

Short communication

## Who would have thought a single Ki67 measurement would predict long-term outcome?

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### Introduction

Recent data from neoadjuvant studies, predominantly for endocrine therapy but also for chemotherapy, indicate that a single measurement of the nuclear proliferation marker Ki67 made in the breast carcinoma during/after neoadjuvant therapy is strongly predictive of long-term disease outcome. In an era when many mega-parameter signatures have been derived with the aim of improving the accuracy of prediction, it is superficially very surprising that a single immunohistochemical measurement can act in this fashion. This provokes a number of questions, and the following three are addressed below: What is the underlying reason for this predictive ability? Given that Ki67 is influenced by treatment/external factors and is not stable in the short term, why does it predict in the long term? What is the best time for measuring Ki67?

### The data

Ki67 is relatively straightforward to measure in formalin-fixed tissue, being clearly expressed in the nuclei of cells that are actively proliferating. The MIB1 antibody has been a preferred diagnostic for many years, but the relatively new antibody SP6 is now our choice because of its more straightforward applicability to image analysis [1].

The IMPACT (Immediate Preoperative Arimidex, tamoxifen, or Combined with Tamoxifen) neoadjuvant trial of anastrozole versus tamoxifen alone or combined reported that reduction in Ki67 at 2 weeks and 12 weeks was greater for the anastrozole alone arm than for either of the other two arms, mimicking the greater clinical benefit (in terms of increased recurrence-free survival (RFS)) seen in the equivalent adjuvant ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial [2]. Most importantly, follow-up of these patients

indicated that the Ki67 level at 2 weeks was a better predictor of RFS than pretreatment levels, to the degree that, in a multivariate model, the 2-week value of Ki67 remained statistically significant but the pretreatment value was no longer significant [3].

Similar data at the end of neoadjuvant treatment have been published for letrozole and tamoxifen in the PO24 trial [4]. These data are important firstly because they add further validity to changing Ki67 at 2 weeks being an intermediate marker of effectiveness, thereby supporting the use of this marker for drug development and studies of mechanisms of resistance. Secondly, the data indicate that prognostic evaluation with Ki67 may be better after presurgical therapy. Lastly, this observation may extend beyond Ki67 such that multiparameter profiling may be of greater value if conducted in on-treatment samples.

These data have underpinned the development of the recently launched POETIC (Peri Operative Endocrine Treatment for Individualising Care CRUK number CRUK/07/015) trial of the presurgical use or not of a nonsteroidal aromatase inhibitor in 4,000 oestrogen receptor-positive breast cancer patients. This study should provide very good power to evaluate the importance of Ki67 on-treatment to predicting long-term clinical outcome.

A particularly striking set of data has been derived from our studies of a single measurement of Ki67 after neoadjuvant chemotherapy in patients not achieving a pathological complete remission. Patients in the highest tertile of Ki67 in the residual tissue at surgery had a median RFS of only about 18 months, while in the lower two tertiles the median RFS was not reached after 7 years [5].

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RFS = recurrence-free survival.

## What is the underlying reason for the prediction provided by on-treatment Ki67?

Firstly, it is notable that Ki67 at baseline is a highly prognostic factor in breast cancer [6]. Many studies confirm that proliferation is a dominant feature of multigene signatures in breast cancer. For example, in a study of seven molecular modules and clinical variables in 628 oestrogen receptor-positive, HER2-negative breast tumours from public databases, Desmedt and colleagues found that only the proliferation module ( $P < 10^{-11}$ ) and the grade ( $P = 0.01$ ) were significant in multivariate analysis [7].

Secondly, it is clear that the suppression of Ki67 by endocrine treatment is profound but variable between patients, apparently reflecting the variable biological impact of oestrogen deprivation. These variable changes result in a pattern of on-treatment Ki67 in which patients with low Ki67 at baseline largely maintain this low proliferation but patients with higher levels may or may not show suppression to that level.

If these changes were associated with the variable impact of a treatment with modest clinical effects, it is unlikely that the on-treatment measurements would be of much greater value than those at baseline. It is clear from studies such as the Oxford overview analysis [8] and recent adjuvant trials of aromatase inhibitors versus tamoxifen [9] that the impact of these treatments in the overall population is very substantial, however, with the recurrent rate being estimated to be reduced by an aromatase inhibitor in oestrogen receptor-positive patients by approximately 50% over the first 5 years.

## When is the best time to measure Ki67?

The change in Ki67 values over the first 2 weeks is highly correlated with changes that occur at 12 weeks after starting neoadjuvant endocrine therapy. A recent (unpublished) comparison from the IMPACT trial of RFS at 2 weeks or 12 weeks in patients who have both measurements available found that, when assessed as a continuous variable, there was near identical prediction provided by each measurement ( $\chi^2 = 11.5$  and  $\chi^2 = 11.8$  at 2 weeks and 12 weeks, respectively). It is notable nonetheless that some patients do show recovery of Ki67 between 2 and 12 weeks. That this recovery is likely to be meaningful is supported by the latter population being enriched with HER2-positive patients. Further work to establish whether this population has a worse RFS than patients showing persistent Ki67 response at 12 weeks is important to allow the extension of the neoadjuvant model to the study of early acquired resistance, both for the purpose of mechanism study and for development of therapeutics.

## Summary and conclusions

If one is interested in assessing prognosis with no account being taken of the effects of treatment, it would appear that the most appropriate time to measure Ki67 is at baseline. If, however, one is interested in also considering the long-term

outcome on a particular endocrine treatment, measurement at 2 weeks or at 12 weeks appears to be of similar value. No account of pretreatment value is needed in these circumstances. If the response of the tumour to a particular treatment is being evaluated, however, then it is the change between the baseline and an on-treatment value that is relevant. A single on-treatment measurement is insufficient for identifying benefit. Lastly, if one is interested in assessing only acquired resistance, the change between 2 weeks and a later time point of at least 12 weeks is needed for the early recovery of Ki67 levels.

## Competing interests

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