Investigation of the Role of Dinutuximab Beta-Based Immunotherapy in the SIOPEN High-Risk Neuroblastoma 1 Trial (HR-NBL1)

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Received: 23 December 2019; Accepted: 25 January 2020; Published: 28 January 2020
Abstract: To explore the effects of immunotherapy in the International Society of Paediatric Oncology Europe Neuroblastoma Group SIOPEN high-risk neuroblastoma 1 trial (HR-NBL1 trial), two cohorts were studied: one prior to and one after the introduction of dinutuximab beta. All patients received standard induction and high-dose therapy (HDT) with autologous stem cell rescue (ASCR); the local control comprised surgery and radiotherapy to the primary tumour site, followed by isotretinoin. A landmark timepoint of 109 days, resulting from the median time between ASCR and initiation of immunotherapy, was used to define patients’ eligibility in the pre-immunotherapy analysis cohort. Median follow-up was 5.8 years (inter-quartile range (IQR): 4.2–8.2 years) for 844 eligible patients balanced for risk factors, such as age, sex, stage 4, MYCN amplification and response prior to HDT. The five-year event-free and overall survival (95% confidence interval (CI) of 466 patients not receiving immunotherapy was 42% (38–47%) and 50% (46–55%) but was 57% (51–62%) and 64% (59–69%) for 378 patients receiving immunotherapy \( p < 0.001 \). A multivariate analysis identified absence of immunotherapy \( p = 0.0002 \), hazard ratio (HR) 1.573; type of HDT \( p = 0.0029 \), HR 1.431); less than complete response prior to maintenance therapy \( p = 0.0043 \), HR 1.494) and >1 metastatic compartment at diagnosis \( p < 0.001 \), HR 2.665) as risk factors for relapse or progression. Results suggest an important role for dinutuximab beta-based immunotherapy within the treatment concepts applied in HR-NBL1/SIOPEN.

Keywords: high-risk neuroblastoma; immunotherapy; dinutuximab beta

1. Introduction

High-risk neuroblastoma defined by metastatic diseases over the age of 12 or 18 months [1] and MYCN amplification at any age remain associated with long-term survival rates of only 40% [2,3]. Treatment approaches comprise intensive induction [4,5], consolidation with high-dose chemotherapy (HDT) and autologous stem cell rescue (ASCR) [3,6], and isotretinoin as maintenance therapy.

As the disialoganglioside GD\(_2\) is expressed on the majority of neuroblastoma cells, with minimal expression on normal cells, it is a suitable target for immunotherapy [7]. Therefore, human/mouse chimeric anti-GD\(_2\) antibody ch14.18, dinutuximab, produced in SP2/0 cells was developed and investigated in clinical trials [8]. In Europe, ch14.18 was re-cloned in Chinese hamster ovarian (CHO) cells (dinutuximab beta) [9] for clinical trials of International Society of Paediatric Oncology Europe Neuroblastoma Group SIOPEN. The tolerability and activity of dinutuximab beta was first evaluated in a dose schedule of 20 mg/m\(^2\) given on five consecutive days by an 8 h infusion [10]. In 2006, SIOPEN opened a randomised trial to compare dinutuximab beta and isotretinoin with isotretinoin alone in patients with high-risk neuroblastoma. However, in 2007, the results of the Children’s Oncology Group (COG) ANBL0032 trial were communicated, followed by publication in 2010 [7], demonstrating that two-year event-free survival (EFS) and overall survival (OS) of patients with high-risk neuroblastoma receiving dinutuximab and cytokines (granulocyte-macrophage colony stimulating factor and interleukin-2), in addition to isotretinoin, were significantly higher by 20% and 11%, respectively [7], compared to those patients receiving isotretinoin alone. Therefore, continuation of the SIOPEN randomised trial was believed to be no longer feasible nor considered ethical, and the study design was modified to allow all patients to receive dinutuximab beta with or without interleukin-2. The altered randomisation opened on 22 October 2009 to investigate the role of subcutaneous interleukin-2 (sc-IL-2) with dinutuximab beta and assigned patients to dinutuximab beta alone or with sc-IL-2 [11]. All patients received oral isotretinoin [12]. The trial showed that the addition of sc-IL-2 to immunotherapy with dinutuximab beta, given as an 8 h infusion, did not improve outcome but increased toxicity.

In this report, we aim to assess the contribution of dinutuximab beta-based immunotherapy to the outcome of patients with high-risk neuroblastoma in the International Society of Paediatric Oncology
Europe Neuroblastoma Group High-Risk Neuroblastoma 1 (HR-NBL1/SIOPEN) trial by investigating the survival of patients in sequential eras with the same eligibility criteria treated with (immunotherapy population (IP), 2009–2013) [12] or without immunotherapy (control population (CP), 2002–2009).

2. Results

2.1. Patient Characteristics

According to the inclusion criteria for the analysis, 844 patients enrolled in 146 SIOPEN member hospitals/institutions in 19 countries were eligible (378 in the IP and 466 in the CP) (Figure 1). Median follow-up was 5.8 years (inter-quartile range (IQR): 4.2 to 8.2 years). The median age of patients at diagnosis was 2.9 years (IQR: 1.8 to 3.8).

![Flow chart for the analysis cohort](image)

**Figure 1.** Flow chart for the analysis cohort. HDT (high-dose chemotherapy); BuMel (high-dose chemotherapy with busulfan and melphalan; CEM (high-dose chemotherapy with carboplatin, etoposide and melphalan); R1 (high-dose chemotherapy randomisation); R2 (immunotherapy randomisation) and IL-2 (interleukin-2).

Both populations were balanced for sex, stage 4, MYCN amplification and response prior to HDT (Table 1).

2.2. Survival

The five-year EFS was 57% (95% CI: 51–62%) for IP, compared to 42% (95% CI: 38–47%) for CP patients ($p < 0.001$) (Figure 2A). The five-year overall survival (OS) for the IP was 64% (95% CI: 59–69%), compared to 50% (95% CI: 46–55%) for CP patients (Figure 2B).
Table 1. Characteristics of the control and immunotherapy populations. N = number; % = percentage; MNA = MYCN amplification; no = not present and yes = present; MC = metastatic compartments; TVD = topotecan, vincristine and doxorubicin; HDT = high-dose chemotherapy; BuMel = high-dose chemotherapy with busulfan and melphalan; CEM = high-dose chemotherapy with carboplatin, etoposide and melphalan; NR = not reported; CR = complete remission; VGPR = very good partial remission; PR = partial remission; CME = complete macroscopic excision and IME = incomplete macroscopic excision.

| Characteristics            | Control Population | Immunotherapy Population |
|----------------------------|--------------------|--------------------------|
|                            | n                  | %                        | n                  | %                        |
| Total                      | 466                | 378                      |
| Sex                        |                    |                          |                    |
| Female                     | 180                | 39%                      | 140                | 32%                      |
| Male                       | 286                | 61%                      | 238                | 63%                      |
| Age                        |                    |                          |                    |
| <1.5 years                 | 64                 | 13%                      | 55                 | 14%                      |
| 1.5–<5 years               | 333                | 71%                      | 254                | 62%                      |
| ≥5 years                   | 69                 | 15%                      | 69                 | 18%                      |
| Median                     | 2.70               | 2.87                     |
| Stage                      |                    |                          |                    |
| Localised                  | 60                 | 13%                      | 32                 | 8%                       |
| Stage 4                    | 406                | 87%                      | 339                | 90%                      |
| Stage 4s                   | 0                  | 0%                       | 7                  | 2%                       |
| MYCN Stage 4               |                    |                          |                    |
| MNA NR                     | 27                 | 6%                       | 16                 | 4%                       |
| MNA no                     | 217                | 57%                      | 197                | 61%                      |
| MNA yes                    | 162                | 43%                      | 126                | 39%                      |
| MC                         |                    |                          |                    |
| NR                         | 23                 | 5%                       | 23                 | 6%                       |
| 0                          | 60                 | 14%                      | 32                 | 9%                       |
| 1                          | 70                 | 16%                      | 35                 | 10%                      |
| 2                          | 136                | 31%                      | 112                | 32%                      |
| 3                          | 120                | 27%                      | 112                | 32%                      |
| >3                         | 57                 | 13%                      | 64                 | 18%                      |
| TVD given                  |                    |                          |                    |
| NR                         | 23                 | 5%                       | 10                 | 3%                       |
| No                         | 391                | 88%                      | 250                | 68%                      |
| Yes                        | 52                 | 12%                      | 118                | 32%                      |
| Surgery                    |                    |                          |                    |
| CME                        | 318                | 76%                      | 261                | 75%                      |
| IME                        | 101                | 24%                      | 87                 | 25%                      |
| Status prior HDT           |                    |                          |                    |
| NR                         | 25                 | 5%                       | 33                 | 9%                       |
| CR                         | 174                | 39%                      | 116                | 34%                      |
| VGPR                       | 159                | 36%                      | 149                | 43%                      |
| PR                         | 108                | 24%                      | 80                 | 23%                      |
| HDT                        |                    |                          |                    |
| BuMel                      | 257                | 55%                      | 348                | 92%                      |
| CEM                        | 209                | 45%                      | 30                 | 8%                       |
| Status prior Maintenance   |                    |                          |                    |
| NR                         | 58                 | 12%                      | 17                 | 4%                       |
| CR                         | 258                | 63%                      | 210                | 58%                      |
| VGPR                       | 93                 | 23%                      | 99                 | 27%                      |
| PR                         | 57                 | 14%                      | 52                 | 14%                      |

The cumulative incidence of relapse/progression (CIR) at five years was 41% (95% CI: 37–47%) for the IP and 57% (95% CI: 53–61%) for the CP patients (p < 0.001). At the last follow-up, 153 patients of the IP had an event versus 272 of CP patients. The cumulative incidence of non-relapse mortality was 2% (95% CI: 1–4%) in the IP and 1% (95% CI < 1–2%) in the CP (Figure 2C).
2.3. Influence of Risk Factors

Disease status prior to maintenance therapy was available in 769/844 (91%) patients (Table 2). Older age; stage 4; involvement of more than one metastatic compartment (MC); disease status prior to maintenance therapy; addition of topotecan, vincristine and doxorubicin (TVD) and use of carboplatin, etoposide and melphalan (CEM) as HDT were associated with lower EFS (Figure 3) in the population analysed. Patients with lymph nodes as their only MC (five-year EFS 60% (95% CI: 36–78%)) had a similar EFS as patients with other isolated metastatic sites (five-year EFS 60% (95% CI: 49–70%)).

![Figure 2.](image)

**Figure 2.** Analysis population comparing control population versus immunotherapy population. (A) Event-free survival, (B) overall survival and (C) cumulative incidence of progression/relapse. CP (control population), IP (immunotherapy population), CIR (cumulative incidence of relapse) and NRM (non-relapse mortality).
Table 2. Outcomes according to risk factors and subgroups. (2A) Event-free survival and (2B) overall survival. Pts (patients); 95% CI (95% confidence interval); p-value (probability value for A: comparison according to risk factor and B: for interaction); MNA (MYCN amplification); - (not present) and + (present); MC (metastatic compartments); TVD (topotecan, vincristine and doxorubicin); HDT (high-dose chemotherapy); BuMel (high-dose chemotherapy with busulfan and melphalan); CEM (high-dose chemotherapy with carboplatin, etoposide and melphalan); CR (complete remission); VGPR (very good partial remission); PR (partial remission); CME (complete macroscopic excision) and IME (incomplete macroscopic excision).

| Characteristics | Total Population | Control Group | Immunotherapy Group | p-Value \( ^{b} \) |
|-----------------|-----------------|---------------|---------------------|----------------|
| (A) Event Free Survival | Events/Pts 5-years EFS (95% CI) | p-value \( ^{a} \) | Events/Pts 5-years EFS (95% CI) | Events/Pts 5-years EFS (95% CI) |
| **Total** | 844 Pts | 466 Pts | 378 Pts |
| **Sex** | | | | |
| female | 164/320 49 (44–55) | 0.803 | 105/180 43 (36–50) | 59/140 57 (49–65) | 0.938 |
| male | 273/524 48 (44–52) | 171/286 41 (36–47) | 102/238 56 (49–62) |
| **Age** | | | | |
| <1.5 years | 48/119 60 (51–68) | 0.007 | 27/64 59 (46–70) | 21/55 61 (47–73) | 0.161 |
| 1.5–<5 years | 302/587 49 (44–53) | 194/333 42 (37–47) | 108/254 57 (50–63) |
| ≥5 years | 87/138 38 (30–47) | 55/99 25 (16–36) | 32/69 53 (40–64) |
| **Stage** | | | | |
| Localised | 25/92 72 (62–80) | <0.001 | 14/60 76 (63–85) | 11/32 66 (47–79) | 0.007 |
| Stage 4 | 410/745 45 (42–49) | 262/406 37 (32–42) | 148/339 56 (50–61) |
| Stage 4s | 2/7 71 (26–92) | – | – | 2/7 71 (26–92) |
| Stage 4 MNA no- | 236/414 43 (38–48) | 0.819 | 147/217 33 (27–39) | 89/197 54 (47–61) | 0.25 |
| Stage 4 MNA yes | 154/288 47 (41–53) | 98/162 42 (34–49) | 56/126 55 (45–63) |
| **MC** | | | | |
| 0 | 25/92 72 (62–80) | <0.001 | 14/60 67 (63–85) | 11/32 66 (47–79) | 0.025 |
| 1 | 43/105 60 (50–69) | 34/70 54 (42–65) | 9/35 71 (50–85) |
| 2 | 136/248 46 (39–52) | 85/136 39 (31–47) | 51/112 55 (45–63) |
| 3 | 134/232 43 (36–49) | 89/120 27 (20–36) | 45/112 60 (50–69) |
| >3 | 76/121 37 (28–46) | 42/57 28 (17–40) | 34/64 45 (32–57) |
| **TVD** | | | | |
| no | 322/641 50 (46–54) | 0.024 | 225/391 44 (39–49) | 97/250 61 (54–67) | 0.732 |
| yes | 100/170 39 (32–47) | 38/52 27 (16–39) | 62/118 45 (34–55) |
| **Surgery** | | | | |
| CME | 288/578 50 (46–54) | 0.123 | 183/318 43 (38–49) | 105/260 59 (52–65) | 0.946 |
| IME | 106/188 45 (38–52) | 66/101 38 (29–48) | 40/87 53 (42–63) |
| **Status Prior HDT** | | | | |
| CR | 134/290 54 (48–59) | 0.022 | 86/174 51 (43–58) | 48/116 57 (47–66) | 0.294 |
Table 2. Cont.

| Characteristics | Total Population | Control Group | Immunotherapy Group | p-value $^b$ |
|----------------|------------------|---------------|---------------------|-------------|
| (A) Event Free Survival | Events/Pts | 5–years EFS (95% CI) | Events/Pts | 5–years EFS (95% CI) | Events/Pts | 5–years EFS (95% CI) |
| HDT | BuMel | 288/605 | 52 (48–56) | <0.001 | 137/257 | 48 (41–54) | 151/348 | 56 (50–61) | 0.055 |
| CEM | 149/239 | 39 (33–45) | | | 139/209 | 35 (29–42) | 10/30 | 67 (47–80) | |
| Status Prior Maintenance | | | | | |
| CR | 225/468 | 52 (48–47) | 0.002 | 144/258 | 46 (39–52) | 81/210 | 61 (53–67) | 0.84 |
| VGPR | 98/192 | 50 (42–56) | | 55/93 | 43 (33–53) | 43/99 | 56 (46–66) | |
| PR | 70/109 | 35 (26–44) | | 42/57 | 26 (15–33) | 28/52 | 45 (32–58) | |
| (B) Overall Survival | Events/Pts | 5-year OS (95% CI) | p-value $^a$ | Events/Pts | 5-year OS (95% CI) | Events/Pts | 5-year OS (95% CI) |
| Sex | | | | | | | | |
| female | 138/320 | 59 (53–64) | 0.536 | 95/180 | 53 (45–60) | 43/140 | 59 (53–64) | 0.488 |
| male | 234/524 | 55 (50–59) | | 150/286 | 49 (43–55) | 84/238 | 55 (50–59) | |
| Age | | | | | | | | |
| <1.5 years | 68/138 | 52 (46–58) | 0.445 | 26/64 | 41 (34–50) | 19/55 | 52 (46–57) | 0.409 |
| 1.5–<5 years | 259/587 | 57 (53–61) | | 177/333 | 50 (45–55) | 82/254 | 57 (53–61) | |
| ≥5 years | 259/587 | 57 (53–61) | 0.001 | 42/69 | 42 (30–54) | 26/69 | 49 (40–58) | 0.003 |
| Stage | localised | 24/92 | 76 (66–83) | | 13/60 | 82 (69–89) | 11/32 | 76 (66–83) | |
| Stage 4 | 347/745 | 54 (50–57) | | 232/406 | 46 (41–51) | 115/339 | 54 (50–57) | |
| Stage 4s | 0 | 0 | | 0 | 1.7 | | | |
| Status Prior HDT | | | | | | | | |
| MC | 0 | 24/92 | 76 (66–83) | <0.001 | 13/60 | 82 (69–89) | 11/32 | 76 (66–83) | 0.013 |
| 1 | 36/105 | 68 (58–76) | | 29/70 | 62 (50–73) | 7/35 | 68 (58–76) | |
| 2 | 109/248 | 56 (50–62) | | 71/136 | 51 (42–59) | 38/112 | 56 (50–62) | |
| 3 | 117/232 | 49 (42–56) | | 81/120 | 36 (27–44) | 36/112 | 49 (42–56) | |
| TVD | >3 | 70/121 | 43 (34–52) | 0.224 | 41/57 | 32 (20–44) | 29/64 | 43 (34–52) | 0.441 |
| TVD no | 281/641 | 57 (53–61) | | 200/391 | 52 (47–57) | 81/250 | 57 (53–61) | |
| Surgery | yes | 78/170 | 52 (44–60) | | 34/52 | 36 (23–49) | 44/118 | 52 (44–60) | |
| CME | 248/578 | 57 (53–61) | 0.218 | 165/318 | 51 (45–56) | 83/260 | 57 (53–61) | 0.765 |
| IME | 91/188 | 52 (45–59) | | 58/101 | 46 (36–55) | 33/87 | 52 (45–59) | |
Table 2. Cont.

| Characteristics | Total Population | Control Group | Immunotherapy Group | p-value |
|-----------------|-----------------|---------------|---------------------|---------|
|                 | Events/Pts | 5-year OS (95% CI) | Events/Pts | 5-year OS (95% CI) | Events/Pts | 5-year OS (95% CI) |         |
| **Status Prior Maintenance** |         |               |         |               |         |               |         |
| CR              | 190/468     | 60 (55–65)      | 129/258   | 54 (47–59)      | 61/210   | 60 (55–65)      | 0.640   |
| VGPR            | 83/192      | 57 (49–64)      | 47/93     | 52 (42–62)      | 36/99    | 57 (49–64)      |         |
| PR              | 61/109      | 43 (33–53)      | 38/57     | 36 (23–48)      | 23/52    | 50 (33–65)      |         |
Figure 3. Influence of risk factors within the analysis population on event-free survival (EFS). (A) EFS and age; (B) EFS and stage; (C) EFS and metastatic compartments (MC) localised and 4s stage vs. stage 4 (one MC vs. multiple MC); (D) response status prior to maintenance phase: CR (complete remission), VGPR (very good partial remission) and PR (partial remission) and (E) EFS and TVD (topotecan, vincristine and doxorubicin). TVD added = yes and TVD not added = no. (F) Type of high-dose chemotherapy = BuMel (busulfan and melphalan) and CEM (carboplatin, etoposide and melphalan).
In the IP, the two-year and five-year EFS rates for 210 patients (81 events) in complete remission (CR) were 68% (95% CI: 61–74%) and 61% (95% CI: 53–57%). In the CP, the two-year and five-year EFS rates for 258 patients in CR (144 events) were 54% (95% CI: 48–60%) and 46% (95% CI: 39–52%).

The impact of immunotherapy on EFS was significantly influenced by stage and MC, and the impact of immunotherapy was stronger in patients with metastatic disease (Table 2). Furthermore, a borderline significant interaction between maintenance treatment and HDT ($p = 0.055$) was observed.

### 2.4. Multivariate Analysis on Analysis Cohort

Patients who had no immunotherapy ($p = < .0001$, cumulative hazard ratio (cHR) 1.75) HDT with CEM ($p = 0.0345$, cHR 1.3); partial remission (PR) prior maintenance therapy ($p = 0.0103$, cHR 1.49); more than one MC at diagnosis ($p < 0.001$, cHR 2.69) and age $> 5$ years ($p = 0.0138$, cHR 1.59) had a higher risk of relapse (Table 3).

**Table 3.** Multivariate analysis of the analysis cohort. cHR (cumulative hazard ratios); 95% CI (95% confidence interval); $p$-value (probability value); MNA (MYCN amplification); MC (metastatic compartments, referring either to bone marrow, skeletal or lymph node involvement); TVD (topotecan, vincristine and doxorubicin); CR (complete remission); VGPR (very good partial remission); PR (partial remission); HDT (high-dose chemotherapy); BuMel (high-dose chemotherapy with busulfan and melphalan); CEM (high-dose chemotherapy with carboplatin, etoposide and melphalan); IP (immunotherapy population) and CP (control population). * test for the global main effect for risk-factors with more than two categories.

| Risk Factor | Characteristics | Pseudo Values for 5-Years EFS | *P*-Value |
|-------------|-----------------|-------------------------------|------------|
| (A) Multivariate Analysis | Immunotherapy vs. Control Cohort | 1.75 (1.36–2.25) | <0.0001 |
| Age (vs. $<1.5$ yrs) | 1.5–5 years | 1.31 (0.92–1.87) | 0.1384 |
| | $>5$ years | 1.59 (1.05–2.42) | 0.0138 |
| Stage 4, 4s and Number of MC (vs. MNA stages 2, 3) | 1 MC | 1.38 (0.80–2.47) | 0.2493 |
| | $>1$MC | 2.69 (1.74–4.15) | <0.0001 |
| TVD | | 1.28 (0.97–1.69) | 0.2478 |
| Status Prior Maintenance (vs. CR) | VGPR | 1.06 (0.81–1.39) | 0.6416 |
| | PR | 1.49 (1.10–2.02) | 0.0103 |
| HDT | CEM vs. BuMel | 1.32 (1.02–1.70) | 0.0345 |
| (B) Subgroup Analysis According to HDT (after adjustment for age, stage, MC, TVD and status prior maintenance treatment) |BUMEL | IP vs. CP | 1.6 (1.2–2.1) | 0.001 |
| CEM | IP vs. CP | 3.0 (1.5–5.8) | 0.002 |
After adjustment for age, stage, MC, TVD and response prior to maintenance therapy (Table 3), a benefit from immunotherapy was confirmed for either HDT (busulfan and melphalan (BuMel) or CEM). Patients receiving BuMel had an adjusted cumulative hazard ratio of 1.6 (1.2–2.1) with an unadjusted five-year EFS for the IP of 56% (95% CI: 50–61%) and 48% (95% CI: 41–54%) for the CP ($p = 0.001$). Patients receiving CEM had an adjusted cumulative hazard ratio of 3.0 (1.5–5.8) and showed an unadjusted five-year EFS of the IP of 67% (95% CI: 47–80%) versus 35% (95% CI: 29–42%) for the CP ($p = 0.002$).

### 2.5. Response to Maintenance Treatments

Thirty-nine of one hundred and eight (36%) CP patients with evaluable diseases prior to maintenance (67 very good partial remission (VGPR) and 41 partial remission (PR)) responded; of whom, 35/108 (32%) achieved CR after isotretinoin. In contrast, 64/130 (49%) of IP patients with evaluable diseases (85 VGPR and 45 PR) prior to immunotherapy responded; of whom, 52/130 (40%) achieved CR after immunotherapy (Table 4, $p = 0.226$).

### Table 4. Response for immunotherapy and control populations. Legend: CR (complete remission), VGPR (very good partial remission), PR (partial remission), SD (stable disease) and PD (progressing disease).

| Response Status before Maintenance | Total | Evaluable | CR | VGPR | PR | SD | PD |
|-----------------------------------|-------|-----------|----|------|----|----|----|
| Immunotherapy and Isotretinoin    |       |           |    |      |    |    |    |
| CR                               | 210   | 188       | 151| 0    | 0  | 0  | 37 |
| <CR Total                        | 151   | 130       | 52 | 43   | 8  | 0  | 0  |
| VGPR                             | 99    | 85        | 36 | 31   | 0  | 0  | 18 |
| PR                               | 52    | 45        | 16 | 12   | 8  | 0  | 9  |
| Isotretinoin                     |       |           |    |      |    |    |    |
| CR                               | 258   | 204       | 163| 0    | 0  | 0  | 40 |
| <CR Total                        | 150   | 108       | 35 | 27   | 18 | 1  | 27 |
| VGPR                             | 93    | 67        | 28 | 23   | 2  | 0  | 14 |
| PR                               | 57    | 41        | 7  | 4    | 16 | 1  | 13 |

### 2.6. Adverse Events and Toxicity

Adverse events (CTC Grades 1 to 4) are summarized in Table 5. Toxicity tended to be higher in the IP population, particularly in those patients who received sc-IL-2. Four patients had non-relapse-related mortality in the IP and four in the CP.
Table 5. Toxicities IL-2 (interleukin 2), non-hem. tox. (non-hematological toxicities), WBC (white blood cells), ECHO LV (left ventricular)/SV (stroke volume), GFR (glomerular filtration rate), central neuro (central neurotoxicity), periph neuro (peripheral neurotoxicity) and liver enzymes: SGOT (serum-glutamat-oxalacetat-transaminase)/SGPT (serum-glutamat-pyruvat-transaminase). Columns in bold show the values for the combined grade 3 and 4 toxicities.

| Toxicities                  | Control | Without IL2 | All          | With IL2 |
|-----------------------------|---------|-------------|--------------|----------|
|                             | Eval    | All         | 0 1 2 3 4 3 + 4 | 0 1 2 3 4 3 + 4 |
| Non-Hem. Tox.               | 317     | 317         | 113 42 116 41 5 46 15% | 186 5 6 53 105 17 122 66% |
| General Condition           | 314     | 314         | 225 69 13 4 3 7 2% | 185 42 70 43 24 6 30 16% |
| Haemoglobin                 | 313     | 313         | 208 39 54 8 4 12 4% | 186 21 2 84 69 10 79 42% |
| WRC                         | 313     | 313         | 235 32 26 15 3 18 6% | 186 35 30 73 42 6 48 26% |
| Granulocytes                | 313     | 313         | 244 23 25 16 5 21 7% | 186 44 26 54 43 19 62 33% |
| Platelets                   | 313     | 313         | 260 13 12 16 12 28 9% | 186 66 25 31 40 24 64 34% |
| Infection                   | 315     | 315         | 220 52 23 19 1 20 6% | 185 79 26 32 47 1 48 26% |
| Fever                       | 314     | 314         | 241 14 54 4 1 5 2% | 185 41 3 116 24 1 25 14% |
| Stomatitis                  | 312     | 312         | 293 10 7 2 0 2 1% | 185 156 18 8 0 3 3 2% |
| Nausea/Vomiting             | 313     | 313         | 284 6 19 4 0 4 1% | 185 88 11 76 9 1 10 5% |
| Diarrhoea                   | 313     | 313         | 286 11 13 3 0 3 1% | 185 93 32 47 10 3 13 7% |
| Constipation                | 312     | 312         | 302 7 3 0 0 0 0% | 185 110 43 32 0 0 0 0% |
| Skin                        | 315     | 315         | 181 50 73 10 1 11 3% | 185 65 46 65 9 0 9 5% |
| Allergy                     | 314     | 314         | 307 4 3 0 0 0 0% | 185 88 50 28 14 5 19 10% |
| Cardiac Function            | 298     | 298         | 298 0 0 0 0 0% | 183 178 0 0 3 1 4 2% |
| Echo LV/SV                  | 298     | 298         | 298 0 0 0 0 0% | 182 181 0 0 0 0 1 1% |
| Hypotension                 | 298     | 298         | 296 2 0 0 0 0 0% | 182 139 22 8 12 1 13 7% |
| Hypertension                | 298     | 298         | 298 0 0 0 0 0% | 182 162 10 3 7 0 7 4% |
| Creatinine                  | 312     | 312         | 301 9 2 0 0 0 0% | 185 167 14 1 3 7 0 3 2% |
| Proteinuria                 | 311     | 311         | 307 4 0 0 0 0% | 184 169 13 2 0 0 0 0% |
| Haematuria                  | 311     | 311         | 305 6 0 0 0 0% | 183 167 11 5 0 0 0 0% |
| GFR                         | 310     | 310         | 302 5 3 0 0 0% | 183 172 6 2 3 0 3 2% |
| Central Neuro               | 311     | 311         | 304 4 0 0 3 3 1% | 185 165 14 3 3 0 3 2% |
| Periph Neuro                | 311     | 311         | 308 1 0 1 1 2 1% | 185 173 8 3 1 0 1 1% |
| Bilirubin                   | 309     | 309         | 301 4 2 2 0 2 1% | 185 169 1 10 4 1 5 3% |
| SGOT/SGPT                   | 311     | 311         | 218 68 19 6 0 6 2% | 185 68 43 43 30 1 31 17% |
| Dilated Pupils              | 22      | 22          | 0 0 0 0 0 0 0% | 123 108 15 0 0 0 0 0% |
| Accommodation Defects       | 22      | 22          | 0 0 0 0 0 0 0% | 121 115 6 0 0 0 0 0% |
| Capillary Leak Syndrome     | 19      | 19          | 18 0 1 0 0 0 0% | 119 91 0 23 5 0 5 4% |
| Cytokine Release Syndrome   | 19      | 19          | 18 1 0 0 0 0 0% | 118 95 8 10 5 0 5 4% |
| Pain related to ch14.18/CHO | 22      | 22          | 0 0 0 0 0 0 0% | 120 113 7 0 0 0 0 0% |
| Papilloedema                | 22      | 22          | 0 0 0 0 0 0 0% | 120 113 7 0 0 0 0 0% |
3. Discussion

This analysis showed superior EFS and OS in the era when dinutuximab beta-based immunotherapy was included in therapy for high-risk neuroblastoma, compared to the previous era in the same trial when isotretinoin alone was the only element of maintenance therapy. Although there are limitations with a historical comparison, this is the first and possibly only demonstration that the addition of dinutuximab beta as immunotherapy improves survival in the high-risk neuroblastoma front-line population treated homogenously in the HR-NBL1/SIOPEN trial. Both the control and immunotherapy populations received the same treatment approach. Furthermore, this analysis is an important contribution, as data on the efficacy of anti-GD2 antibody-based immunotherapy are limited to one prospective randomised trial and a few retrospective analyses. Ethical concerns precluded a randomised comparison of immunotherapy after the results of the COG ANBL0032 trial emerged. A randomised trial of dinutuximab beta and isotretinoin compared to isotretinoin alone would have produced more robust data, but this was believed not to be ethically feasible within the SIOPEN community.

As the two cohorts were from the same trial and using the same criteria; in particular, HDT within nine months from diagnosis and no progression at 109 days after ASCR as a starting point for survival, with an unchanged supportive care protocol guidance, this analysis provides important data supporting the benefit of dinutuximab beta-based immunotherapy. The landmark time identified was the median time observed between ASCR and initiation of dinutuximab beta; thus, only patients without progressive diseases at this timepoint were included in the pre-immunotherapy CP. The introduction of this landmark was important in order to exclude early relapse before immunotherapy could be commenced. Both populations, IP and CP, were balanced for age, sex, stage 4, MYCN amplification and response prior to HDT.

The benefit of immunotherapy was further underpinned by multivariate analysis. After adjustment for risk factors (for example, age, stage, MC at diagnosis, need for TVD and response prior to maintenance therapy) a positive impact on outcome was observed with either BuMel or CEM. This is particularly important in view of a higher percentage of patients receiving CEM in the control group, as we previously have shown superior outcomes for patients treated with BuMel [6]. Hence, the superior outcomes in the immunotherapy group are unlikely to be solely related to BuMel. These conclusions are supported by the recent publication from COG [13] demonstrating that immunotherapy improves survival even after optimised HDT regimens.

Response prior to immunotherapy is an important prognostic factor. Patients treated in CR in the IP had a two-year EFS of 68%, compared to 54% in the CP. Acknowledging that comparisons across trials are challenging, these results are similar to the previous report of dinutuximab in combination with IL-2 and GM-CSF in patients in CR, resulting in a two-year EFS of 66% for patients treated by immunotherapy, compared to only 46% in patients with an isotretinoin maintenance treatment [7]. Further support in favour of dinutuximab beta-based immunotherapy within the HR-NBL1/SIOPEN trial comes from improved response rates in patients with residual diseases at the site of the primary tumour or metaiodobenzylguanidine (mIBG)-positive skeletal disease following immunotherapy; a 49% response rate and a 40% CR rate was observed in patients treated with immunotherapy, as compared to a 36% overall response and 32% CR rate in the control population.

The increasing role for immunotherapy, with anti-GD2 antibodies, in the therapy of patients with high-risk neuroblastoma is further highlighted by the recent demonstration of the efficacy of combining anti-GD2 antibodies with chemotherapy, either at relapse or at initial presentation [14,15].

In summary, this report describes the effects of including dinutuximab beta in high-risk neuroblastoma maintenance therapy and shows a clear survival benefit. This provides an important baseline to further build immunotherapy strategies in this challenging patient population.
4. Materials and Methods

4.1. Trial Eligibility

HR-NBL1/SIOOPEN, an international, randomised, multiarm, open-label, phase 3 trial for high-risk neuroblastoma, opened on 24 June 2002 and is registered with ClinTrials.gov, number NCT01704716, and EudraCT, number 2006-001489-17. All randomisations of the HR-NBL1/SIOOPEN trial are closed, and three have been published [5,6,12]. SIOOPEN institutions recruited patients after approval of the trial by national regulatory authorities and ethical committees. Parents/guardians and patients provided written informed consent or assent, when applicable.

The International Neuroblastoma Staging System criteria (INSS) and International Neuroblastoma Response Criteria (INRC) [16] were used to classify the disease and to evaluate responses to therapy. Untreated patients with INSS stage 4 metastatic neuroblastoma aged 1-20 years or INSS stage 2–4 neuroblastoma with MYCN amplification, as determined in SIOOPEN reference laboratories [17], any age up to 20 years, were eligible. The SIOOPEN-R-NET web-based system (https://www.siopen-r-net.org) randomly assigned eligible patients in real-time.

4.2. Eligibility for the Analysis Cohort and Treatments Given

This analysis included all patients registered in the HR-NBL1/SIOOPEN trial between 2002 and 2013 who met the criteria: (i) HDT within 9 months from diagnosis and (ii) no progression at 109 days after ASCR. The median time between ASCR and initiation of dinutuximab beta was 109 days; therefore, only patients without progressive diseases at this landmark timepoint were included in the pre-immunotherapy control population (CP). The 18 patients randomised to receive dinutuximab beta and isotretinoin between 2006 and 2009 were excluded from this analysis. Two cohorts were compared, a CP between 2002–2009 who did not receive dinutuximab beta and an IP between 2009 and 2013 who was randomised to receive dinutuximab beta with or without sc-IL2 [12]. As the addition of IL-2 to immunotherapy with dinutuximab beta did not improve outcome, both randomised arms were considered as one group for the purposes of this analysis [12]. The CP comprised patients who received HDT randomised to BuMel or CEM [6] but did not receive immunotherapy.

All patients received rapid COJEC induction [4,5] (Figure 4). Between 2002 to 2010, patients who had a bone marrow complete response (CR) and a metastatic CR or a metastatic partial response (PR) defined by INSS criteria but at least 50% reduction in skeletal metaiodobenzylguanidine (mIBG) positivity from baseline and three or fewer areas of abnormal uptake on $^{123}$I-mIBG scintigraphy were eligible for HDT randomisation comparing BuMel with CEM [6]. Thereafter, BuMel became the standard of care. Patients who did not achieve a metastatic response to fulfil the HDT eligibility criteria received two courses of topotecan, vincristine and doxorubicin (TVD) [18] prior to HDT. Local treatment of the primary tumour comprised attempted total surgical resection and radiotherapy (21 Gy) to the primary tumour site between 60 and 90 days after ASCR. There was no dose modification in the event of incomplete tumour excision; neither were metastatic sites systematically irradiated.
All patients received 6 cycles of oral isotretinoin over two weeks [3] after local irradiation. From 2009, patients were randomised between day 60 to 90 after ASCR to receive five courses of dinutuximab beta at a dose of 20 mg/m²/day as an 8 h infusion for 5 consecutive days (total dose of 100 mg/m² per cycle, days 8 to 12) with or without 6 × 10⁶ IU/m²/day of sc-IL-2 on days 1 to 5 and days 8 to 12 of each immunotherapy cycle [12].
Patients had full disease evaluations prior to and after 2 and 5 courses of maintenance treatment. This included whole body $^{123}$I-mIBG scintigraphy, CT or MRI scans of the primary tumour and any other evaluable site of the disease; bone marrow examination with both aspirates and trephines obtained from two sites and measurement of urinary catecholamine metabolites. Response was assessed by the 1993 INRC based on local institution reporting. The only evaluable diseases prior to immunotherapy were mIBG-positive skeletal disease or diseases detectable on CT/MRI scans prior to randomisation, as patients with bone marrow involvement were not eligible.

4.3. Statistical Analysis

4.3.1. Establishment of the Analysis Cohort

The number of MC at diagnosis was calculated according to the number of MC at diagnosis either in the bone marrow, skeleton or other sites, with a possible range from one to six. Characteristics of the CP were compared to the IP by the chi-square test to assess the balance of risk factors between the two populations.

4.3.2. Outcome Parameters

Follow-up commenced at 109 days for the CP cohort and from the first dose of dinutuximab beta for the IP cohort. EFS and OS were estimated using the Kaplan and Meier method and compared with the log-rank test [19]. CIR was estimated [20], taking into account the competing risk of death without relapse/progression. For cumulative incidence of non-relapse mortality, relapse or progression was considered as a competing event. The statistical comparison of cumulative incidences used Gray’s methodology [21]. EFS, OS, cumulative incidence of relapse/progression and cumulative incidence of non-relapse mortality are presented as 5-year point estimates with confidence intervals (CI), as previously described [20,22]. Two-year EFS was determined to facilitate comparisons with published data [7]. $p$-values of less than 0.05 were considered to indicate statistical significance. In spite of the limitations of subgroup analysis (including multiple testing and lack of power), the cohorts were assessed according to baseline, pre and post-HDT risk factors (Table 2). A formal test for interaction was performed within a Cox model.

4.3.3. Multivariate Analysis

Multivariate analysis of treatment and risk factors was undertaken on the analysis cohort. In the presence of nonproportional hazards, as detected for age and MYCN amplification, the pseudo-value regression [22] for a 5-year EFS approach was chosen. The aim of this analysis was to adjust the comparison between the two populations (IP and CP) for potential confounders and risk factors, such as age, stage, addition of TVD, disease status prior to maintenance and HDT/ASCR. Using the same approach, a subgroup analysis was performed in order to separately evaluate the value of immunotherapy in patients with BuMel and CEM.

The data cut-off time of this analysis was July 31, 2017. Median follow-up was calculated using the inverse Kaplan Meier estimate. The statistical evaluation and power calculation were done with SAS 9.4 and Module LRI of Pass 2002, respectively.
5. Conclusions

This report shows that the introduction of dinutuximab beta is associated with a survival benefit for children with high-risk neuroblastoma. Similar results were reported for dinutuximab in one randomized trial and one nonrandomized investigation [23–25]. However, dinutuximab beta is a different molecule with a separate development pathway, and the demonstration of its beneficial effects on treatment outcome is an important finding.

Given the absence of a beneficial effect by adding sc-IL-2 to an 8 h infusion of dinutuximab beta [12], the standard treatment recommended by SIOPEN is dinutuximab beta with isotretinoin for maintenance therapy of high-risk neuroblastoma. The benefits might be less in some subgroups (<1.5 years, MYCN-amplified localised disease) and needs close monitoring in future studies. Modifications of the length of the dinutuximab beta schedule and immunotherapy combination strategies may optimise the benefits of immunotherapy in high-risk neuroblastoma to improve survival.

Author Contributions: Conceptualization: R.L. (Ruth Ladenstein), U.P., H.N.L. Study design: R.L. (Ruth Ladenstein), U.P., H.N.L. Resources: R.L. (Ruth Ladenstein), R.L. (Roberto Luksch), V.C., S.A., G.L., P.B., J.M.M., C.O., T.T., G.C.F.C., G.S., E.R., H.S., M.B.-P., G.S., H.L., P.A., K.H., M.R.C., M.N.G., A.G. Quality control of data and algorithms: R.L. (Ruth Ladenstein), U.P. Data analysis and interpretation: R.L. (Ruth Ladenstein), U.P., H.N.L. Formal analysis: U.P. Writing-original draft preparation: R.L. (Ruth Ladenstein), U.P., A.D.J.P., H.N.L. Writing-review and editing: R.L. (Ruth Ladenstein), U.P., H.N.L., A.D.J.P., D.V.-C.M.N.G. Project administration: R.L. (Ruth Ladenstein). All authors have read and agreed to the published version of the manuscript.

Funding: The funding of the European Commission 5th Framework Grant (SIOPEN-R-NET EC grant No. QLRI-CT-2002-01768, www.siopen-r-net.org) is disclosed as a source of funding in the author form. Pierre Fabre Médicament provided Busilvex® (Paris, France) and APEIRON (Vienna, Austria) provided dinutuximab beta (ch14.18/CHO), along with the St. Anna Kinderkrebshilfswerk (Vienna, Austria). The European Commission, Pierre Fabre Médicament and APEIRON had no involvement in the conduct of the research and preparation of the article. The St. Anna Kinderkrebshilfswerk was the academic sponsor of the trial. In addition, this work was supported by these charities, as follows (in alphabetic order): France: Fondation ARC (Association pour la Recherche sur le Cancer), Villejuif; SFC (Société Française de Lutte contre les Cancers et Leucémies de l’Enfant et de l’Adolescent), Paris; Enfant Cancers et Santé, Montfaucon and Enfant et Cancer Association Hubert Gouin, Divonne-les-Bains. Israel: Hayim Association-For Children with Cancer in Israel, Ramat Gan. Italy: Fondazione Italiana per la Lotta al Neuroblastoma O.N.L.U.S. c/o Istituto G. Gaslini. Genova: Associazione Bianca Garavaglia O.N.L.U.S., Busto Arsizio. Switzerland: Oncosuisse, Bern; Swiss Cancer League, Bern; Fond’action contre le Cancer, Lausanne and FORCE (Fondation Recherche sur le Cancer de l’Enfant), Ecublens. UK: Cancer Research UK, London and The Neuroblastoma Society, London.

Acknowledgments: Re-cloning and production of the ch14.18 monoclonal antibody was done at Polymun, Vienna, Austria and was enabled by a SIOPEN fundraising effort in 2001. APEIRON provided additional products at a later stage. Neither Polymun nor APEIRON had a role in the study design or analysis. The authors express their gratitude and appreciation to SIOPEN investigators, treating physicians, clinical research and care teams and most importantly to patients and families participating in the trial. The authors are indebted to Ingrid Pribill for her important technical assistance in trial management and to Mag. Claudia Zeiner-Koglin for editorial assistance.

Conflicts of Interest: The academic data supported APEIRON to obtain the dinutuximab beta product licensure in May 2017 in the European Union (EMA). SIOPEN and CCR established a contract with APEIRON regarding the provision of academic data. Ruth Ladenstein and Holger Lode acted as consultants for APEIRON on behalf of SIOPEN for the ch14.18/CHO development. The other authors declare no conflict of interest.
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