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Studying When to Add Biological Therapy to Cytotoxic Therapy in Advanced Cancer Care Using Real World Data: How to Avoid Design Choices That Generate Immortal Time

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Received: 3 November 2025 | **Revised:** 16 April 2026 | **Accepted:** 7 May 2026

Keywords: clone censor weighting | immortal time | sequential emulation | target trial emulation

ABSTRACT

Background: Since real-world data lack information on treatment assignment (usually the only information available is treatment prescription/administration) and early outcomes can happen in advanced cancer, some design choices may introduce immortal time due to selection or due to treatment strategy misclassification.

Objective: To illustrate design choices that would biassedly introduce immortal time and propose unbiased alternatives, using as a case study the estimation of the effects of different monoclonal antibody (mAb) sequencing strategies on overall survival in colorectal cancer patients.

Methods: We specified a target trial to estimate the effect on overall survival of initiating mAb within 8 weeks of starting first line chemotherapy versus initiating mAb with second line chemotherapy. The first biased design choice would be requiring the initiation of second line chemotherapy for eligibility. This was avoided by aligning eligibility with the initiation of first line chemotherapy. The second biased design choice would be excluding from the first line mAb strategy those who die during

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the grace period without starting mAb. This was avoided separately via cloning, censoring and weighting and by sequential emulation.

Results: There were 1014 eligible patients in GEMCAD 1401. The CCW approach estimated a 4-year survival difference (second—first line mAb) of 4.3% (95% CI) (−3.3%; 10.0%) and a risk ratio (RR) of 0.95 (0.88; 1.04). Sequential trial emulation estimated a survival difference of −2.8% (−7.5%; 1.2%) and a RR of 1.04 (0.98; 1.10). Biased analyses that introduced immortal time estimated an implausible early survival benefit for mAb initiation with second-line (6-month RR of 0.13 (0.03; 0.28)).

Conclusions: Our results are compatible with no effect on survival by mAb sequencing. Misalignment of time zero, treatment assignment, and eligibility introduced immortal time.

1 | Introduction

Treatment for patients with advanced cancer involves receiving drugs in sequences as "lines of treatment", consisting of one or several drugs received for a pre-specified duration or until tumor progression, toxicity, or a decision to stop treatment is reached. When a drug with a new mechanism of action is available as an add-on for cytotoxic therapy, the optimal sequencing may be unknown because pivotal clinical trials usually evaluate its efficacy vs. the standard of care, rather than comparing early use (e.g., to achieve an early control of the tumor) vs. later use (e.g., to delay toxicity). Such is the case for the use of monoclonal antibodies (mAb) for the treatment of advanced colorectal cancer: their effect on improving cancer outcomes compared with the standard of care is well-established [1–5], but the added value of administering them earlier vs. later is unknown.

In the absence of clinical trials to guide decisions on mAb sequencing, causal inference can rely on real-world data (RWD), for example, by emulating a target trial [6]. Causal inference from RWD can be challenging due to features inherent to the data (e.g., unmeasured confounding or measurement error) and due to features related to some design choices, which is the focus of this article. Since RWD lack information on treatment assignment (usually the only information available is treatment prescription or administration) and early outcomes can happen in advanced cancer, some design choices may introduce immortal time due to selection or due to treatment strategy misclassification [7]. Here we use sequencing of mAb in advanced colorectal cancer as a case study to illustrate biased design choices that introduce immortal time, followed by a proposal and implementation of solutions. The manuscript is structured as follows. First, we articulate the research question by specifying the target trial. Second, we identify two design choices that, due to the nature of the research question, would biasedly introduce immortal time. Third, we use RWD (an observational registry of patients with advanced colorectal cancer) to emulate the target trial avoiding the introduction of immortal time and compare the results with those obtained using other biased design choices.

2 | Specification of the Target Trial

To articulate the research question, we outline the protocol for the target trial [6] (see also Table 1) that would estimate the effect of adding mAb to first line chemotherapy versus deferring their addition to second-line chemotherapy on overall survival in patients with metastatic colorectal cancer (mCRC).

2.1 | Eligibility Criteria

Diagnosis of mCRC between June 2014 and June 2018, treatment-naïve for metastatic disease, no previous history of cancer in the last 3 years, an ECOG performance status of 0–2, no history of metastatic resection, and a candidate to start chemotherapy (e.g., FOLFOX, CAPOX, FOLFIRI or FOLFOXIRI).

2.2 | Treatment Strategies

The strategies to be compared would be (1) addition of mAb within 8 weeks (grace period) of initiating first line chemotherapy (provided there is no contraindication, defined as disease progression, worsening of ECOG PS to 3–4, hospital admission, onset of treatment toxicity or adverse events, or a decision to stop cytotoxic therapy during the grace period), ("first line mAb strategy") and (2) deferral of initiation of mAb concomitantly with second line chemotherapy, ("second line mAb strategy"). In both strategies, the duration of mAb treatment would be at the discretion of the treating physician.

2.3 | Treatment Assignment

Patients would be randomly assigned to a strategy at baseline and be aware of the strategy they have been assigned to.

2.4 | Outcomes

Death due to any cause, grade 3–4 toxicity, hospitalization for any cause.

2.5 | Follow-Up

Patients would be followed from the time of treatment assignment (baseline) to the earliest of death, last contact with the registry, or June 2021 (administrative end of study).

2.6 | Causal Contrast

The per-protocol effect of adhering to the specified treatment strategies throughout the study follow-up.

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TABLE 1 | Specification of the target trial protocol components.

Protocol component	Description of target trial	Description of the emulation using the GEMCAD 1401 registry
Eligibility criteria	• Histologically confirmed colorectal metastatic adenocarcinoma • No previous systemic oncologic therapy for metastatic disease • Patients with less than 6-months disease-free interval from adjuvant therapy are eligible[a] • Patients with resectable liver or lung metastatic disease are eligible[a] • No previous history of cancer in the 3 years before inclusion • ECOG performance status 0–2[a] • No history of resection of metastases • Initiator of any of the following chemotherapy regimens: FOLFOX, CAPOX, FOLFIRI or FOLFOXIRI Baseline is the time of initiation of first-line chemotherapy	Same as for the target trial Patients enter the registry when initiating any of the following chemotherapy regimens: FOLFOX, CAPOX, FOLFIRI or FOLFOXIRI
Treatment strategies	1. Initiation of monoclonal antibodies (mAb) on top of first line chemotherapy. Patients must start mAb within 8 weeks (grace period) of starting first-line chemotherapy, unless they develop any of the following contraindications during the grace period: disease progression, worsening of ECOG performance status to 3–4, hospital admission, onset of treatment toxicity or adverse events, or a decision to stop cytotoxic treatment during that time 2. Initiation of mAb on top of second line chemotherapy[b]. Patients must start mAb the week of starting second-line chemotherapy (not earlier), unless they do not have a disease progression, their ECOG performance status worsens to 3–4, they have a surgery or a hospital admission, they develop toxicity or an adverse event, or they make a decision to stop cytotoxic therapy prior to the initiation of second line chemotherapy In both strategies, the duration of mAb treatment will be at the discretion of the treating physician	Same as for the target trial
Treatment assignment	Patients are randomized to the treatment strategy and are aware of the assigned strategy	Same as for the target trial except each individual was assigned to all the treatment strategies at baseline with which their data were compatible
Follow-up	Follow-up starts at the time of treatment assignment (baseline) and ends at the earliest of death, date of last contact with the registry, or administrative end of study (June 2021)	Same as for the target trial
Outcome	Death from any cause	Same as target trial
Causal contrast	Per protocol effect (i.e., effect of adhering to the specified treatment strategies throughout follow-up)	Observational analogue of the per protocol effect
Statistical analysis	Per protocol analysis: Individuals are censored if and when they deviate from their assigned treatment strategy. Inverse probability weighting is used to adjust for baseline and post-baseline prognostic factors associated with adherence. Weights are estimated as a function of baseline variables (age, ECOG performance status, Charlson Comorbidity Index, RAS mutational status, BRAF mutational status, microsatellite instability status, sidedness of primary tumor, primary surgery, number of organs affected by metastasis, number of lesions and size of metastasis in liver, number of lesions and size of metastasis in lung, peritoneal involvement, node involvement, LDH level within normal range), and variables evaluated at each computed tomography evaluation (ECOG performance status, Charlson Comorbidity Index, toxicity, LDH level within normal range)	Same as for the target trial. Deaths in the first 8 weeks are accounted for via 2 approaches: • CCW design: the per-protocol analysis will be conducted in an expanded data set that included 2 clones of each eligible individual, created at the beginning of the grace period • Sequential emulation design: the per protocol analysis will be conducted in a sequence of 8 cohorts, starting at each of the 8 weeks of the grace period

[a]These are major differences between randomized clinical trials comparing mAb vs. no mAb.
[b]Change of therapy line is considered when FOLFOX or CAPOX is changed to FOLFIRI, or FOLFIRI is changed to FOLFOX or CAPOX.

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2.7 | Statistical Analysis

For each outcome, the per-protocol effect would be estimated by fitting a pooled logistic regression model containing an indicator of treatment strategy, a flexible function of follow-up time (restricted cubic splines with knots at times 3, 34, 80, and 169 weeks) and a product term between treatment strategy and follow-up time. This model would be fitted to the data after censoring patients if and when they deviate from their assigned treatment strategy [8]. Specifically, patients in the first line mAb strategy would be censored at the end of the grace period if they have not started mAb by then (in the absence of a contraindication defined as disease progression, worsening of ECOG PS to 3–4, hospital admission, onset of treatment toxicity or adverse events, or a decision to stop cytotoxic therapy during the grace period) and patients in the second line mAb strategy would be censored at the earliest of the following: initiation of mAb before initiating second-line chemotherapy or initiation of second line chemotherapy if mAb is not started the same week. Time-varying non-stabilized inverse-probability weights would be used to adjust for baseline and time-varying confounding [9]. The inverse probability weights would be a function of baseline (age, ECOG performance status, Charlson Comorbidity Index, RAS mutational status, BRAF mutational status, microsatellite instability status, sidedness of primary tumor, primary surgery, number of organs affected by metastasis, number of lesions and size of metastasis in liver, number of lesions and size of metastasis in lung, peritoneal involvement, node involvement, LDH level within normal range) and time-varying covariates (ECOG performance status, Charlson Comorbidity Index, toxicity, LDH level within normal range). The predicted values from these models would be used to estimate the 48-month risk of each outcome under each treatment strategy. The estimated weights would be truncated at the 99th percentile to avoid undue influence of outliers. Risk of grade 3–4 toxicity and of hospitalization would be estimated without elimination of competing events [10]. Effects would be estimated as risk differences and ratios, bounded by percentile-based 95% confidence intervals computed using bootstrapping with 500 samples.

3 | Emulation of the Target Trial

We emulated this target trial using the GEMCAD 1401 registry database (ClinicalTrials.gov identifier: NCT02254941 [11]) which collected data prospectively from patients with advanced colorectal cancer initiating first-line treatment from 47 Spanish centers from June 2014 to June 2018 (1106 patients enrolled). GEMCAD 1401 was designed with several objectives, one of them being the evaluation of different sequences of treatment. The registry collected tumor samples and recorded the following information at baseline and over follow up: demographic and clinical characteristics, tumor response, treatment toxicities (the occurrence of grade 3–4 toxicity, whether it prompted stopping or switching treatments and, if so, the organ affected), hospitalizations and reasons for stopping treatment.

Due to the nature of the specified treatment strategies, the emulation of the target trial can be at risk of creating immortal time due to selection and due to treatment strategy misclassification [7]. We elaborate on these challenges in the corresponding components of the emulation of the target trial.

3.1 | Eligibility Criteria

Same as for the target trial. The time of the application of the eligibility criteria is key to avoid the first challenge, which is the potential for immortal time due to selection. In our target trial emulation, patients assigned to the strategy "initiation of mAb concomitantly with second line chemotherapy" will only have the opportunity to initiate mAb if they have initiated second line chemotherapy (i.e., they have documented disease progression and are fit enough to continue treatment). If we (incorrectly) included in the analysis only those who initiated a second line of treatment, the period between initiating first line chemotherapy (baseline) and initiating second line of treatment would be immortal. The solution to avoid introducing immortal time by design is aligning the application of eligibility criteria with treatment assignment and the beginning of follow-up. In our application, this means that patients who, within the study period, do not start neither mAb in the first line grace period nor a second line of treatment because they die or never present an indication to do so, are compliant with the second-line mAb strategy and should be included and contribute follow-up from the study baseline.

3.2 | Treatment Strategies

Same as for the target trial.

3.3 | Treatment Assignment

Given that GEMCAD 1401 does not contain information on treatment assignment decisions, only on treatment administered, patients need to be classified into treatment strategies according to the latter. This opens the door for the second challenge, which is the introduction of immortal time due to misclassification into treatment strategies. In the real world there can be a waiting (or grace) period from the time a treatment decision is made to the time a treatment is administered, for several reasons. For example, in our case study, mAb may not be initiated at the exact same time as first-line chemotherapy because of administrative delays or a need for approval by payers, for additional testing, or to accommodate further consideration by patients (the length of the grace period, 8 weeks, was proposed by practicing oncologists in the research team based on usual care; Figure S1 shows the distribution of time from initiation of first-line chemotherapy to initiation of mAb). In our target trial, the grace period to add mAb to first-line chemotherapy is 8 weeks, which implies that treatment strategies are not distinguishable at time zero. A form of misclassification of treatment assignment that would introduce immortal time would consist of considering those patients who die during the grace period without initiating mAb as non-compliant with the first line mAb strategy. This would effectively grant immortal time to the remaining patients in the first line mAb strategy, that is, the time it took them to initiate mAb within the grace period.

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For the current emulation, we apply two possible solutions to avoid the introduction of immortal time due to misclassification when using a grace period: cloning-censoring-weighting (CCW) [12, 13] and changing the causal question to make the treatment strategies distinguishable at time zero. CCW consists of assigning patients to all treatment strategies their baseline data are compatible with (in our case, effectively creating two copies of each individual). The second approach consists of comparing treatment strategies that are distinguishable at time zero by reformulating the first-line mAb strategy as "Initiate mAb concomitantly with first line chemotherapy", without a grace period. Therefore, individuals who initiate mAb concomitantly with first line chemotherapy are classified into the first line strategy, and into the second line strategy otherwise. In both approaches, as in the target trial, individuals are censored when they deviate from their assigned strategy and time-varying non-stabilized inverse-probability weights are applied to adjust for this artificial censoring (see Statistical Analysis).

3.4 | Outcomes and Follow-Up

Same as for the target trial.

3.5 | Causal Contrast

Observational analogue of the per-protocol effect, that is, the effect under complete adherence to the treatment strategies.

3.6 | Statistical Analysis

For the analysis in which the research question involves an 8-week grace period to initiate mAb as first line therapy, the analysis is the same as in the target trial except that we created two individuals (clones) per eligible patient and assigned one to each treatment strategy. For the analysis that reformulates the first line mAb treatment strategy to make it distinguishable at baseline (eliminating the grace period), we emulated a series of trials starting at each of the 8 weeks following the initiation of first-line chemotherapy, applying eligibility criteria and classifying individuals into treatment strategies based on information from the corresponding baseline week. The 8 cohorts are pooled for the outcome analysis [14]. Time to event probabilities are standardized to the joint distribution of the baseline covariates. See Supporting Methods for details on the computation of weights and outcome models.

3.7 | Sensitivity Analyses

We assessed the robustness of our estimates to several analytic decisions, including: (1) changing the length of the grace period of the CCW analysis to 4 weeks (vs. 8 weeks in the primary analysis), (2) stabilizing the weights of the CCW analysis to emulate a uniform initiation of mAb during the grace period (i.e., the proportion of eligible patients initiating mAb each week of the grace period is the same [12]), (3) emulating a series of trials starting at each of the 56 days (instead of 8 weeks) following the initiation of first-line chemotherapy and classifying patients into treatment strategies based on information on the baseline day, and (4) truncating weights at the 99.5 and 97.5 percentiles (vs. the 99th percentile in the primary analysis).

3.8 | Biased Analyses Introducing Immortal Time

To visualize the impact of immortal time due to selection bias and due to treatment strategy assignment misclassification on the estimated risk of death in this application, we used a Kaplan–Meier estimator to plot the overall survival of the cohort from eligibility (natural history) and compared it to that in a selection of patients who initiated second-line chemotherapy and in a group of misclassified patients where only those who initiated mAb within the grace period were classified into the first-line mAb strategy (i.e., omitting deaths that prevented first-line mAb initiation). Additionally, we compared the survival of the selection of patients who initiated second-line chemotherapy and mAb concomitantly versus those who received mAb with first-line chemotherapy by adjusting for baseline variables via standardized parametric survival analysis as described above. All analyses were conducted using R version 4.3.1. Code is available at https://github.com/jvilaguilera/GEMCAD1401.

4 | Results

Between June 2014 and June 2018, 1106 individuals initiating first-line chemotherapy for metastatic colorectal cancer were registered in GEMCAD 1401, of whom 1014 were eligible for this study.

4.1 | Emulation of a Target Trial Comparing Strategies Indistinguishable at Baseline via CCW

The 1014 eligible patients contributed 16,617 person-months to the first-line mAb strategy and 8637 person-months to the second-line mAb strategy. Figure S2 shows the selection process. Table 2 shows the baseline characteristics of the eligible patients, and of those who started treatment under each of the treatment strategies. Eligible patients had a median age of 66 years, 92% had an ECOG performance score of 1 or 2, and 20% had more than 2 Charlson comorbidities. Individuals compliant with the first-line mAb strategy were younger and had a better ECOG PS than those compliant with the second-line mAb strategy. Mean follow-up was 16.4 months (min: 1 month, max: 69 months) for individuals compliant with the first-line mAb strategy and 8.5 months (min: 1 month, max: 85 months) for individuals compliant with the second-line mAb strategy. Table S1 describes the censoring reasons per treatment strategy. Table S2 describes the distribution of mAb types.

During follow-up, there were 504 deaths under the first line mAb strategy and 226 under the second line mAb strategy. The estimated 4-year overall survival was 19.6% (95% CI 16.4%; 23.5%) for the first line mAb strategy and 23.9% (17.6%; 30.0%) for the second line mAb strategy, which corresponds to a 4-year survival difference (second—first line mAb) of 4.3% (−3.4%; 10.1%) and a 4-year risk ratio of 0.95 (0.87; 1.04) (Figure 1). The

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TABLE 2 | Baseline characteristics of eligible individuals by treatment strategy. Clone-censor-weighting analysis, GEMCAD 1401 (2014–2018).

	Baseline overall sample $N=1014$	First-line mAb strategy. Individuals who remained uncensored by the end of the grace period. $N=675$	Second-line mAb strategy. Individuals who remained uncensored by the time of second line chemotherapy initiation. $N=306$
Females, *n* (%)	387 (38.2)	261 (38.7)	119 (38.9)
Median age, years (IQR)	65.9 (58.0–72.7)	64.9 (56.9–71.2)	68.1 (61.0–75.6)
ECOG Performance Status, *n* (%)			
0	414 (40.8)	296 (43.9)	97 (31.7)
1	517 (51.0)	339 (50.2)	157 (51.3)
2	83 (8.2)	40 (5.9)	52 (17.0)
Charlson comorbidity index, *n* (%)			
< 3	808 (79.7)	540 (80.0)	247 (80.7)
≥ 3	206 (20.3)	135 (20.0)	59 (19.3)
RAS mutational status, *n* (%)			
Wild type	437 (43.1)	322 (47.7)	109 (35.6)
Mutant	519 (51.2)	315 (46.7)	174 (56.9)
Not evaluated	58 (5.7)	38 (5.6)	23 (7.5)
BRAF mutational status, *n* (%)			
Wild type	532 (52.5)	345 (51.1)	177 (57.8)
Mutant	58 (5.7)	44 (6.5)	16 (5.2)
Not evaluated	424 (41.8)	286 (42.4)	113 (36.9)
MSS/MSI, *n* (%)			
MSI	19 (1.9)	12 (1.8)	6 (2.0)
MSS	417 (41.1)	266 (39.4)	146 (47.7)
Not evaluated	578 (57.0)	397 (58.8)	154 (51.3)
Sidedness primary tumor, *n* (%)			
Left	714 (70.4)	482 (71.4)	204 (66.7)
Right	300 (29.6)	193 (28.6)	102 (33.3)
Primary surgery, *n* (%)	458 (45.2)	296 (43.9)	130 (42.5)
Number of organs affected by metastasis, *n* (%)			
1	482 (47.5)	320 (47.4)	139 (45.4)
> 1	532 (52.5)	355 (52.6)	167 (54.6)
Liver involvement, *n* (%)	754 (74.4)	503 (74.5)	227 (74.2)
Lung involvement, *n* (%)	377 (37.2)	248 (36.7)	117 (38.2)
Peritoneal involvement, *n* (%)	241 (23.8)	175 (25.9)	72 (23.5)
Node involvement, *n* (%)	310 (30.6)	206 (30.5)	104 (34.0)
Number of lesions in liver, *n* (%)			
0	260 (25.6)	172 (25.5)	79 (25.8)
1	107 (10.6)	68 (10.1)	31 (10.1)

(Continues)

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TABLE 2 | (Continued)

	Baseline overall sample $N=1014$	First-line mAb strategy. Individuals who remained uncensored by the end of the grace period. $N=675$	Second-line mAb strategy. Individuals who remained uncensored by the time of second line chemotherapy initiation. $N=306$
2–9	421 (41.5)	280 (41.5)	119 (38.9)
>9	226 (22.3)	155 (23.0)	77 (25.2)
Maximum size of liver metastasis, *n* (%)			
NA	260 (25.6)	172 (25.5)	79 (25.8)
≤5 cm	518 (51.1)	349 (51.7)	146 (47.7)
>5 cm	236 (23.3)	154 (22.8)	81 (26.5)
Number of lesions in lung, *n* (%)			
0	637 (62.8)	427 (63.3)	189 (61.8)
1	68 (6.7)	49 (7.3)	16 (5.2)
2–9	216 (21.3)	142 (21.0)	63 (20.6)
>9	93 (9.2)	57 (8.4)	38 (12.4)
Maximum size of lung metastasis, *n* (%)			
NA	637 (62.8)	427 (63.3)	189 (61.8)
≤5 cm	363 (35.8)	239 (35.4)	109 (35.6)
>5 cm	14 (1.4)	9 (1.3)	8 (2.6)
LDH level within normal range, *n* (%)			
Yes	390 (38.5)	266 (39.4)	101 (33.0)
No	466 (46.0)	295 (43.7)	168 (54.9)
Not performed	158 (15.6)	114 (16.9)	37 (12.1)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; mAb, monoclonal antibody; MSI, microsatellite instability; MSS, microsatellite stability.

estimated 4-year risk of grade 3–4 toxicity was 42.5% (37.5%; 46.6%) for the first line mAb strategy and 33.6% (28.1%; 39.3%) for the second line mAb strategy. The estimated 4-year risks of hospitalization were 63.4% (56.3%; 67.3%) for first line mAb and 63.0% (56.3%; 68.5%) for second line mAb (Table S4).

4.2 | Emulation of a Target Trial Comparing Strategies Distinguishable at Baseline via Sequential Emulation

Figure S3 shows the flowchart of patient selection into the 8 cohorts and Table 3 shows the baseline characteristics of the 4167 eligible individuals (corresponding to 1014 unique patients). Eligible individuals in this analysis had a median age of 67 years, 88% had an ECOG performance score of 1 or 2, and 21% had more than 2 Charlson comorbidities. Individuals in the first line mAb group were on average younger and had fewer Charlson comorbidities than those in the second line mAb group. Mean follow-up was 25.2 months (min: 1 month, max: 69 months) for individuals in the first line mAb group and 24.7 months (min: 1 month, max: 86 months) for individuals in the second line mAb group. Table S1 describes the censoring reasons per treatment strategy.

During follow-up, there were 453 (all unique) deaths under the first line mAb strategy and 1665 (232 unique) under the second line mAb strategy. The estimated 4-year overall survival was 25.1% (20.7%; 28.9%) for the first line mAb strategy and 22.5% (20.3%; 24.3%) for the second line mAb strategy, which corresponds to a 4-year survival difference (second—first line mAb) of −2.6% (−6.8%; 1.9%) and a 4-year risk ratio of 1.03 (0.98; 1.09) (Figure 2).

4.3 | Sensitivity Analyses

The length of the grace period in the CCW analysis is arbitrary, and research questions corresponding to different lengths may be relevant for different decision makers or health care systems. We emulated a target trial using a 4-week grace period to start first line mAb. The estimated 4-year survival difference (second—first line mAb) was 4.3% (−3.2%; 15.1%) and the 4-year risk ratio was 0.94 (0.82; 1.04).

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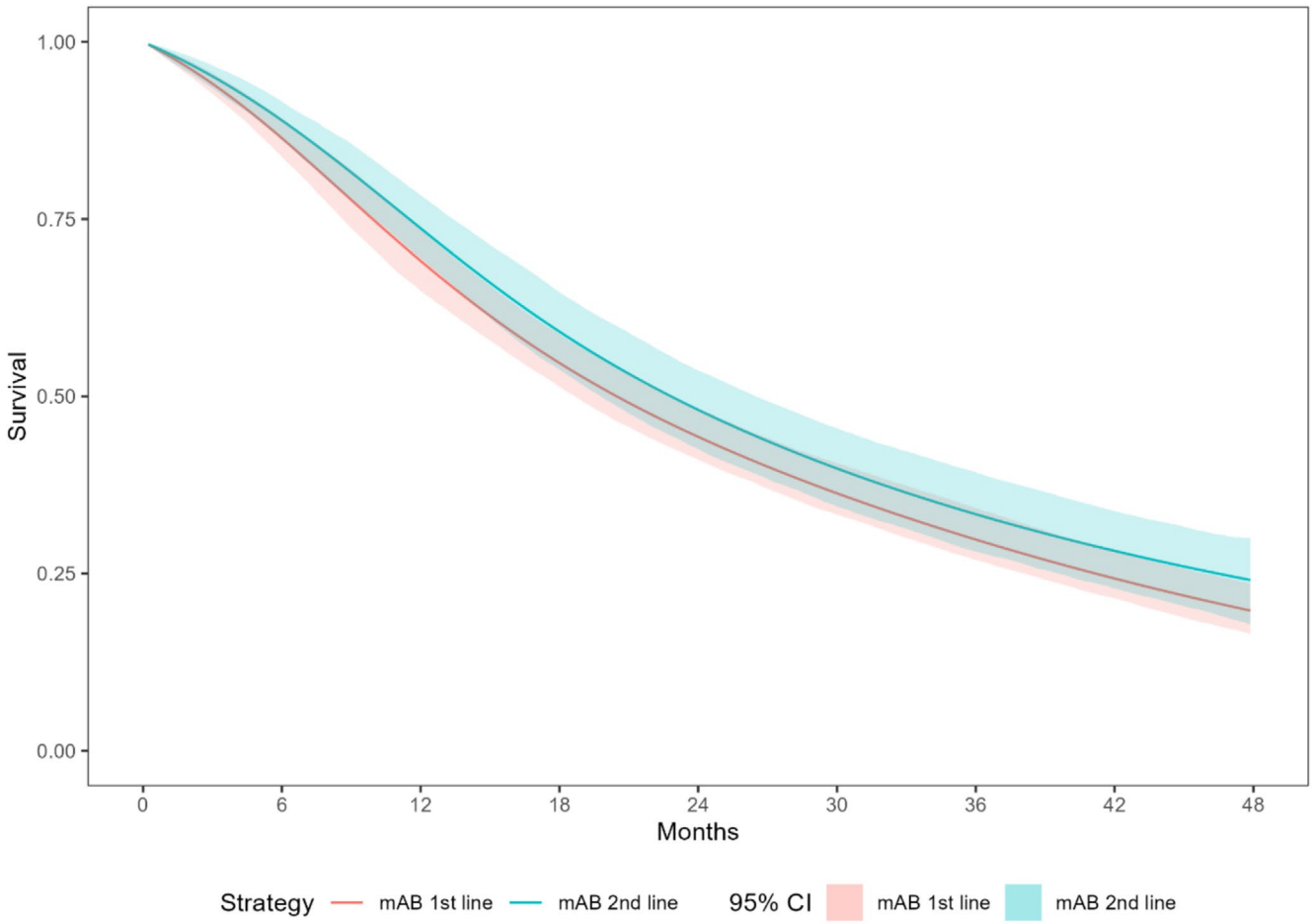

FIGURE 1 | Standardized overall survival curves comparing first line vs. second line monoclonal antibodies, cloning-censoring-weighting method, GEMCAD 1401 (2014–2018).

In the CCW analysis, the first line mAb strategy, which specifies that mAb must be initiated within 8 weeks of starting first line chemotherapy, can have multiple versions, corresponding to all the possible patterns of initiation of mAb during the grace period [12]. The main analysis evaluates a pattern that is the one observed in the data (where most of the patients initiate mAb early in the grace period, Figure S1) and creates a pseudo-population where patients initiate mAb at the end of the grace period if they have not done so by then. We explored a different pattern, consisting of a uniform mAb initiation during the grace period (Supporting Methods). The estimated 4-year survival difference (second—first line mAb) was 8.6% (0.6%; 15.6%) and the 4-year risk ratio was 0.90 (0.82; 0.99). Risks of grade 3–4 toxicity and of hospitalization were materially the same in the sequential emulation analysis as in the CCW analysis.

In the sequential trial emulation analysis, we classified individuals into treatment strategies based on information from the corresponding baseline week. If the risk of the outcome is not negligible in the weekly time interval, this could introduce immortal time because only patients who live through the baseline week could initiate mAB any day of that week. To evaluate whether our choice of 7-day intervals for the sequential emulation was sensitive to this, we repeated the analysis decreasing the interval to 1 day, effectively emulating a sequence of 56 trials. The estimated 4-year survival difference was −2.3% (−5.9%; 2.5%) and the 4-year risk ratio was 1.03 (0.97; 1.09).

Results of the main analysis (both CCW and sequential trial emulation) were robust to different truncations of extreme weights (Table S3).

4.4 | Analyses Introducing Immortal Time

Figure 3 shows the natural course of the 1014 eligible patients, together with the survival (from the study baseline) of patients who initiated mAB concomitantly with second-line chemotherapy ($n = 123$). The latter represents a selection of the eligible patients based on post-baseline information and thus biasedly granted with immortal time. Additionally, Figure 3 depicts the survival of patients who initiated mAb within the grace period ($n = 610$). If individuals who died before the end of the grace period are not considered compliant with the "mAb as first line" strategy, then these patients are misclassified and the "mAb as first line" group would also be granted with immortal time (the time from initiation of first-line chemotherapy to initiation of mAb). In both cases, the introduction of immortal time translates into an apparently early improvement in survival relative to the natural course of the cohort, compatible with bias rather

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TABLE 3 | Baseline characteristics of eligible individuals by treatment strategy. Sequential emulation analysis, GEMCAD 1401 (2014–2018).

	Baseline overall sample $N = 4167$	First-line mAb strategy $N = 610$	Second-line mAb strategy $N = 3557$
Females, *n* (%)	1567 (37.6)	237 (38.9)	1330 (37.4)
Median age, years (IQR)	67.1 (59.7–74.4)	64.5 (56.6–71.0)	67.6 (60.2–74.9)
ECOG performance status, *n* (%)			
0	1513 (36.3%)	287 (47.0%)	1226 (34.5%)
1	2167 (52.0%)	300 (49.2%)	1867 (52.5%)
2	487 (11.7%)	23 (3.8%)	464 (13.0%)
Charlson comorbidity index, *n* (%)			
< 3	3279 (78.7%)	490 (80.3%)	2789 (78.4%)
≥ 3	888 (21.3%)	120 (19.7%)	768 (21.6%)
RAS mutational status, *n* (%)			
Wild type	1578 (37.9%)	299 (49.0%)	1279 (36%)
Mutant	2317 (55.6%)	282 (46.2%)	2035 (57.2%)
Not evaluated	272 (6.5%)	29 (4.8%)	243 (6.8%)
BRAF mutational status, *n* (%)			
Wild type	2222 (53.3%)	317 (52.0%)	1905 (53.6%)
Mutant	205 (4.9%)	38 (6.2%)	167 (4.7%)
Not evaluated	1740 (41.8%)	255 (41.8%)	1485 (41.7%)
MSS/MSI, *n* (%)			
MSI	80 (1.9%)	10 (1.6%)	70 (2.0%)
MSS	1750 (42.0%)	243 (39.8%)	1507 (42.4%)
Not evaluated	2337 (56.1%)	357 (58.6%)	1980 (55.7%)
Sidedness primary tumor, *n* (%)			
Left	2910 (69.8%)	441 (72.3%)	2469 (69.4%)
Right	1257 (30.2%)	169 (27.7%)	1088 (30.6%)
Primary surgery, *n* (%)	1876 (45.0%)	282 (46.2%)	1594 (44.8%)
Number of organs affected by metastasis, *n* (%)			
1	1944 (46.7%)	294 (48.2%)	1650 (46.4%)
> 1	2223 (53.3%)	316 (51.8%)	1907 (53.6%)
Liver involvement, *n* (%)	3116 (74.8%)	452 (74.1%)	2664 (74.9%)
Lung involvement, *n* (%)	1576 (37.8%)	220 (36.1%)	1356 (38.1%)
Peritoneal involvement, *n* (%)	912 (21.9%)	154 (25.2%)	758 (21.3%)
Node involvement, *n* (%)	1318 (31.6%)	182 (29.8%)	1136 (31.9%)
Number of lesions in liver, *n* (%)			
0	1051 (25.2%)	158 (25.9%)	893 (25.1%)
1	462 (11.1%)	61 (10%)	401 (11.3%)
2–9	1713 (41.1%)	261 (42.8%)	1452 (40.8%)
> 9	941 (22.6%)	130 (21.2%)	811 (22.8%)

(Continues)

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TABLE 3 | (Continued)

	Baseline overall sample $N = 4167$	First-line mAb strategy $N = 610$	Second-line mAb strategy $N = 3557$
Maximum size of liver metastasis, *n* (%)			
NA	1051 (25.2%)	158 (25.9%)	893 (25.1%)
≤ 5 cm	2103 (50.5%)	320 (52.5%)	1783 (50.1%)
> 5 cm	1013 (24.3%)	132 (21.6%)	881 (24.8%)
Number of lesions in lung, *n* (%)			
0	2591 (62.2%)	390 (63.8%)	2201 (61.9%)
1	268 (6.4%)	46 (7.5%)	223 (6.3%)
2–9	887 (21.3%)	129 (21.1%)	758 (21.2%)
> 9	421 (10.1%)	45 (7.4%)	375 (10.6%)
Maximum size of lung metastasis, *n* (%)			
NA	2591 (62.2%)	390 (63.9%)	2201 (61.9%)
≤ 5 cm	1508 (36.2%)	215 (35.2%)	1293 (36.4%)
> 5 cm	68 (1.6%)	5 (0.8%)	63 (1.8%)
LDH level within normal range, *n* (%)			
Yes	1557 (37.4%)	246 (40.3%)	1311 (36.9%)
No	2050 (49.2%)	259 (42.5%)	1791 (50.4%)
Not performed	560 (13.4%)	105 (17.2%)	455 (12.8%)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; mAb, monoclonal antibody; MSI, microsatellite instability; MSS, microsatellite stability.

than with a true causal effect of therapy. Adjustment for baseline variables does not eliminate the bias: the 6-month baseline-adjusted RR was 0.13 (0.04; 0.27) (Figure S4 and Table S5).

5 | Discussion

Using the GEMCAD 1401 registry data we emulated a target trial comparing the addition of mAb at earlier vs. later lines of treatment. Our estimates suggest that the addition of mAb to first line chemotherapy treatment compared with deferring their addition to second line did not have a meaningful effect on the 4-year overall survival of patients. We showcase the importance of aligning eligibility and treatment assignment with time zero [14, 15] and propose design solutions to successfully emulate a target trial and avoid introducing immortal time due to selection and due to treatment strategy misclassification [7].

Immortal time due to selection can arise in both RCTs and observational studies when evaluating the effect of different sequences of treatment under complete adherence if patients are assigned to the deferred treatment strategy based on post-baseline conditions (e.g., tumor progression and fitness to initiate a second line of treatment). Specifying the treatment strategy of the target trial and aligning the application of eligibility criteria with time zero avoids immortal time due to selection.

Immortal time due to treatment strategy misclassification, instead, is a problem that is more likely to happen in observational studies than in RCTs because in RCTs information on treatment assignment exists, as opposed to observational studies, where usually only information on prescription, dispensation or administration is available. In research questions, like the current one, involving a grace period to initiate treatment, individuals' baseline data may be consistent with more than one treatment strategy. Scenarios with early events (as can be the case in advanced cancer treatment studies) or early losses to follow-up open the door for the misclassification of patients into treatment strategies. CCW and the reformulation of treatment strategies so that they are distinguishable at baseline are two approaches that avoid the problem of immortal time due to treatment strategy misclassification [7, 15]. The preference of one approach vs. another should be based on whether changing the research question is clinically relevant, and on the strength of the identifying assumptions of each approach, which may differ and will be specific to each application. In our case, the CCW approach assumed exchangeability over the grace period conditional on baseline and time-varying variables, and the sequential emulation approach assumed baseline exchangeability in each of the 8 cohorts, conditional on baseline variables. To illustrate the impact of immortal time due to selection and misclassification, we conducted a biased analysis. This analysis introduced immortal time by imposing post-baseline selection (requiring patients to start second line chemotherapy) and by misclassifying patients into treatment strategies (excluding from the first line mAb strategy those who die in the grace period without starting mAb), yielding implausible early survival benefits.

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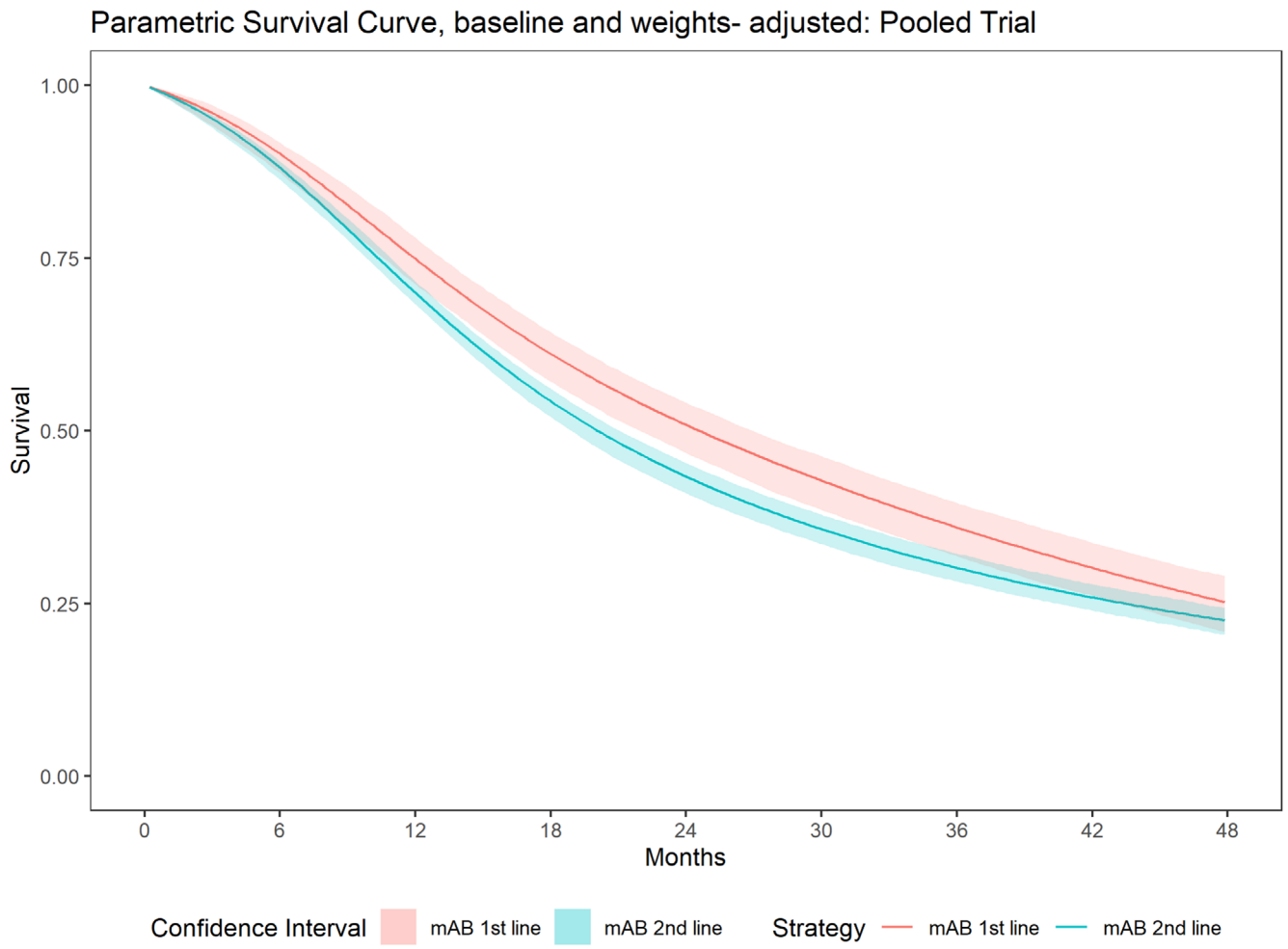

FIGURE 2 | Standardized overall survival curves comparing first line vs. second line monoclonal antibody strategies, sequential emulation method, GEMCAD 1401 (2014–2018).

Another difference between causal analyses conducted in RCTs vs. in observational data is that in RCTs, the positivity assumption holds by design, whereas in RWD, such assumption will only hold if the treatment strategies are represented across all patient subgroups defined by combinations of covariate levels [16]. In the centers contributing data to GEMCAD1401 there was heterogeneity in mAb administration because reimbursement policies for mAb varied by center, which benefited the current analysis by providing different patterns of use in the study population.

In both cases, an explicit specification of treatment strategies as part of the causal estimand is needed to appropriately estimate the effect under complete adherence [17, 18]. The effect under complete adherence can only be estimated if all the clinical scenarios in which treatment is indicated or contraindicated are known. In our application, the occurrence of contraindications for mAb during follow-up was an important factor to assess adherence (e.g., a patient who never received mAb during follow-up is considered compliant with the second line mAb strategy if they develop a contraindication to continue treatment after first line therapy).

Besides its methodological pertinence, this work is clinically relevant because the evaluation of the effects of mAb timing across sequences of treatment is important given the trade-off between early use of mAb to achieve tumor control or later use to delay potential toxicity as shown in our analysis and associated costs. Anti-EGFR mAb (cetuximab, panitumumab) can induce skin toxicity like rashes and pruritus [19], and antiangiogenic mAb (bevacizumab, aflibercept) can induce toxicity in the form of hypertension, thromboembolic events, proteinuria, bleeding, and gastrointestinal perforation [20, 21], which merits careful consideration of treatment strategies that minimize toxicity while maintaining clinical efficacy.

While this study's design choices prevent the introduction of immortal time, the potential for unmeasured confounding, as in any observational study, cannot be ruled out. For example, if doctors deferred mAb initiation in frailer patients and we failed to capture and adjust for frailty, the survival of the second line mAb strategy would appear artificially worse. However, it is reasonable to expect that frailty would also affect the risk of hospitalization for any cause, which was very similar across treatment strategies in our analyses, supporting the assumption of no unmeasured confounding.

In conclusion, our results are compatible with no effect on survival by the sequencing of mAb in patients with advanced colorectal cancer who start treatment for metastatic disease. We

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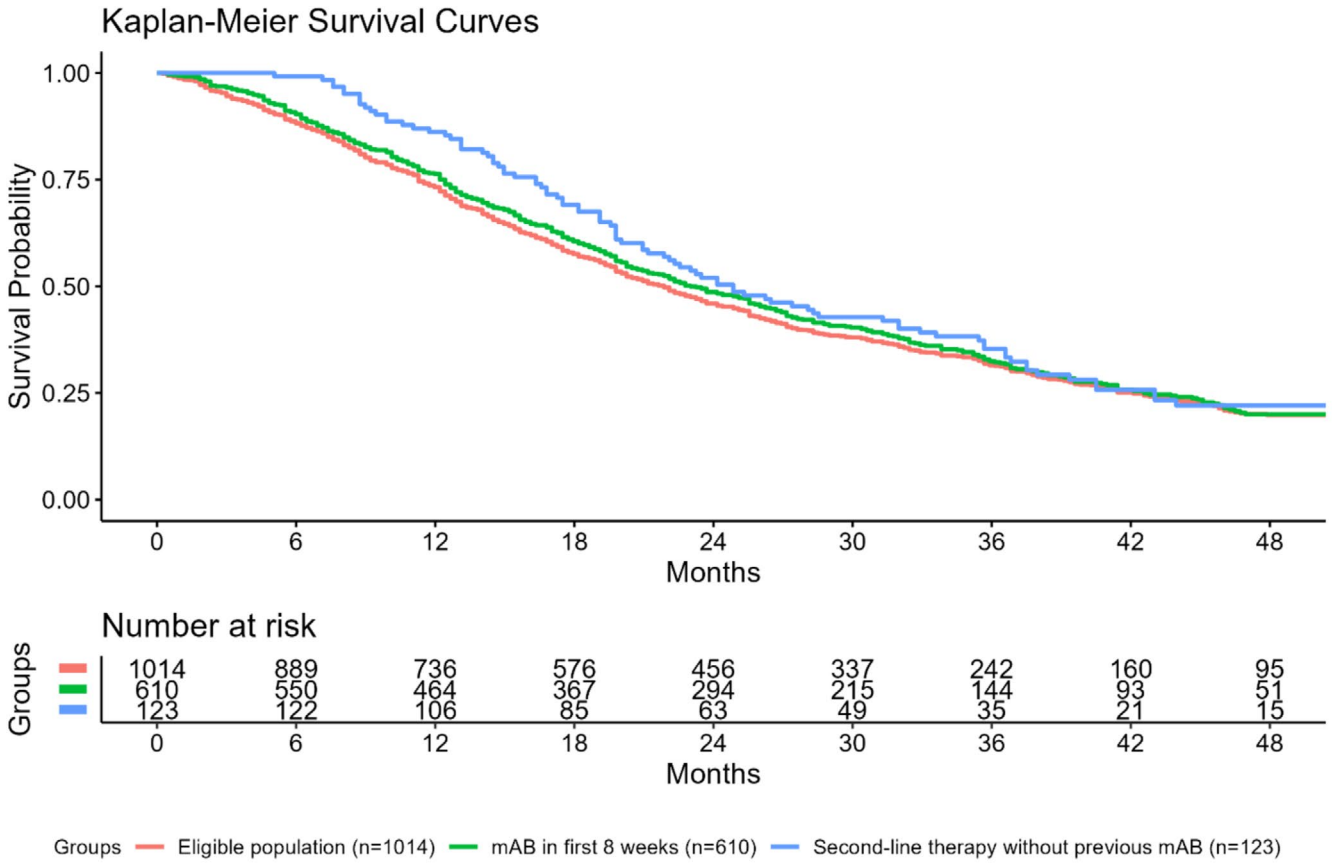

FIGURE 3 | Kaplan–Meier estimation of the natural survival course of eligible patients, among patients who initiated mAb within the grace period, and among a selection of patients who initiated second-line chemotherapy, GEMCAD 1401 (2014–2018).

showcase how aligning time zero and treatment assignment with eligibility avoided introducing immortal time due to selection and using CCW when treatment strategies are not distinguishable at baseline or changing the research question to make them distinguishable at baseline avoided introducing immortal time due to treatment strategy misclassification.

Author Contributions

Xabier Garcia-Albeniz: conceptualization (lead), formal analysis (lead), methodology (lead), supervision (lead), writing – original draft (lead). **Julia Vila-Guilera:** formal analysis (equal), methodology (equal), writing – original draft (equal), writing – review and editing (equal). **Manuel Zamparini:** formal analysis (equal), methodology (equal), writing – original draft (equal), writing – review and editing (equal). **Helena Oliveres:** writing – review and editing (equal). **Vicente Alonso-Orduna:** writing – review and editing (equal). **Jaime Feliu:** funding acquisition (equal), resources (equal), writing – review and editing (equal). **Ana Fernández-Montes:** writing – review and editing (equal). **Marta Martín-Richard:** writing – review and editing (equal). **Elisa Gálvez-Munoz:** writing – review and editing (equal). **Ana Ruiz-Casado:** writing – review and editing (equal). **Alfonso Yubero-Esteban:** writing – review and editing (equal). **Jorge Aparicio:** writing – review and editing (equal). **Julia Alcaide-Garcia:** writing – review and editing (equal). **Javier Gallego Plazas:** writing – review and editing (equal). **Carlos Fernandez-Martos:** writing – review and editing (equal). **Rosa Gallego-Sanchez:** writing – review and editing (equal). **Mónica Guillot Morales:** writing – review and editing (equal). **Rubén Leno:** writing – review and editing (equal). **Francis Esposito:** writing – review and editing (equal). **Sandra Lopez-Prades:** writing – review and editing (equal). **Míriam Cuatrecasas:** writing – review and editing (equal). **Barbra A. Dickerman:** methodology (equal), writing – review and editing (equal). **Joan Maurel:** conceptualization (equal), data curation (lead), funding acquisition (equal), project administration (lead), resources (equal), supervision (equal), writing – review and editing (equal).

Funding

This work was supported by the Spanish Fondo de Investigación en Salud (FIS PI 13/01659).

Ethics Statement

This study was approved by the Ethics Committee of the Hospital Clinic de Barcelona.

Consent

All patients provided written informed consent.

Conflicts of Interest

J.V.-G., M.Z., and X.G.-A. are full-time employees of RTI Health Solutions, a unit of RTI International, a non-profit organization that conducts work for government, public, and private organizations, including pharmaceutical companies. M.G.M. has a speaker role at Servier, MSD, Roche, Merck, and Incyte, she has received accommodation support and travel reimbursement from Servier and MSD, and educational grants from MSD. J.M. has received personal fees from Advanced Medical, Amgen (including travel and congress support), and Incyte Biosciences International; has served on advisory boards for Cancer Expert Now, Delcath Systems, GSK, and MSD; and has a consulting agreement with Incyte; has received institutional research funding as Coordinating PI from Amgen, Guardant, Incyte, Merck, and Terumo; and holds a

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leadership role in GEMCAD. J.G.P. has an advisory and speaker role at Merck, travel expenses funded by Amgen, and an educational grant at Roche and Amgen. F.E. has been employed by AstraZeneca since March 2023, however involvement in this study predates this employment and AstraZeneca had no involvement in the study. A.R.-C. has a speaker role at Amgen, Roche, Sanofi, and Merck, an advisory role in Amgen, and travel expenses funded by Roche. J.A.-G. is an invited speaker at Amgen, Merck, and Servier and provides congress support at Amgen, Merck, MSD, and Servier. J.A. is an invited speaker at Amgen, MSD, Pierre Fabre, Servier, and Takeda. V.A.-O. serves on advisory boards at Amgen, Novartis, Servier, and Merck; has received funding for travel expenses by F. Hoffmann-La Roche Ltd. and Merck.

Data Availability Statement

Data are owned by Grupo Español Multidisciplinar en Cáncer Digestivo (GEMCAD). Please contact GEMCAD with data access requirements at: "secretaria@gemcad.org".

References

1. D. J. Browning, P. K. Kaiser, P. J. Rosenfeld, and M. W. Stewart, "Aflibercept for Age-Related Macular Degeneration: A Game-Changer or Quiet Addition?," *American Journal of Ophthalmology* 154, no. 2 (2012): 222–226, https://doi.org/10.1016/j.ajo.2012.04.020.

2. C. Gravalos, J. Cassinello, P. García-Alfonso, and A. Jimeno, "Integration of Panitumumab Into the Treatment of Colorectal Cancer," *Critical Reviews in Oncology/Hematology* 74, no. 1 (2010): 16–26, https://doi.org/10.1016/j.critrevonc.2009.06.005.

3. M. F. Mulcahy and A. B. Benson, 3rd, "Bevacizumab in the Treatment of Colorectal Cancer," *Expert Opinion on Biological Therapy* 5, no. 7 (2005): 997–1005, https://doi.org/10.1517/14712598.5.7.997.

4. H. Piessevaux, M. Buyse, W. De Roock, et al., "Radiological Tumor Size Decrease at Week 6 Is a Potent Predictor of Outcome in Chemorefractory Metastatic Colorectal Cancer Treated With Cetuximab (BOND Trial)," *Annals of Oncology* 20, no. 8 (2009): 1375–1382, https://doi.org/10.1093/annonc/mdp011.

5. L. B. Saltz, H. J. Lenz, H. L. Kindler, et al., "Randomized Phase II Trial of Cetuximab, Bevacizumab, and Irinotecan Compared With Cetuximab and Bevacizumab Alone in Irinotecan-Refractory Colorectal Cancer: The BOND-2 Study," *Journal of Clinical Oncology* 25, no. 29 (2007): 4557–4561, https://doi.org/10.1200/jco.2007.12.0949.

6. M. A. Hernán and J. M. Robins, "Using Big Data to Emulate a Target Trial When a Randomized Trial Is Not Available," *American Journal of Epidemiology* 183, no. 8 (2016): 758–764, https://doi.org/10.1093/aje/kwv254.

7. M. A. Hernán, J. A. C. Sterne, J. P. T. Higgins, I. Shrier, and S. Hernández-Díaz, "A Structural Description of Biases That Generate Immortal Time," *Epidemiology* 36, no. 1 (2025): 107–114, https://doi.org/10.1097/ede.0000000000001808.

8. M. A. Hernán and J. M. Robins, "Per-Protocol Analyses of Pragmatic Trials," *New England Journal of Medicine* 377, no. 14 (2017): 1391–1398, https://doi.org/10.1056/NEJMsm1605385.

9. M. A. Hernán, E. Lanoy, D. Costagliola, and J. M. Robins, "Comparison of Dynamic Treatment Regimes via Inverse Probability Weighting," *Basic & Clinical Pharmacology & Toxicology* 98, no. 3 (2006): 237–242, https://doi.org/10.1111/j.1742-7843.2006.pto_329.x.

10. J. G. Young, M. J. Stensrud, E. J. Tchetgen Tchetgen, and M. A. Hernán, "A Causal Framework for Classical Statistical Estimands in Failure-Time Settings With Competing Events," *Statistics in Medicine* 39, no. 8 (2020): 1199–1236, https://doi.org/10.1002/sim.8471.

11. ClinicalTrials.gov. Grupo Espanol Multidisciplinario del Cancer Digestivo, "Observational Study to Evaluate the Use of Targeted Therapies in Metastatic Colorectal Cancer. NCT02254941," (2022), https://clinicaltrials.gov/study/NCT02254941?tab=history&a=3.

12. L. E. Cain, J. M. Robins, E. Lanoy, R. Logan, D. Costagliola, and M. A. Hernán, "When to Start Treatment? A Systematic Approach to the Comparison of Dynamic Regimes Using Observational Data," *International Journal of Biostatistics* 6, no. 2 (2010): 18, https://doi.org/10.2202/1557-4679.1212.

13. C. E. Gaber, A. A. Ghazarian, P. D. Strassle, et al., "De-Mystifying the Clone-Censor-Weight Method for Causal Research Using Observational Data: A Primer for Cancer Researchers," *Cancer Medicine* 13, no. 23 (2024): e70461, https://doi.org/10.1002/cam4.70461.

14. X. García-Albéniz, J. Hsu, and M. A. Hernán, "The Value of Explicitly Emulating a Target Trial When Using Real World Evidence: An Application to Colorectal Cancer Screening," *European Journal of Epidemiology* 32, no. 6 (2017): 495–500, https://doi.org/10.1007/s10654-017-0287-2.

15. M. A. Hernán, B. C. Sauer, S. Hernández-Díaz, R. Platt, and I. Shrier, "Specifying a Target Trial Prevents Immortal Time Bias and Other Self-Inflicted Injuries in Observational Analyses," *Journal of Clinical Epidemiology* 79 (2016): 70–75, https://doi.org/10.1016/j.jclinepi.2016.04.014.

16. Y. Zhu, R. A. Hubbard, J. Chubak, J. Roy, and N. Mitra, "Core Concepts in Pharmacoepidemiology: Violations of the Positivity Assumption in the Causal Analysis of Observational Data: Consequences and Statistical Approaches," *Pharmacoepidemiology and Drug Safety* 30, no. 11 (2021): 1471–1485, https://doi.org/10.1002/pds.5338.

17. T. J. VanderWeele, "Concerning the Consistency Assumption in Causal Inference," *Epidemiology* 20, no. 6 (2009): 880–883, https://doi.org/10.1097/EDE.0b013e3181bd5638.

18. M. A. Hernán, "Does Water Kill? A Call for Less Casual Causal Inferences," *Annals of Epidemiology* 26, no. 10 (2016): 674–680, https://doi.org/10.1016/j.annepidem.2016.08.016.

19. J. Li and H. Yan, "Skin Toxicity With Anti-EGFR Monoclonal Antibody in Cancer Patients: A Meta-Analysis of 65 Randomized Controlled Trials," *Cancer Chemotherapy and Pharmacology* 82, no. 4 (2018): 571–583, https://doi.org/10.1007/s00280-018-3644-2.

20. A. Chitkara, N. Kaur, A. Desai, et al., "Risks of Hypertension and Thromboembolism in Patients Receiving Bevacizumab With Chemotherapy for Colorectal Cancer: A Systematic Review and Meta-Analysis," *Cancer Medicine* 12, no. 24 (2023): 21579–21591, https://doi.org/10.1002/cam4.6662.

21. C. Y. Qu, Y. Zheng, M. Zhou, et al., "Value of Bevacizumab in Treatment of Colorectal Cancer: A Meta-Analysis," *World Journal of Gastroenterology* 21, no. 16 (2015): 5072–5080, https://doi.org/10.3748/wjg.v21.i16.5072.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Follow-up, censoring reasons and events, by arm and by study design, GEMCAD 1401 (2014–2018). **Table S2:** Type of monoclonal antibody received in each treatment line, by arm and by study design, GEMCAD 1401 (2014–2018). **Table S3:** Summary of main and sensitivity analyses, GEMCAD 1401 (2014–2018). **Table S4:** Four-year risk of grade 3–4 toxicity and of hospitalization by any cause (total effect), by mAb treatment strategy. Clone-censor-weighting analysis*, GEMCAD 1401 (2014–2018). **Table S5:** Biased analysis due to the introduction of immortal time: overall survival comparing first line vs. second line monoclonal antibodies, GEMCAD 1401 (2014–2018). **Figure S1:** Distribution of time from initiation of first line chemotherapy to initiation of mAb in eligible patients, GEMCAD 1401 (2014–2018). **Figure S2:** Flowchart for selection of eligible individuals, clone censor weight analysis, GEMCAD 1401 (2014–2018). **Figure S3:** Flowchart for selection of eligible individuals, sequential trials analysis, GEMCAD 1401 (2014–2018). **Figure S4:** Standardized overall survival curves comparing first line vs. second line monoclonal antibodies, where immortal time is introduced via selection and treatment strategy misclassification, GEMCAD 1401 (2014–2018).

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