

Prospects for achieving treatment-free remission in chronic myeloid leukaemia

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Summary

In addition to the best possible overall survival, discontinuation of the tyrosine kinase-inhibitor (TKI) treatment [treatment free remission (TFR)] without observing a recurrence of the disease has become a major goal of the therapy of chronic myelogenous leukemia (CML). Many clinical studies have demonstrated that TFR is possible, although for the moment limited to a fraction of the CML patients able to achieve a stable deep molecular response (DMR). The factors associated to the possibility of remaining in TFR or of losing it, have been investigated by a number of controlled and observation clinical trials and although total TKI treatment duration, DMR duration and stability and, more recently, also the depth of the molecular response obtained at the time of discontinuation have been shown to be significant elements, most of the factors associated with a higher possibility of a successful discontinuation still remain elusive and are here reviewed.

Keywords: CML, treatment-free remission, tyrosine kinase inhibitors.

Introduction

Chronic myeloid leukaemia (CML) is probably the best example of the success of precision oncology. Beginning with the description of the Philadelphia chromosome (Ph1 chromosome) in 1960 (Nowell & Hungerford, 1960), there has been continuous progress in the laboratory and clinic, bringing us to what some term the *cure*, or at least an *operational cure*, of CML in some patients at least (Goldman & Melo, 2003; Goldman & Gordon, 2006).

Twenty years ago almost everyone with chronic phase CML progressed to the acute phase (or blast phase) after a

median of three to four years (Goldman, 2010). Death followed quickly, usually within six months to one year. Only two therapies showed a promise: interferon and allogeneic bone marrow transplant, therapies only available to or really effective in fewer than 20% of people (Goldman, 2010). To say that the prognosis of people with CML has now changed dramatically is an understatement. Imatinib, the first tyrosine kinase-inhibitor (TKI), was introduced to the clinic in 2002 (Kantarjian *et al.*, 2002). Several recent studies claim that 10 years after starting TKI therapy, survival of people with CML is almost similar to that of a sex- and age-matched population without CML (Hehlmann *et al.*, 2017; Hochhaus *et al.*, 2017a). These impressive data are achieved not only with imatinib but also with second and third generation TKIs, such as nilotinib (Weisberg *et al.*, 2006), dasatinib (Shah *et al.*, 2004), bosutinib (Rensing Rix *et al.*, 2009) radotinib (Kim *et al.*, 2014), ponatinib (O'Hare *et al.*, 2009) and others which will soon follow. These second and third generation TKIs are used in the 35-40 percent of persons failing or intolerant to imatinib and their use has been registered also as first-line therapy in addition to imatinib (Saglio *et al.*, 2010; Kantarjian *et al.*, 2010; Cortes *et al.*, 2018a).

Because of the success of TKI therapy, few patients with CML now receive an allotransplant (Innes, Milojkovic & Apperley, 2016). However, the enormous success of TKI therapy should not overshadow the fact that there remain problems to solve. For example, although there are now few CML-related deaths, most patients seemingly need to continue TKI therapy for the rest of their lives. Although this therapy is mostly safe, especially imatinib, there are chronic side effects adversely affecting quality of life. Furthermore, the follow-up interval of TKI-treated patients is still relatively short, a maximum of 20 years, and we cannot exclude long-term safety concerns (Steeegmann *et al.*, 2016). These considerations are reasons why trying to achieve a therapy-free remission (TFR) has become an important goal in the treatment of people with CML.

TFR is possible

In 2004 a few anecdotal reports started appearing of people with CML discontinuing TKI therapy for diverse reasons, but

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with no leukaemia recurrence (Mauro, Druker & Maziarz, 2004). These were followed by two clinical studies, STIM and TWISTER, which reported that TFR, the possibility of discontinuing TKI therapy without observing clinical leukaemia recurrence, was possible (Rousselot *et al.*, 2007; Mahon *et al.*, 2010; Ross *et al.*, 2010). In these studies, 40–50% of people with CML with a stable complete molecular response (CMR), defined as undetectable BCRABL1 fusion transcripts at a reverse quantitative polymerase chain reaction (RQ PCR), with a sensitivity of at least a 4.5 log-reduction in BCRABL1 transcripts ($MR^{4.5}$) according to the International Scale (IS, (Hughes *et al.*, 2006) for ≥ 2 years were without leukaemia recurrence after stopping imatinib. With additional follow-up, median TFR duration is now >7 –8 years (Etienne *et al.*, 2017; Ross *et al.*, 2018). These data indicate that at least in some people with CML, TKI therapy can be stopped and a *cure* (at least an *operational cure*; see below) achieved without life-long TKI therapy. Interestingly, using ultra-high sensitivity detection techniques, such as DNA-based PCR, it is evident that most people in TFR have at least some residual leukaemia cells (Ross *et al.*, 2010). Whether these cells have the biological ability to cause CML recurrence, or whether they simply don't during the observation interval, perhaps for stochastic reasons or both, is unknown. This situation, where the (theoretical) potential for leukaemia recurrence exists but does not occur during the observation interval, is termed *operational cure* (Goldman & Gordon, 2006). Another indication that most people in TFR have residual leukaemia cells is fluctuating concentrations of BCRABL1 transcripts without loss of major molecular response (MMR), defined as $0.1\%^{15}$ BCRABL1 transcripts, and no need to restart the TKI therapy immediately (Rousselot *et al.*, 2011). In most studies, TKI therapy is restarted only when the BCRABL1 transcript concentration is $> 0.1\%^{15}$. This strategy, albeit arbitrary, increases the proportion of patients off-therapy.

There are > 50 other studies of TKI discontinuation (Saussele *et al.*, 2016; Breccia & Foà, 2018; Cortes, Rea & Lipton, 2019; Chen *et al.*, 2019). Most studies enrolled patients treated with imatinib, but some included patients receiving second generation TKIs (Breccia & Foà, 2018; Cortes, Rea & Lipton, 2019). The current EUROSKI trial of the European Leukemia Net (ELN) is considered the most informative (Saussele *et al.*, 2018). Several observational studies of TKI discontinuation are also reported (Hernández-Boluda *et al.*, 2018; Fava *et al.*, 2019; Shen *et al.*, 2019; Dengler *et al.*, 2019). The sum of these data are that 30–70% of patients with a good, sustained response to TKI therapy achieve TFR and are the base for the recommendations regarding TKI discontinuation (Hughes & Ross, 2016; Hochhaus *et al.*, 2017c; Rea *et al.*, 2018; Saglio *et al.*, 2018; Shah, 2019).

An important consideration in the context of TKI discontinuation is safety. Reassuringly, among an estimated 4000 patients *at risk*, there are only two reports of CML

progression to acute phase and, surprisingly, both progressions occurred when patients (after failing TFR) had restarted the TKI treatment for some months and had regained MMR in both cases, and in one case also a deep molecular response (DMR) (Rousselot *et al.*, 2014; Papalexandri *et al.*, 2018).

As a general aspect, in spite of some differences in the trials' design – such as the depth and duration of the molecular response at the moment of the discontinuation, the definition of the TFR loss, and the criteria to restart the TKI and other therapies – all the studies show that TFR is possible in a percentage of patients ranging approximately 30–70%, but also that it can never be certain nor totally excluded, even when the conditions are apparently not ideal, as in patients who have achieved only MMR and not DMR (Dragani *et al.*, 2019; Clark *et al.*, 2019).

This of course raises important questions that need to be investigated and understood if we want to extend the TFR option to a number of CML patients other than those who can actually benefit from it; at present this can be roughly estimated to represent only 15–20% of the total CML population. In this study, we comment on the major issues which, in our opinion, are relevant regarding what we know and what we need to know concerning TFR. One of the intriguing issues is certainly represented by the dynamics of the TFR loss.

The dynamics of the TFR loss

Showing an almost biphasic behaviour pattern, the shape of the curve of the TFR loss is in all studies similar and for certain aspects rather surprising. The vast majority of the molecular recurrences are observed within the first six months from discontinuation, few in the subsequent six months, and very rarely after the first year (Etienne *et al.*, 2017; Ross *et al.*, 2018). Furthermore, using very sensitive methods of measurable residual disease detection by digital PCR, it has been documented that most of the patients who do not relapse after discontinuation of the TKI therapy, show a progressive decrease of the number of BCRABL1 transcripts, which can become undetectable at the end even with a degree of sensitivity of $>MR^5$ (Goh *et al.*, 2011; Ross *et al.*, 2018).

The reasons for the recurrence of the two clearly different types of leukaemia are probably related to the different types of cells responsible for it. The cells responsible for the early relapse after discontinuation are probably residual leukaemic cells (maybe leukaemic progenitor cells), controlled, but certainly not eliminated, by the TKI therapy and certainly endowed with a high degree of clonogenic potential as they are able to reproduce the leukaemic phenotype in a very short period of time (Michor *et al.*, 2005). We do not know at the moment if these cells were already representing the majority of the cell population present at diagnosis, or if they were hidden subclones originated by the natural tendency of the BCR-ABL1 clone to progress and accumulate other

molecular lesions in addition to the BCR-ABL1 rearrangement. Next-generation sequencing (NGS) studies have already provided evidence of this subclonal complexity, particularly apparent in CML patients with a Sokal's high risk score (Branford *et al.*, 2018). An expanded longitudinal genomic analysis at diagnosis, in cases resistant to TKI therapy and at disease transformation will certainly increase our knowledge in this regard (Branford *et al.*, 2019). Extending this analysis to include the patients who lose TFR shortly after discontinuation will certainly be important, but due to the low burden of leukaemic cells present in these cases, some procedures to enrich the leukaemic population will be needed. For this purpose, the selective expression of some surface antigens like CD26 in the Ph⁺ (Philadelphia chromosome positive) population could be very useful (Valent *et al.*, 2014).

Conversely, could the cellular origin of the late relapses show a much lower incidence of the early relapses?

A possible hypothesis is that these cases could really be caused by the persistence of quiescent leukaemic stem cells (LSCs), insensitive to the TKI therapy, but still able in some circumstances to resume their clonogenic activity (Graham *et al.*, 2002; Holyoake & Vetrie, 2017). In this hypothesis, every CML patient who discontinued the TKI therapy could have leukaemia recurrence, if followed-up for long enough – this is consistent with the observations made with A-bomb survivors in which the radiation-induced CML showed a very variable and in certain cases a very long latency time after exposure (Radivoyevitch *et al.*, 2014). Another possibility is that the immune system could keep residual LSCs under control and that a failure of this control could leave LSCs free to re-expand (Tarafdar *et al.*, 2017). Even after transplants from genetically-identical twins, a small anti-leukaemia effect has been observed in CML and this supports the idea that an autologous immunologic control may subsist for CML cells after discontinuation, particularly when the residual disease is small (Gale *et al.*, 1994), but it is apparently in contrast with the observation that the CML incidence is not increased in patients with inherited, congenital or acquired immune deficiency diseases, nor in immune-suppressed recipients of solid organ transplants (Gale & Opelz, 2017).

The biology of the CML LSCs has been thoroughly investigated in recent years and a huge number of papers on this topic have been published (for review see (Holyoake & Vetrie, 2017; Houshmand *et al.*, 2019). So far however, in spite of the large amount of data generated and available in the literature, in part also contradictory, the real role and the behaviour of the LSCs' persistence during the TKI therapy remain elusive. As a consequence, the LSCs' relevance in determining the persistence of CML patients in TFR cannot be established at the moment, but the general hope is that if we are able to identify and quantify CML LSCs more accurately in the future, we will possibly be able to obtain the information needed to provide the TFR option to more patients with CML.

Relevance of the depth and duration of the molecular response

Therapy-free remission studies enrolled subjects with DMR, defined as $\geq \text{MR}^4$ according to the International Scale (Hughes *et al.*, 2006). The recommended duration of the DMR is usually ≥ 2 years (Hughes & Ross, 2016; Hochhaus *et al.*, 2017c; Rea *et al.*, 2018; Shah, 2019; Saglio *et al.*, 2018).

However, in many trials, subjects had a $\geq \text{MR}^{4.5}$ or undetectable *BCRABL1* transcripts. In some studies there was no correlation between depth of molecular response and probability of TFR (Saussele *et al.*, 2018), whereas others using more sensitive detection methods, such as digital PCR, report a correlation (Mori *et al.*, 2015; Ross *et al.*, 2018; Nicolini *et al.*, 2019; Bernardi *et al.*, 2019). However, sustained TFR is also reported in patients with MMR only (Clark *et al.*, 2017; Dragani *et al.*, 2019; Clark *et al.*, 2019). The depth of the molecular response is therefore a requirement, but it does not appear to represent the only determinant element for successful TFR.

Another controversial issue is the correlation between the duration of the deep molecular response and the success of TFR. One study reported a correlation between the duration of DMR and the probability of remaining in TFR (Saussele *et al.*, 2018). However, to determine whether the duration of TKI therapy correlates with the probability of the success of TFR can only be deduced in a randomised trial – consequently, only ongoing studies designed to explore this aspect will allow for recommendations to be made regarding the optimum duration of TKI therapy for an attempt to achieve TFR.

We also know that the faster the initial response, the higher the probability of achieving and maintaining DMR (Marin *et al.*, 2012; Hughes *et al.*, 2014; Hanfstein *et al.*, 2014).

It is likely that depth and rapidity of response are confounded variables, which are correlated with a degree of subclonal complexity and clinical risk score (Pfirrmann *et al.*, 2015). At diagnosis, subclones with additional non-*BCRABL1* mutations may not be detectable because of low variable allele frequencies (VAF). However, increasing numbers and the molecular complexity of these subclones are correlated with rate and depth of response to TKI therapy which is, in turn, associated with the probability of achieving and maintaining a DMR. This vision is also supported by the observations that a delay in starting the TKI therapy after diagnosis, allowing for a longer interval that can favour the natural tendency of the Ph⁺ clone to evolve and to generate resistant subclones, is associated with a lower probability of response (Scerni *et al.*, 2009); a higher degree of subclonal complexity has been found to correlate with the risk of the disease (Branford *et al.*, 2018).

There is however a further degree of complexity of the Ph⁺ clone that we must consider, and at the moment that is not easy to incorporate in a general perspective. In some

cases at least, the BCR-ABL1 rearrangement does not seem to be the primary event responsible for the leukaemia process, but only a secondary event subsequent to a previous lesion of the haematopoietic stem cell compartment which, even in association with a deep BCR-ABL1 molecular response, determines a residual clonal haematopoiesis (Kim *et al.*, 2017). This feature, already envisaged by Fialkow (Fialkow *et al.*, 1981), was already supported by the finding that 4–5% of CML patients who achieved a complete cytogenetic remission (CCyR), show other chromosomal abnormalities (Fabarius *et al.*, 2007; Issa *et al.*, 2017). These molecular defects are those typically found in other examples of Clonal Haematopoiesis of Indeterminate Potential (CHIP), and at the moment these lesions do not appear to influence the outcome of the patients in response to TKI therapy (Kim *et al.*, 2017), but we do not know if they can play a role in influencing the chances of remaining in TFR.

Immune aspects

Some data support a role of the immune system in controlling residual CML cells after stopping TK-therapy. One observation is the progressive decline in concentration of *BCRABL1* transcripts after TKI therapy is discontinued (Goh *et al.*, 2011; Ross *et al.*, 2018).

Alternative explanations are dilution of the leukaemia clone by recovery of normal haematopoiesis, and/or senescence or exhaustion of residual CML progenitor or stem cells. In some instances of TKI discontinuation there is an initial increase in the concentration of *BCRABL1* transcripts followed by a decrease. Again the immune system has been invoked as the aetiology but there are no supporting data.

Interactions of the immune system with CML LSCs are the focus of considerable study (Barrett, 2008; Yong *et al.*, 2008; Yong *et al.*, 2009). The immune system is impaired in newly-diagnosed patients with CML. TKI therapy may restore immune function (Hughes & Yong, 2017) but TKIs are also reported to cause immune suppression (Zitvogel *et al.*, 2016). Mechanisms of immune control associated with response to TKI therapy and with successful TKI discontinuation are reported and, although a clear *cause and effect* has not been demonstrated, there are several reports of a correlation between concentrations of diverse types of natural killer (NK) cells and success of achieving TFR (Hughes & Yong, 2017; Ilander & Mustjoki, 2017; Ilander *et al.*, 2017; Rea *et al.*, 2017; Schutz *et al.*, 2017). Recently, a correlation between innate CD8 + T-cells has also been reported (Caysials *et al.*, 2019).

Another important aspect influencing the CML response and the TFR outcome concerns the haplotypes of killer-cell immunoglobulin-like receptors (KIRs), which endow NK-cells with inhibitory and activating functions (Kelly & Trowsdale, 2017). Patients who are homozygous for the KIR A haplotype have better molecular response and higher rates of successful TFR compared with other KIR haplotypes (La

Nasa *et al.*, 2013; Caocci *et al.*, 2015). The KIR2DL5B genotype reportedly correlates with a lower probability of MMR and DMR in patients receiving TKI therapy, and also in a lower chance of achieving TFR (Yeung *et al.*, 2015; Dumas *et al.*, 2019). These data suggest NK-cell anti-CML activity is modulated by KIR haplotype and polymorphisms of HLA molecules with which they associate. This picture is complicated by the effects of TKIs on the immune system. For example, dasatinib activates cytotoxic lymphocytes and dysregulates Tregs in about 30% of patients with CML. However, the clinical relevance of these effects is still unknown at the moment (Iriyama *et al.*, 2015; Schiffer *et al.*, 2016; Hughes & Yong, 2017). Imatinib has also been shown to have potent immune modulating effects *in vitro* and *in vivo* (Wolf *et al.*, 2007; Hildebrandt *et al.*, 2011).

In contrast to these claims of a role for the immune system in achieving or maintaining TFR, there is little or no increased risk of CML in children with severe combined immune deficiency, in adults with acquired immune deficiency or HIV, or in immune-suppressed recipients of solid organ transplants (Gale & Opelz, 2017).

In conclusion, several observations suggest an involvement of the immune system on the expansion and on the survival of the CML leukaemic cells and in particular of the CML LSCs, but its exact role in different contexts at the moment is not defined and needs further investigation.

Ways to extend the TFR option

Due to the logistical requirements (the first of which is the availability of a reliable and frequent molecular monitoring) and the stringent recommendations for trying TKI therapy discontinuation (CML patients must have a minimum of five years of TKI treatment and a sustained DMR for at least two years), no more than 30–40% of the patients treated with imatinib and 40–50% of those treated with second generation TKIs can be expected to reach the possibility to try to stop the TKI therapy (Hochhaus *et al.*, 2016; Cortes *et al.*, 2016, 2018b). This means that a successful TFR can be estimated in no more than 15% of the patients treated with imatinib and in 25–30% of those treated with second generation TKIs. Therefore, a still rather limited portion of the CML population (and only in countries with a well organised healthcare system) can hope to achieve this goal. To extend the TFR option to a larger percentage of CML patients, some aspects of the TFR phenomenon need to be further clarified and this should only be done combining good biological and translational research with clinical trials, which in part are already ongoing or planned.

In our opinion, the first point to explore is indeed whether the early molecular recurrences that occur in approximately 50% of the patients who discontinue the treatment represents a 'last and extreme' form of resistance to the TKI therapy, due to the regrowth of some subclones of the original Ph+ population endowed with molecular alterations,

which allow them to survive for a long time despite the switching off of BCR-ABL1 TK activity initiated by the TKI therapy. This hypothesis needs to be supported by genomic molecular analysis with NGS studies of the cells raised after discontinuation, with respect to the original population at the diagnosis of the patients losing TFR. A therapy with more potent TKIs, able to overcome mechanisms of resistance and washing out resistant subclones (at least in part) could in theory limit this type of recurrence. At the moment, although good results have been obtained with second generation TKIs, the data do not show a clear superiority of second generation TKIs in increasing the relative rate of successful TFR with respect to imatinib (Hochhaus *et al.*, 2017b). However, as a higher number of patients treated as first-line with second TKIs achieve these conditions, and in a shorter period of time with respect to those treated with imatinib (Hochhaus *et al.*, 2016; Cortes *et al.*, 2016; Cortes *et al.*, 2018b), this is expected to translate to a higher absolute number of patients finally achieving a successful TFR if first treated with second generation TKIs. Furthermore, patients who were not achieving a sustained DMR with imatinib, achieved this degree of response after switching to nilotinib. Therefore they could try to discontinue the treatment, obtaining a successful TFR in approximately 50% of the cases (Hochhaus *et al.*, 2017b; Mahon *et al.*, 2018).

It would be good to see whether even more potent TKIs like ponatinib or asciminib used as first-line therapy could allow a higher number of patients to achieve TFR. At the moment we know only that first-line ponatinib therapy allows a patient to reach a high percentage of DMR in a short period of time, but the trial was suspended due to the toxicity of the drug at the dosage of 45 mg per day, as established in the PACE trial where ponatinib was tested as third-line therapy (Lipton *et al.*, 2016; Cortes *et al.*, 2018b). It would be worthwhile to see if ponatinib at a lower and less toxic dosage would be able to induce a faster and higher achievement of TFR. Asciminib, a new, potent and specific inhibitor with a different mechanism of action in suppressing the BCR-ABL1 TK activity, still awaits testing as first-line therapy, but the possibility to be used not only as a single agent, but also in combination with other TKIs, offers other potential opportunities to explore (Schoepfer *et al.*, 2018).

Trials testing the combination between TKI therapy and a-interferon have been performed in the past and new ones are presently ongoing (Palandri *et al.*, 2010; Simonsson *et al.*, 2011; Nicolini *et al.*, 2015). The rationale of these trials is to test the combination of agents that have been demonstrated to work in CML. However, although we know the TKI function well, we still do not exactly know the mechanisms through which a-interferon is active in CML (Goldman, 2010). One of the many suggested actions is however the idea that a-interferon may stimulate the immune system to control CML cells, including those which are residual when TKI therapy is stopped. At the moment the TFR trials are not conclusive about the role of a-interferon in inducing a

higher rate of successful TFR in patients who were previously pretreated with a-interferon alone, and one of the aspects to solve remains establishing the best timing to introduce a-interferon in combination with TKIs, whether at an early phase of treatment, or later on in patients already in DMR, awaiting discontinuation of the therapy or continuing it as a maintenance therapy (Burchert *et al.*, 2010).

After stopping the TKI therapy in some patients, a slight raise of the BCR-ABL1 transcript has been observed, followed by a subsequent return to lower levels – this could suggest that the previous TKI therapy may in some way also initially delay the onset of an efficacious immune control of the Ph+ cells after discontinuation. The results of the DESTINY trial, in which a complete discontinuation of the treatment was preceded by a 12 month period of gradual treatment-withdrawal, show that for patients stopping the therapy in stable MR4 at 36 months, a TFR rate of 72% – much higher therefore than the results obtained in studies planning an abrupt discontinuation – may indeed support this hypothesis, suggesting also that different approaches and schedules of discontinuation treatment and of the subsequent molecular monitoring, could not only be more convenient for patients and doctors, but also have a higher rate of success (Clark *et al.*, 2019).

Immunotherapy approaches can indeed complement very well what can be obtained by TKIs, as they could also hit targets which could be expressed by quiescent CML leukaemic cells, which are not affected by TKI treatment (Landberg *et al.*, 2018). Several markers distinguishing BCR-ABL1-positive CML cells with respect to their normal counterparts have been revealed, like CD26 (Valent *et al.*, 2014). These markers could be potential targets of immunotherapy approaches, using specific monoclonal antibodies or adoptive cellular therapies like CAR-T cells. However, although we could envisage in the future the development of similar approaches, at the moment proof of absolute safety should be obtained before clinical trials using these types of therapy can be proposed and accepted for patients who are doing very well simply by continuing TKI therapy.

Similar limitations apply also for PD-1/PD-L1 checkpoint inhibitors as well as for other molecules affecting the pathways involved in the stem cells' survival and maintenance, such as the Hedgehog, the Wnt/beta-catenin and the Notch pathways (Holyoake & Vetrie, 2017). Indeed, when tested in CML patients resistant to TKIs, some of these drugs have been shown to be toxic and the related trials were discontinued, making their potential use in CML patients in DMR totally unsuitable.

Conclusions

At present only a restricted number of CML patients can achieve a sustained TFR. Although this goal is particularly appealing for young patients and for those without other comorbidities, we believe that in the future it will

progressively become the therapy goal for all CML patients as long as we are able to develop ways of implementing the rate of successful discontinuation with a minimum discomfort to the patients.

We must also consider however that at the moment the clinical and logistic procedures leading to TFR are rather complex and demanding for both patients and doctors, and is therefore limited to those CML patients being treated in countries with well organised and efficient healthcare systems, whereas for many logical reasons TFR could be particularly appealing to countries of low or medium income, where the CML population is also generally younger.

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