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Neuroblastoma in Infants: Long-Term Survival From INES Protocols A SIOPEN Study

Julie Savagner[1] | Caroline Munzer[1] | Gudrun Schleiermacher[2,3] | Alberto Garaventa[4] | Riccardo Haupt[4] | Stefania Sorrentino[4] | Katia Mazzocco[5] | Andrea Di Cataldo[6] | Adela Canete[7] | Vanessa Segura[8] | Desiree Ramal[8] | Kate Wheeler[9] | Ruth Ladenstein[10] | Ana Forjaz de Lacerda[11] | Benedicte Brichard[12] | Maja Beck-Popovic[13] | Dominique Valteau-Couanet[14] | Claudia Pasqualini[14] | Carole Coze[15] | Benoit Dumont[16] | Anne Sophie Defachelles[17] | Marion Gambart[1]

[1]Pediatric Oncology Department, Hôpital des enfants, Toulouse, France | [2]SIREDO Department for Pediatric Oncology, Institut Curie, Paris, France | [3]RTOP Translational Research in Pediatric Oncology, U830 INSERM, PSL, Institut Curie, Paris, France | [4]Department of Hematology/Oncology, Unit of Pediatric Oncology, IRCCS Istituto Giannina Gaslini, Genoa, Italy | [5]Pathology Unit, IRCCS Istituto Giannina Gaslini, Genoa, Italy | [6]Department of Clinical and Experimental Medicine, Unit of Pediatric Hematology-Oncology, University Hospital, Catania, Italy | [7]Department of Pediatrics, Pediatric Oncohematology Unit, Hospital Universitario y Politécnico La Fe-IIs La Fe, Obstetrics and Gynecology, Universidad de Valencia, Valencia, Spain | [8]Clinical and Translational Research in Cancer Group, Pediatric Oncology, Instituto de Investigación Sanitaria La Fe, Valencia, Spain | [9]Department of Pediatric Oncology, Children's Hospital Oxford, University of Oxford, John Radcliffe Hospital, Oxford, UK | [10]St Anna Children's Hospital and Department of Pediatrics of the Medical University, Vienna, Austria | [11]Department of Pediatric and Adolescent Oncology, Portuguese Institute of Oncology, Lisbon, Portugal | [12]Pediatric Hematology and Oncology Department, Cliniques Universitaires Saint-Luc, Université Catholique de Louvain, Brussels, Belgium | [13]Department of Women, Mother and Child, Pediatric Hematology-Oncology Unit, University Hospital Lausanne, Lausanne, Switzerland | [14]Department of Pediatrics, Institut Gustave-Roussy, Villejuif, France | [15]Pediatric Hematology-Oncology Department, Hôpital pour enfant de La Timone, AP-HM, Aix- Marseille University, Marseille, France | [16]Pediatric Hematology-Oncology Department, Institut d'Hématologie et Oncologie Pédiatrique IHOPe, Lyon, France | [17]Department of Pediatric Oncology, Centre Oscar Lambret, Lille, France

Correspondence: Julie Savagner (julie.savagner@hotmail.fr)

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ABSTRACT

Objectives: Neuroblastoma is the most common extracranial solid tumor in infants, with a possibility of spontaneous regression even in disseminated disease. Despite an overall good prognosis, relapse can worsen the outcome for some patients. A long-term analysis is crucial to identify subgroups of patients with poorer prognosis, assessing the risks of late relapse, progression or long-term toxicity associated with multimodal treatment in very young children.

Methods: Estimation of the 10-year event-free and overall survivals in 750 infants under 12 months with neuroblastoma, enrolled in the prospective INES protocols between 1999 and 2004. Follow-up data from INES patients were updated, and survival analyses were performed in order to determine prognostic factors such as age, stage, genomic profile, or MYCN amplification.

Results: Overall, 10-year overall survival was 91.1% ± 1.0%, and 10-year event-free survival was 82.4% ± 1.4%, with significantly better outcomes in infants under 6 months compared with those aged 6–12 months, even considering the MYCN-amplified tumors only. MYCN amplification was the strongest prognostic factor and was correlated with lower survival in patients with metastatic disease.

Abbreviations: CNS, central nervous system; COG, Children's Oncology Group; CRF, Case Report Form; EFS, event-free survival; HR, hazard ratio; INES, Infant Neuroblastoma European Study; INES-FU, INES follow-up; INPC, International Neuroblastoma Pathology Classification; INRG-SS, International Neuroblastoma Risk Group Staging System; INSS, International Neuroblastoma Staging System; MKI, mitosis–karyorrhexis index; MYCN, N-Myc proto-oncogene; MYCNA, MYCN amplification or MYCN-amplified; NCA, Numerical chromosome alterations; OS, Overall survival; SCA, Segmental chromosome alterations; SIOPEN, European SIOP Neuroblastoma Group.

Discussion: Survival in patients less than 12 months remains excellent and stable even at long term, as a 10-year follow-up did not change the number of events. However, survival in MYCN-amplified tumors remained poor. Patients with metastatic tumors require accurate risk stratification. For each treatment group, there was no significant difference in long-term outcomes compared with previous publications from INES. No lethal toxicity affecting long-term survival occurred.

1 | Introduction

Neuroblastoma is the most frequent solid extracranial tumor in infancy, representing 8% of all childhood cancers, with approximately 50% diagnosed during the first year of life [1]. Infants under 12 months old at diagnosis represent a distinctive population, as spontaneous regression can sometimes occur, but they are especially vulnerable to antimitotic treatment [2–4]. Their survival rates tend to be higher than in older children [5]; however, patients with MYCN-amplified (MYCNA) tumors still have a poor prognosis [6–8]. Stratification of these patients into different risk groups is a major concern. Identification of prognostic factors allows us to deescalate or intensify their treatment compared with former recommendations [9, 10], leading to decreased toxicity and improved outcome for low- and intermediate-risk patients, as well as higher survival rates for high-risk patients.

The INES protocols (Supporting File S2) were launched by the SIOPEN group from 1999 to 2004, recruiting 750 infants under 1 year diagnosed with neuroblastoma at any stage (except for non-MYCNA INSS stage 1) in nine European countries (Austria, Belgium, France, Italy, Portugal, Spain, Switzerland, and the United Kingdom). They were sorted into four trials (99.1, 99.2, 99.3, and 99.4) in an attempt to identify the most appropriate therapy. Infants who could not be included in these trials, mainly due to INSS stage 1 status or missing data at enrolment, were enrolled in the 99.9 arm. The results of each protocol have been published and contributed to improving neuroblastoma management [11–13].

The 99.1 trial for inoperable localized neuroblastoma without MYCNA proposed low-dose chemotherapy in infants under 12 months with the aim to enable complete surgical resection [14, 15]. One hundred twenty patients were enrolled, confirming the possibility of avoiding intensive chemotherapy, with 26% of infants treated by low-dose chemotherapy only, and 60% of them without anthracycline [11]. Five-year OS was 99% ± 1%, and 5-year EFS was 90% ± 3%.

Trials 99.2 and 99.3 included patients with metastatic non-MYCNA tumors. At the time of the INES study, some infants were classified as INSS stage 4, although—apart from having a large primary tumor that crossed the midline—they would otherwise have been considered as stage 4S, as they had metastases confined to skin, marrow, nodes, or liver [5, 8].

Trial 99.2 included all stage 4S infants and those with stage 4 with a primary tumor extending across the midline or positive skeletal scintigraphy but no detectable changes to bone on plain radiographs and/or CT, who were to be observed in the absence of symptoms. Trial 99.3 included infants with tumor dissemination to the lung (intended as parenchymal lesions or pleural effusion), CNS, or skeleton (defined by abnormal X-ray and/or CT) to be treated with a minimum of four chemotherapy courses. For 125 infants from 99.2, 5-year OS was 96% and 5-year EFS was 88%, with no difference in survival in children presenting life-threatening symptoms. Regarding the 45 infants from 99.3, 5-year OS was 96% and 5-year EFS was 87% [12].

The 99.4 trial was designed for all stage MYCNA neuroblastoma (excluding INSS stage 1), with patients treated as high risk using intensive multimodal therapy. This included: four courses of chemotherapy for induction, followed by leukapheresis and delayed surgery; two additional chemotherapy courses were administered, followed by a single course of high-dose busulfan and melphalan with peripheral stem-cell support; finally, radiotherapy to the primary site was given to all patients. Thirty-five infants were enrolled, among which 97% had metastatic disease (24 INSS stage 4 and 10 stage 4S). Two-year OS was 30% ± 8% and 2-year EFS was 29% ± 7%, with survival of 20% of INSS stage 4 and 59% of stage 4S [13].

Tumor genetic analyses from INES 99.1–99.3 studies have already been published by Schleiermacher et al., illustrating the prognostic impact of genomic profiles: any segmental chromosome alteration being associated with a poorer outcome [16]. This follow-up study was established to assess long-term survival in infants.

2 | Methods

2.1 | Data Collection

The INES-FU protocol (Supporting File S3) and Case Report Form (CRF; Supporting File S1) were sent to national coordinators to collect data from all institutions that enrolled patients in the INES studies. They were invited to complete CRFs based on each patient's last examination or follow-up, noting any events such as progression, relapse, or secondary malignancies. No supplementary investigation was required. Data were gathered between April 2014 and March 2016. For patients without 10-year updates, the last known follow-up status (minimum 5-year updates) was used. The INES-FU protocol was an update of INES. Parents or guardians had provided written informed consent for INES, including long-term follow-up. The study was approved by local institutional ethics committees for all INES protocols.

2.2 | Patients and Tumor Assessment

Eligible patients were infants aged under 365 days with newly diagnosed neuroblastoma enrolled in INES protocols [11–13]. Staging was initially performed in accordance with the INSS; all patients were reclassified in compliance with the INRG-

SS in order to match current research [17]. Original diagnostic imaging was reviewed for Image Defined Risk Factors when available, and patients were reclassified based on the initial description of the disease and its extent when imaging was not accessible. Reference laboratories performed MYCN copy-number determination using fluorescence in situ hybridization, and genomic profile analysis by CGH arrays, when possible, using DNA from frozen tumor tissue. Numerical chromosomal aberrations (NCA) were defined for only numerical changes, while segmental chromosomal aberrations (SCA) were defined for segmental changes with or without numerical aberrations.

Patients were sorted into four trials according to their stage and MYCN status: 99.1 for inoperable localized tumors without MYCNA, 99.2 and 99.3 for metastatic and non-MYCNA tumors, and 99.4 for MYCNA neuroblastoma. Non-eligible infants were enrolled in the 99.9 arm and treated according to the trials with a risk-adapted approach. All patients from the INES 99.1–99.4 studies and the 99.9 arm were analyzed.

2.3 | Statistical Analyses

Descriptive statistics were reported as absolute frequencies and percentages for qualitative variables, while medians and ranges were used for continuous variables. EFS was defined as the time from diagnosis to first event (progression or relapse, secondary cancer, death from any cause) or until last follow-up. OS was defined as the time from diagnosis to death or last follow-up. Survivals were estimated using the Kaplan–Meier method and reported at the 10-year time point; differences between groups were assessed using the log-rank test. Standard errors (±) were calculated using Greenwood's formula. With the aim of determining prognostic factors in multivariate analyses, hazard ratios (HR) and 95% confidence intervals were estimated using the Cox regression model; potential interactions were tested. Per-group analyses were performed in intent to treat. *P*-values of less than 0.05 were considered statistically significant. All reported *p*-values were two-sided. All analyses were performed using the SAS software (version 9.4, Cary, NC, USA).

3 | Results

3.1 | Patient Characteristics

Data from the 750 patients enrolled in the INES 99.1–99.4 studies and the 99.9 arm were analyzed. General characteristics are summarized in Table 1. The mean age at diagnosis was 6 months; 61.7% were under 6 months, and one-third was under 1 month. We received follow-up forms from 539 patients for INES-FU, including data from 23 who died during the first five years of follow-up, representing 75.1% of the entire INES population. Median survival follow-up was 9.8 years (0.3–16). One hundred nineteen infants were treated in trial 99.1, 133 in 99.2, 48 in 99.3, 46 in 99.4, and 404 in 99.9, which represents a large proportion of the INES population (53.9%).

The initial tumor site was most frequently adrenal (54.5%). Approximately 40% of patients had metastases at diagnosis, mostly in the liver (26.8%), then in the bone marrow (17.7%). Other metastatic sites were rare. The distribution of metastases

TABLE 1 | Patients and tumor characteristics at diagnosis in infants analyzed in the INES-FU study.

	Patients	
Characteristics	**No.**	**%**
Total,	750	100.0
Age		
<6 months old	463	61.7
≥6 months old	287	38.3
Initial tumor location		
Adrenal	409	54.5
Abdominal	129	17.2
Thoracic	90	12.0
Cervical	28	3.7
Pelvic	23	3.1
Contiguous	58	7.7
Not available	*13*	*1.7*
Metastases		
Absence	448	59.7
Presence	301	40.1
Not available	*1*	*0.1*
Sites of metastases		
Bone marrow	133	17.7
Bone	45	6.0
CNS	25	3.3
Cutaneous	47	6.3
Distant lymph nodes	32	4.3
Liver	201	26.8
Pleural or lung	42	5.6
INRG-SS		
L1 stage	269	35.9
L2 stage	191	25.5
M stage	98	13.1
Ms stage	187	24.9
Not available	*5*	*0.7*
Initial histology (INPC)		
Ganglioneuroblastoma	15	2.0
Neuroblastoma	665	88.7
Neuroblastoma, NOS	114	15.2
Neuroblastoma, undifferentiated	90	12.0
Neuroblastoma, poorly differentiated	398	53.1
Neuroblastoma, differentiating	63	8.4
Neuroblastic tumor, not classifiable	14	1.9
Not available	*56*	*7.5*
MKI status (INPC)		
Low/intermediate	302	40.3
High	54	7.2
Not available	*394*	*52.5*
Treatment group		
Trial 99.1	119	15.9
Trial 99.2	133	17.7
Trial 99.3	48	6.4
Trial 99.4	46	6.1
Arm 99.9	404	53.9

(Continues)

TABLE 1 | (Continued)

Characteristics	Patients	
	No.	**%**
MYCN status		
Non-amplified	653	87.1
Amplified	61	8.1
Not available	*36*	*4.8*
Ploidy		
Di/Tetraploid	94	12.5
Pseudo-triploid	196	26.1
Not available	*460*	*61.3*
Genomic profile		
NCA	162	21.6
SCA	48	6.4
Not available	*540*	*72.0*

CNS: central nervous system, INPC: International Neuroblastoma Pathology Classification, INRG-SS: International Neuroblastoma Risk Group Staging System, NCA: numerical chromosome alterations, NOS: not otherwise specified, SCA: segmental chromosome alterations.

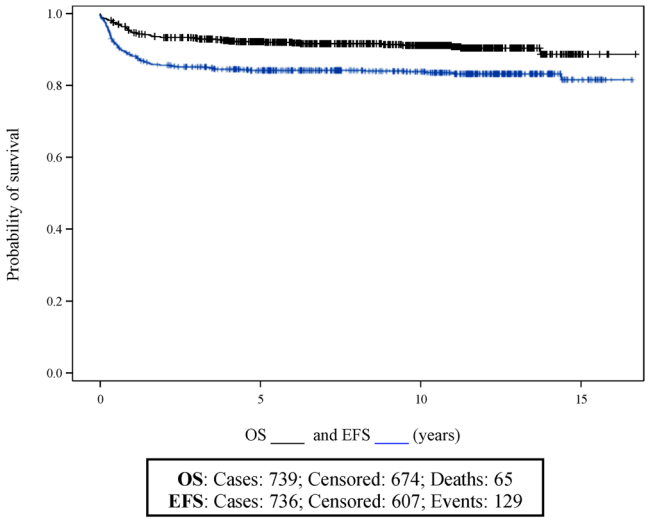

FIGURE 1 | Kaplan–Meier survival analysis in the total population: OS for 739 patients, and EFS for 736 patients.

was different according to age, with more liver and cutaneous dissemination in younger infants and more bone, lung/pleural, CNS, and distant lymph nodes in the 6–12-month population. Bone marrow involvement was similar in both age groups. Patients with MYCNA tumors have more dissemination in the bone marrow (69.1% vs. 43.2% in non-amplified; $p < 0.001$), bones (51.2% vs. 22.4% in non-amplified; $p < 0.001$), CNS (12.2% vs. 3.1% in non-amplified; $p < 0.001$), distant lymph nodes (20.9% vs. 9.7% in non-amplified; $p < 0.01$), and lung/pleura (20.0% vs. 7.1% in non-amplified; $p < 0.001$).

As previously described in this population [16], genomic profile was obtained for 210 infants from the 99.1–99.3 trials, of which 77.1% were NCA and 22.9% SCA profiles. SCA profiles prevailed in disseminated diseases (81.3%), while NCA profiles were found in both localized (46.3%) and metastatic neuroblastoma (53.7%).

The MYCN status was available for 714 patients (95.2% of INES); 61 tumors were amplified (8.5%), with 23 in the <6 months group and 38 in the ≥6 months group. Amongst MYCNA tumors, 44 were included in the 99.4 trial, and the remaining 17 were in the 99.9 arm. Regarding INRG staging, 47.54% were M stage, 26.2% were Ms, 19.7% were L1, and 6.6% were L2. In other words, 30.85% of M-stage tumors were amplified (vs. 8.6% of Ms, 4.5% of L1, and 2.1% of L2).

The distribution of patients in the 99.9 arm was similar to the trials with regard to initial tumor site, histology, and age S4. However, metastatic forms were less frequent in 99.9 (16.8% vs. 40.1%), including a high number of patients with INRG L1 neuroblastoma (63.4%). Seventeen of the 61 patients with MYCNA tumors were part of 99.9, as they could not be enrolled in 99.4.

3.2 | Survival Analyses

As presented in Figure 1, in the total population, 10-year OS was 91.1% ± 1.0% and 10-year EFS 82.4% ± 1.4%, indicating long-term stability in survival as compared with previous publications [11–13]. Seven hundred thirty-nine patients were analyzed for OS, with 65 deaths from any cause. As for late deaths, only eight patients died more than five years after diagnosis: seven from their diseases and one from a car accident. OS without death from any other cause (not neuroblastoma) remained equivalent, with a 10-year OS of 91.5% ± 1.0%. Seven hundred thirty-six patients were assessed for EFS, with 129 events. Only five events, excluding deaths, occurred more than five years after diagnosis; five patients had late relapse (including one paravertebral ganglioneuroma occurring 9.68 years after diagnosis). There was no secondary cancer.

The main survival data are summarized in Table 2. Survival rates were higher in infants aged under 6 months compared with those aged 6–12 months. We did not find any difference in survival while comparing infants under 3 months with older patients, even in Ms stage's diseases.

Considering INRG-SS (See Figure 2A and B/), there was a clear contrast for both 10-year OS and EFS, with lower survival in Ms stages compared with localized L1 and L2 stages, and an even lower survival in M stages. The presence of metastases was significantly associated with a poorer prognosis. Among M-stage patients, there was no difference in survival comparing metastatic sites other than Ms sites (bone marrow, cutaneous, or liver), meaning there was no difference in terms of outcome between metastatic diseases localized in distant lymph nodes vs. lungs/pleura vs. bones. This difference was also found considering treatment groups (Figure 2C and D). Results from 99.9 were compared with each trial according to specific characteristics: data from patients with MYCNA tumors were compared with 99.4, with no difference in survival (99.9 MYCNA: OS 39.2% ± 12% and EFS 35.3% ± 11%; $p = 0.82$). Likewise, there were no differences comparing localized patients from trial 99.1 (99.9 localized non-amplified: OS 97.5% ± 1% and EFS 90.4% ± 2%; $p = 0.72$) and metastatic patients from trials 99.2 and 99.3 (99.9 Ms-stage non-amplified: OS 82.6% ± 7% and EFS 78.42% ± 9%; 99.9 M-stage non-amplified: OS 84.9% ± 9% and EFS 78.8% ± 10%; $p = 0.21$).

TABLE 2 | Results of the univariate survival data according to Kaplan–Meier analysis for OS and EFS, and of the multivariate Cox model analysis on survival for OS, in the total population.

Patient and tumor characteristics		Univariate survival analyses					Multivariate survival analyses		
		OS			EFS		OS		
		No.	10-year OS (%)	*p*	10-year EFS (%)	*p*	HR	95% CI	*p*
Total		750	91.1 ± 1.0		82.4 ± 1.4				
Age	<6 months	463	93.4 ± 1.2	0.033	84.5 ± 1.8	0.093			
	≥6 months	287	87.6 ± 2.1		79.3 ± 2.4				
Initial tumor location	Adrenal	403	88.0 ± 1.7	0.006	80.3 ± 2.0	0.048			
	Abdominal	127	94.9 ± 2.0		86.6 ± 3.2				
	Cervical	28	100.0 ± 0.0		74.3 ± 8.4				
	Thoracic	90	98.9 ± 1.1		90.5 ± 3.3				
	Pelvic	23	100.0 ± 0.0		91.3 ± 5.9				
	Contiguous	56	86.9 ± 4.7		76.2 ± 5.8				
Metastasis	Absence	455	97.3 ± 0.8	0.001	88.5 ± 1.5	0.001			
	Presence	284	83.0 ± 2.3		72.2 ± 2.7				
INRG stage	L1	264	98.9 ± 0.7	0.001	92.3 ± 1.7	0.001	1		
	L2	188	95.2 ± 1.7		84.1 ± 2.7		3.65	0.8 to 16.5	0.093
	M	97	67.8 ± 4.9		62.3 ± 4.9		16.07	4.1 to 62.8	0.001
	Ms	187	88.2 ± 2.5		77.3 ± 3.2		5.93	1.5 to 22.5	0.009
MYCN status	Non-amplified	653	95.9 ± 0.8	0.001	86.6 ± 1.4	0.001	1		
	Amplified	61	43.5 ± 6.5		42.6 ± 6.5		9.30	3.8 to 22.6	0.001
Treatment group	99.1	119	99.1 ± 0.9	0.001	85.7 ± 3.4	0.001			
	99.2	133	92.5 ± 2.5		84.2 ± 3.2				
	99.3	48	95.8 ± 2.9		85.4 ± 5.1				
	99.4	46	48.4 ± 7.5		47.7 ± 7.5				
	99.9	404	92.6 ± 1.4		84.2 ± 1.9				
MKI	Low/inter	297	95.0 ± 1.4	0.001	86.0 ± 2.1	0.166			
	High	54	82.7 ± 5.3		79.3 ± 5.6				
Ploidy	Di/tetraploid	94	87.7 ± 3.5	0.008	79.7 ± 4.1	0.103			
	Pseudo-triploid	193	96.6 ± 1.4		87.2 ± 2.5				
Genomic profile	NCA	162	98.8 ± 0.9	0.019	90.3 ± 2.4	0.001			
	SCA	48	88.8 ± 4.8		70.8 ± 6.6				

CI: confidence interval, EFS: event-free survival, INRG: International Neuroblastoma Risk Group, MKI: mitosis–karyorrhexis index, NCA: numerical chromosome alterations, OS: overall survival, SCA: segmental chromosome alterations.

Regarding MYCN status, its amplification was significantly correlated with poorer survival. As shown in Figure 3–C and D, age at diagnosis appeared to be a prognostic factor in the MYCNA population, as children aged < 6 months displayed a better 10-year OS (50.9% ± 10.7% vs. 39.1% ± 7.9% in MYCNA tumors in patients aged 6–12 months; $p < 0.001$) and a better 10-year EFS (51.8% ± 10.5% vs. 36.8% ± 7.8%; $p < 0.001$). On the other hand, survival in non-amplified infants is similar in both age groups for OS and EFS. Survival according to MYCN status and INRG staging suggested lower OS and EFS in each stage in case of amplification (for L1 stages: 10-year OS of 83% ± 11% vs. 99% ± 1% in non-MYCNA, and 10-year EFS of 75% ± 13% vs. 93% ± 2%, $p < 0.001$; for L2 stages: 10-year OS of 75% ± 22% vs. 96% ± 2% in non-MYCNA, and 10-year EFS of 75% ± 22% vs. 84% ± 3%, $p < 0.001$). As seen in Figure 4, inequality in survival was particularly notable for Ms stages, with 10-year OS at 56% ± 12% in the MYCNA population vs. 91% ± 2% ($p < 0.001$) and 10-year EFS at 56% ± 12% vs. 80% ± 3% ($p < 0.001$); similarly for M stages, with 10-year OS at 15.1% ± 7% in the MYCNA population vs. 93.6% ± 3% ($p < 0.001$) and 10-year EFS at 17% ± 7% vs. 84% ± 5% ($p < 0.001$). One hundred seventeen Ms-stage patients were analyzed, 16 of which had MYCN amplification, and 94 M-stage patients, of which 29 were amplified.

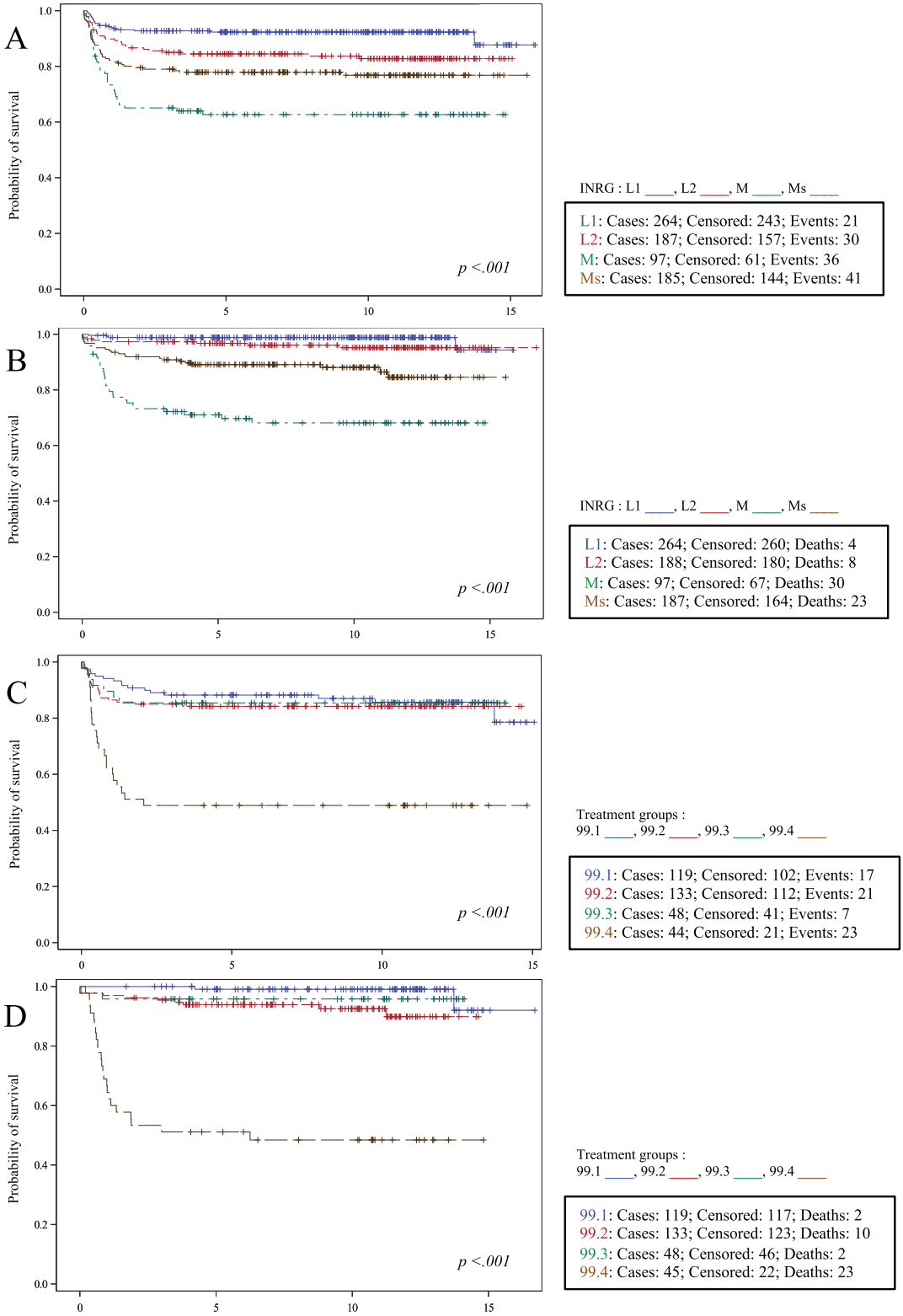

FIGURE 2 | Kaplan–Meier survival analysis according to INRG classification in A (OS) and B (EFS); and according to trials 99.1–99.2–99.3 and 99.4 in C (OS) and D (EFS) (739 patients were analyzed for OS and 736 patients for EFS.

There is no significant difference between stages in the non-amplified population, even with a metastatic disease (10-year OS: 99 ± 1% for L1, 96% ± 2% for L2, 93% ± 3% for M, and 91% ± 2% for Ms; $p = 0.71$). In infants whose tumors harbor MYCNA, there was no difference by age; however, the results tend to show a better survival in younger children (10-year OS 51% ± 11% in < 6 months vs. 39% ± 8% in > 6 months; $p = 0.37$). SCA were correlated with lower survival at 10 years for both OS and EFS (respectively 88.8 ± 4.8 and 70.8 ± 6.6), in comparison with NCA (10-year OS and EFS: 98.8 ± 0.9 and 90.3 ± 2.4; $p = 0.02$). Age at diagnosis tends to

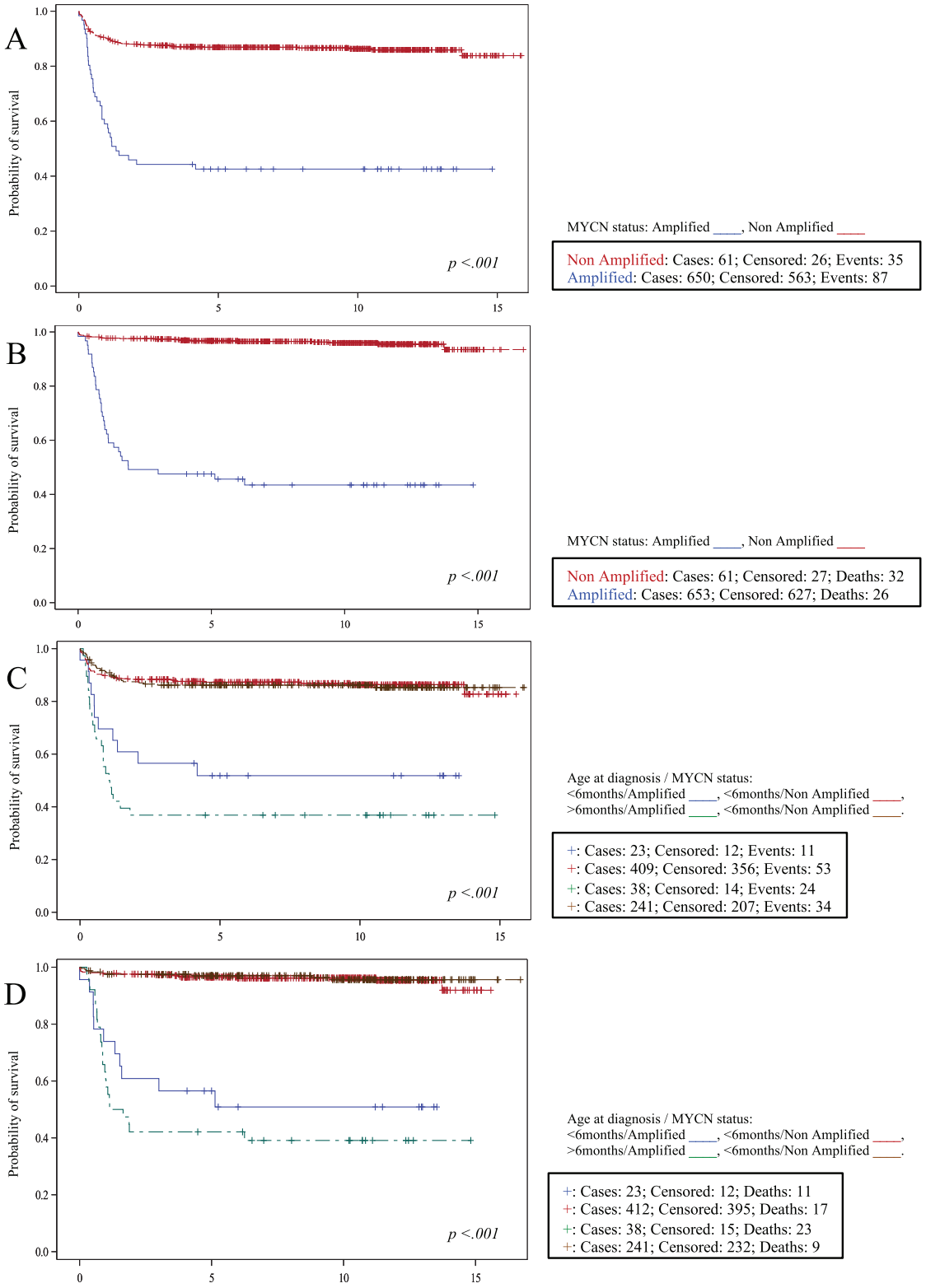

FIGURE 3 | Kaplan–Meier survival analysis according to MYCN status in A (OS) and B (EFS); and according to MYCN status and age < or ≥ 6 months old in C (OS) and D (EFS). 714 patients were analyzed for OS and EFS (of which 61 were MYCNA: 23 <6 months and 38 ≥ 6 months old at diagnosis). Non Ampl: no MYCN amplification; Ampl: MYCN amplification.

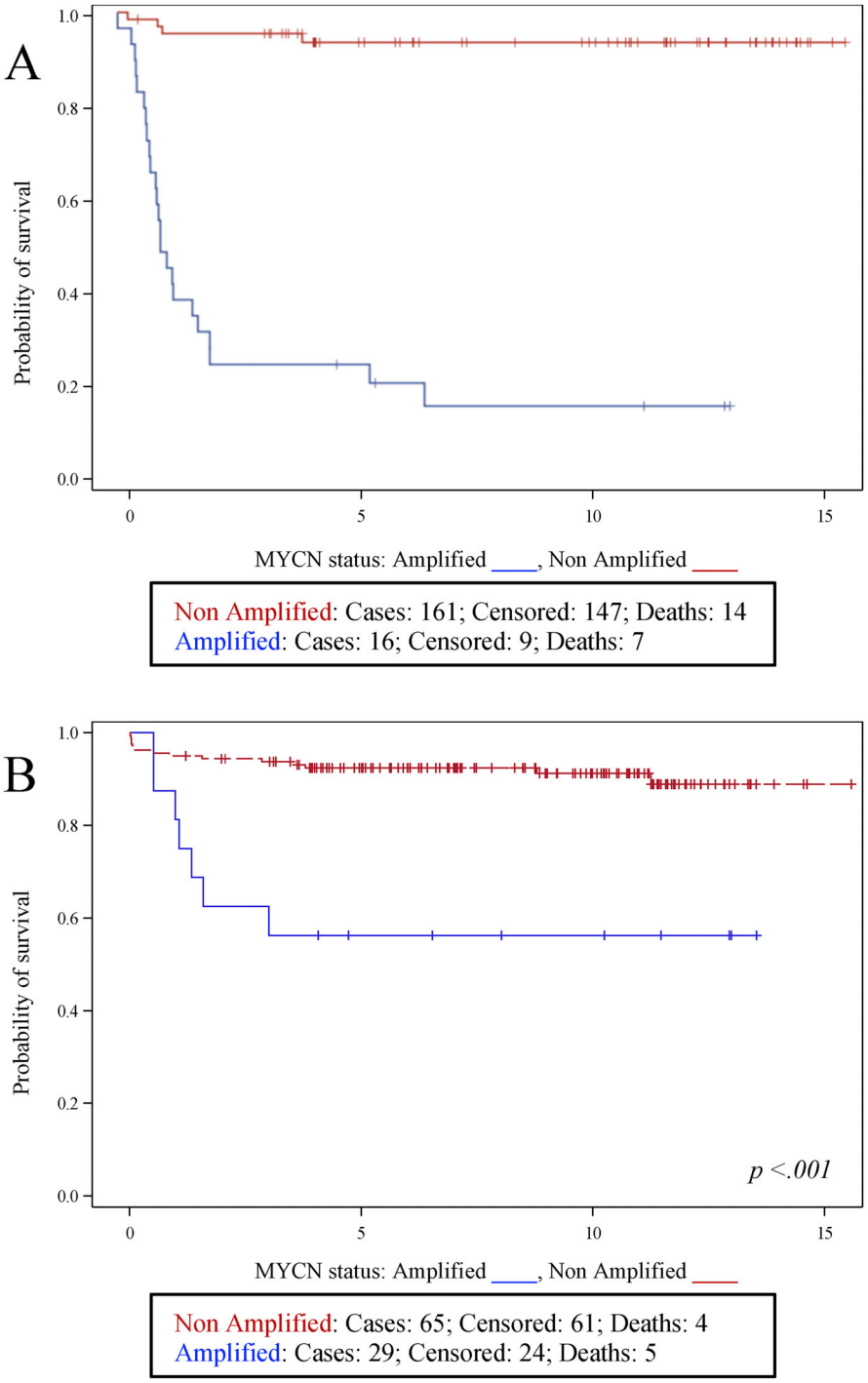

FIGURE 4 | Kaplan–Meier survival analysis regarding OS according to MYCN status in INRG classification: M stage (A) and INRG Ms stage (B). 94 patients were analyzed for M stage, with 29 MYCNA; 177 patients were analyzed for M stage, of which 16 were MYCNA.

impact outcome according to genomic profile, with a lower survival rate in younger patients, but without reaching significance.

3.3 | Prognostic Factors

Many known factors were significant in univariate analyses, as seen in Table 2. Multivariate analyses were performed in 343 patients for whom complete data were available to estimate which factors had an impact on prognosis, with the following results: MYCN amplification with an HR of 9.77 for OS and 3.48 for EFS. INRG staging was also found to be significant, with an HR in the Ms stage of 5.93 and in the M stage of 16.07, both for OS.

4 | Discussion

In accordance with previous publications on the INES population, survival in infants appeared to be excellent and remained stable over time. Our median survival follow-up was 9.8 years,

while it ranged from 4.3 to 6.1 years in the initial publications focusing on each INES protocol [11–13]. Survival rates were close at 10 years compared with 5 years, with a decrease of 1% for both OS and EFS (91.1% ± 1.0% vs. 92.2% ± 1.0% at 5 years for OS; 82.4% ± 1.4% vs. 83.1% ± 1.4% for EFS). In most cases, children died or relapsed from their diseases during the first three years following diagnosis. We did not find any lethal toxicity affecting long-term survival, and there was no secondary cancer. For both the localized and metastatic populations, even with MYCNA, survival rates tended to stabilize over time. However, the five-year follow-up appeared to be sufficient in our study for most infants, as the number of late events or deaths was very low. Despite such results, long-term examination must be pursued for those young patients, with a substantial risk of relapse or progression and a strong probability of late sequelae related to the disease or treatment, with no visible impact on survival [18]. Late effects data were not included in this paper and need to be evaluated in order to provide a complete statement on long-term follow-up in our population.

For each treatment group, we did not find any significant difference in long-term survival, in comparison with what has already been published by INES. We decided to include all infants in our study, even those who were not eligible because of a lack of inclusion criteria at the time of recruitment (99.9). For this reason, our survival results differed from Rubie, De Bernardi, and Canete's publications focusing on trials.

Inclusion of the population from 99.9 in the analysis was essential, as it differs from those from trials 99.1–99.4, with a higher proportion of low-risk tumors: 50.7% of INSS 1 tumors receiving first-line surgery, 16.8% of metastatic neuroblastoma (40.1% in total population), and only 4.2% of MYCNA tumors (8.5% in total population). Survival rates of the 99.9 arm were slightly higher than the rates from the total population; however, this difference disappeared while comparing each group of infants by stage as defined in the trials, reinforcing the necessity for treatment stratification.

Some long-term results from INES metastatic patients have already been published by Di Cataldo [19], but they only referred to the Spanish and Italian population, accounting for 13.1% (98/750) of all INES infants. Survival rates were worse for patients not included in the trials in Di Cataldo's publication (99.9 arm). We showed better survival rates for the same metastatic population (10-year OS of 82% vs. 74% in said paper and 10-year EFS of 72% vs. 70%), potentially due to the limited number of data in this paper. Only MYCN status was found to be prognostic with 16 amplified patients, as we confirmed here in a larger population. The difference in survival can also be explained by an intention-to-treat analysis.

The number of updated data from initial INES patients concerned only 563 of the 750 initially enrolled; the 187 remaining patients, for which only a 5-year update was available, were analyzed in order to assess the sufficiency of our sampling. Both populations were significantly similar with regard to stage, age, presence of metastases, and histology; the only differences found were the MYCN status (with more MYCNA in the 10-year updated population; $p = 0.042$) and ploidy (with less pseudo-triploid tumors in the 10-year updated population; $p = 0.039$), leading to a potential underestimation of survival. In order to minimize the number of patients with missing data, each participating center was sent reminders to increase the response rate.

Infants with low- or intermediate-risk neuroblastoma presented high survival rates, as their current treatment strategies are based on LINES protocols. Low-risk patients (localized and Ms diseases) can benefit from the wait-and-see strategy in the absence of threatening symptoms, as there is a possibility of spontaneous regression; alternatively, they can be treated with surgery alone or the least aggressive chemotherapy that is effective [20]. The Ms population can have a critical presentation at diagnosis and require emergency treatment. We found Ms more frequently in children under 6 months, with lower survival than for localized stages; observation should therefore be considered carefully [21]. The unfavorable prognosis of Ms patients under three months of age has been established for decades [15] but was not confirmed in our findings.

The INRG-SS did not match the distribution between 99.2 and 99.3 trials, as the classification was published afterward in 2009 [22]. The treatment strategies were planned according to each protocol: 99.2 included all stage INSS 4S infants and stage 4 with a primary tumor extending across the midline or positive skeletal scintigraphy but no overt bone lesion evidenced by radiography or CT-scan, while 99.3 included infants with tumor dissemination to the lung, or CNS or skeleton documented by abnormalities on radiographs or CT-scans. In different series [23, 24], the proportion of stage M and MS disease differed according to the inclusion of patients with scintigraphic positivity over the bone marrow or skeleton, into either stage M or MS. Previous publications described that positive bone scintigraphy not associated with changes in the cortical bone documented on plain radiographs and/or computed tomography had a better outcome compared with other stage M infants [8].

As previously published [16], SCA profiles were associated with poorer outcomes in non-MYCNA neuroblastoma in this study with longer follow-up, especially for 1p deletion and 2p gain [25–28].

Neuroblastoma in infancy differs from that in older children, as metastatic tumors without MYCNA are not considered high risks. In our study, they showed survival rates over 85% for both OS and EFS; comparable in 99.2 and 99.3 trials. Among metastatic patients under 1 year old, the prognosis tends to stay the same in infants under or over 6 months, with an intermediate survival and the requirement of 4–6 cycles of chemotherapy until the disappearance of metastases for INRG-M patients [29]. Infants from the 99.2 trial received chemotherapy when they presented with life-threatening symptoms, and the remaining patients could only be observed, decreasing the overall treatment burden.

MYCNA neuroblastomas are rare in infants, with very few studies focusing on them, INES being one of the first to evaluate specifically their outcome. Most trials include these young children with all other high-risk patients, failing to distinguish in terms of survival or toxicity assessment [30]. Early investigations on MYCN amplification are fundamental in the management of these

patients [31, 32]. In our study, MYCNA tumors were predominant in older infants and correlated with the dissemination of the disease (73.8% of MYCNA), especially for M-stage locations, with a more aggressive behavior acquired by amplified neuroblastic cells [33]. MYCN status remained the strongest prognostic factor in all age groups; however, survival tended to be slightly higher in MYCNA tumors in infants under 6 months.

The more favorable outcome in younger infants with MYCN-amplified tumors in our study has to be interpreted carefully, as the number of patients in the MYCNA population was very limited and could lead to potential bias. In our population of young children, MYCNA was very significant even in metastatic stages, with a striking difference in survival, possibly applicable for older children in future research [34].

While comparing our data to other reports outside Europe, the Children's Oncology Group has reported the risk of late mortality and subsequent malignant neoplasms among five-year survivors of neuroblastoma diagnosed < 1 year between 1970 and 1999. Their follow-up was much longer (25 years), but comparisons are impossible as they had no stage and tumor biology data available. The COG study observed 28 late deaths with a 25-year cumulative incidence of late mortality of 2.1%, including eight due to late cancer recurrence and three due to second cancer [35].

INES-FU could help with the development of future clinical trials and strengthen international collaboration in research for this kind of rare disease. Our study emphasized the importance of stratification by age, especially for infants under six months. Survival in this population is significantly better than in older children, as they presented a different type of disease, with more Ms stages and fewer MYCNA tumors. In these patients, good knowledge of late effects is important to provide appropriate guidelines. A proper broad-based analysis should be performed for high-risk infants aged under six months to help improve therapy adaptation, manage adverse events, and optimize follow-up.

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Conflicts of Interest

All authors declare that there are no conflicts of interest.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supporting File S1: pbc31982-sup-0001-SuppMat.pdf. **Supporting File S2**: pbc31982-sup-0002-SuppMat.pdf. **Supporting File S3**: pbc31982-sup-0003-SuppMat.pdf. **Supporting Table 1:** Characteristics of the patients enrolled in the INES-FU 99.9 arm (not eligible for trials because of a lack of inclusion criteria at time of recruitment), compared with patients included in the trials and the overall population.