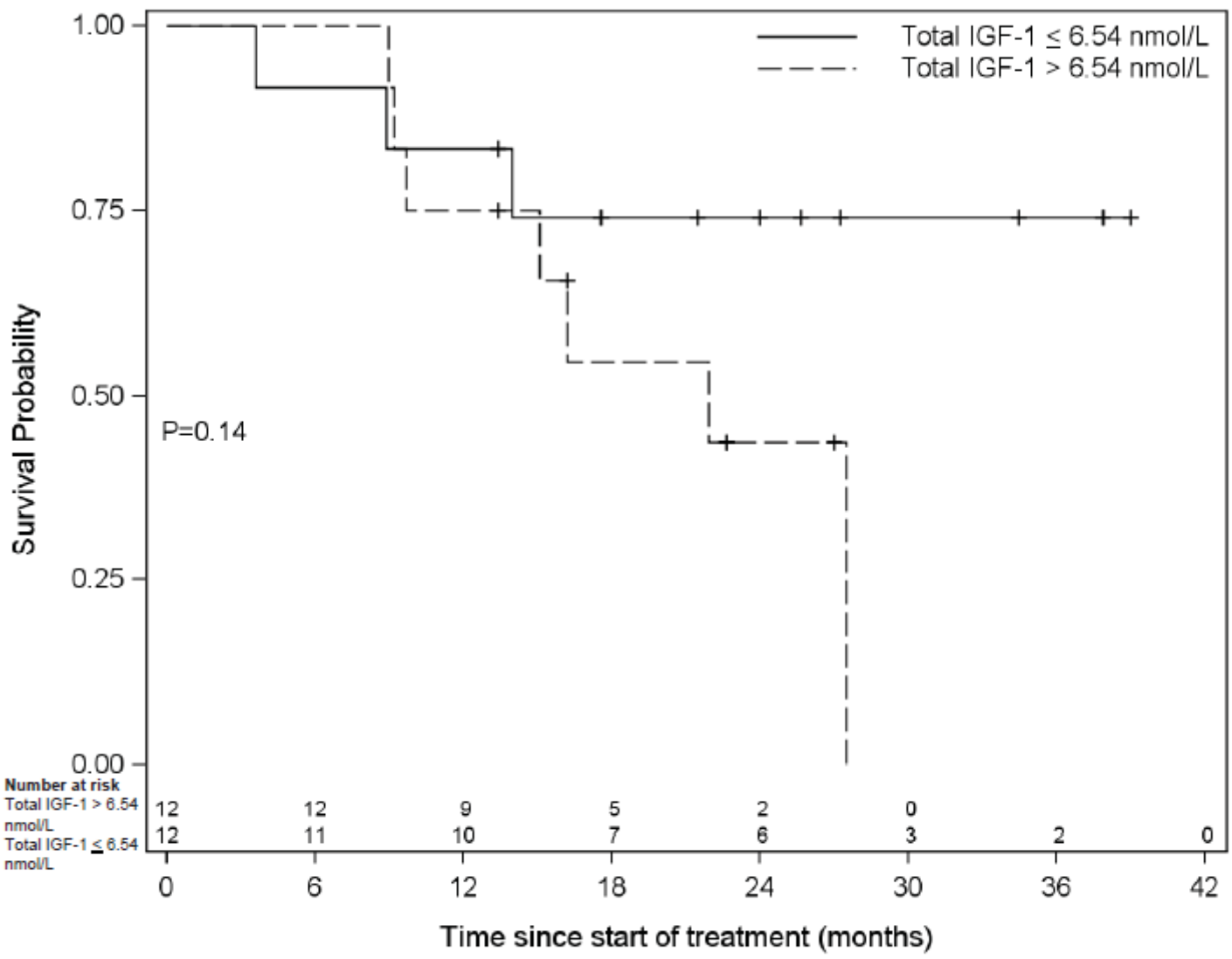


Supplemental Figure 2 : **Kaplan-Meier curves for overall survival in patients with thymoma according to amounts of (A) free IGF-1 (≤0·056 nmol/L *vs* >0·056 nmol/L) and (B) total IGF-1 (≤6·54 nmol/L *vs* >6·54 nmol/L) before treatment**

(A)



(B)


1.00
0.75
0.50
0.25
0.00
Survival Probability
Total IGF-1 ≤ 6.54 nmol/L
Total IGF-1 > 6.54 nmol/L
P=0.14
Number at risk
Total IGF-1 > 6.54 nmol/L
Total IGF-1 ≤ 6.54 nmol/L
12 12 9 5 2 0
12 11 10 7 6 3 2 0
0 6 12 18 24 30 36 42
Time since start of treatment (months)

Supplemental Figure 3: **Association between amounts of IGF-1 and pre-existing or new or worsening autoimmune disease**
(A) Free IGF-1 and pre-existing autoimmune disease (p=0·51). (B) Free IGF-1 and new or worsening autoimmune disease (p=0·94).
(C) Total IGF-1 and pre-existing autoimmune disease (p=0·68). (D) Total IGF-1 and new or worsening autoimmune disease (p=0·82).