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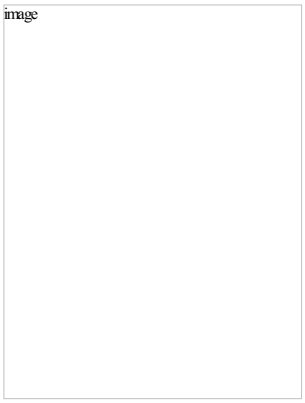

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Differential effects of two therapeutic cancer vaccines on short- and long- term survival populations among patients with advanced lung cancer

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ABSTRACT

Background. Progress in immunotherapy has revolutionized the treatment landscape for advanced lung cancer, with emerging evidence of patients experiencing long-term survivals. The goal of this study was to explore the existence of short-term and long-term survival populations and to assess the effect of immunotherapy on them.

Methods. Data from two randomized, multicenter, controlled clinical trials was used to evaluate the effect of two therapeutic vaccines (anti-idiotypic vaccine VAXIRA and anti-EGF vaccine CIMAVAX) on survival curves in advanced non-small cell lung cancer patients. Data were fitted to Kaplan-Meier, standard Weibull survival and two-component Weibull mixture models. Bayesian Information Criterion was used for model selection.

Results. VAXIRA did not modify, neither the fraction of patients with long-term survivals (0.18 in the control group vs 0.19 with VAXIRA, p=0.88), nor the median overall survival (OS) of the patients in the short-term survival subpopulation (6.8 vs 7.8 months, p=0.24). However, this vaccine showed great benefit for the patients belonging to the subpopulation of patients with long-term survival (33.8 vs 76.6 months, p<0.0001). CIMAVAX showed impact in the OS of both, short- and long- term, survival populations (6.8 vs 8.8 months, p=0.005 and 33.8 vs 61.8 months, p=0.007). It also increased the proportion of patients with long-term survival (from 0.18 to 0.28, p=0.02).

Conclusions. The study shows that therapeutic vaccines produce differential effects on short- and long-term survival populations and illustrates the application of advanced statistical methods to deal with the long-term evolution of patients with advanced lung cancer in the era of immunotherapy.

Keywords: Long-term survival, non-small-cell lung cancer, immunotherapy, survival mixture models

BACKGROUND

Advances in immunotherapy and targeted therapy have revolutionized the treatment landscape for advanced non-small-cell lung cancer, raising survival expectations beyond those historically anticipated with this disease.[1, 2] While with immunotherapy changes in the median survival times have been less dramatic, a change in the shape of survival curves, characterized by a stable plateau at the end of the curve with heavy censoring in the tail has started to be observed. [3] This means that a proportion of patients remain alive even after a long follow up, suggesting the existence of a subgroup of long-term survivors. Such stable plateaus or tails are not captured by standard statistical procedures for the analysis of survival curves that typically assume all study participants are equally susceptible to the event of interest. [3] Median survival differences, for example, may not capture differences in the tail of the curves. Novel models for survival data analysis, such as two-components mixture models - frequently used in statistical literature, although rarely in the evaluation of treatment effects in clinical trials - have been employed to account for the heterogeneous structure of the data. These models could allow investigators to evaluate whether a new therapy is associated with an increase or a decrease in the probability of being a long-term survivor or with an improvement or detriment in survival for those who are in short-term or long-term survival subpopulations.

We have previously shown there exists a subgroup with long-term survival among patients with advanced non-small cell lung cancer (NSCLC) reported by the National Cancer Registry of Cuba. [4] A complex mixture model of two populations, rather than a simpler model assuming only one homogeneous population, explained the overall survival (OS) data. The National Cancer Registry of Cuba study provided evidence of the existence of a long-term survival population, consisting of around 10% of all reported cases, with a survival time greater than 24 months. In the present study, a two-component mixture model was used to explore the existence of two populations among patients with advanced lung cancer and to assess the effect of immunotherapy on short-term and long-term survival populations.

METHODS

Data

Data from two randomized, multicenter, controlled clinical trials in patients with advanced NSCLC were used. The first, a phase II/III trial (hereby referred to as Trial I), evaluated the efficacy of Vaxira, an anti-idiotype vaccine targeting the NeuGcGM3 tumor-associated ganglioside, as switch maintenance therapy. The second, a phase III trial (hereby referred to as Trial II), evaluated the efficacy of CIMAvaxEGF, an EGF-based cancer vaccine compared with best supportive care (Control).

For both clinical trials, eligible patients were those aged 18 years or older with histologically or cytological confirmed stage IIIb or IV NSCLC, and with an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2. All patients had received 4 to 6 cycles of platinum-based chemotherapy before random assignment and had finished first-line chemotherapy at least 4 weeks before entering in the trial. Pregnancy or lactation, secondary malignancies, or history of hypersensitivity to foreign proteins rendered patients ineligible. In Trial I patients were randomized 1:1 to receive racotumomab-alum (group A) or placebo (group B). Randomization was balanced according to gender, clinical stage, performance status, race, and response to first-line treatment. In trial II patients were randomly assigned to the CIMAVAX, or a control group, treated with best supportive care. In both trials the primary endpoint was OS. Additional details of study designs have been reported elsewhere. [5, 6]

The patient recruitment period was from September 27, 2006 to June 28, 2010 for Trial I, and from July 5, 2006, until January 3, 2012, for Trial II. All patients were followed from the date of their inclusion in the study until December 31, 2013. An independent CRO (CENCEC) was responsible for conducting both clinical trials and the follow up for vital status (alive, dead, emigrated or lost to follow up). During the monthly monitoring visits, follow-up information was sought in the patient's medical records. The official database of the hospital contained the date on which a patient was last known to have been alive. The exact date for death was verified from the Cuban mortality registry. The analysis included control group patients in each trial and all patients in the vaccination groups who received at least one vaccine dose. A total of 87, 250 and 202 patients were analyzed in the Vaxira, CIMAvaxEGF and control groups respectively. In both trials, demographic and baseline characteristics were similar in control and treated/vaccination groups.

Modeling approach

Standard survival analysis

The Kaplan-Meier survival estimation to identify the existence or not of a plateau at the end of the curve was done. This is a straightforward way to identify whether a particular dataset might include a subset of long-term survivors.

Evidence for existence of two different populations.

Testing the hypothesis of the existence of a different subgroup of long-term survivors, requires procedures to assess if survival data are fit best by unimodal or bimodal distributions. Various parametric distributions have been considered for the survival function. Among these, we selected the Weibull distribution, a fairly flexible approach that has been found to provide a good description of many types of lifetime data and is widely used in biomedical applications. [7, 8] A standard Weibull parametric survival model (model 0) and two-component Weibull–Weibull mixture model (model 1) were fitted. The survival function for overall population survival time T, was given by,

$$\text{Model 0: } S(t)=W(t \mid \lambda_1,s_1)$$

$$\text{Model 1: } S(t)=\pi_1\, W(t \mid \lambda_1,s_1)+ \pi_2\, W(t \mid \lambda_2,s_2)$$

Where $W(t \mid \lambda, s)$ is a Weibull distribution function. The parameters π_k, (where k=1 or 2), with the restriction that $0 < \pi_k \leq 1$ and $\pi_1 + \pi_2=1$, are the mixing proportions for the kth population. The parameters π_1 and π_2 in model 1 can be interpreted as the proportion of short-term and long-term survivors, respectively. Moreover λ_k and s_k, are the scale and shape parameters, respectively, for the Weibull distribution. To incorporate the effect of treatment in this model, we considered three variants:

I) The vaccine produces an effect on the parameters for the median OS of the populations, but not on the mixing proportion ($\lambda_k= \beta_{0k} + \beta_{1k}\bullet IT_1+ \beta_{2k}\bullet IT_2$; $\pi_1=\text{logit}(z_0)$ and $\pi_2=1- \pi_1$)

II) The vaccine produces an effect on the mixing proportion, but not on the median OS of the populations ($\lambda_2= \lambda_1+ \alpha$; $\pi_1= \text{logit}(z_0 + z_1\bullet IT_1+z_2\bullet IT_2)$ and $\pi_2=1- \pi_1$)

III) The vaccine produces an effect on the parameters for the median OS and on the mixing proportion of the populations ($\lambda_k= \beta_{0k} + \beta_{1k}\bullet IT_1+ \beta_{2k} \bullet IT_2$; $\pi_1=\text{logit}(z_0 + z_1\bullet IT_1+z_2\bullet IT_2)$ and $\pi_2=1- \pi_1$

Where IT_1 and IT_2 are dummy variables for the treatment group (Control: $IT_1=IT_2=0$; Vaxira: $IT_1=1$ and CIMAvaxEGF: $IT_2=1$).

From these parameters the median OS is given by,

$$\text{Median}_k = \lambda_k\, (\log(2)^{(1/S}k^{)})$$

The maximum likelihood estimators of the parameters for the one component Weibull model or all variants of two components mixture models were found by maximizing the likelihood function. All analyses were conducted using the NLMIXED procedure in SAS. We compared the parametric models using the Bayesian information criterion (BIC= $-Log(likelihood)+\frac{p}{2}\log(n)$, where p is the number of parameters and n is the sample size)[9] to find the most probable model for any given data. The model with the smallest BIC value was considered the best fit to the observed data.

For Model 1, where we assume the existence of two populations, the intersection of the estimated density functions has been taken as a cut-point. The mutually exclusive populations defined by this cut- point are called short-term and long-term survival populations. All patients with estimated survival less than this cut-point have been classified in the short-term survival population, while any patient with a survival estimate above the cut-point is considered to belong to the long-term survival population.

Effect of immunotherapies in short- and long-term survival populations.

A novel immunotherapy treatment being evaluated could either have no effect in the parameters for both populations or could modify the proportion of patients belonging to each group, or the median survival of each subgroup, or simultaneously, any of these effects. To assess the effect of the two vaccine therapies, Vaxira and CIMAvaxEGF, we allowed the models parameters to depend on the treatment. **Table 1** summarizes model assumptions and hypothesis testing considered.

<**Insert Table 1**>

Ethics

The trials were approved by local ethics review boards and the Cuban Regulatory Agency. The trials were conducted in accordance with the principles of the declaration of Helsinki and Good Clinical Practice guidelines. They were registered at the Cuban Registry of Clinical Trials (http://www.who.int/ictrp/network/rpcec/en/; Cuban Public Registry of Clinical Trials (Spanish acronym: RPCEC), Trial 1 number RPCEC00000009, Trial 2 number RPCEC00000161)).

RESULTS

Demographic and baseline characteristics of the patients included in the study were similar in all groups (**Table 2**).

<**Insert Table 2**>

Conventional Kaplan-Meier survival analysis

Figure 1 shows the Kaplan-Meier curves for all groups. The median OS of the CIMAvaxEGF group reached 11.2 (95% CI 8.5- 12.2) while the Vaxira and control groups achieved median overall survivals of 8.2 (95% CI, 5.6–10.9); and 7.5 (95% CI 6.9- 10.9), months, respectively. Ten patients from the Vaxira, 10 patients from control groups and 38 patients from the CIMAvaxEGF group were alive at the end of the study and were consequently censored. There were significant global differences in survival between the treatment groups using log-rank test ($p< 0.0001$).

<**Insert Figure 1**>

Evidence of the existence of two different populations.

The analyses carried out consistently confirmed there is a subgroup with long-term survival. First, the BIC for the two-component Weibull-Weibull survival model decreases by more than 100 compared to the one-component Weibull model (BIC: 3613 vs 3736). The more complex two–components model is therefore the preferred model, thus suggesting the existence of two populations of patients. Second, the proportion of long term survivors was significantly larger than zero (π_2=0.2, $p<0.001$). Finally, the difference between the median OS estimated for short- and long-term survival populations ($Median_1$=7.8, $Median_2$=52.8) was also significantly greater than zero ($Median_1$- $Median_2$=45.1, $p<0.0001$).

The differential effects of therapeutic vaccines on short and long-term survival populations.

Of all the models used to evaluate the effect of the vaccine therapies, the best fit was attained for variant III of model 1, that considered the impact of the vaccines simultaneously on the proportion of the long-term survivors and on the median survival of short- plus long-term survival populations (Variant I: BIC=3609, Variant II: BIC=3608 and Variant III: BIC=3601). However, both evaluated vaccine therapies, CIMAvaxEGF and Vaxira differentially impacted the overall survival of patients. On one hand, the Vaxira group and

control group had the same proportion for long-term survivors, indicating that administration of this vaccine did not increase the proportion of the population with long-term survival. Although no significant differences were observed in the median OS of the short-term survival populations of the Vaxira and the control group, the median OS of the long-term survivors was statistically significantly longer for those receiving Vaxira with an increase in OS from almost 3 years in the control group to more than 6 years in the Vaxira group. On the other hand, CIMAvaxEGF induced an increase of the proportion of patients with long-term survival from 0.18 to 0.28, and at the same time led to a significant increase in the median OS of both the short- and long-term survival populations (**Table 3**).

<**Insert Table 3**>

The density distribution survival curves for short-term and long-term survival populations for all groups are shown in **Figure 2**. The density peak at around 5 months for the short-term survival population in all groups indicates that most patients died early. However, in the long-term survival population the density is flattened. The figure shows that none of the patients in the short-term survival population survived more than 24 months for all the groups, whereas more than 50% of patients in the long-term survival population are still alive.

<**Insert Figure 2**>

Finally, **Figure 3** shows the Weibull mixture model survival curves by group. Note that, although for the short-term survival population few differences are observed among the curves, for the long-term survivors an impact of the vaccines can be seen clearly.

<**Insert Figure 3**>

DISCUSSION

The results of the present study can be interpreted from three different points of view: the existence of a subgroup of long term survivors within the population of patients with lung cancer, the differential effects of therapeutic vaccines on different patient subgroups, and the inadequacy of current statistical methods to deal with the transition of advanced cancer to a chronic disease.

Amongst patients with lung cancer those with long-term survivals, defined as surviving more than two years after a diagnosis of advanced disease, comprise only 4 to 6% of all patients. [10] [11] Our study confirms that among patients with advanced lung cancer there is a subgroup that survives long-term. Amongst our 337 patients with stage IIIb-IV NSCLC who had received a first line chemotherapy and were treated with a vaccine on a clinical trial, 86 (25%) were alive after 2 years. This survival is somewhat greater than that reported in some previous studies of long-term survivors (12.8% in the study of Satoh et al.[12]; 15.9% in the study of Giroux et al.[10]; and 16.1% in the study of Lee et al.[13], but, is similar to the 2-year OS rate in the study of Kaira et al.[11], although many patients with tumors harboring EGFR mutations had been treated with gefitinib as either a second or third line therapy after receiving a cytotoxic regimen. While all these studies show evidence for the existence of long-term survivors, all used conventional statistical methods in their analysis. Mixture survival models such as the ones we have used have been widely used in the statistical literature but are infrequently used in clinical research and this limits the comparability of our findings.

In our study, the fit of two components mixture models to the data of clinical trials with two different therapeutic vaccines allowed us to evaluate separately the effects of the vaccines on the short-term and the long-term survival populations. It was shown that these vaccines could have differential impact on the survival of cancer patients, either increasing the median survival of each subpopulation or increasing the proportion of patients in the long-term survival subpopulation. In our case, the anti-idiotypic vaccine VAXIRA did not increase the percentage of patients surviving long-term, nor statistically improve the median survival time of the patients in the short-term survival subpopulation. However, this vaccine showed great benefit for the patients belonging to the long-term survival subpopulation. On the other hand, the anti-EGF vaccine CIMAVAX behaved differently, showing an impact in the median OS of both subpopulations, and also increasing the proportion of patients with long-term survival. To our knowledge, this is the first study showing evidence of differential effects of a vaccine therapy on the short-term and long-term survival subpopulations.

At the present time, it is very difficult to interpret these finding in terms of cellular and molecular biology. The interaction of the tumor with the immune system is highly complex, and includes diverse effector mechanisms such as antibodies, CD4 and CD8 T-cells, NK cells and macrophages, [14] together with diverse ways through which the tumor can escape immune rejection, such as regulatory T-cells, myeloid-derived suppressor cells, immunosuppressive cytokines, and mediators of chronic inflammation.[15, 16] It is to be expected that these processes have different kinetics, and that different vaccines impact them in diverse ways. T-cell mediated adaptive immunity is thought to be a major mechanism of antitumor immunity. However, some tumors demonstrate a robust infiltration of T-lymphocytes, whereas others show a minimal infiltration or none. In contrast, innate immunity cells such as macrophages, granulocytes, and immature myeloid cells are almost universally seen infiltrating tumors. [17, 18]

The phenomena of antigen spreading adds further complexity to the process, because the immune system could react not only to the antigen introduced by the vaccine itself, but to other tumor antigens which are presented to the system as cancer cells undergo immunogenic apoptosis.[19] The kinetics of toxicities is illustrative, as some antitumor antibodies (such as anti-Her1 or anti-Her2 antibodies) targeting tumor cells directly show immediate cytotoxic effects, whereas antibodies targeting regulatory loops including anti-checkpoint antibodies, such as nivolumab, the onset of adverse events could take weeks or months.

Although we considered data from two randomized, multicenter controlled clinical trials, this re-analysis was unplanned and retrospectively conducted. Standard statistical methods such as log-rank and Cox proportional hazard model, have been declared not ideal for immunotherapy trials in which it is known that delayed clinical effects may occur.[20, 21, 3] An alternative approach would be to use two mixture distribution models, where one can interpret the two components as defining two groups with different intrinsic mortality patterns. These models allow separate interpretation of the effect of the therapy on the mixing proportion and on the failure time distribution of the short- and long- term survival populations via two sets of regression coefficients. Further research is needed to explore how the treatment effect and the short-term and long-term division of the population depend on the distribution assumed in the survival model.

As survival rates continue to improve, long-term survival is becoming an increasingly important endpoint when planning clinical trials in oncology. The potential benefit of immunotherapy to increase the proportion of patients in the long-term survival subpopulation should be specifically considered. Additionally, the duration of the plateau at the end of the

survival curves could be an important outcome to evaluate the long-term impact of immunotherapies. Further research is needed to characterize patient subpopulations and their correlation with biomarkers, and to explore the impact of vaccines and other immunotherapies in their outcomes. Finally, this study illustrates the need to refine and improve statistical methods to deal with potentially different subpopulations among patients with advanced lung cancer when analyzing clinical trial data. Novel statistical methods could be useful tools to monitor the transformation of advanced cancer into a chronic disease, and to design clinical research intending to accelerate it.

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Conflict of Interest The authors declared that they have no conflict of interest

Authors' contributions

LS, PL, and AL conceived the study, participated in data analysis, and drafted the manuscript. PCR, SA, EN and TC conceived and design the clinical trials and participate in the clinical interpretation of the data. CV and PL participated in data management and quality control of data. LS, LM and ZS developed the methodology and Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis). All authors participated in the interpretation of the data and critically revised subsequent drafts of the manuscript.

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Table 1: Hypotheses tested for each model

Variant[A]	Hypothesis	Explanation	Mean structure	Mixing proportions
I	H_0: β_{1k}, β_{2k}=0, k=1,2	There is an effect of the therapy on the parameters for median overall survival, but not on the mixing proportion parameters.	$\lambda_k = \beta_{0k} + \beta_{1k} \bullet IT_1 + \beta_{2k} \bullet IT_2$ k=1,2	π_1=logit(z_0) π_2=1 - π_1
II	H_0: α=0 H_0: z_1, z_2=0	There is an effect of the therapy on the mixing proportion parameters, but not on the parameters for median overall survival.	$\lambda_2 = \lambda_1 + \alpha$	π_1=logit(z_0 + $z_1 \bullet IT_1 + z_2 \bullet IT_2$) π_2=1 - π_1
III	H_0: β_{1k}, β_{2k}=0 H_0: z_1, z_2=0	There is an effect of the therapy on the mixing proportion parameters and on the parameters for median overall survival.	$\lambda_{1k} = \beta_{0k} + \beta_{1k} \bullet IT_1 + \beta_{2k} \bullet IT_2$ k=1,2	π_1=logit(z_0 + $z_1 \bullet IT_1 + z_2 \bullet IT_2$) π_2=1 - π_1

[A]To incorporate the effect of treatment in this model, we considered three variants

Definition of terms:

H_0, the null hypothesis

IT_1 and IT_2 are dummy variables for the treatment groups (Control: IT_1=IT_2=0; Vaxira: IT_1=1 and CIMAvaxEGF: IT_2=1)

k=1,2 represent the short- and long-term survival populations

π_1 and π_2 = the proportion of short-term and long-term survivors, respectively; (where k=1 or 2), with the restriction that $0 < \pi_k \leq 1$ and $\pi_1 + \pi_2 = 1$, are the mixing proportions for the kth population.

λ_k and s_k, are the scale and shape parameters, respectively, for the Weibull distribution

Table 2. Demographic and clinic characteristics of the patients

Characteristic	Control (*N*=202)	Vaxira (*N*=87)	CIMAvaxEGF (*N*=250)	*P*-value
Gender				
Male	132 (65.3%)	59 (67.8%)	165 (62.4%)	0.92
Female	70 (34.7%)	28 (32.2%)	85 (37.6%)	
Age				
≤60	90 (45.6%)	38 (43.7%)	112 (44.8%)	0.86
>60	107 (54.4%)	49 (56.3%)	138 (55.2%)	
Race				
White	148 (73.2%)	70 (80.5%)	174 (69.6%)	
Afro	29 (14.5%)	11 (12.6%)	42 (16.8%)	0.35
Other	25 (12.3%)	6 (6.9%)	34 (13.6%)	
ECOG				
0	75 (37.1%)	40 (46.0%)	96 (38.4%)	
1	111 (54.9%)	45 (51.7%)	135 (54.0%)	0.33
2	16 (7.9%)	2 (2.3%)	19 (7.6%)	
Disease stage				
IIIb	132 (65.3%)	48 (55.1%)	155 (62.0%)	0.26
IV	70 (34.7%)	39 (44.9%)	95 (38.1%)	

Table 3: Parameters of Weibull distribution and estimated median survival times and mixing proportions for the short-term and long-term survival populations

Group	*N*	Short term survival population					Long term survival population						
		N_1	λ_1	S_1	Median$_1$	*P*-value[a]	π_1	λ_2	S_2	Median$_2$	*P*-value[a]	π_2	*P*-value[b]
Control	202	164	8.71	1.49	6.8	-	0.82	44.1	1.53	33.8	-	0.18	-
Vaxira	87	70	9.9	1.49	7.8	0.24	0.81	98.7	1.53	76.6	**<0.001**	0.19	0.88
CIMAvax EGF	250	180	11.3	1.49	8.8	**0.005**	0.72	80.1	1.53	61.8	**0.007**	0.28	**0.02**

N_1 *and* N_2 = Number of patients in the short-term and long-term subpopulations, respectively

π_1, π_2 = Mixing proportions of patients in the short- term and long-term survival populations, respectively

λ_1,S_1 and λ_2,S_2 = Parameters of Weibull distribution

[a] *P*-value of the differences in OS median between vaccine and control group

[b] *P* -value of the differences in the proportion of long-term survivors between each vaccine therapy group and the control group

VITAE

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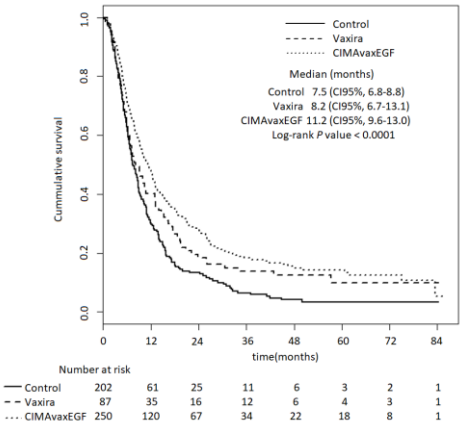
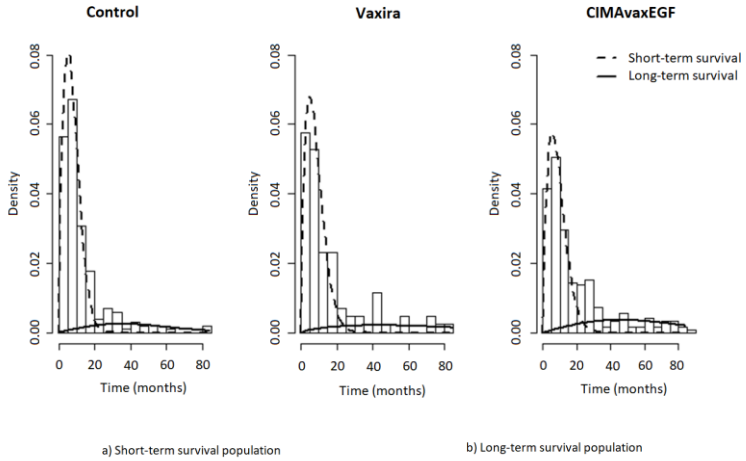
Conflict of Interest The authors declared that they have no conflict of interest





a) Short-term survival population

b) Long-term survival population