Data S1. The regionally-stratified Kaplan-Meier curves from Figure 1

This section has the details of how the regionally-stratified Kaplan-Meier curves of the PML-incidence in Ho et al.'s pooled study-cohort[1] were obtained. First of all, to estimate the number of U.S. patients in the subcohort of those with a positive JCV-serostatus and no prior IS-exposure, I (B.T.) started with the proportions from Ho et al.'s reply to my published correspondence.[2] As the authors noted, the JCV-seroprevalence and prior IS-exposure among U.S. patients in their pooled cohort were 57% and 12%, respectively, i.e., 88% had *not* had prior IS-exposure. Further, from Table 1 of their article,[1] there were 26,538 U.S. patients in the cohort; hence, of these, about 26,538 × 57% × 88% = 13,311 had a positive JCV-serostatus but no prior IS-exposure.* Similarly, there were 10,711 × 62% × 83% = 5512 European patients in this constellation.† So the proportion of U.S. patients among all JCV-seropositive patients without prior IS-exposure in the pooled study-cohort was

$$13{,}311 \div (13{,}311 + 5512) = 70.7\%.$$

Of course, this quantity presumably varied with duration of treatment, slightly anyway—however, as no further data are available, for the purpose of this analysis, I assumed it to be constant.‡ I then applied the preceding percentage to the numbers of JCV-seropositive at-risk subjects with no prior IS-exposure from Figure 2A of Ho et al.'s publication. For instance, there were 9755 such patients having had 36+ infusions; therefore, approximately 9755 × 70.7% = 6897 were based in the U.S., and so the remaining 9755 – 6897 = 2858 were in Europe.

As for estimating the geographic prevalence of PML, I proceeded by considering each of the four trials that were pooled in turn and deducing from various sources how many U.S. PML-patients there must have been who had not had prior IS-exposure. To begin with, again from Ho et al.'s paper, there were

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* This calculation assumes, probably incorrectly, that there is no correlation between JCV-serostatus and prior-IS status; indeed, that correlation is almost certainly a positive one as the JCV-seroprevalence naturally increases with age, so that the above figure is an overestimate. However, the same of course applies to the European subcohort, too, and since both the JCV-seroprevalence and the prior IS-exposure are higher in Europe, the magnitude of this effect is even bigger there. Hence the number of European patients in the cohort will, if anything, be overestimated, and therefore the European risk of PML will be underestimated.

† This pretends that *all* ex-U.S. patients were in fact in Europe, even though a number of patients in Ho et al.'s pooled cohort were from Argentina, Australia, Canada, Israel, Mexico, or New Zealand.[1] However, it is known that the incidence of natalizumab-associated PML in these countries falls somewhere between the incidence in the U.S. and that in Europe,[3] so that, again, a genuinely European-only estimate will actually be higher.

‡ A quick inspection of Table 1 in Ho et al.'s article[1] reveals that the median number of natalizumab-infusions in both the STRATIFY-2 and TYGRIS trials was 44, whereas in TOP—which had no U.S. enrollment—the median number of infusions was only 29. On the other hand, in STRATA, where just 33% of participants were U.S.-based, the median was actually 65, although this was by far the smallest of the four trials included in Ho et al.'s pooled cohort (n = 1094). By contrast, STRATIFY-2 (the largest study; n = 24,402) was U.S.-only, of course. On balance, the continental exposures do not seem to have differed very much; if at all, the proportion of U.S. patients *grew* during the later years of treatment (because of TOP), thus once more causing the risk of PML in Europe to be understated.

66 cases of PML in STRATIFY-2, one of them in a JCV-seronegative patient.[1] Of the 65 JCV-seropositive patients (who were necessarily all from the U.S.), about 51 had not had prior IS-exposure.[§] Next, in STRATA, among the 18 participants developing PML during the study,[1] at least five had received prior IS-exposure.[4] With the remaining 13, conservatively assuming that Europeans have just double the risk, about three must have been from the U.S.,[¶] given that U.S. enrollment in STRATA was only one-third.[1] Likewise, of the 44 PML-cases in TYGRIS, three affected patients in the U.S., all of them with no prior IS-exposure;[5] thus, it follows that there were

$$51 + 3 + 3 + 0 \;=\; 57$$

cases in total in JCV-seropositive U.S. patients without prior IS-exposure in the pooled cohort—there were no cases in TOP, of course, as that study had no U.S. enrollment. Further, of the 120 cases in the pooled cohort with a positive JCV-serostatus and no prior IS-exposure, eleven occurred in those having had >72 natalizumab-infusions.[1] Consequently, of the U.S. cases, about $11 \div 120 \times 57 = 5.2$ fell in that range, leaving 52 during infusions 1–72. (The remaining 109 – 52 = 57 were therefore in Europe.) The temporal distribution of cases was then approximated via the data from Figure 2A in Ho et al.'s paper, in such a way that the case-numbers increase roughly uniformly in the U.S. and Europe:

Interval of therapy (infusions)	Overall cases of PML as per Figure 2A[1]	U.S. cases	European cases
1–12	2	0	2
13–24	8	4	4
25–36	18	8	10
37–48	34	16	18
49–60	27	14	13
61–72	20	10	10

Data S2. Properly computing the incidence of PML in TYGRIS

i) Why the statistical method is inadequate

The problem with the method employed is perhaps best illustrated by example. Consider therefore the question of the incidence of PML during natalizumab-infusions 49–72. In the U.S. subcohort from TYGRIS consisting of all study-participants known to be JCV-seropositive with no prior IS-exposure, there were 430 having received 49+ infusions.[5] Among them, two developed PML sometime during

§ In Ho et al.'s cohort, prior IS-exposure increased the risk of PML by a factor of around two;[1] e.g., during infusions 1–48, the risk with prior IS-exposure was 12.6 per 1000 vs. 6.3 per 1000 without. Since 12% of STRATIFY-2 participants were in the former category,[1] there would have been only $65 \div (88\% + 12\% \times 2) = 58.0$ cases of PML in that trial if prior IS-exposure did not influence the risk at all; of these, $58 \times 88\% = 51.0$ occurred in individuals without such pre-therapies.

¶ There were approximately $13 \times 33\% \div (33\% + 67\% \times 2) = 2.6$ cases of PML in U.S. subjects.

infusions 49–72; hence, the investigators estimated the incidence in this constellation at 2/430, or 4.7 per 1000.[5] However, only a fraction of these 430 patients had in fact completed 72 infusions: While the exact number is not disclosed, just 4/2207 patients in the *overall* U.S. subcohort had received 73+ infusions. Thus, via interpolation, the number of JCV-seropositive patients without prior IS-exposure having received 72+ infusions was approximately 26; finally applying the lifetable-method, the PML-incidence was not 2/430 but rather 2/{(430+26)/2} = 2/228, or 8.8 per 1000—not quite twice higher.

ii) Imputing the missing values on the JCV antibody-status

The other shortcoming with the assessment of the incidence of PML in TYGRIS is that all participants with unknown JCV-serostatus were simply excluded.[5] (Technically, instead of effectively considering such individuals as 'negative', Foley et al. should have imputed the missing values, exactly as Ho et al. did during the course of their analysis.[1]) For instance, in the Euro-Canadian subcohort (*n* = 4301) from TYGRIS, the JCV-serostatus was unavailable for 1697 patients not developing PML, while among the remaining 2563, 1627 (63.5%) were positive.[5] Assuming that the seroprevalence was similar in those with unknown status, approximately 63.5% × 1697 = 1078 patients should further have been included in the at-risk population, thereby increasing the overall number by 1078/(1627 + 41) = 64.6%, and consequently lowering the incidence-estimates concerned by roughly 40%. To work one specific case, using the same statistical method as Foley et al.,[5] the total PML-incidence—i.e., with or without prior IS-exposure—in Europe and Canada during natalizumab-infusions 1–24 was not 2/1668 (1.2 per 1000) but just 2/2746 (0.7 per 1000).

References

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