Short-term and long-term prognostic value of histological response and intensified chemotherapy in osteosarcoma: a retrospective reanalysis of the BO06 trial

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Short-term and long-term prognostic value of histological response and intensified chemotherapy in osteosarcoma: a retrospective reanalysis of the BO06 trial

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ABSTRACT
Cure rate models accounting for cured and uncured patients, provide additional insights into long and short-term survival. We aim to evaluate the prognostic value of histological response and chemotherapy intensification on the cure fraction and progression-free survival (PFS) for the uncured patients.

Design Retrospective analysis of a randomised controlled trial, MRC BO06 (EORTC 60931).

Setting Population-based study but proposed methodology can be applied to other trial designs.

Participants A total of 497 patients with resectable highgrade osteosarcoma, of which 118 were excluded because chemotherapy was not started, histological response was not reported, abnormal dose was reported or had disease progression during treatment.

Intervention(s) Two regimens with the same anticipated cumulative dose (doxorubicin 6×75 mg/m²/week; cisplatin 6×100 mg/m²/week) over different time schedules: every 3 weeks in regimen-C and every 2 weeks in regimen-DI.

Primary and secondary outcome measures The primary outcome is PFS computed from end of treatment because cure, if it occurs, may happen at any time during treatment. A mixture cure model is used to study the effect of histological response and intensified chemotherapy on the cure status and PFS for the uncured patients.

Results Histological response is a strong prognostic factor for the cure status (OR 3.00, 95% CI 1.75 to 5.17), but it has no clear effect on PFS for the uncured patients (HR 0.78, 95% CI 0.53 to 1.16). The cure fractions are 55% (46%–63%) and 29% (22%–35%), respectively, among patients with good and poor histological response (GR, PR). The intensified regimen was associated with a higher cure fraction among PR (OR 1.90, 95% CI 0.93 to 3.89), with no evidence of effect for GR (OR 0.78, 95% CI 0.38 to 1.59).

Conclusions Accounting for cured patients is valuable in distinguishing the covariates effects on cure and PFS. Estimating cure chances based on these prognostic factors is relevant for counselling patients and can have an impact on treatment decisions.

Trial registration number ISRCTN86294690.

STRENGTHS AND LIMITATIONS OF THIS STUDY
⇒ By accounting for cured and uncured patients, this study provides additional insights into curing and life-prolonging effects of histological response and chemotherapy intensification.
⇒ In addition to survival probabilities for the entire cohort, as in the traditional framework, the methodology provides estimation of the cure fraction, that is, the chance that a given patient is cured, and of the progression-free survival for the uncured patients.
⇒ The method contributes to understanding the role of chemotherapy intensification, which is not clear when no distinction is made between curing and life-prolonging effects.
⇒ Since the actual received dose intensity differs considerably from the intended one as result of delays and reductions related to toxicity, the prognostic value of the treatment arm might not properly represent the prognostic value of the intensified chemotherapy.
⇒ The patients for whom histological response was not reported are excluded from the analysis.

BACKGROUND
Osteosarcoma is a malignant bone tumour that occurs mostly in children, adolescents and young adults. Surgery alone is insufficient for curation of osteosarcoma patients and the introduction of adjuvant chemotherapy has led to significant improvements in survival.1 2 Current treatment for osteosarcoma includes neoadjuvant chemotherapy in combination with adequate surgery and different treatment schedules have been used in the past to establish an optimal chemotherapeutical design.3 4 5 Recently, in a randomised controlled international collaborative study, the European and American Osteosarcoma Study Group (EURAMOS) concluded that the three-drug regimen, consisting of
methotrexate, adriamycin (doxorubicin) and cisplatin is the standard treatment. In the experimental arms no survival benefit of introducing Interferon-α–2b, an immune-modulator drug, for good responders (GR, ≤10% viable tumour cells after induction chemotherapy), or intensifying treatment by adding Ifosfamide and Etoposide for poor responders (PR, ≥10% viable tumour cells after induction chemotherapy) could be established. With respect to dose intensity, defined as given dose in mg/m² per time unit, a phase-III study (BO06) in osteosarcoma that randomised patients between a conventional dose-intensity (CI) regimen or dose-intensified (DI) regimen with the same drug combination (doxorubicin plus cisplatin) in both arms, showed no difference in outcome. Despite the fact that the group of patients with the DI-regimen had a higher proportion of GR, no better event free or overall survival was seen in this group. It is noteworthy that histological response, as assessed by histological examination of the surgical specimen, is used as a key prognostic factor for survival of patients with osteosarcoma. Among PR, 5-year progression-free survival (PFS) is around 50% and among GR it is over 70%. However, histological response has been a debate as a surrogate marker for outcome in osteosarcoma. So, if this actor is considered to be important, its interpretation is difficult, particularly in relationship to DI, and it is of interest to understand how this, and other factors contribute to better survival of PR.

In osteosarcoma, there is an ongoing debate about the effect of doses and dose intensities, which is an issue for other cancer types as well. All these studies have identified the presence of long-term disease-free survivors among osteosarcoma patients, who are practically considered as ‘cured’. Late relapse after 5 years follow-up occurs only in less than 5% of the patients. This suggests, at the moment that primary treatment is completed, a patient actually belongs to one of two categories: the cured ones and the uncured, who will experience disease progression within their lifetime. However, the identification of cured patients can only be done after a patient has been observed to remain disease-free for many years after treatment. In particular for patients alive and progression-free who have only been followed for a few years, the category to which they belong is not known. Hence, all the patients are usually studied together as one group. However, in the presence of a significant fraction of cured patients, the effect of a treatment or other prognostic factors may relate either to the probability of never experiencing progression or to the time free from progression for the uncured. This distinction is not captured by the traditional methods, that is, it is not possible to identify whether survival has improved since the treatment is curing more patients or because the uncured are living longer. As a result, certain effects might not be identified.

For this reason, cure rate models have started to be adopted in oncological studies as an alternative statistical modelling approach, which by accounting for cured and uncured patients, can provide additional insights into long and short term survival patterns. Through these models, it is possible to identify prognostic factors with a cure or a life-prolonging effect. The mixture cure model simultaneously studies these effects for the combined group of cured and uncured patients by assuming a regression model (typically logistic) for the effect of the prognostic factors on the cure probability and a regression model (eg, Cox) for the PFS of the uncured patients. Hence, compared with commonly used statistical techniques such as the Cox regression model that assume the same model for the survival of all patients, the cure model consists of two components (eg, logistic and Cox). This allows us to estimate not only survival probabilities as in the traditional framework, but also a cure fraction, the chance that a given patient is cured, and the PFS for the uncured patients. Such information may be used to select patients at high risk of progression for more aggressive chemotherapeutical strategies and protect those with high chances of being cured from the toxic side effects.

In this study, the BO06 clinical trial is revisited from a new point of view focusing on two different survival outcomes: the cure fraction and the PFS for the uncured patients. By accounting for cured patients, the prognostic value of histological response and intensification of chemotherapy is evaluated on each of these outcomes with the aim to reveal new insights into the complex nature of such effects.

**METHODS**

**Patients and chemotherapy information**

Data from the MRC BO06/EORTC 80931 randomised controlled trial (RCT) for patients with localised resectable high-grade osteosarcoma, diagnosed between May 1993 and September 2002 were considered. Patients were randomly assigned to the conventional two-drug regimen (Reg-C), consisting of six 3-week cycles of doxorubicin (DOX, 75 mg/m²) and cisplatin (CDDP, 100 mg/m²) or to the DI regimen (Reg-DI), consisting of same doses administered 2-weekly and supported by G-CSF (5 µg/kg daily). The 2-week cycles of Reg-DI correspond to increasing the planned dose intensity of DOX and CDDP by a factor 1.5 compared with the conventional 3-week cycles. For both groups, surgery was scheduled at week 6 since the start of treatment, that is, after two cycles for Reg-C and after 3 cycles for Reg-DI, and postoperative chemotherapy was intended to resume 3 weeks after surgery. However, the received course of chemotherapy was often different from the intended one as a result of delays and reductions related to treatment-induced toxicity. The dataset consists of 497 patients (245 assigned to Reg-C and 252 to Reg-DI) aged 40 years or less. More details about the patients and chemotherapy can be found in the primary publication of the trial.
Patient involvement
There was no patient or public involvement in the original RCT, which was run before this was recognised as good practice, nor in this study; patient and public involvement is not common in methodological studies.

Sample selection and follow-up
The outcome of interest is progression-free survival (PFS), that is, time free from any relapse (including distant metastasis) or death. PFS is computed from end of treatment because cure, if it occurs, may happen at any time during treatment and assuming that patients are either cured or not at early stages postdiagnosis it would not be appropriate. In addition, covariates such as histological response and received dose or DI are not known yet at time of randomisation. End of treatment is computed from the starting date of the last chemo cycle received, by adding the planned duration of the last cycle (14 or 21 days depending on the treatment arm). If the first follow-up visit after treatment occurs before this date, the first follow-up visit is considered as the end of treatment (meaning that the treatment was interrupted or completed earlier than planned). For patients who do not receive any additional chemotherapy after surgery the date of surgery is considered as the end of treatment. Patients who did not receive chemotherapy, reported an abnormal dosage of one or both agents (more than 1.25×prescribed dose), did not have surgery or histological response was not reported, died or had disease progression during the treatment period, were excluded from the original dataset. The consort diagram is given in figure 1. A total of 379 patients were included in the analysis. Since the focus of the study is investigating cure or cancer progression after primary treatment, the overall survival is not further considered in this study because it incorporates also the effects of further treatments after cancer relapse.

Statistical analysis
A mixture cure model is used to assess the association of several variables of interest with cure and survival time for the uncured patients. This model assumes that the probability of survival without progression until time \( t \), given covariates \( x \) and \( z \), is

\[
S(t; x, z) = \pi(x) + \{1 - \pi(x)\} S_u(t; z),
\]

where \( \pi(x) \) and \( S_u(t; z) \) denote the probability of being cured given the covariate \( x \) and the probability of survival without progression until time \( t \) given the covariate \( z \), if the patient is not cured. The covariates of the two components in the mixture cure model can be the same or different, allowing for certain prognostic factors to have an affect only on one of these two outcomes. The probability of being cured \( \pi(x) \) is modelled by logistic regression and the PFS for the uncured patients \( S_u(t; z) \) is modelled by a Cox proportional hazards model. The proportional hazards assumption for the uncured was assessed through a Kolmogorov-type supremum test based on martingale residuals. p values for the estimated univariate and multivariate models were 0.8, 0.85 and 0.71 respectively. A more detailed description about the model is provided in online supplemental material.

We used the R package smcure (V.2.0) to compute the estimates of the parameters and their standard errors in the R statistical software environment. The prognostic values of histological response, allocated treatment and received DI are evaluated through univariate and multivariate mixture cure models. The
effects of the variables on the cure fraction and on thePFS for the uncured patients are summarised throughORs and HRs respectively. An OR larger than 1 meanshigher cure fraction with respect to the reference cate-
gory, while a HR of less than 1 indicates a reduced risk for
the uncured patients (longer PFS). In addition, the Wald-
type CIs are computed at the 95% level. For visual inspec-
tion, Kaplan-Meier curves from end of treatment for the
whole population and stratified according to histological
response and treatment group are also reported.

**RESULTS**

Based on the reverse Kaplan-Meier method, median
follow-up time was 60 months (quartiles 52–67 months,
range 0–116 months) from end of treatment. A total
of 105 patients (57%) from Reg-C and 96 (49%) from
Reg-DI experienced cancer relapse or death (all deaths
were cancer related) during the follow-up period. Only
three patients (1%) experienced disease progression
after 5 years. The largest observed progression time
was 5.3 and 7.7 years in Reg-C and Reg-DI, respectively.
The estimated PFS for all patients (figure 2A) reaches a
plateau around 7 years or earlier, indicating a consistent
fraction of cured patients. There are 178 (47%) censored
patients who were reported alive with no progression at
the last contact. In total 159 patients (93%) from Reg-C
and 172 (88%) from Reg-DI received the complete 6
chemotherapy cycles, while 19 patients (9 and 10 from
Reg-C and Reg-DI respectively) did not receive any chemo-
therapy after surgery. GR was observed in 68 (37%) and
in 97 (50%) Reg-C and Reg-DI patients, respectively. The
Kaplan-Meier estimator from end of treatment, stratified
by histological response, is shown in figure 2B.

The estimated parameters together with 95% CIs and p
values for a univariable logistic/Cox mixture cure model
based on histological response are given in figure 3A.
Histological response is found to be strongly prognostic
for the cure fraction (OR 3.00, 95% CI 1.75 to 5.17) in
favour of GR. For the uncured patients, there is no clear
indication that GR is associated with longer PFS (HR
0.78, 95% CI 0.53 to 1.16). The estimated cure fractions
and PFS at three or 5 years for the uncured patients with
GR or PR are shown in table 1. Apart from separating the
long-term from the short-term effect, the mixture cure
model provides estimation of PFS for cured and uncured
patients combined, as usually done in survival analysis.
The estimated 3-year and 5-year combined PFS are given
in the last two rows of table 1.

Parameter estimates together with 95% CIs and p
values for a univariable logistic/Cox mixture cure model
based on the allocated treatment are given in figure 3B.
There is no evidence of a statistically significant effect of
the allocated regimen on the cure fraction (OR 1.35, 95%
CI 0.80 to 2.29, reference Reg-C). Among the uncured
patients, the HR is 0.88 (95% CI 0.59 to 1.30) in favour of Reg-DI. These estimates suggest that the intensified treatment might be associated with better cure chances and longer PFS, but the CIs are too wide for clear conclusions in both components.

A multivariable cure model is also considered to assess the effect of the treatment group after conditioning on histological response. A logistic/Cox cure model was fitted with allocated treatment, histological response and an interaction term as covariates for the cure model. Only histological response was added to the survival model of the uncured patients to limit the number of parameters that need to be estimated. Parameter estimates together with 95% CIs and p values for the fitted model are provided in figure 3C. GR was found to be strongly associated with good chances of being cured as in the univariate model. Moreover, being allocated to the intensified treatment group (Reg-DI) seems to have a positive effect on the cure fraction among PR (OR 1.90, 95% CI 0.93 to 3.89) reference Reg-C). Among GR, there is no indication for such a positive effect (OR 0.78, 95% CI 0.38 to 1.59, reference Reg-C). The estimated cure fractions according to allocated treatment and histological response are given in figure 4. Slightly lower survival for the Reg-DI group versus the Reg-C group among patients with GR can also be observed in the stratified Kaplan-Meier estimator (figure 2D). For the uncured patients, GR is associated with better PFS (HR 0.78, 95% CI 0.54 to 1.13) compared with the reference group of PR, but not significantly. Estimated PFS curves for the uncured patients with GR

<table>
<thead>
<tr>
<th>Table 1</th>
<th>The estimated cure fractions, PFS at 3 and 5 years for the uncured patients, PFS at 3 and 5 years for cured and uncured patients combined according to histological response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good histological response</td>
<td>Poor histological response</td>
</tr>
<tr>
<td>Cure fraction</td>
<td>55% (46% to 63%)</td>
</tr>
<tr>
<td>3 years PFS if uncured</td>
<td>18% (8% to 29%)</td>
</tr>
<tr>
<td>5 years PFS if uncured</td>
<td>10% (0% to 20%)</td>
</tr>
<tr>
<td>3 years PFS combined</td>
<td>63% (57% to 69%)</td>
</tr>
<tr>
<td>5 years PFS combined</td>
<td>60% (53% to 66%)</td>
</tr>
</tbody>
</table>

Model based 95% CIs are reported.
PFS, progression-free survival.
and PR are given in figure 4. PFS probabilities at 3 and 5 years for the uncured patients are estimated from a univariable model and are as in table 1. Including also the allocated treatment group as a covariate for the survival model of the uncured patients did not show any evidence of an effect of treatment on PFS. A similar behaviour is observed if, instead of the allocated treatment, we use the received postoperative DI defined as in the primary analysis of the trial8 (details can be found in online supplemental material). This sensitivity analysis was performed since the effect of the preoperative treatment can already be captured in the histological response. Prediction accuracy of cure probabilities from the multivariate model is captured in the histological response. Prediction accuracy of cure probabilities from the multivariate model is estimated through a type of Brier score based on inverse probability of censoring weights45 (details can be found in online supplemental material).

DISCUSSION
The aim of the MRC BO06/EORTC 80931 clinical trial was to investigate whether increasing the DI would improve the survival of patients with nonmetastatic limb osteosarcoma. The initial analysis of the trial9 showed that intensified chemotherapy results in higher chances of GR, mainly related to the increased number of cycles and amount of dose received before surgery. However, even though GR, as prognostic factor, is associated with better survival, no significant better survival was shown in the DI-regimen with a higher proportion of GRs, due to a more intensified treatment. This outcome makes the value of this marker debatable, even or even better, makes the interpretation of this marker more complex. Here a new point of view is provided on the same data by using a cure model framework that distinguishes between short-term and long-term effects of covariates.

Previous studies had suggested that a fraction of osteosarcoma patients get cured by treatment, that is, do not experience cancer progression during their lifetime.8 10 However, as a result of censoring, it cannot be determined whether patients with relatively short follow-up and last observed alive without showing signs of the disease are cured or not. Compared with the traditional survival analysis methods, which estimate only the survival for the entire cohort, mixture cure models also allow us to estimate the chances of being cured and the PFS if a patient is not cured. From a clinical point of view, the cure fraction might be more informative than 5-year survival rates, in particular for young patients, being the most common patient group with osteosarcoma. Most importantly, enabling the investigation of separate covariate effects on cure and on PFS for the uncured patients, cure models provide a more detailed information of these effects.21 27 28

This study showed that the prognostic effect of histological response on survival is mainly a result of more patients being cured when they have GR. In other words, the chances of being cured after treatment for GR are considerably higher compared with those for patients with PR, while the effect of histological response on PFS for the uncured patients was not so clear. With a univariate analysis, there is no evidence of a significant effect of the allocated treatment on cure or on PFS for the uncured patients. However, in a multivariate model accounting for histological response and allowing for interaction between the treatment arm and histological response, Reg-DI seemed to have a positive effect on the cure fraction among PR, consistent with the findings of Bishop et al.10 The results suggest that, for GR the intensified treatment might have no effect on cure status, that is, GR in Reg-DI do not have lower chances of being cured than GR in Reg-C, which is also consistent with the findings of Bishop et al.10 One explanation might be the following. Since a GR in Reg-C is less common, given that it has been achieved suggests that these responding osteosarcoma cells do not reflect the self-renewing tumorigenic stem cell, at least less than in poor responsive osteosarcoma, hence have a different biological behaviour with higher chances of getting cured.15 Patients who respond poorly even despite high DI (Reg-DI) have less chemotherapy susceptible osteosarcoma cells with likely a different molecular profile, resulting in worse cure possibilities. In the initial analysis of the trial,9 a multivariate Cox regression model, accounting for histological response, did not find any significant effect for the treatment group or the
interaction term. This might be due to a failure to separate short and long-term effect when cured and uncured patients are considered together as one group with the same survival pattern. Not accounting for the presence of cured patients hides some of the treatment effects. Here the highest cure fraction (59%) is observed among GR in Reg-C, while the lowest cure fraction (23%) corresponds to PR in Reg-C.

The focus of this study was on the prognostic value of histological response and allocated treatment to be in line with the focus of the initial analysis of the trial. Since the actual received DI differ considerably from the intended ones as result of delays and reductions related to toxicity, the prognostic value of the treatment arm might not properly represent the prognostic value of the intensified chemotherapy. Nevertheless, replacing the allocated treatment by the post-operative received DI gave similar results. It is however important to note that, in order to capture different effects on cure and short-term survival, mixture cure models require more parameters compared with the Cox regression model. As a result, they are less powerful in detecting a modest effect and CIs are wider. In addition, we emphasise that for an appropriate use of cure models, it is required to have a long follow-up for a considerable proportion of patients because otherwise it is not possible to correctly identify the cure fraction. In our study, this is confirmed by the plateau in the Kaplan-Meier survival estimator and the medical belief that patients surviving more than 5 years can be practically considered as cured. It would also be interesting to analyse other osteosarcoma datasets using the cure model in order to see whether the obtained results are consistent and yield a better understanding of the effect of different therapeutic options. We are working at the moment with the EURAMOS bone tumour clinical trial. The new insights provided by the mixture cure model are relevant for both patient and clinical perspective. They can help in identifying patients at a higher risk of progression, who might benefit from an intensified chemotherapy, and avoid that patients with good chances of being cured undergo a more toxic and aggressive treatment.

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**Contributors** EM and MF planned and designed the study. EM and MF conducted the statistical analysis and reported the results. EM, NvG, JA, HG and MF interpreted the results. EM drafted the first version of the manuscript. EM, NvG, JA, HG and MF contributed to the manuscript drafting/revision and gave their final approval of the submitted version. MF acts as guarantor.

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**Competing interests** None declared.

**Patient and public involvement** Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

**Patient consent for publication** Not applicable.

**Ethics approval** Permission to recruit patients to the MRC B006/EORTC 80931 protocol was provided by the appropriate national and local regulatory and local committees. Link-anonymised data were used for the purposes of this study, and the use of the data was consistent with the consent taken. Because it involved retrospective review, the project was exempt from Human Subjects protection requirements by the Institutional Review Board of the European Organisation for the Research and Treatment of Cancer.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data availability statement** Data may be obtained from a third party and are not publicly available. The data that support the findings of this study are available from MRC B006/EORTC 80931 collaborators and European Osteosarcoma Intergroup but restrictions apply to the availability of these data, which were used under licence for the current study, and so are not publicly available.

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Supplementary Material

Short- and long-term prognostic value of histologic response and intensified chemotherapy in osteosarcoma - a retrospective reanalysis of the BO06 trial

E. Musta, N. van Geloven, J. Anninga, A.J. Gelderblom, M. Fiocco

Mixture cure model

The idea behind the mixture cure model is to assume that the population consists of two groups: the cured and the susceptible and to combine two different models for simultaneous estimation of the probability of being cured and of the time-to-event if uncured. Two, possibly different, sets of covariates X and Z are used to model the effects of certain prognostic factors separately on cure and time-to-event. If we denote by B an unobserved variable which indicates the cure status, i.e. B=0 if the patient is cured and B=1 otherwise, then the probability of being cured is

\[ \pi(x) = \mathbb{P}(B = 0 \mid X = x) \]

and the survival function of the uncured patients is given by

\[ S_u(t \mid z) = \mathbb{P}(T > t \mid B = 1, Z = z) . \]

It follows that the survival function of a general patient (where the cure status is unknown) is

\[ S(t \mid x, z) = \pi(x) + (1 - \pi(x))S_u(t \mid z) \]

and the survival reaches a plateau

\[ \lim_{t \to \infty} S(t \mid x, z) = \pi(x) > 0. \]

Various models (parametric and non-parametric) can be used for both the components but the most common choice is to assume a logistic model for the cure fraction

\[ \pi(x) = \frac{e^{\gamma'x}}{1 + e^{\gamma'x}} \]

and a Cox proportional hazards model for the time-to-event of the uncured patients

\[ S_u(t \mid z) = \exp(-\Lambda_0(t)e^{\beta'x}) \]

for some parameters \( \beta, \gamma \) and a nonparametric cumulative baseline hazard \( \Lambda_0 \). This model seems to be appropriate for our purposes since it is a good balance between simplicity and flexibility. Note that the proportional hazards assumption is only made at the level of the susceptible patients and not for the whole population. As in the usual logistic and Cox models, the effects of the covariates X and Z are captured through the coefficients \( \gamma, \beta \) and for interpretation purposes we can compute the odds ratio for being cured and the hazard ratio for the risk of uncured patients.

Since the baseline hazard \( \Lambda_0 \) is left unspecified and estimated nonparametrically, for the model to be identifiable we need sufficient follow-up: the Kaplan-Meier estimator reaches a plateau at a time \( \tau \) smaller than the maximum follow-up. In other words, the chances for the event of interest to happen beyond the duration of the study should be negligible. Otherwise, the cure rate cannot be correctly identified. We do not need to specify the time \( \tau \) beyond which the patient can be considered cured, but just to have evidence that there are sufficient observed follow-up times larger than \( \tau \).

Estimation of the model is carried out through the maximum likelihood principle. Differently from the common Cox model, because of the unobserved cure status B, it is not possible to derive explicit expressions for the estimators but an iterative Expectation-Maximization algorithm is used to estimate simultaneously \( \beta, \gamma \) and \( \Lambda_0 \). The standard errors of the estimated parameters and confidence intervals for the survival at 3 and 5 years are obtained via a nonparametric bootstrap procedure. For our analysis we use 1000 bootstrap samples.
**Prediction accuracy**

We consider the multivariate mixture cure model in which histologic response, allocated treatment and an interaction factor are included as covariates for the cure probability model and only histologic response is included as risk factor for the PFS model. We assess prediction accuracy of cure probabilities through a type of Brier score based on inverse probability of censoring weights. As proposed in [43], we estimate the prediction error through 10-fold cross validation. For 50 replications of the cross validation, with random allocation of the 10-folds, the average Brier score is 0.235 with standard deviation 0.003 for the multivariate model compared to the Brier score of 0.289 with standard deviation (0.007) for the null model (without any covariate). This corresponds to a relative Brier score of 1-0.235/0.289=19%, which can be interpreted as about 19% explained variance. Hence, including histologic response and the allocated treatment as covariates helps in prediction of the cure probabilities.

**Received dose-intensity**

Here we evaluate the effect of the received dose-intensity on cure and PFS for the uncured patients accounting for histologic response through a multivariate mixture cure model. Since the effect of chemotherapy intensification during the pre-operative phase is already captured in the observed histologic response, to avoid strong dependencies between variables we consider only the received post-operative dose intensity (Post-DI). As in the initial analysis of the trial, the post-operative dose intensity for both agents (DOX and CDDP) is defined by the following formula

\[
\text{Post-DI} = \frac{\text{Received dose after surgery/Expected dose after surgery}}{\text{Actual duration of post−operative chemotherapy/Expected duration of post−operative chemotherapy}}
\]

where calculation of the expected dose (mg/m²) and duration (days) was based on the protocol for Reg-C, i.e. the expected duration of the post-operative chemotherapy is 84 days (4×3-week cycles) and the expected dose after surgery is 700 mg/m² (4×75 mg/m² DOX + 4×100 mg/m² CDDP). The actual duration of post-operative chemotherapy is computed as the period from the start of the first cycle after surgery until the end of the treatment. The received dose after surgery is computed as the sum of the received doses of DOX and CDDP during all the post-operative chemotherapy cycles, standardized by the body surface area.

For this analysis we further exclude 4 patients, for which at least one of the doses during the post-operative chemotherapy was not reported and 19 patients that interrupted the treatment after surgery, i.e. did not receive any post-operative chemotherapy. As a result, we consider in total 356 patients. Histograms of the Post-DI for each treatment arm and according to histologic response are given in Figure S1. To avoid too many parameters in the model, we consider histologic response, the Post-DI and an interaction factor between them as covariates for the cure status while only the histologic response is used as a prognostic factor for the PFS of the uncured patients. However, including the Post-DI also in the model for the survival of the uncured patients does not find its effect significant. Post-DI is centered at 1, which is the expected post-DI for a patient of Reg-C that receives all the doses as planned. Moreover, a patient of Reg-DI that receives all the doses as planned would have Post-DI=1.5.

We again observe that good histologic response is highly associated with good chances of being cured when Post-DI=1 (Table S1). An increase by 0.5 units in Post-DI seems to have a positive effect in the cure fraction among PR, while among GR we observe an opposite effect (Table S1). However, the latter two effects are not found statistically significant and a larger sample would be needed to confirm the results. The estimated cure rates for different combinations of histologic response and Post-DI are given in Table S2. For the uncured patients, as in the main paper, good histologic response is associated with longer PFS (HR: 0.79 [0.55 - 1.14]) compared to the reference group of PR.
Fig. S1. Histogram of the Post-DI according to treatment arm (A and B) and according histologic response (C and D).

<table>
<thead>
<tr>
<th></th>
<th>Post-DI</th>
<th>Post-DI+0.5</th>
<th>OR [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor histologic response</td>
<td></td>
<td></td>
<td>1.41 [0.61 – 3.25]</td>
</tr>
<tr>
<td>Good histologic response</td>
<td>Post-DI</td>
<td></td>
<td>0.83 [0.37 – 1.85]</td>
</tr>
<tr>
<td></td>
<td>Post-DI+0.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table S1. Odds ratios (OR) together with 95% confidence intervals for the effect on the cure fraction of histologic response and of an increase by 0.5 units of the received Post-DI compared to the standard post-DI=1. A patient of Reg-DI who receives all doses as planned would have Post-DI=1.5.

<table>
<thead>
<tr>
<th>Post-DI</th>
<th>Good histologic response</th>
<th>Poor histologic response</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>58% [41%, 77%]</td>
<td>24% [11%, 39%]</td>
</tr>
<tr>
<td>1</td>
<td>53% [45%, 62%]</td>
<td>30% [22%, 37%]</td>
</tr>
<tr>
<td>1.5</td>
<td>48% [28%, 71%]</td>
<td>38% [17%, 59%]</td>
</tr>
</tbody>
</table>

Table S2. The estimated cure probabilities (and 95% confidence intervals) according to histologic response and Post-DI.