Haploinsufficiency of UNC13D Increases the Risk of Lymphoma

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BACKGROUND: Experimental models have demonstrated that immune surveillance by cytotoxic lymphocytes can protect from spontaneous neoplasms and cancer. In humans, defective lymphocyte cytotoxicity is associated with the development of hemophagocytic lymphohistiocytosis, a hyperinflammatory syndrome. However, to the best of the authors’ knowledge, the degree to which human lymphocyte cytotoxicity protects from cancer remains unclear. In the current study, the authors examined the risk of lymphoma attributable to haploinsufficiency in a gene required for lymphocyte cytotoxicity. METHODS: The authors exploited a founder effect of an UNC13D inversion, which abolishes Munc13-4 expression and causes hemophagocytic lymphohistiocytosis in an autosomal recessive manner. Within 2 epidemiological screening programs in northern Sweden, an area demonstrating a founder effect of this specific UNC13D mutation, all individuals with a diagnosis of lymphoma (487 patients) and matched controls (1844 controls) were assessed using polymerase chain reaction for carrier status. RESULTS: Among 487 individuals with lymphoma, 15 (3.1%) were heterozygous carriers of the UNC13D inversion, compared with 18 controls (1.0%) (odds ratio, 3.0; P = .002). It is interesting to note that a higher risk of lymphoma was attributed to female carriers (odds ratio, 3.7; P = .004). CONCLUSIONS: Establishing a high regional prevalence of the UNC13D inversion, the authors have reported an overrepresentation of this mutation in individuals with lymphoma. Therefore, the results of the current study indicate that haploinsufficiency of a gene required for lymphocyte cytotoxicity can predispose patients to lymphoma, suggesting the importance of cytotoxic lymphocyte-mediated surveillance of cancer. Furthermore, the results of the current study suggest that female carriers are more susceptible to lymphoma. Cancer 2019;125:1848-1854. © 2019 The Authors. Cancer published by Wiley Periodicals, Inc. on behalf of American Cancer Society. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

KEYWORDS: cancer, hemophagocytic lymphohistiocytosis, immune surveillance, lymphocyte cytotoxicity, lymphoma.

INTRODUCTION

A role for the immune system in protecting the host from cancer first was hypothesized by Ehrlich in 1909. In the 1950s, Burnet and Thomas reintroduced the concept and coined the term “immune surveillance.”1,2 Over the years, the theory has been heavily debated. Whereas an increased frequency of cancer was observed in immunocompromised individuals, immunodeficient animal models failed to prove a protective role of immune cells in the elimination of nonviral induced cancers.3 However, accumulating experimental evidence now supports the immune system’s role in general and cytotoxic lymphocytes in particular in the surveillance and eradication of neoplastic cells.4,5 In experimental models, spontaneous and induced lymphomas are observed at a higher frequency in Pff1 knockout mice, which lack perforin, a pivotal protein for lymphocyte cytotoxicity.6-8 However, to the best of our knowledge, the development of lymphomas has not been reported in UNC13D-deficient mice nor that of other mouse knockouts of genes required for perforin-mediated cytotoxicity. In humans, the extent of infiltration of cytotoxic lymphocytes in solid tumors has been established as a positive prognostic factor for outcome and survival.9 However, to our knowledge, genetic evidence of a link between impaired lymphocyte cytotoxicity and cancer in humans has remained elusive.

Humans with autosomal recessive null mutations in perforin (PRF1) or other genes required for lymphocyte cytotoxicity (including UNC13D and syntaxin-binding protein 1 [STXBP2]) invariably present with hemophagocytic lymphohistiocytosis (HLH), a life-threatening disorder of immune dysregulation and hyperinflammation.10 In contrast,
individuals carrying hypomorphic mutations in such genes may present atypically and have been anecdotaly described with various hematologic malignancies.\textsuperscript{11-14} Even haploinsufficiency of genes required for lymphocyte cytotoxicity has been suggested to contribute to cancer predisposition.\textsuperscript{15-18} Given that mutations are rare, it is difficult to perform systematic, unbiased studies of the contribution of cytotoxic lymphocytes to cancer immune surveillance in the human population. Nonetheless, a registry-based study indicated a higher incidence of malignancies in first-degree relatives of patients with primary HLH (ie, presumed heterozygous carriers of disease-causing mutations in genes required for lymphocyte cytotoxicity).\textsuperscript{19} However, to the best of our knowledge, other studies have failed to find convincing evidence of the impairment of immune surveillance due to monoallelic mutations in \textit{PRF1}.\textsuperscript{20,21} Thus, it remains unclear whether genetic impairments in immune surveillance predispose an individual to cancer and, if so, to what degree.

In Sweden, a founder mutation in \textit{UNC13D} represents the most common cause of primary HLH, constituting >50% of the mutated alleles in infants with HLH.\textsuperscript{22} This mutation, a 253-kilobase inversion that disrupts the \textit{UNC13D} locus, results in protein degradation, defective cytotoxic lymphocyte degranulation, and cytotoxicity in individuals with biallelic mutations. In northern Sweden, all patients with familial HLH that we diagnosed between 2005 and 2011 were found to be homozygous for the \textit{UNC13D} inversion,\textsuperscript{22} thereby establishing this particular mutation as the predominant genetic factor predisposing to defective lymphocyte cytotoxicity in the area.

In the current study, taking advantage of a high prevalence of the \textit{UNC13D} inversion in Västerbotten county in northern Sweden, we established an increased frequency of a monoallelic genetic lesion in patients with lymphoma, a malignancy that may be associated with immune dysfunction. The results appear to indicate that haploinsufficiency in genes required for cytotoxic lymphocyte-mediated immune surveillance are associated with a higher incidence of lymphoma.

\textbf{MATERIALS AND METHODS}

\textbf{Study Population}

The current study was designed as a retrospective case-control study nested within 2 prospective cohorts included in the Northern Sweden Health and Disease Study (NSHDS). The NSHDS comprises 3 subcohorts, 2 of which, the Västerbotten Intervention Programme (VIP) cohort and the Mammary Screening Cohort (MA), were used in the current study.\textsuperscript{23} The VIP was established in 1985 to reduce morbidity and mortality in cardiovascular diseases, with all residents of Västerbotten county being invited for screening, blood sampling, and health counselling at ages 40 years, 50 years, and 60 years.\textsuperscript{23} In June 2016, the VIP cohort comprised 114,809 individuals, with an equal distribution between men and women. Between 1990 and 2006, the participation rate was 65% for women and 58% for men.\textsuperscript{24} The MA cohort consists of blood samples and data collected in connection with mammography screenings performed between 1995 and 2006. In total, 28,790 women aged 18 to 82 years were included, 95% of whom were sampled between ages 48 and 70 years. A majority of the individuals in MA also are included in the VIP cohort.

The Swedish Cancer Registry compiles data regarding all invasive cancers in Sweden and dates back to 1958. The registry holds a high overall level of completeness, with approximately 98% of the cancers confirmed morphologically. All individuals in the VIP and MA cohorts with a lymphoma diagnosis according to the seventh revision of the \textit{International Classification of Diseases} (ICD-7 codes 200.0-202.2) in the Swedish Cancer Registry on December 31, 2013, were included in the study. Patients diagnosed after 1992 had an \textit{International Classification of Diseases for Oncology, Second Edition} (ICD-O-2) and Systematized Nomenclature of Medicine (SNOMED) classification, whereas patients diagnosed earlier had only ICD-9 or ICD-7 classifications. Individuals with a previous cancer diagnosis prior to the lymphoma event were excluded to eliminate cases of treatment-related lymphomas. For each match with the cancer registry, available classification of the lymphoma and the date of diagnosis were retrieved.

For each individual with lymphoma, 4 unique controls were selected randomly from the NSHDS, and matched for year of birth (±6 months), sex, and subcohort (MA or VIP, respectively). In 12 cases, only 3 controls per case fulfilled the matching criteria, and for 1 case only 2 matched controls were available. To enable use of conditional logistic regression, controls for whom follow-up was >1 year shorter than that of their respective case were excluded from the study (91 controls). When the date of diagnosis was specified only by year and month, the first of the respective month was chosen.

\textbf{Genetic Analyses}

Constitutional DNA from individuals with lymphoma and controls was prepared from EDTA blood according to standard procedures. The DNA was diluted to
10 ng/μL and randomly distributed over the plates. Each plate had a positive control that was heterozygous for the inversion. For the detection of the UNCI3D inversion, 5 μM of forward primer 5′CCCTGAGATGGCCACATT-3′ and 5 μM of reverse primer 5′CCTTCCATCTTGCACACCCA-3′ were used to amplify a segment including one of the breakpoints, thus yielding product only in carriers of the inversion. DNA from 6 donors was pooled using 10 ng (10 ng/μL) of DNA from each donor. Positive assays were validated individually using the same assay. Polymerase chain reaction (PCR) products were analyzed by electrophoresis on 2% agarose gels, and visualized on ImageMaster VDS-CL (Amersham Biosciences, GE Healthcare Life Sciences, Uppsala, Sweden) using Software Bis303 PC (Pharmacia Biotech, Piscataway, NJ). All samples that were positive for the inversion thereafter were re-run using the multiplex PCR previously described by Meeths et al\textsuperscript{22} to discriminate between heterozygous and homozygous individuals. The multiplex PCR amplifies 2 products of 725 base pairs and 922 base pairs, respectively, in homozygous carriers of the inversion and 2 products of 466 base pairs and 1220 base pairs, respectively, in individuals who were wild-type carriers. In heterozygous carriers of the inversion, all 4 products are amplified. PCR conditions are available on request.

**Statistical Analysis**

An a priori calculation of statistical power demonstrated that a 3-fold increased frequency of the mutations among lymphoma cases would have an 80% probability of being detected at \( P \leq .05 \). Data were analyzed using IBM SPSS statistical software (version 23.0; IBM Corporation, Armonk, New York) and R statistical software (version 3.3.2; R Foundation for Statistical Computing, Vienna, Austria). Odds ratios (ORs) for lymphoma were estimated using the conditional logistic regression model, taking the matched design into account. The Mann-Whitney \( U \) test for independent samples was applied to assess age differences between carriers of the inversion and wild-type carriers. All tests were 2-sided and findings with a level of \( P \leq .05 \) for statistical significance were established a priori.

**Ethical Considerations**

The study was approved by the Regional Ethical Review Board in Stockholm and in accordance with the Declaration of Helsinki. All participants had provided informed consent to health research prior to inclusion in the NSHDRS.

**RESULTS**

**Characterization of the Cohort**

In total, we included 487 individuals with lymphoma (251 males and 236 females) and 1844 controls (941 males and 903 females) from 2 different cohorts within the NSHDRS. Of these, 2018 individuals (422 cases and 1596 controls) were from the VIP cohort and 313 individuals, all of whom were female (65 cases and 248 controls), were from the MA. The median age at diagnosis was 62 years for lymphoma cases (range, 3-82 years; mean, 60 years [standard deviation (SD), ±13.7]). One control was excluded due to missing data in the Swedish population and death registries. Among the cases, 423 patients had non-Hodgkin lymphoma, 49 patients had Hodgkin lymphoma, and 15 patients had unclassified lymphoma (Table 1).

**Genetic Analyses**

The UNCI3D inversion was established in a heterozygous state in 33 individuals; no individual was found to have a homozygous inversion. It is interesting to note that a significantly higher carrier frequency of the inversion was identified in the lymphoma cohort compared with the matched controls. In the lymphoma cohort, 3.1% (15 of 487 cases) were carriers compared with 1.0% among the matched controls (18 of 1844 controls) (OR, 3.0; 95% confidence interval [95% CI], 1.5-6.0 [\( P = .002 \)]) (Table 2).

Ten of the heterozygous carriers in the lymphoma group were females and 5 were males, giving a female-to-male ratio of 2:1. Among the controls, the female-to-male ratio was 1.3:1 (10 females and 8 males). Correspondingly, the carrier frequency among females with lymphoma was 4.2% (10 of 236 female cases) compared with 1.1% among their controls (10 of 903 female controls) (OR, 3.7; 95% CI, 1.5-8.9 [\( P = .004 \)]). The difference among the males and their controls was not statistically significant (OR, 2.2; 95% CI, 0.7-6.8 [\( P = .17 \)]) (Table 2). No significant difference in the OR could be observed between females and males. There was no significant difference in age at the time of the lymphoma diagnosis noted between heterozygous carriers of the inversion compared with individuals without the inversion (median age, 65 years [range, 50-79 years; mean, 65 years (SD, ±7.5 years)] and median age, 62 years [range, 3-82 years; mean, 59 years (SD, ±13.8 years)], respectively; \( P = .12 \)).

Analyzing non-Hodgkin lymphoma separately demonstrated a carrier frequency of 3.5% (15 of 423 cases) among cases compared with 1.0% (16 of 1594 controls) among their controls (OR, 3.4; 95% CI, 1.7-7.0...
Unc-13 homolog D (UNC13D) mutation increases cancer risk.

**DISCUSSION**

In the current study, taking advantage of endemic immunogenetic features and 2 comprehensive regional epidemiological screening programs, we genotyped DNA samples from patients with lymphoma and controls and linked results to data from the national Swedish Cancer Registry. Supporting the concept of cytotoxic lymphocyte-mediated cancer immunosurveillance in humans, we demonstrate that haploinsufficiency of UNC13D, a gene required for lymphocyte cytotoxicity, may represent a risk factor for lymphoma. It is interesting to note that this effect appeared most pronounced in women.

In a recent retrospective cohort study, we described a 3-fold increased cancer incidence rate ratio in first-degree female relatives of Swedish patients with primary HLH, whereas the cancer incidence was not significantly found to be increased in males. This unexpected finding had several caveats. Not all patients had a molecular diagnosis. Moreover, first-degree relatives were assumed to be heterozygous carriers of a mutation, but genetic data were largely lacking. The fact that all these individuals had had a severely ill child could possibly bias the results. Due to a limited sample size, no conclusions could be made regarding specific types of cancer. We believe our

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**TABLE 1. Lymphoma Classification**

| Lymphoma Classification | ICD-O (ICD-9) Code | Total | No. of Inv/wt | Frequency of Inv/wt |
|-------------------------|-------------------|-------|--------------|---------------------|
| All lymphoid neoplasms  |                   | 487   | 15           | 3.1%                |
| Non-Hodgkin lymphoma    |                   | 423   | 15           | 3.5%                |
| B-cell lymphoma (unspecified) | C85.1          | 84    | 1            | 1.2%                |
| Follicular lymphoma (other specified and NOS) | C82.9, C82.7     | 82    | 3            | 3.7%                |
| Burkitt lymphoma        | C83.7            | 5     | 0            |                     |
| Waldenstrom macroglobulinemia | C88.0          | 4     | 1            | 25%                 |
| Immunoblastic lymphoma  | C83.4            | 2     | 0            |                     |
| Anaplastic large cell lymphoma | C83.6          | 2     | 0            |                     |
| Other nonfollicular lymphoma | C83.8          | 127   | 5            | 3.9%                |
| Non-Hodgkin lymphoma NOS | C85.0, C85.9 (202.8, 200.1) | 92    | 4            | 4.3%                |
| Peripheral T-cell lymphoma | C84.4          | 8     | 1            | 12.5%               |
| Mycosis fungoides       | C84.0 (202.1)    | 5     | 0            |                     |
| T-cell lymphoma (other and NOS) | C84.5          | 12    | 0            |                     |
| Hodgkin lymphoma        |                   | 49    | 0            |                     |
| Classic or unspecified  | C81.9 (201.9)    | 33    | 0            |                     |
| Nodular sclerosis       | C81.1            | 8     | 0            |                     |
| Mixed cellularity       | C81.2            | 5     | 0            |                     |
| Nodular lymphocyte predominant | C81.0          | 3     | 0            |                     |
| Unclassified lymphomas  |                   | 15    | 0            |                     |
| Malignant immunoproliferative diseases (other) | C88.7          | 11    | 0            |                     |
| Nonspecified malignant neoplasms of lymphoid and histiocytic tissue | D76.0 (202.9) | 2 | 0 |                     |
| Lymphomas with ICD-7 classification | 2 | 0 |                     |

**Abbreviations:** Inv/wt, heterozygous carrier; ICD-O, International Classification of Diseases for Oncology; ICD-7, International Classification of Diseases, Seventh Revision; ICD-9, International Classification of Diseases, Ninth Revision; NOS, not otherwise specified. Bold type indicates the main classification of lymphomas, and normal type the sub-classification.

*aIndividuals with lymphoma were grouped according to ICD-9 (67 patients) or, when available, ICD-O (418 patients).

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**TABLE 2. UNC13D Inversion in Individuals With Lymphoma and Matched Controls**

|           | Inv/wt | wt/wt | Carrier Frequency, % | OR | 95% CI | P     |
|-----------|--------|-------|----------------------|----|--------|-------|
| All       | 15     | 472   | 3.1                  | 3.0 | 1.5-6.0 | .002  |
| Females   | 10     | 226   | 4.2                  | 3.7 | 1.5-8.9 | .004  |
| Males     | 5      | 246   | 2.0                  | 2.2 | 0.7-6.8 | .17   |
| NHL       | 15     | 408   | 3.5                  | 3.4 | 1.7-7.0 | .0006 |
| HL        | 0      | 49    | 0.0                  | 0.0 | 0-inf  | 1.0   |

**Abbreviations:** 95% CI, 95% confidence interval; HL, Hodgkin lymphoma; inf, infinity; Inv/wt, heterozygous carrier of UNC13D inversion; NHL, non-Hodgkin lymphoma; OR, odds ratio; UNC13D, Unc-13 homolog D; wt/wt, no UNC13D inversions.

([P = .0006]) (Table 2). There were no carriers found among the 49 cases with Hodgkin lymphoma but 2 cases were noted among their 191 controls (numbers were not sufficient for meaningful statistical calculations) (Table 2).

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**DISCUSSION**

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In a recent retrospective cohort study, we described a 3-fold increased cancer incidence rate ratio in first-degree female relatives of Swedish patients with primary HLH, whereas the cancer incidence was not significantly found to be increased in males. This unexpected finding had several caveats. Not all patients had a molecular diagnosis. Moreover, first-degree relatives were assumed to be heterozygous carriers of a mutation, but genetic data were largely lacking. The fact that all these individuals had had a severely ill child could possibly bias the results. Due to a limited sample size, no conclusions could be made regarding specific types of cancer. We believe our
current approach, in which we screened 487 patients with lymphoma from a region noted for a founder effect of an UNC13D inversion, is less biased. This aberration represents the predominant cause of autosomal recessive, early-onset HLH in northern Sweden.22 With an allele frequency of the UNC13D inversion higher than that originally predicted using national incidence figures,25 the relatively isolated population in northern Sweden provided a unique opportunity to evaluate the impact of haploinsufficiency in a gene required for lymphocyte cytotoxicity and immune surveillance of cancer.

Establishing a higher carrier frequency of the UNC13D inversion in individuals with lymphoma (OR, 3.0; \( P = .002 \)), the results of the current study greatly strengthen the theory that haploinsufficiency of autosomal genes required for lymphocyte cytotoxicity predispose an individual to cancer. With a female predominance, women with impaired lymphocyte cytotoxicity may be at greater risk of developing cancer compared with men. The current study was not sufficiently powered to establish a difference in the OR between males and females. Nonetheless, combined, the results of the current study and that of relatives of patients with primary HLH suggest that impaired lymphocyte cytotoxicity may demonstrate a stronger predisposition to cancer in women compared with men. Significant sex biases are a hallmark of several infectious and immunological diseases.26 Although immune-related diseases such as rheumatoid arthritis and systemic lupus erythematosus are highly overrepresented among women of all ages, lymphomas generally are more common in men. There also is growing evidence that inflammatory and autoimmune disorders are risk factors for the development of lymphoma, and that the severity of disease correlates with a higher cancer risk.27 With respect to lymphocyte cytotoxicity, to the best of our knowledge sex differences have not been described. Speculatively, factors involved in sex bias in the immune response together with haploinsufficiency in the cytotoxic pathway with low-grade inflammation could contribute to an increased risk of developing cancer over time. The observations from the current study hopefully will prompt more thorough investigations of potential sex differences in cytotoxic lymphocyte function.

HLH generally has been regarded as a recessive disease associated with monogenic mutations in autosomal genes required for lymphocyte cytotoxicity and equally affects men and women. Lately, studies have provided evidence of more complicated inheritance models of mutations. In mice, the accumulation of 2 or 3 monoallelic mutations in different genes appeared to increase the risk of developing immunopathology after stimulation with lymphocytic choriomeningitis virus infection.28 In humans, patients with monoallelic mutations in 2 different genes involved in the same pathway have been described, but an additional effect of other, unidentified mutations cannot be ruled out.29 House et al reported that primary natural killer cells from healthy carriers of p.A91V in PRF1 have at least a 35% reduction in natural killer cell cytotoxicity.30 In contrast, a functional effect of the more severe UNC13D inversion was not experimentally detected.19 Together, findings suggest that haploinsufficiency of cytotoxic lymphocyte function is of significance, in which even monoallelic aberrations in a single gene may contribute to pathogenicity and several weak alleles could contribute to the risk of developing disease. In the current study, we could not exclude the fact that additional loss-of-function mutations in UNC13D or mutations in other genes associated with HLH also contributed to the development of cancer in affected individuals. Nonetheless, all children from the Västerbotten region presenting with HLH during the period between 2005 and 2011 were homozygous for the UNC13D inversion, indicating that this mutation is the singular most significant cause of defective cytotoxicity in the endemic population. The current study findings are likely generalizable to individuals with UNC13D haploinsufficiency and plausible to those with other genes that cause primary HLH, including PRF1, STX11, STXBP2, and RAB27A. It is interesting to note that monoallelic mutations in these genes also have been assigned pathogenicity in secondary HLH (eg, macrophage activation syndrome [MAS-HLH], also called autoimmune-associated HLH).31-35 Speculatively, such monoallelic mutations may serve as genetic risk factors that together with other genetic events and environmental factors can reach the threshold for the development of either secondary HLH34 or, as in the current study, cancer.

It is interesting to note that no significant difference in age at the time of the lymphoma diagnosis was noted between individuals with the monoallelic inversion and those without, indicating a low-grade impairment that only very gradually increases the risk of lymphoma. All 15 patients with lymphoma who carried the inversion were found among the cases with non-Hodgkin lymphoma (423 cases). Because cases of Hodgkin lymphoma were scarce in the current study cohort (49 cases), reliable statistical calculations could be performed only in the group of patients with non-Hodgkin lymphoma,
in which a statistically significant difference between cases and controls was observed (OR, 3.4; \( P = 0.0006 \)). However, due to the small sample size, we were unable to conclude that the inversion is not a risk factor for the development of Hodgkin lymphoma, but this could be an interesting hypothesis for further studies.

The current study has limitations related to the relatively small sample size and the fact that the lymphoma diagnoses were not validated. Future studies with larger materials and a more precise classification of the lymphomas will be valuable for confirming and further exploring the role of mutations affecting lymphocyte-mediated cytotoxicity as a risk factor for the development of lymphoma. In addition, this could possibly reveal significant differences between subgroups of patients that were not possible to establish in the current study. Furthermore, broader genetic screening accompanied by functional studies of lymphocyte-mediated cytotoxicity could contribute to models of the impact of different genetic lesions.

We consider the findings of the current study to be important with respect to understanding that, and to what extent, haploinsufficiency of genes required for lymphocyte cytotoxicity can contribute to disease, and we believe it is appropriate in the future to inform relatives of patients with \textit{UNC13D} mutations about this risk, and possibly also relatives of patients with other mutations that may cause primary HLH. In view of the rapidly developing field of genomics, the findings of the current study also may bear significance in regard to novel algorithms aimed at predicting disease risk based on personal genome analyses.

In the current study, 3.1% of individuals with lymphoma were found to be carriers of an \textit{UNC13D} inversion compared with 1.0% of their matched controls. The results of the current study strongly support the idea of cytotoxic lymphocyte-mediated immune surveillance being an important mechanism for cancer control, perhaps particularly in women. Further studies are warranted to understand the potential relationship between sex and cytotoxic lymphocyte-mediated immune surveillance.

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**CONFLICT OF INTEREST DISCLOSURES**

Yenan T. Bryceson has received grants from the Swedish Cancer Society, the Swedish Research Council, the Swedish Childhood Cancer Foundation, and the European Research Council for work performed as part of the current study. Jan-Inge Henter has received grants from the Swedish Childhood Cancer Foundation, the Swedish Research Council, the Swedish Cancer Society, and the Swedish Cancer and Allergy Foundation for work performed as part of the current study. Marie Meeths has received a grant from the Swedish Childhood Cancer Foundation for work performed as part of the current study. The other authors made no disclosures.

**AUTHOR CONTRIBUTIONS**

Alexandra Löfstedt: Data curation, formal analysis, investigation, methodology, project administration, writing–original draft, and writing–review and editing. Clas Ahlén: Conceptualization, methodology, resources, and writing–review and editing. Bianca Tesi: Formal analysis and writing–review and editing. Ingvart A. Bergdahl: Methodology, resources, and writing–review and editing. Magnus Nordenskjöld: Methodology, validation, formal analysis, resources, funding acquisition, supervision, and writing–review and editing. Yenan T. Bryceson: Conceptualization, methodology, supervision, and writing–review and editing. Jan-Inge Henter: Conceptualization, methodology, funding acquisition, supervision, and writing–review and editing. Marie Meeths: Conceptualization, methodology, formal analysis, investigation, validation, funding acquisition, supervision, and writing–review and editing. All authors approved the final version of the article as submitted and agreed to be accountable for all aspects of the work.

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