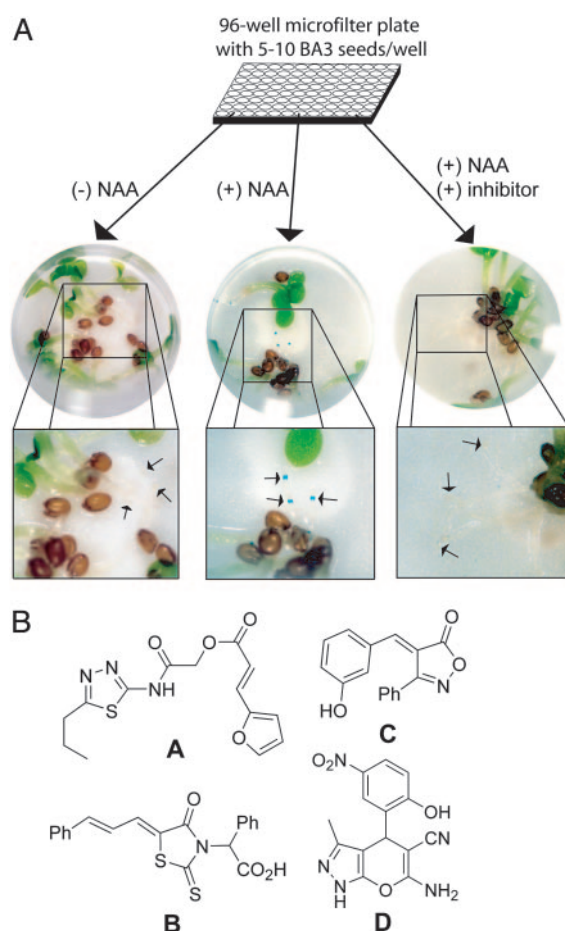


## Corrections

**PLANT BIOLOGY.** For the article “Identification of inhibitors of auxin transcriptional activation by means of chemical genetics in *Arabidopsis*,” by Joshua I. Armstrong, Shialou Yuan, Joseph M. Dale, Vanessa N. Tanner, and Athanasios Theologis, which appeared in issue 41, October 12, 2004, of *Proc. Natl. Acad. Sci. USA* (**101**, 14978–14983; first published October 4, 2004; 10.1073/pnas.0404312101), Fig. 1 contains an erroneous depiction of compound **D**. This problem appears to be a result of an internal database error early in our studies that propagated the incorrect structure throughout our figure preparation process. This correction does not affect the conclusions of the article. The corrected figure and its legend appear below.



**Fig. 1.** A high-throughput screen for auxin signaling inhibitors. (A) BA3 seeds expressing GUS from an auxin-sensitive promoter were arrayed into 96-well microfilter plates (5–10 seeds per well) and grown in liquid culture for 5 days. Incubation of the seedlings with 5  $\mu$ M NAA results in the tissue-specific expression of GUS in the root elongation zone, easily visualized after incubation with 5-bromo-4-chloro-3-indolyl  $\beta$ -D-glucuronide (X-gluc). The inclusion of an inhibitor of auxin signaling prevents GUS expression. (B) Structures of the four inhibitors, compounds **A–D**, chosen for detailed analysis.

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**MEDICAL SCIENCES.** For the article “Large-scale cDNA transfection screening for genes related to cancer development and progression,” by Dafang Wan, Yi Gong, Wenxin Qin, Pingping Zhang, Jinjun Li, Lin Wei, Xiaomei Zhou, Hongnian Li, Xiaokun Qiu, Fei Zhong, Liping He, Jian Yu, Genfu Yao, Huiqiu Jiang, Lianfang Qian, Ye Yu, Huiqun Shu, Xianlian Chen, Huili Xu, Minglei Guo, Zhimei Pan, Yan Chen, Chao Ge, Shengli Yang, and Jianren Gu, which appeared in issue 44, November 2, 2004, of *Proc. Natl. Acad. Sci. USA* (**101**, 15724–15729; first published October 21, 2004; 10.1073/pnas.0404089101), the authors note that on page 15725, in paragraphs 2 and 3 under the subheading *Measurement of Colony Formation of SMMC7721 Cells and Focus Formation of NIH 3T3 Cells*, “JCL-1/breast-cancer-associated gene” incorrectly appeared as “JCL-1/bacillus Calmette–Guérin 1.”

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**MEDICAL SCIENCES.** For the article “Humanized anti-CD25 (daclizumab) inhibits disease activity in multiple sclerosis patients failing to respond to interferon  $\beta$ ,” by Bibiana Bielekova, Nancy Richert, Thomas Howard, Gregg Blevins, Silva Markovic-Plese, Jennifer McCartin, Jens Würfel, Joan Ohayon, Thomas A. Waldmann, Henry F. McFarland, and Roland Martin, which appeared in issue 23, June 8, 2004, of *Proc. Natl. Acad. Sci. USA* (**101**, 8705–8708; first published May 25, 2004; 10.1073/pnas.0402653101), the authors request that Joseph A. Frank, Experimental Neuroimaging Section, Laboratory of Diagnostic Radiology Research, National Institutes of Health, Bethesda, MD 20892-1074, be added to the author list between Jennifer McCartin and Jens Würfel. The online version has been corrected. The corrected author and affiliation lines appear below.

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# Humanized anti-CD25 (daclizumab) inhibits disease activity in multiple sclerosis patients failing to respond to interferon $\beta$

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Contributed by Thomas A. Waldmann, April 14, 2004

**Identifying effective treatment combinations for MS patients failing standard therapy is an important goal. We report the results of a phase II open label baseline-to-treatment trial of a humanized monoclonal antibody against CD25 (daclizumab) in 10 multiple sclerosis patients with incomplete response to IFN- $\beta$  therapy and high brain inflammatory and clinical disease activity. Daclizumab was very well tolerated and led to a 78% reduction in new contrast-enhancing lesions and to a significant improvement in several clinical outcome measures.**

**M**ultiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the CNS with suspected autoimmune pathogenesis. The available treatments for MS are only partially effective, and considerable numbers of treated patients retain inflammatory CNS activity with contrast-enhancing MRI lesions (CEL) and continue to accumulate clinical disability.

Limiting T cell expansion by blocking IL-2 signaling by means of its high-affinity receptor that is expressed on activated T cells (i.e., blocking IL-2R $\alpha$ -chain, CD25) inhibits solid-organ graft rejection (1–3) and helps to restore tolerance in immune-mediated uveitis (4). Based on analogies of pathogenesis between these conditions and aberrant T cell activity in MS, we tested the effect of add-on therapy of daclizumab in MS patients with incomplete clinical and MRI response to IFN- $\beta$  therapy.

## Materials and Methods

**Trial Design.** Eleven patients with relapsing-remitting (RR) or secondary progressive (SP) MS were treated in this open-label baseline vs. treatment phase II trial (Table 1). Inclusion criteria included the following: age 18–65 yr and expanded disability status scale (EDSS) (5) 1.0–6.5. Exclusion criteria included the following: primary-progressive MS and concurrent medical conditions that could influence the immune system or accumulation of disability. Patients who were previously treated with therapies other than IFN- $\beta$  had to discontinue these therapies for at least 12 weeks. Failure to respond to IFN- $\beta$  was defined as follows: at least one MS exacerbation or progression of sustained disability by at least 1 EDSS point during the preceding 18 months on therapy. Patients were followed by monthly clinical and MRI examinations on IFN- $\beta$  monotherapy for 4 months. To initiate daclizumab dosing, we stipulated at least 0.67 new CEL/month during this baseline period. Daclizumab was administered i.v. at 1 mg/kg/dose 2 weeks apart for the first 2 doses (month 0 & 0.5) and every 4 weeks thereafter for a total of seven infusions. MS exacerbations were defined by Schumacher's criteria (6) and treated by i.v. methylprednisolone (IVMP) therapy (1g/day  $\times$  5 days). MRI scans and clinical ratings within 28 days of IVMP were disregarded and substituted by data from the following month. Both baseline and treatment phases were extended appropriately by 1 month per MS exacerbation to yield 4 baseline and 6.5 treatment months that were not affected by IVMP.

Primary outcome measures were new CEL and total number of CEL at baseline (IFN- $\beta$ ) vs. combination therapy (IFN- $\beta$  plus daclizumab). Secondary outcomes (MRI) were as follows: T2 lesion volume (T2LV), volume of CEL, and T1-hypointensities [black hole volume (BHV)]. Secondary outcomes (clinical measures) were as follows: exacerbation rate (cumulative number of exacerbations/cumulative baseline or treatment months), change in EDSS and Scripps Neurological Rating Scale (Scripps NRS) (7), change in ambulation index, timed 25-foot walk, and 9-hole peg test (9-HPT), all baseline vs. treatment. The trial was approved by the National Institute of Neurological Disorders and Stroke institutional review board, and informed consent was obtained from every patient.

**MRI Collection and Analysis.** Contiguous axial MRI images (3 mm  $\times$  42 axial slices) were acquired at 1.5 Tesla with T2-weighted/proton density (PD)/fast spin echo (FSE), fluid attenuation inversion recovery (FLAIR)-, and T1-weighted sequences before and after contrast (Magnevist 0.1 mmol/kg; Berlex Laboratories, Cedar Knolls, NJ) administration as described (8). CEL were recorded on hard copy films by consensus of two neuroradiologists. T2 lesion volume was determined by a semiautomated thresholding technique (PV-WAVE) (9). Black hole volume and volume of CEL were determined from registered images (10) by using a semiautomated thresholding program (Jeff Solomon, MRIPS, National Institutes of Health) on MEDX (Sensor Systems, Sterling, VA) applied to postcontrast T1WI, after verification of lesion colocalization on T2WI or FLAIR images.

**Statistical Analysis.** Statistics were based on nonparametric comparisons of group medians by Signed Rank Test with predetermined *P* value  $<$  0.05 for statistical significance.

## Results

The primary trial objective was to assess safety and tolerability of daclizumab in MS. A total of 11 screened patients proceeded to the dosing phase, and all received seven doses of daclizumab without any serious adverse event (Table 1). We observed an increase in the number of infections during the treatment phase (1 in 50 at baseline vs. 5 in 67 cumulative months at treatment), but all of these were mild urinary and upper respiratory tract infections that are common in MS patients. Furthermore, 2 transient elevations of liver function

Abbreviations: CEL, contrast-enhancing lesions; EDSS, expanded disability status scale; IVMP, intravenous methylprednisolone; MS, multiple sclerosis; NRS, neurological rating scale.

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**Table 1. Patient characteristics: demographic data, clinical data, and adverse events**

Patient code	Sex/race/age	MS type*	IFN-β <sup>†</sup> formulation/neutral. Ab	MS duration, y <sup>‡</sup>	End-baseline EDSS <sup>§</sup>	End-therapy EDSS <sup>§</sup>	Baseline NRS (mean) <sup>¶</sup>	Therapy NRS (mean) <sup>¶</sup>	Baseline exac. rate No./mo <sup>  </sup>	Therapy exac. rate No./mo <sup>  </sup>	Adverse events during baseline			Adverse events during therapy		
											I**	L**	O**	I**	L**	O**
MS-Z1	F/W/38	SP	A/-	8.0	4.5	5.0	68.3	63.6	0/4	0/6.5	1			1		
MS-Z2	F/W/27	SP	A/-	3.3	2.5	2.0	81.5	84.9	1/5	0/6.5						
MS-Z3	M/W/36	SP	B/-	9.3	6.0	6.0	60.0	81.8	2/6	0/6.5						1
MS-Z4	F/W/49	RR	B/+	24.0	3.5	3.0	66.3	78.3	2/6	1/7.5		1	1	1	1	1
MS-Z5	F/B/51	SP	B/-	10.3	6.0	6.0	56.5	59.0	0/4	1/7.5				1		
MS-Z6	F/W/42	RR	B/-	7.9	3.0	3.0	83.5	86.6	2/6	0/6.5						1
MS-Z7	M/W/33	RR	B/-	1.9	2.5	0.0	85.0	97.0	0/4	0/6.5	1	1		1		
MS-Z8 <sup>††</sup>	F/W/48	SP	B/-	6.0	3.5	3.5	69.7	72.4	0/4	1/7.5						
MS-Z9	M/W/23	RR	B/-	1.0	2.0	2.0	94.8	89.6	2/6	0/6.5	1				1	
MS-Z10	F/W/29	RR	A/-	5.5	3.0	1.5	62.7	89.9	1/5	0/6.5						1
MS-Z11	F/W/40	RR	B/+	6.7	1.5	1.0	93.3	98.0	0/4	0/6.5				2		
Group medians or cumulative values				7.6	3.0	2.5	74.9	85.8	10/50	2/67	1	2	2	5	2	4

\*RR, relapsing-remitting MS; SP, secondary progressive MS.

<sup>†</sup>A, Avonex; B, Betaserone; neutral. Ab, neutralizing antibodies; -, negative; +, positive.

<sup>‡</sup>Disease duration in years was calculated from the first symptom attributable to MS.

<sup>§</sup>EDSS at the end of baseline or treatment period that was sustained > 3 months = measure of sustained disability.

<sup>¶</sup>Average Scripps NRS score from baseline and treatment periods = clinical measure that includes monthly variations in clinical status.

<sup>||</sup>Exacerbation rate (exac. rate) is displayed as number of exacerbations per number of months during baseline or treatment periods. Note that, when exacerbation occurred, the baseline or treatment period was extended by 1 month per 1 exacerbation because the MRI and clinical values within 28 days of IVMP therapy were excluded from the analysis and were replaced by the next month's values.

\*\*I, infections (four urinary tract infections, two upper respiratory tract infections); L, abnormal laboratory values (transient elevation of LFTs); O, others (transient headache, constipation, breast tenderness, iron-deficiency anemia, exacerbation of ongoing depression, surgery for kidney stones).

<sup>††</sup>Patient MS-Z8 received higher dose of daclizumab (2mg/kg i.v. q 2w) and was excluded from final analysis.

**Table 2. Results for the primary and secondary outcome measures**

Outcome measures	Baseline* group median average ± SD	Treatment* group median average ± SD	P value <sup>†</sup> % improvement <sup>‡</sup>
Primary outcome measures: brain MRI			
Number of new CEL	1.38	0.75	P = 0.004
	2.92 ± 3.16	0.63 ± 0.48	78.42%
Number of total CEL	1.50	1.00	P = 0.002
	3.58 ± 3.95	1.06 ± 1.22	70.39%
Secondary outcome measures: brain MRI			
Volume of CEL (GdLV)	0.121	0.043	P = 0.014
	0.231 ± 0.302	0.063 ± 0.080	72.72%
Volume of T2 hyperintense lesions	4.195	3.471	NS
	4.793 ± 3.554	4.654 ± 3.414	2.90%
Volume of T1 hypointense lesions (black hole volume)	1.443	1.393	NS
	1.912 ± 1.879	1.907 ± 1.980	0.26%
Secondary outcome measures: clinical measures			
Exacerbation rate (no. of exacerbations per patient/year)	2.40	0.00	P = 0.047
	1.92 ± 1.78	0.37 ± 0.78	80.72%
Kurtzke Expanded Disability Status Scale (EDSS; from 0 = best to 10 = worst)	2.94	2.72	NS
	3.31 ± 1.72	3.07 ± 1.90	7.25%
Scripps NRS (from 100 = best to 0 = worst)	74.88	85.63	P = 0.048
	75.48 ± 13.79	82.54 ± 13.22	9.35%
Ambulation index (from 0 = best to 10 = worst)	1.25	1.06	NS
	1.80 ± 1.61	1.58 ± 1.45	12.22%
Timed 25-foot walk, s (average of two attempts per each clinical visit)	4.33	4.31	NS
	5.09 ± 2.13	4.86 ± 1.82	4.52%
9-hole peg test, (s)	22.07	20.65	P = 0.006
	22.96 ± 5.27	21.86 ± 5.26	4.79%

\*For each patient, an average of 4 baseline months is compared with an average of 6.5 treatment months. MRI and clinical data collected within 28 days of IVMP therapy were disregarded and substituted by subsequent month data. The baseline and therapy periods were extended appropriately to yield full 4 months vs. 6.5 months comparison for each patient.

<sup>†</sup>P value is based on nonparametric paired signed rank test.

<sup>‡</sup>Percentage of improvement is calculated from group averages.



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