Pierre Fabre outside the submitted work. Dr Ring listed as inventors of the patent entitled "Arenavirus particles to treat solid tumors" (patent number WO2018/185307 A1) describing the application of artLCMV vectors in the treatment of tumors, outside the submitted work. Dr Joerger reports advisory role (institutional): Novartis, AstraZeneca, Basilea Pharmaceutica, Bayer, BMS, Debiopharm, MSD, Roche, and Sanofi, research funding: Swiss Cancer Research, and travel grants: Roche, Sanofi, and Takeda. Dr Schroeder reports institutional grants from Illumina and research grants from BMS Stiftung Immunonkologie outside the submitted work. Dr Cozzio has served as adviser for and/or received speaking fees from AbbVie, Almirall, Amgen, BMS, Eli Lilly, Galderma, LEO Pharma, Janssen-Cilag, Novartis, Pfizer, Pierre Fabre Pharma, Sanofi, and UCB, outside the submitted work. Dr Leiter reports research support from MSD, consulting fees and honoraria from Sun Pharma, Sanofi (personal and institutional), MSD (personal and institutional), Novartis, Roche, and Almirall Hermal, support for attending meeting from Sun Pharma and participation on a Data Safety Monitoring Board or Advisory Board from Sun Pharma, Sanofi, MSD, Novartis, Roche, and Almirall Hermal, outside the submitted work. Dr Thomas reports honoraria for talks or advisory boards from BMS and Pierre Fabre and also reports research funding (institutional) from BMS, Pfizer, MSD, Amgen, argenx, LEO, Novartis, UCB, 4SC, AstraZeneca, BioNtech, Genentech, Roche, Biotech, CureVac, HUYA, Incyte, Idera, Iovance, InflaRx, CerpassRx, Kartos, Nektar, Philogen, Pierre Fabre, Regeneron, Replimune, and Sanofi. Dr Garbe reports personal fees from Amgen, MSD, and Philogen and grants and personal fees from Novartis, NeraCare, BMS, Roche, and Sanofi, outside the submitted work. Dr Forchhammer received personal fees from Recordati, Kyowa Kirin, and Takeda Pharmaceuticals (speakers' honoraria) as well as institutional grants from NeraCare, SkylineDx, and BioNTech outside the submitted work. Dr Levesque has received project-specific research funding outside the scope of this work from Novartis, Roche, Molecular Partners, and Oncobit. Dr Mangana has intermittent projects focused consultancy or advisory relationships with Merck/Pfizer, MSD, Amgen, Novartis, Roche, BMS, and Pierre Fabre and has received travel support from Ultrasun, L'Oreal, MSD, BMS, and Pierre Fabre outside of the submitted work. Dr Dummer reports research funding to their institution for clinical studies from MSD and consulting or advisory roles with MSD, Novartis, Roche, BMS, Amgen, Takeda, Pierre Fabre, Sun Pharma, Sanofi, Catalym, Second Genome, Regeneron, Alligator, T3 Pharma, MaxiVAX, Pfizer, and touchIME outside the submitted work. Dr Schürch is on the Scientific Advisory Board of and has received research funding from Enable Medicine, Inc, both outside the current work. Dr Forschner served as consultant to Roche, Novartis, MSD, BMS, and Pierre Fabre, received travel support from Roche, Novartis, BMS, and Pierre Fabre, received speaker fees from Roche, Novartis, BMS, MSD, and CeGaT and also reports institutional research grants from BMS Stiftung Immunonkologie. Dr Flatz reports grants or contracts from Hookipa Pharma, SAKK/Immunophotonics, DFG grant (Deutsche Forschungsgemeinschaft), Philogen, and Mundipharma, consulting fees from Philogen, Sanofi, Novartis, and BMS, participation on Data Safety Board University of Basel, outside the submitted work. The other authors have no conflicts of interest to declare.

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Serum levels of S-100 protein are directly proportional to the size, number, thickness and degree of cellularity of congenital melanocytic nevi

To the Editor: Some patients with congenital melanocytic nevi (CMN) present progressive growth and thickening, extracutaneous involvement (neurocutaneous melanocytosis, NCM) or neoplastic transformation (melanoma); and others remain stable or even regress. There are no markers to assess progression or follow-up. Recently, we found S-100, a protein which acts on cell differentiation and proliferation, elevated in CMN.[1] S-100 is a ligand of the RAGE pathway (related to the MAPK-pathway), and low serum levels of soluble-RAGE were related to poor survival in melanoma.[2] Also SOX10, expressed in melanocytes with high specificity, is useful in detection, prognosis and treatment assessment of melanoma.[3] We explored if S-100, RAGE and SOX10 serum levels vary in children's CMN and assessed clinical or pathological correlations.

Table I. Characteristics of CMN patients and lesions and clinical and histopathological correlation with serum protein levels

Patients (N = 64)		S-100 (μg/L): mean (IQR)	P value
Age, median (IQR)	9 (5-12)		
Male, *n* (%)	21 (32.81)		
Neurocutaneous melanosis, *n* (%)			.843
No	56 (87.5)	0.31 (0.13-0.55)	
Yes	8 (12.5)	0.27 (0.14-0.46)	
Subcutaneous tissue alteration, *n* (%)			**.042**
No	55 (85.9)	**0.19 (0.10-0.38)**	
Yes	9 (14.1)	**0.35 (0.19-0.71)**	
Size classification depending on PAS *n* (%)			**.030**
M1 1.5-10 cm	11 (17.19)	**0.09 (0.05-0.14)**	
M2 >10-20 cm	8 (12.50)	**0.19 (0.14-0.45)**	
L1 >20-30 cm	11 (17.19)	0.31 (0.16-0.58)	
L2 >30-40 cm	5 (7.81)	0.31 (0.11-0.71)	
G1 >40-60 cm	12 (18.75)	0.17 (0.08-0.51)	
G2 >60 cm	17 (26.56)	0.31 (0.19-0.43)	
No. of satellite nevi *n* (%)			**.039**
No satellites	23 (35.94)	**0.14 (0.09-0.25)**	
<20 satellites	14 (21.88)	**0.28 (0.12-0.52)**	
20-50 satellites	11 (17.19)	**0.49 (0.15-1.35)**	
>50 satellites	16 (25.00)	**0.31 (0.19-0.46)**	
Dermal or subcutaneous nodules *n* (%)			.087
None	41 (64.06)	0.19 (0.10-0.32)	
Scattered	14 (21.88)	0.27 (0.14-0.52)	
Extensive	9 (14.06)	0.53 (0.19-1.21)	
Hypertrichosis *n* (%)			**.043**
None	8 (12.50)	**0.09 (0.05-0.19)**	
Notable	25 (39.06)	0.27 (0.11-0.75)	
Marked	31 (48.44)	0.22 (0.15-0.49)	
General location #*n* (%)			.588
Head	25 (39.06)	0.16 (0.09-0.32)	
Trunk	10 (15.63)	0.26 (0.09-0.76)	
Extremities	7 (10.94)	0.15 (0.13-0.78)	
Head + trunk	4 (6.25)	0.22 (0.09-1.08)	
Trunk + extremities	16 (25.00)	0.32 (0.16-0.50)	
Head + trunk + extremities	2 (3.13)	0.38 (0.22-0.53)	

Histopathological analysis (N = 25)	Melanocyte extent	Serum protein	Rho	P value
Deep dermis	Melan-A	S-100	**0.41**	**.043**
		SOX10	**0.64**	**<.001**
		RAGE	0.18	.384
	S-100	S-100	**0.44**	**.028**
		SOX10	**0.58**	**.002**
		RAGE	−0.17	.421
Subcutaneous tissue	Melan-A	S-100	0.34	.107
		SOX10	0.29	.170
		RAGE	−0.20	.334
	S-100	S-100	**0.61**	**.001**
		SOX10	0.38	.080
		RAGE	−0.06	.765

Bold *P*-values indicate statistically significant results; bold S-100 levels and IQR in clinical features indicate the subgroups significantly different.

Regarding histopathological analysis, Melan-A$^+$ and S-100$^+$ areas and the number of Ki-67$^+$ cells were studied using ImageJ software in dermis and with conventional semi-quantitative scales in subcutaneous tissue. In addition to results presented in the table, a positive correlation between serum SOX10 and subcutaneous Ki67$^+$ (Rho 0.438 $P = .029$) and a negative correlation between serum RAGE and lesion thickness (Rho −0.62 $P = .001$) were found. Supplementary Methods Material, available via Mendeley at https://data.mendeley.com/datasets/mzr3rvxdkh/1.

CMN, Congenital melanocytic nevus; *G1 and G2*, giant; *IQR*, interquartile range; *L1 and L2*, large; *M1 and M2*, medium; *PAS*, projected adult size.

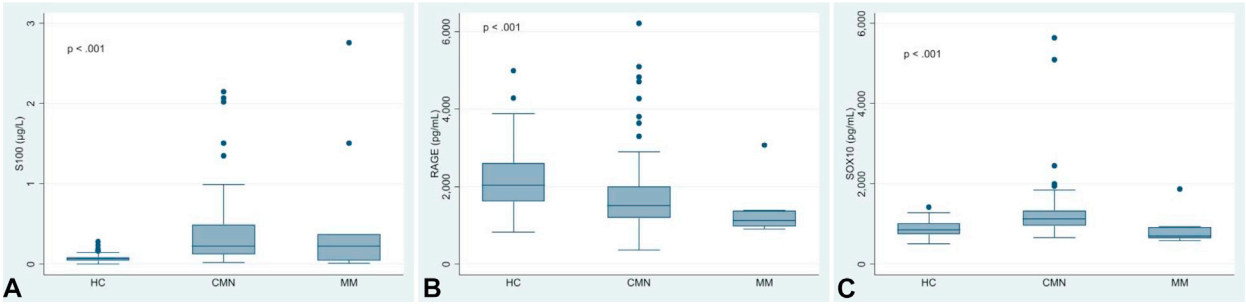

Fig 1. Serum determinations of S-100, RAGE and SOX10. HC: healthy children (27 men [42.18%] $P = .215$ vs CMN; median age: 9 [IQR 6-12] $P = .464$ vs CMN). MM: metastatic melanoma patients with disease progression (4 men [40%]; median age: 62.5 [IQR 57-74]). Healthy controls were children seen at the emergency department who underwent serologic testing for suspected infections, and metastatic melanoma patients were undergoing laboratory review. **A,** Comparison of levels of ***S-100*** between HC, CMN and MM. CMN patients showed higher levels of S-100 (mean 0.38; standard deviation [SD] 0.43 ug/L; mean for HC 0.08 [SD 0.05] ug/L; and mean for MM 0.56 [SD 0.88] ug/L; $P < .001$). **B,** Comparison of levels of ***RAGE*** between HC, CMN and MM. CMN patients showed lower levels than HC and higher than MM (mean for CMN 1805.10 [SD] 1098.89 pg/mL; mean for HC 2189.07 [SD 857.20] pg/mL; and mean for MM 1337.85 [SD 634.71] pg/mL; $P < .001$). **C,** Comparison of levels of ***SOX10*** between HC, CMN and MM. SOX10 was higher in CMN than in HC patients (mean for CMN 1316.84 [SD] 768.49 pg/mL; mean for (HC) 895.21 [SD 222.39] pg/mL; $P < .001$). When comparing SOX10 between CMN and MM (mean for MM 858.73 [SD] 379.54 pg/mL) no significant differences were found ($P = .200$). Some of the CMN patients had already undergone surgeries when they entered the study. The percentage (median, IQR) of the CMN present/maintained when the blood samples were collected was 85, 50-100. The most frequent surgeries performed when obtaining samples were expanders and tissue removal + flap (36%), tissue excision (32%) and expanders placement (19.5%). Samples were taken before starting the procedure.

This prospective, case-control, multicenter study enrolled consecutive patients with medium-to-giant-CMNs who underwent surgery (April 2019-December 2020). Left over serum samples of sex-and-age-matched healthy controls (HC) and a small group of metastatic melanoma patients were collected. The study was approved by the University Clinic of Navarra institutional review board and participants provided informed consent. CMN categorization was performed according to Krengel et al[4] criteria. S-100 was determined with an electrochemiluminescence-immunoassay; RAGE and SOX10 with ELISA kits. Representative tissue specimens taken during certain patients' surgery were analyzed for nevomelanocytic extent and proliferation. Supplementary Material, available via Mendeley at https://data.mendeley.com/datasets/mzr3rvxdkh/1.

64 CMN (Table I), 64 HC and 10 metastatic melanoma patients were recruited. CMN presented the highest levels of S-100; and lower levels of RAGE and higher levels of SOX10 than HC (Fig 1). Serial analyses were performed in 11 CMN showing that S-100 and SOX10 tended to decrease (correlation S-100-SOX10 Rho 0.253, $P = .362$) while RAGE increased (correlation S-100-RAGE Rho −0.432, $P = .107$) across time and treatments (months between determinations: median 21, 7-36). S-100 showed association with size, satellite nevi, hypertrichosis and subcutaneous tissue alterations (Table I and Supplementary Fig 2, available via Mendeley at https://data.mendeley.com/datasets/mzr3rvxdkh/1), and multivariate analysis showed that extensive dermal nodules, >20 satellites, or at least notable hypertrichosis increased S-100 values (coefficients 0.35, $P = .011$; .34, $P = .008$; and .20, $P = .041$). Higher serum levels of S-100 and SOX10 correlated with higher Melan-A$^+$ and S-100$^+$deep dermis areas; and S-100 serum levels correlated with S-100$^+$subcutaneous-areas (Table I). Serum SOX10 and subcutaneous Ki67$^+$ presented positive correlation (Rho 0.438 $P = .029$) and serum RAGE presented negative correlation with lesion thickness (Rho −0.62 $P = .001$).

Findings indicate higher nevomelanocytes populations result in greater S-100 release into the blood, as patients with larger, multiple, deeper and more cellular lesions presented higher levels. CMNs' size and satellites were reported to correlate with melanoma and NCM though no definitive conclusions could be made in regard to these complications in the present study due to sample size. However, it seems plausible that in those children affected by

CMN suffering overgrowth, melanoma, or NCM progression, serial determinations of serum S-100 could help in early detection and treatment, being complementary to physical examination and MRI. Usefulness of serum S-100 in melanoma, even within normal ranges, was highlighted recently.[5]

Long-term studies are needed to understand serum proteins variations across time in different settings. S-100 could be an easy, noninvasive, affordable analysis in CMN characterization and follow-up.

We are grateful to our patients and relatives for their cooperation and kindness and to all the colleagues who helped us with the study.

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Funding sources: The research has been partially funded by grants from Fundación Ramón Areces and Instituto de Salud Carlos III, from Spain; and by the Marjorie K. Harmer *endowment for research in Pediatric Pathology (MRM), from US. The funders had no role in the design and conduct of the study; in the collection, analysis, and interpretation of the data; in the decision of the submission of the manuscript; or in the preparation, review, or approval of the manuscript.*

IRB approval status: Reviewed and approved by the institutional review board at the University Clinic of Navarra.

Patient consent: No identifiable patient photographs or other identifiable material are included in the present article. Informed consent was obtained by the authors from all patients.

Key words: benign skin tumor; biomarkers; congenital melanocytic nevus; giant nevus; melanoma; neurocutaneous melanocytosis; S-100; serum; skin neoplasm; subcutaneous tissue.

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Conflicts of interest

None disclosed.

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https://doi.org/10.1016/j.jaad.2023.06.059

Time spent outdoors and sun protective behaviors among sexual minority women

To the Editor: Sexual minority women (SMW; eg, lesbian, bisexual), a National Institutes of Health US health disparity population,[1] may have a lower prevalence of indoor tanning and skin cancers compared to heterosexual women[2,3]; however, the relationship between sun protective behaviors (SPBs), sexual orientation, and sexual behavior is not well-studied. This cross-sectional study compared SPBs in women by sexual orientation and sexual behavior using nationally representative National Health and Nutrition Examination Survey data pooled from 2009 to 2016.

Self-reported sexual orientation was dichotomized into SMW (lesbian, bisexual) and heterosexual; sexual behavior was dichotomized into