A one-mutation mathematical model can explain the age incidence of acute myeloid leukemia with mutated nucleophosmin (*NPM1*)

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ABSTRACT

Acute myeloid leukemia with mutated *NPM1* gene and aberrant cytoplasmic expression of nucleophosmin (NPMc$^+$ acute myeloid leukemia) shows distinctive biological and clinical features. Experimental evidence of the oncogenic potential of the nucleophosmin mutant is, however, still lacking, and it is unclear whether other genetic lesion(s), e.g. *FLT3* internal tandem duplication, cooperate with *NPM1* mutations in acute myeloid leukemia development. An analysis of age-specific incidence, together with mathematical modeling of acute myeloid leukemia epidemiology, can help to uncover the number of genetic events needed to cause leukemia. We collected data on age at diagnosis of acute myeloid leukemia patients from five European Centers in Germany, The Netherlands and Italy, and determined the age-specific incidence of AML with mutated *NPM1* (a total of 1,444 cases) for each country. Linear regression of the curves representing age-specific rates of diagnosis per year showed similar slopes of about 4 on a double logarithmic scale. We then adapted a previously designed mathematical model of hematopoietic tumorigenesis to analyze the age incidence of acute myeloid leukemia with mutated *NPM1* and found that a one-mutation model can explain the incidence curve of this leukemia entity. This model fits with the hypothesis that NPMc$^+$ acute myeloid leukemia arises from an *NPM1* mutation with haploinsufficiency of the wild-type *NPM1* allele.

Key words: acute myeloid leukemia, nucleophosmin, mutation.

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The online version of this article contains a supplemental appendix.

Introduction

The nucleophosmin (*NPM1*) gene, which encodes a nucleolar multifunctional protein, is frequently translocated or mutated in hematologic malignancies.[1,2] Mutation of *NPM1*[1] is one of the most common genetic alterations in adult acute myeloid leukemia (AML), occurring in about one-third of patients and accounting for 50-60% of all AML cases with normal karyotype.[3] Since *NPM1* mutations were first discovered in AML in 2005,[1] about 40 mutation variants have been identified.[3] Despite molecular heterogeneity, all variants lead to common changes at the C-terminus of the NPM1 protein4 which cause an increased nuclear export of the nucleophosmin leukemic mutant and its aberrant accumulation in the cytoplasm of leukemic cells;[4-6] hence the term NPMc+ (cytoplasmic-positive) AML.[1,3] AML with mutated NPM1 shows distinctive biological and clinical features,[3] including a unique gene expression profile,[7,8] a distinct microRNA signature,[9] frequent CD34-negativity (more than 95% of cases),[1,3] increased incidence of FLT3-ITD mutations (about 40% of cases),[1] good response to induction therapy[1] and a favorable prognosis (in the absence of *FLT3-ITD*).[10-15] These findings strongly suggest that AML with mutated *NPM1* represents a new disease entity. Experimental evidence of the oncogenic potential of the nucleophosmin mutant is, however, still lacking, and it is unclear whether other genetic lesion(s), such as *FLT3-ITD*, cooperate with *NPM1* mutations in generating the leukemic phenotype. The multi-step theory of carcinogenesis was conceived after mathematical modeling demonstrated that the increasing cancer incidence with age can be explained by several stochastic events needed for tumorigenesis.[16-19] A recently developed population genetics model[20] was used to study the age specific incidence of chronic myeloid leukemia and found that the data are consistent with the hypothesis that the BCR-ABL fusion oncogene alone is sufficient to cause the chronic phase of the disease. Later on, Vickers demonstrated that the age of onset of polycythemia vera is in accordance with the assumption of a single rate-limiting mutation and a small number of stem cell divisions per year.[21]

To investigate the age-specific incidence of AML with mutated *NPM1*, we adapted the one-mutation model that was originally designed to describe chronic myeloid leukemia age distribution.[20] The model fits the NPMc+ AML age-specific incidence curve assuming plausible parameter values, supporting the hypothesis that a single genetic event, the NPM1 mutation, is sufficient to cause leukemia. The role of *NPM1* mutations in AML development is discussed in the light of these findings.

Design and Methods

Patients

National registry-based AML incidence data with details of *NPM1* mutation status are not available. Therefore, we collected data sets at five major European Institutions involved in the diagnosis and treatment of AML patients: (i) the Laboratory of Cytogenetic and Molecular Diagnostics, University Hospital Ulm, representing the German-Austrian AML study Group (AMLSG); (ii) the Laboratory of Hemopathology, Institute of Hematology, University of Perugia, representing the Gruppo Italiano Malattie Ematologiche dell' Adulto (GIMEMA); (iii) the Laboratory for Molecular Diagnostics, University Hospital Carl Gustav Carus, Dresden, Germany, representing the Deutsche Studieninitiative Leukämie (DSIL); (iv) the Munich Leukemia Laboratory (MLL), Munich, Germany; and (v) the Department of Hematology, Erasmus University Medical Center, Rotterdam, The Netherlands.

A total of 1,444 AML patients (age range: 20-59; median 47) carrying a mutated NPM1 gene were included in this study (n=476 from AMLSG; n=354 from GIMEMA; n=251 from DSIL; n=223 from MLL; and n=140 from The Netherlands). Exclusion criteria were: i) patients under 20 years of age because few cases were available, due to the low frequency of NPM1 mutations in this age group[22]; and ii) patients over 59 years of age who are often treated in local hospitals. Consequently, those patients referred to major institutions for diagnosis and treatment may not be representative of the population of AML patients in this age group.

Information on *FLT3* status was available in 1,386/1,444 AML patients with mutated *NPM1* (96%). *FLT3-ITD* was detected in 553/1,386 cases (40%). For analysis, the 1,444 *NPM1*-mutated AML patients were stratified in 5-year age classes.

For this study, we assume that the mutational event needed to develop NPMc+ AML occurs independently of local exposure to environmental leukemogenic factors and that the age specific rates of *NPM1*-mutated AML patients 20-59 years in age reflect those of the general population in the three European countries included in the study.

Modeling age specific incidence

The mathematical model by Michor *et al.*[20] was adapted to analyze the AML incidence data. Our model is based on the following considerations: (i) we consider a population of N hematopoietic stem cells. Initially, all cells are wild type and proliferate according to a stochastic process known as the Moran model:[23] every τ days, a cell is chosen at random proportional to fitness to divide; its offspring replaces another randomly chosen cell. The population size is strictly constant; (ii) a wild-type cell gives rise to a mutated cell at rate u per cell division. A mutated cell has a relative growth rate (fitness) of r. If r=1, the mutation is neutral as compared to wild type cells; if r<1, the mutant is disadvantageous, and if r>1, the mutant has a proliferation advantage over the wild type cell. We assume that an *NPM1* mutation confers a fitness advantage to the cell, r>1; (iii) Our model adheres to standard Moran process until a surviving mutant cell appears; thereafter, clonal growth is initiated that continues until the mutated cell population reaches population size $\bar{N}$. Unlike the model designed by Michor *et al.*,[20] which assumes a constant population

size of N cells, our model allows the mutant clone to expand until a maximal size, $\bar{N}$. This change is intended to account for the marked expansion of the initial cell compartment which is peculiar to AML; (iv) the AML detection rate is proportional to the number of mutated cells present; if there are N_m mutated cells, the rate of diagnosis is $q\,N_m$. From assumptions (i) and (ii) it follows that the waiting time for the first successful (=surviving) mutation has a negative exponential distribution, $b=Nu(1-1/r)$. Let a be the time since the occurrence of the first surviving mutation. Then assumption (3) states that the number of mutated cells, N_m, grows according to

$$\dot{N}_m(a) = cN_m(a)(1-N_m(a)/\bar{N}$$

where $c=(r-1)/\tau$ and $N_m(0)=1/(1-1/r)$. To account for the significant expansion of the mutated clone, we assume $\bar{N}>>N$. Finally, if (q) is the proportionality constant between the rate of detection and the number of mutated cells (assumption iv), then the probability of diagnosis[20] at time t is given by

(1)

$$P(t) = \int_0^t \left[1 - \left(1 + \frac{e^{cz}-1}{\bar{N}(1-1/r)}\right)^{-q\bar{N}/c}\right] e^{-b(t-z)} b\,dz$$

We compared the predictions of equation (1) with the direct computer simulation of the stochastic process. The simulation is performed by first determining the time at which the first surviving mutated cell arises in a population of N wild type cells; this time follows a negative exponential distribution with mean 1/b. Once such a cell has emerged, the branching process of clonal expansion is simulated by choosing a cell for reproduction or for death at random at each time step. The probability that the number of wild type cells, N, increases by one is given by

(2a)

$$\Pr[(N, N_m) \to (N+1, N_m)] = N(1-u)/\Gamma$$

where $\Gamma = (1+d)N + (r+d)N_m$. Here d denotes the death rate of both wild type and mutated cells. The probability that the number of mutated cells, N_m, increases by one is given by

(2b)

$$\Pr[(N, N_m) \to (N, N_m+1)] = (Nu + rN_m)/\Gamma$$

The probabilities that the numbers of wild type and mutated cells decrease by one are respectively given by

(2c)

$$\Pr[(N, N_m) \to (N-1, N_m)] = dN/\Gamma$$

(2d)

$$\Pr[(N, N_m) \to (N, N_m-1)] = dN_m/\Gamma$$

A patient is diagnosed at rate qN_m and is entered into the incidence data base of his age class. *Online Supplementary Figure 1* shows the fit of equation (1) and system (2). Under particular circumstances, i.e. when the waiting time for the first successful mutation is long and clonal expansion occurs fast and reaches large cell numbers, the incidence data can be a kinked curve. A more detailed mathematical investigation of such situations is forthcoming (*Michor F. et al., in preparation*) but will not be discussed here since the experimentally determined incidence data is a straight line on a doubly logarithmic plot.

Finally, we compared equation (1) with the experimental data, which allowed us to quantify AML-specific parameters.

Statistical analysis

The χ^2 test (α<0.05) was used to assess independence of the age distribution of cases by center of diagnosis. The likelihood ratio test, comparing a Poisson regression model including age, country, and age x country interaction terms with the nested model without the interaction term was performed to evaluate dependence of age specific *NPM1*-mutated AML rates on the country.

Results

Age specific rates of acute myeloid leukemia with mutated NPM1 are similar in different countries

First, we determined whether age specific incidence curves of AML with *NPM1* mutations were comparable in Italy, Germany and The Netherlands. The AML cases registered by each center do not provide a precise estimate of incidence, since the population that is referred to each study center for diagnosis cannot be identified. However, it is important to note that the slope of the incidence curve is needed for our purpose, not population-based incidence figures. Therefore, population data from the U.S. Census Bureau website (*http://www.census.gov/ipc/www/idb*, accessed on February 12, 2008) were used to obtain demographic data (person-years) for each country. In each country the number of AML cases was stratified into age classes. The cases in each age class were divided by the total population in that age class, which provided the age specific rate of diagnoses per year per million inhabitants.

Chi-square testing of the age distribution of cases on center of diagnosis was not significant (p=0.48) indicating that, although absolute incidence levels vary because they reflect the percentage of the general population that is covered by participating centers, number of cases by age class does not differ among study centers (Table 1). The likelihood ratio test comparing the Poisson model which includes a country x age class interaction with the simpler model without the interaction term (*Online Supplementary Table 1*) was non-significant (p=0.85). Together, these findings provide evidence that AML data from the three countries are comparable. In particular, AML incidence curves analyzed via linear regression all showed a slope of about 4 on a log-log scale (Figure 1).

The one-mutation model fits the incidence curve of acute myeloid leukemia with mutated NPM1

We next adapted the one-mutation mathematical model that was originally designed to describe chronic myeloid leukemia epidemiology[20] to investigate age specific incidence data in AML with *NPM1* mutations. The model provided adequate data fitting and generated slopes similar to real age specific incidence curves from patients (Figure 2) from Germany, The Netherlands, and Italy. The corresponding χ^2 and p values for the three countries were 0.02027 (p=0.9899), 0.00862 (p=0.9956), and 0.15275 (p=0.9264). The fitting procedure provided estimates of the parameters in each country (Table 2) generating numbers that are biologically plausible (*see below*).

The initial hematopoietic stem cell (HSC) compartment was quantified as 1.03×10^{4}-1.14×10^{4}. This fits with experimental findings[24] suggesting that, although humans require more blood cells per lifetime than mice (because of their larger size and longer life expectancy), the total number of human HSCs is equivalent to the total number of HSCs in mice, which has been shown to be of about 11,400±5,400.[24]

The maximum number of mutated cells generated by the model was about $\bar{N}=10^{13}$. This number is consistent with the high tumor burden observed in leukemia patients, if one assumes that, under physiological conditions, the amount of human nucleated marrow cells per kg body weight has been calculated to be approximately 2.1×10^{10} (1.5×10^{12} in a subject of 70 kg).[25] The relative fitness of mutated cells spanned the range 1.38-1.61. The mean cell generation time (i.e. the time needed for a cell to divide), was between 2.67 and three days, which concurs with early experimental findings[26] and with clinical data.[3] In the *NPM1*-mutated AML case, the rate of cancer detection per mutated cell was found to be in the range of 7.77×10^{-5}-1.58×10^{-4} days. This implies that the total rate of detection (qN) is in the range of 0.26-1.78, which is higher than previous estimates in chronic myeloid leukemia.[20] Leukemic clones are initiated by single *NPM1* mutations occurring at rates ranging from 2.43×10^{-9} to 4.86×10^{-9} days per cell division. Taken together, these estimates imply that for a single individual the waiting time for the appearance of a surviving mutation is on average $1/(Nu(1-1/r))$, which is about 5532, 9940 and 8779 days for Germany, The Netherlands and Italy respectively.

Table 1. Cumulative incidence of the NPM1-mutated acute myeloid leukemia in Germany (DE), the Netherlands (NL) and Italy (IT).*

Age class	*Cum P (DE)*	*Cum P (NL)*	*Cum P (IT)*
20-24	8.87×10^{-7}	9.73×10^{-7}	5.92×10^{-7}
25-29	2.55×10^{-6}	2.07×10^{-6}	3.58×10^{-6}
30-34	4.34×10^{-6}	4.36×10^{-6}	6.29×10^{-6}
35-39	8.17×10^{-6}	7.72×10^{-6}	1.10×10^{-5}
40-44	1.35×10^{-5}	1.29×10^{-5}	1.76×10^{-5}
45-49	1.93×10^{-5}	1.84×10^{-5}	2.56×10^{-5}
50-54	3.05×10^{-5}	2.45×10^{-5}	3.72×10^{-5}
55-59	3.81×10^{-5}	3.49×10^{-5}	4.85×10^{-5}

**Corresponding curves have a slope of about 4 on a doubly logarithmic scale (slope DE=4.038, slope NL=4.043, slope IT=4.332) as seen in Figure 1.*

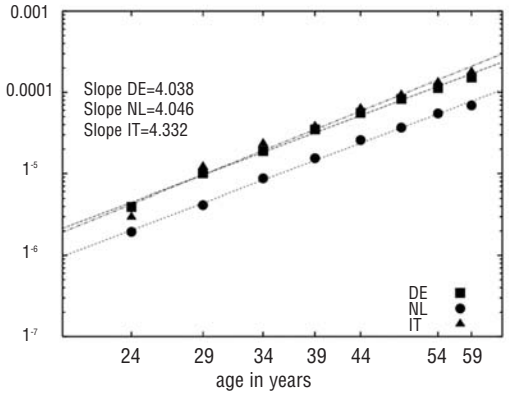

Figure 1. Incidence data for the NPMc+ acute myeloid leukemia in Germany (DE), The Netherlands (NL) and Italy (IT). Linear regression shows a slope of about 4 on a doubly logarithmic scale.

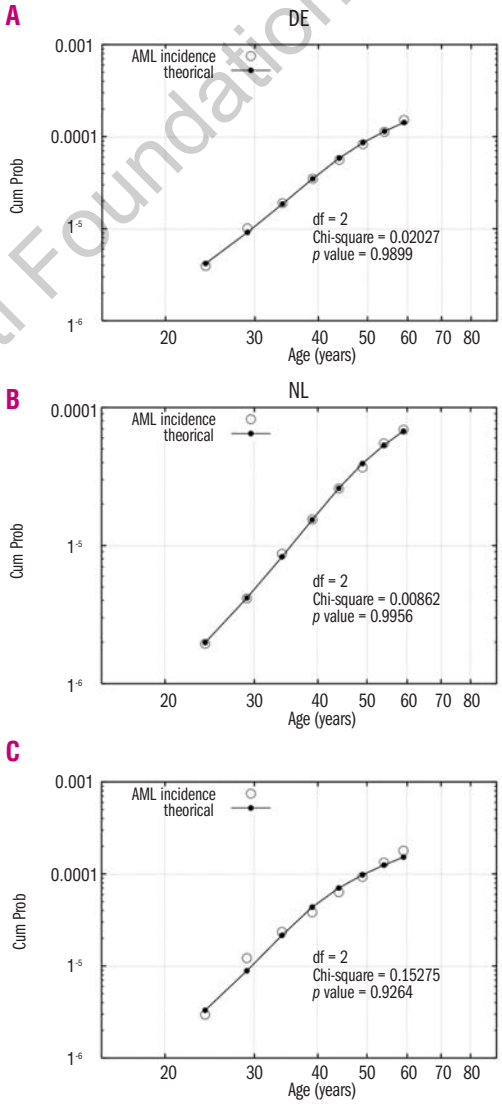

Figure 2. The incidence data can be fit to the one-mutation model assuming plausible parameter values for Germany (A), The Netherlands (B) and Italy (C). χ^2 and corresponding p-values reported as labels in the corresponding figures show the quality of the fit.

Table 2. Parameters of the one-mutation model for all *NPM1* mutated acute myeloid leukemias.

Parameter	*Definition*	*Germany*	*The Netherlands*	*Italy*
r	Relative fitness	1.42	1. 38	1.61
τ	Mean cell generation time	2.79	2.67	3.00
q	Rate of cancer detection per mutated cell	1.58×10^{-4}	1.56×10^{-4}	7.77×10^{-5}
N	Standard number of hematopoietic stem cells	1.10×10^{4}	1.14×10^{4}	1.03×10^{4}
u	Mutation probability per cell division	4.86×10^{-9}	2.43×10^{-9}	4.19×10^{-9}
$\bar{N}$	Maximum number of mutated cells	1.00×10^{13}	1.00×10^{13}	1.00×10^{13}

Values were obtained via minimization of the least squares function in eq. (2).

FLT3 gene status does not influence the age specific incidence of acute myeloid leukemia with mutated *NPM1*

Internal tandem duplication (ITD) at the *FLT3* gene locus has been implicated as a cooperating genetic alteration in various AML subtypes.[27,28] Since *FLT3-ITD* frequently associates with *NPM1* mutations[1] and appears to abrogate the favorable prognostic effect of *NPM1* mutations in AML1,[15,29] we determined whether the age incidence of *NPM1*-mutated AMLs with *FLT3-ITD* differs from cases with wild-type *FLT3*. No significant difference emerged in the slopes of FLT3-ITD-positive and -negative AML with mutated *NPM1* (Figure 3). The quality of fit with the model-generated data was adequate and similar to the quality of fit for all AMLs with *NPM1* mutations (Figure 4). The one-mutation model parameters for fitting *FLT3-ITD* positive and *FLT3-ITD* negative AML with mutated *NPM1* are reported in Table 3. The slopes of the three groups (*NPM1* mutated, *NPM1* mutated/FLT3-ITD, *NPM1*-mutated/FLT3 wild-type) are not significantly different according to the Mann-Witney U test ($p>0.05$) (*Online Supplementary Table 2*).

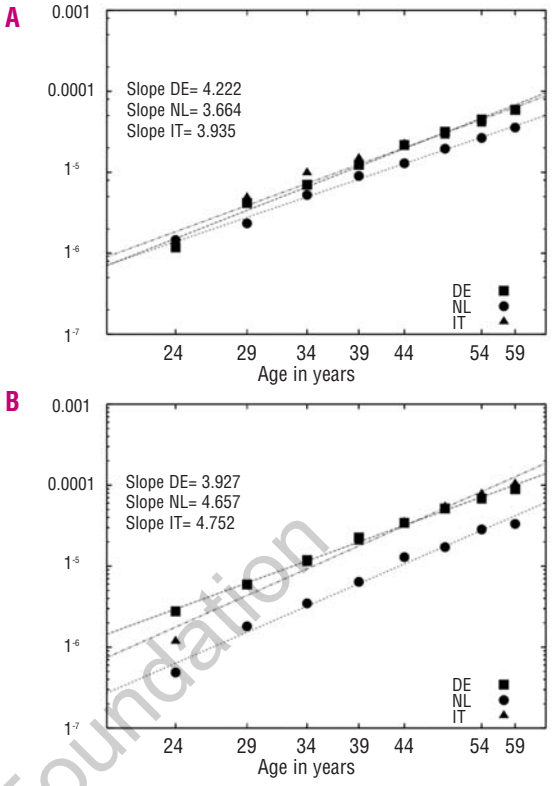

Figure 3. Incidence data for the NPMc$^+$ acute myeloid leukemia bearing *FLT3*-ITD (A) or *FLT3* wild-type for Germany (DE), The Netherlands (NL) and Italy (IT) (B).

Discussion

In this study, we adapted a one-mutation mathematical model that was originally designed to describe chronic myeloid leukemia epidemiology[20] to investigate the age specific incidence data in AML with mutated *NPM1*. The model fits the NPMc$^+$ AML age specific incidence curve for plausible parameter choices, supporting the hypothesis that a single genetic event, the *NPM1* mutation, is sufficient to cause this type of leukemia. However, evidence derived from *in vitro* functional studies and experimental models are required to confirm or refute this hypothesis.

Our findings add to the body of evidence that the *NPM1* mutation is a founder genetic lesion in NPMc$^+$ AML: i) cytoplasmic mutated nucleophosmin is specific for AML[1,30,31] and clinically shows close association with AML of *de novo* origin[1,32-34]; ii) all *NPM1* mutations generate changes at the C-terminus of nucleophosmin protein which appear to maximise nuclear export of NPM leukemic mutants,[3,35-37] pointing to cytoplasmic dislocation of the mutants as the central event for leukemogenesis; iii) *NPM1* mutations are mutually exclusive with other recurrent genetic abnormalities,[1,38] with the exception of rare cases in which both *NPM1* and CEPBA (or *FLT3-ITD*) mutations are found;[15] iv) they are stable during the course of the disease[39,40] as the same type of *NPM1* mutation is consistently detected at relapse in medullary and extramedullary sites;[40] and v) quantitative real-time PCR shows that *NPM1* mutations disappear at complete remission.[41,42]

The major finding in the present study is that the one-mutation mathematical model can explain the age specific incidence in NPMc$^+$ AML. This hypothesis is in contrast to current concepts in AML development which, like other human cancers, is believed to be a consequence of more than one oncogenic hit.[43] Indeed, several animal models of AML clearly point to leukemogenesis as a multi-step process.[43] Moreover, *in vitro* findings that the *NPM1* leukemic mutant specifically cooperates with the E1A adenovirus to transform primary MEFs in soft agar[44] suggest that *NPM1* mutations need to act in close concert with other oncogenic hits. In MEF cells, this mutual cooperation involves the *NPM1*

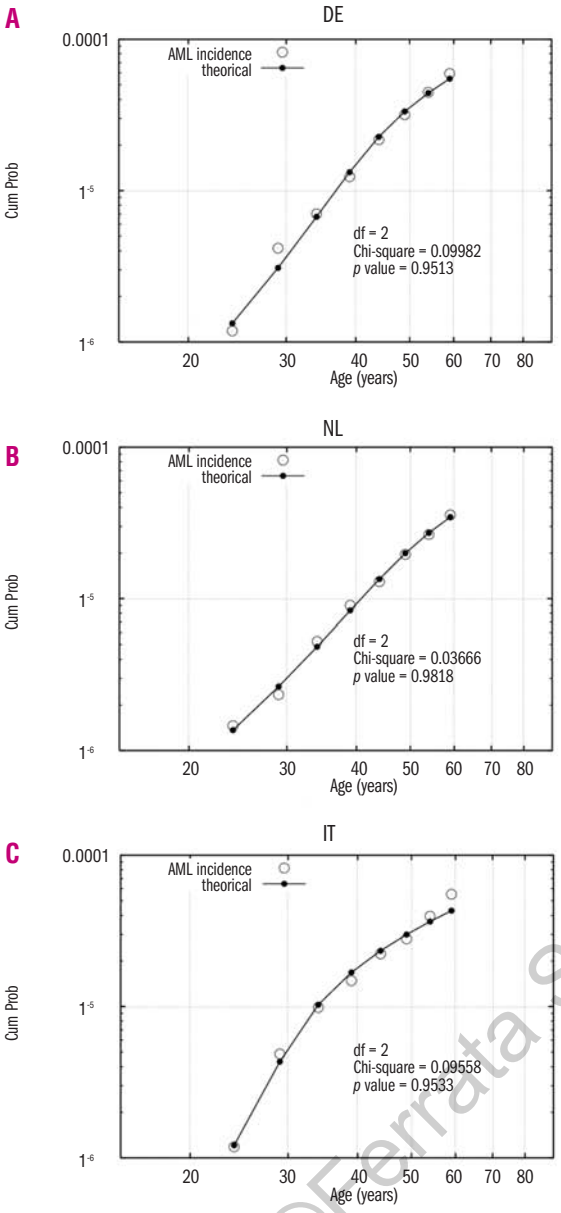

Figure 4. **Fitting the incidence data with the one-mutation model (A) leads to plausible parameter values in all cases (NPMc⁺ acute myeloid leukemia with *FLT3*-ITD or *FLT3* wild-type) for all three countries (Germany, The Netherlands (B) and Italy (C)). χ^2 and corresponding *p*-values reported as labels in the corresponding figures show the quality of the fit.**

mutant inhibiting the E1A-elicited p19(Arf) induction and E1A overcoming *NPM1* mutant-induced cellular senescence.[44] Furthermore, an activating mutation of the *FLT3* gene (*FLT3*-ITD) leading to an internal tandem duplication of the juxtamembrane portion of *FLT3*, a receptor which plays an important role in controlling proliferation and/or survival of hematopoietic progenitors, has been implicated as a cooperating genetic alteration in various AML subtypes.[27,28] Since *FLT3-ITD* has been detected in about 40% of AML with mutated *NPM1*,[1] it has been suggested that it may play an important role also in this leukemia subtype.

Table 3. **Parameters of the one-mutation model for the *NPM1*-mutated/*FLT3*-ITD and *NPM1*-mutated/*FLT3* wild-type (wt) subsets.**

Parameter	*definition*	*Germany*		*The Netherlands*		*Italy*	
		ITD	*wt*	*ITD*	*wt*	*ITD*	*wt*
N	Standard number of hematopoietic stem cells ($\times 10^4$)	1.00	1.11	1.09	1.11	1.10	1.10
$\bar{N}$	Maximum number of mutated cells ($\times 10^{12}$)	9.99	10.00	10.00	10.00	9.99	9.99
r	Relative fitness	1.57	1.40	1.34	1.54	1.85	1.62
τ	Mean cell generation time (days)	3.41	2.79	2.87	2.90	2.86	2.90
q	Rate of cancer detection per mutated cell($\times 10^{-4}$)	1.29	1.84	2.54	0.64	0.32	0.43
u	Mutation probability per cell division ($\times 10^{-9}$)	2.03	2.96	1.52	0.97	0.73	2.39

The findings of this paper suggest that the role of *FLT3-ITD* as a cooperative mutation in the pathogenesis of NPMc⁺ AML should be interpreted with caution. In fact, no difference can be detected between the slopes of the age specific incidence of *FLT3*-ITD-positive and -negative NPMc⁺ AML, supporting the view that NPMc⁺ AML is a homogeneous group irrespective of the FLT3 mutational status. This is consistent with the observation that the unique gene expression profile of AML with mutated *NPM1*, i.e. upregulation of *HOX* genes and downregulation of CD34,[7,8] does not appear to be significantly influenced by the *FLT3* gene status. This is also in keeping with the clinical observation that FLT3-ITD can appear or disappear in *NPM1*-mutated AML patients during the course of the disease.[39] Moreover, in oncogenic cooperation tests, the *NPM1* leukemic mutant and *FLT3*-ITD did not cooperate to transform mouse embryonic fibroblasts (MEFs).[44] Hypothetically, *FLT3-ITD* may not be necessary for the development of AML but rather provide a selective advantage for leukemic cells that already harbor the *NPM1* mutation. Unfortunately, there is as yet no experimental mouse model to prove or disprove this hypothesis. However, this interpretation would at least fit with the clinical observation that *FLT3*-ITD appears to abrogate the favorable prognostic impact of *NPM1* mutations,[29] suggesting that it may play a role at later stages of NPMc⁺ AML, leading to a more aggressive AML phenotype.

Thus, how can we reconcile the results of our one-mutation mathematical model with current evidence that favor the hypothesis that AML is the result of more than one oncogenic hit[43] ? One possible explanation is that NPMc⁺ AML arises from the concerted action of an *NPM1* mutation and another leukemogenic event

occurring at the same time. Since the *NPM1* mutant has intrinsic oncogenic properties[44] and in knock-out mice NPM haploinsufficiency results in a MDS-like syndrome[45] and in overt leukemia,[46] an attractive hypothesis would be that these alterations act together to cause NPMc+ AML.[3,47] Indeed, *NPM1* mutations are associated with haploinsufficiency of wild-type NPM in leukemic cells, since mutations are always monoallelic[3] and lead to dislocation of functionally active wild-type NPM from the nucleoli to the cytoplasm through formation of heterodimers with the *NPM1* leukemic mutant.[4]

However, other scenarios cannot be excluded with certainty only on the basis of the mathematical model. *NPM1* and yet undiscovered mutation(s) may act synergistically such that their actions cannot be discerned when investigating incidence data. Moreover, even though *NPM1* mutations may be sufficient to cause leukemia, secondary mutations (e.g. *FLT3-ITD*) could increase the fitness of leukemic cells and/or result in the development of more aggressive AML stages. Finally, it is still possible that cancer incidence data cannot be used to identify the number of genetic changes necessary to cause cancer. Therefore, further experimental studies are warranted to clarify the oncogenic role of *NPM1* mutations and other putative cooperating genetic lesions in NPMc+ AML.

Authorship and Disclosures

AL and BF had the original idea, coordinated the whole project and wrote the paper; FC and AC adapted the one-mutation mathematical model to the study of AML with mutated NPM1 and helped write the manuscript. FS performed the statistical analyses on incident cases before fitting the one-mutation model; RFS collected molecular and clinical data from patients of the AMLSG study and helped write the manuscript; SA was involved in designing the GIMEMA study and collecting clinical data from patients; CT performed molecular analyses of patients from DSIL and helped write the manuscript; SS performed mutational analysis in patients from the Munich Leukemia Laboratory (MLL) and helped write the manuscript; PJMV carried out molecular studies on AML patients from The Netherlands and reviewed the manuscript; KD collected molecular and clinical data from patients of AMLSG study and helped write the manuscript; MFM recruited patients in the GIMEMA study and reviewed the manuscript; MS designed and coordinated the clinical study (DSIL); JK collected molecular and clinical data from patients of the AMLSG study; AG collected clinical data from patients of the AMLSG study and coordinated the clinical study (AMLSG); MPM and NB performed immunohistochemical studies on the GIMEMA patients; BL recruited patients from The Netherlands and reviewed the manuscript; TH coordinated the study of patients from the Munich Leukemia Laboratory (MLL) and helped write the manuscript; GE designed and coordinated the clinical study (DSIL); FM designed and coordinated the clinical study (GIMEMA); HD designed and coordinated the clinical study (AMLSG); FM carried out computational simulation studies and helped write the manuscript.

The authors reported no potential conflicts of interest.

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